BEHAVIORAL HEALTH EFFECTS OF PESTICIDES EXPOSURE AMONG CHILDREN LIVING IN PATHUMTHANI PROVINCE THAILAND

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ผลจากการรับสัมผัสสารกำจัดศัตรูพืชต่อพฤติกรรมของเด็กในพื้นที่เกษตรกรรม จังหวัดปทุมธานี ประเทศไทย

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จุฑาสิริ โรหิตรัตนะ : ผลจากการรับสัมผัสสารกำจัดศัตรูพืชต่อพฤติกรรมของเด็กในพื้นที่เกษตรกรรม จังหวัดปทุมธานี ประเทศไทย. (BEHAVIORAL HEALTH EFFECTS OF PESTICIDES EXPOSURE AMONG CHILDREN LIVING IN PATHUMTHANI PROVINCE THAILAND) อ.ที่ปรึกษาวิทยานิพนธ์หลัก: ผศ. ดร.วัฒน์สิทธิ์ ศิริวงศ์, อ.ที่ปรึกษาวิทยานิพนธ์ร่วม: ศ. ดร.แนนซี่ ฟีลด์เลอร์, ศ. ดร.มาร์ค เกรกอรี่ ร็อบสัน, 146 หน้า.

อัตราการเกิดโรคสมาธิสั้นในเด็กไทยกำลังเพิ่มขึ้นอย่างต่อเนื่อง ปัจจัยเสี่ยงของการเกิดโรคอาจมีสาเหตุมาจากกรรมพันธุ์และสิ่งแวดล้อม ในพื้นที่สังคมเกษตรกรรมมีการใช้สารกำจัดศัตรูพืชกลุ่มออร์กาโนฟอสเฟตและไพรีทรอยด์อย่างกว้างขวาง ซึ่งสารเหล่านี้อาจเป็นปัจจัยที่มีผลต่อระบบประสาทพฤติกรรมในเด็ก ซึ่งปัจจุบันยังขาดงานวิจัยทางระบาดวิทยาและงานวิจัยเชิงลึก วัตถุประสงค์ของงานวิจัยนี้ เพื่อหาความสัมพันธ์ระหว่างอาการสมาธิสั้นและการรับสัมผัสสารกำจัดศัตรพืชกลุ่มออร์กาโนฟอสเฟตและไพรีทรอยด์ในเด็กที่อาศัยอยู่ในพื้นที่เกษตรกรรมนาข้าวและเลี้ยงสัตว์น้ำ งานวิจัยแบบภาคตัดขวาง โดยมีการเก็บข้อมูล 3 ครั้ง คือ (1) การทดสอบนำร่องเพื่อทดสอบเครื่องมือ (2) การเก็บตัวอย่างในช่วงที่มีการใช้สารกำจัดศัตรูพืชมาก (ฤดูฝน) และ (3) ช่วงที่มีการใช้สารกำจัดศัตรูพืชน้อย (ฤดูแล้ง) ผู้เข้าร่วมเด็กจำนวน 53 คนอายุระหว่าง 6-8 ปีถูกทดสอบสมาธิต่อเนื่องด้วยวิธี Continuous Performance Test (CPT) โดยใช้โปรแกรมคอมพิวเตอร์ Behavioral Assessment and Research System (BARS) ร่วมกับผู้ปกครองเด็กจะตอบแบบสอบถาม Conners Attention Deficit/Hyperactivity Disorder (ADHD) เกี่ยวกับพฤติกรรมของเด็ก พร้อมเก็บตัวอย่างปัสสาวะและเลือดของเด็กเพื่อหาสารกำจัดศัตรูพืชที่ได้รับสัมผัส (dialkylphosphate; DAP, 3-phenoxybenzyl alcohol; 3-PBA, acetylcholinesterase; AChE, และ pseudocholinesterase; PChE) ผลการศึกษาพบว่าแม้จะตรวจพบความเข้มข้นของสารอนุพันธ์กลุ่มออร์กาโนฟอสเฟตในปัสสาวะของเด็กที่อาศัยในพื้นที่นาข้าวมากกว่าเด็กที่อาศัยในพื้นที่เพาะเลี้ยงสัตว์น้ำอย่างมีนัยสำคัญในทุกครั้งที่เก็บตัวอย่าง (Mann-Whiney U test, $p<0.05$) แต่ผลการทดสอบระบบประสาททางพฤติกรรมส่วนใหญ่ไม่มีความแตกต่างกันทั้งในระหว่างกลุ่มและฤดู จากการวิเคราะห์ความถดถอยโดยมีการปรับตัวแปรที่อาจมีผลร่วม ได้แก่ อายุ ระดับการศึกษาของผู้ปกครอง และรายได้ของครอบครัว ไม่พบความสัมพันธ์อย่างมีนัยสำคัญระหว่างความเข้มข้นของสารอนุพันธ์กลุ่มออร์กาโนฟอสเฟตในปัสสาวะและระดับคลอรีนเอสเตอเรสในเลือดของเด็กกับอาการสมาธิสั้น อย่างไรก็ตามงานวิจัยนี้เป็นการทดสอบนำร่องของงานวิจัยพฤติกรรมของเด็กที่อาศัยอยู่ในพื้นที่เกษตรกรรมและได้ทดสอบความเที่ยงตรงของเครื่องมือทดสอบอาการสมาธิสั้นในเด็กโดยวัดจากผลของ CPT และ Conners ADHD ($r=0.29$, $p=0.03$) ซึ่งสามารถนำไปประยุกต์ใช้ในงานวิจัยในอนาคตที่ควรมีระยะเวลาการศึกษาที่เพิ่มขึ้นและเพิ่มจำนวนกลุ่มประชากร เพื่อจะสามารถเห็นผลกระทบต่อเด็กที่รับสัมผัสสารกำจัดศัตรูพืชในระยะยาวได้ชัดเจนยิ่งขึ้น

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ปีการศึกษา 2556 ลายมือชื่อ อ.ที่ปรึกษาวิทยานิพนธ์หลัก ....

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ลายมือชื่อ อ.ที่ปรึกษาวิทยานิพนธ์ร่วม ....

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KEYWORDS: CHILDREN / BEHAVIORAL HEALTH EFFECTS / ATTENTION DEFICIT/HYPERACTIVITY DISORDER (ADHD) / ORGANOPHOSPHATE (OP) / PYRETHROID (PYR)

JUTHASIRI ROHITRATTANA: BEHAVIORAL HEALTH EFFECTS OF PESTICIDES EXPOSURE AMONG CHILDREN LIVING IN PATHUMTHANI PROVINCE THAILAND. ADVISOR: ASST. PROF. WATTASIT SIRIWONG, Ph.D., CO-ADVISOR: PROF. NANCY FIEDLER, Ph.D., PROF. MARK GREGORY ROBSON, Ph.D., 146 pp.

The prevalence of attention deficit/hyperactivity disorder (ADHD) is rising in Thai children. The possible causes of this developmental disorder include environmental and genetic risk factors. Organophosphate (OP) and pyrethroid (PYR) are insecticides popularly used in agricultural areas. The epidemiological evidence on their potential neurobehavioral effects in children is lacking. This study aimed to clarify the relationship of levels of OP and PYR exposure and ADHD behaviors compared between children living in rice farming area and children living in aquacultural farming area. The cross-sectional study was done in 3 sessions: pilot, high (wet season) and low (dry season) pesticide use periods. Participants (N=53) aged between 6-8 years old were recruited. The first morning void of urine samples and blood cholinesterase were collected. Participants assessed the continuous performance test (CPT) form the Behavioral Assessment and Research System (BARS) and their parents completed the Conners ADHD questionnaires. Although the concentrations of urinary OP metabolites in participants living in rice area were significantly higher than participants living in aquacultural areas (Mann-Whiney U test, $p<0.05$), most of neurobehavioral health effects were not different between groups in every season. From the multiple linear regression (adjusted for age, parent education, and family income), both concentrations of urinary OP metabolite (dialkylphosphate; DAP), urinary PYR metabolite (3-phenoxybenzyl alcohol; 3-PBA), and blood cholinesterase levels (acetylcholinesterase; AChE and pseudocholinesterase; PChE) were not significantly associated with CPT scores and ADHD symptoms. However, this study showed the validity of the behavioral tests by the significant correlation between CPT and Conners questionnaires ($r=0.29$, $p=0.03$) as a reflection of ADHD behaviors. As a pilot study of research project on behavioral health effects of children living in agricultural area in Thailand, the longitudinal study with larger study population should be conducted on potential neurobehavioral effects of long-term exposure of OP and PYR in children.

Field of Study: Public Health	Student's Signature ....
Academic Year: 2013	Advisor's Signature ....
	Co-Advisor's Signature ....
	Co-Advisor's Signature ....

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CHAPTER I

INTRODUCTION

1.1 Background and rationale

Agricultural sectors play an important role in Thailand. With increasing food demands, agricultural practices need to be intensive. Due to the high application of pesticide use for agricultural purposes, the pesticides are widely used throughout Thailand and dispersion to nearby residential areas is common (1). Organophosphate (OP) insecticide is one of the most popular agents used for crop protection due to its broad spectrum toxicity. OPs are known as nervous system toxicants or cholinesterase inhibitor. This mode of action leads to over stimulation of nerve function and neurotransmitter inhibitors.

Urinary metabolites are biomarkers for OP pesticides. The urinary dialkylphosphate (DAP) metabolites of OP pesticides have been quantified in human urine as a dosimeter for exposure and bodily adsorption of pesticides. The six common DAP metabolites are dimethylphosphate (DMP), diethylphosphate (DEP), dimethylthiophosphate (DMTP), dimethyldithiophosphate (DMDTP), diethylthiophosphate (DETP), and diethyldithiophosphate (DEDTP).

Another pesticide group serving as a substitute for OPs is the pyrethroid group (PYR) because it is less toxic to mammals when compared to OP. PYR is easily broken down in the environment, especially when exposed to the sunlight (2). However, there are some cholinergic activities relating to PYR exposure in animal tests (3).

PYR half-life in blood or plasma ranges from 2.5 to 12 hours which are less accumulated than OP compound. The PYR metabolite forms in urine are 3-PBA, DCCA, Br_2CA, etc depend on the parent compounds (4).

Children can be exposed to pesticides and other hazardous chemicals through multiple pathways and by multiple routes (5, 6). Levels of pesticide exposures in agricultural communities have a strong association with difference in seasonal pesticide application periods (7). Farm children are more likely to experience high exposure to pesticide spraying in dry relative to the wet season (8). The distance from sites where pesticides are applied and households seem to affect the concentration of pesticide metabolites in children. Children who have a parent working in agricultural fields had significantly higher pesticide metabolite concentrations than children whose parents work in other occupations and had higher take-home exposure of pesticide from farmer parents (9, 10). Therefore, children living in agricultural area tend to have more pesticide exposure.

Children living in agricultural areas are exposed to pesticide residues via inhalation, ingestion and dermal contact. Playing activities both indoors and outdoors may enhance pesticide exposure. Mouthing behavior in young children with toys or environments (e.g. soil eating) may increase exposure to the deposited pesticides on the object surfaces (11).

In Thailand, there are plenty of pesticide applications not only for agricultural purposes but also for pest control in households. Especially in rice farming communities, the depression of blood cholinesterase levels were significantly associated with farmers (12). However, children are more vulnerable to pesticide exposure than adults because they are still developing. Most pesticide research has been done among adults with direct exposure to the pesticides but only few studies focused on indirect exposure, especially in children (6, 13, 14).

Because OP and PYR disrupt cholinergic signaling, they are hypothesized to be associated with attention-deficit/hyperactivity disorder (ADHD) (15). In an animal study, OPs were shown to cause hyperactivity and cognitive deficits (16). OP exposure is associated with delays in mental development, behavioral problems and poor short-term memory and motor skill among children (17-19). Only few studies have evaluated the association between urinary DAP levels and ADHD behavior (20) and the continuous performance test (CPT) in children (21). Low subclinical levels of chlorpyrifos were associated with persistent long-term cognitive dysfunction and defects including concentration and short-term memory (22).

Very few studies have addressed the health effects of OP and PYR exposure among children living in agricultural areas in Thailand. For example, some studies reported that children are routinely exposed to OP pesticides in Thailand (1), but there have been no studies of the potential health effects of this exposure especially related to neurobehavior (23). The acute and chronic neurobehavioral effects of pesticide exposure among children will be examined with emphasis on behaviors associated with ADHD.

This study will help to clarify the relationship between blood cholinesterase and urinary OP and PYR metabolite levels and ADHD behaviors in children by the appropriate ADHD evaluation tools (e.g. CONNERS questionnaires and CPT).

1.2 Research objectives

1.2.1 Main objective

- To compare ADHD behavior within subjects at three different time points (low, high, and low pesticide use)

1.2.2 Specific objective

- After control for covariates, determine the association between levels of cholinesterase in blood and OP and PYR metabolites in urine with ADHD behavior in children.
- To investigate the relationship between concentrations of pesticide metabolites in urine samples and participants' environments and activities.

1.3 Research hypothesis

1.3.1 Group main effect (rice participants vs. aquacultural participants)

H_0: Rice farming participants will show similar levels of pesticide exposure and ADHD symptoms to aquacultural farming participants

H_A: Rice farming participants will show significantly greater levels of pesticide exposure and ADHD symptoms than aquacultural farming participants

H_1: Rice farming participants will show significantly greater concentrations of OP and PYR metabolites in their urine and significantly lower cholinesterase in blood than aquacultural farming participants.

H_2: Rice farming participants will have significantly more ADHD symptoms than aquacultural farming participants and perform significantly worse on the continuous performance test.

H_3: Environment and activities of rice farming participants will have significantly increased the concentrations of pesticide metabolite in urine samples.

1.3.2 Within subject time effect (time 1: low pesticide use; time 2: high pesticide use; time 3: low pesticide use)

H_0: Participants will report similar ADHD symptoms regardless of season.

H_A: Participants will report significantly different ADHD symptoms with regard to season.

H_1: In the same participants, ADHD symptoms will have significantly different in Conners and CPT scores with regard to season.

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1.3.3 Group x Time interaction

H_0: Rice farming participants at high pesticide use period will have similar ADHD symptoms and levels of pesticide exposure to aquacultural farming participants. No differences between rice farming participants and aquacultural farming participants will be seen at low pesticide use periods.

H_A: Rice farming participants at high pesticide use period will have significantly greater ADHD symptoms and levels of pesticide exposure than aquacultural farming participants.

H_1: Rice farming participants at high pesticide use period will have significantly greater ADHD symptoms and more errors on CPT than aquacultural farming participants.

1.4 Scope of This Study

The behavioral health effects in this study refer to the attention deficit and hyperactivity disorder (ADHD) because its prevalence in school-aged children are rising in Thailand.

The levels of OP and PYR exposure are of interest in this study due to their high intensity use in rice farming areas.

1.5 Expected Benefit from this study

Although it might be difficult to detect ADHD symptoms of pesticide exposure in this study due to small sample size and limited sampling times, the present study will provide an appropriate tool for screening of ADHD symptoms in the future.

The results from this study will be useful for more understanding of the OP and PYR exposure pathway of children living in agricultural area. If the results show high risk of OP & PYR pesticide exposure, the recommendation to reduce the risk will be advised to protect children’s health.

The neurobehavioral tests can determine how pesticide exposure affects cognitive function and mental health of children. The acute effect, assumed in high pesticide use period, can be detected by OP & PYR metabolite in urine. Moreover, the chronic effect can be determined by showing the continuous neurobehavioral deficits. This result can raise awareness and be used to reduce the risk from long-term pesticide exposure of children living in agricultural areas.

1.6 Conceptual Framework

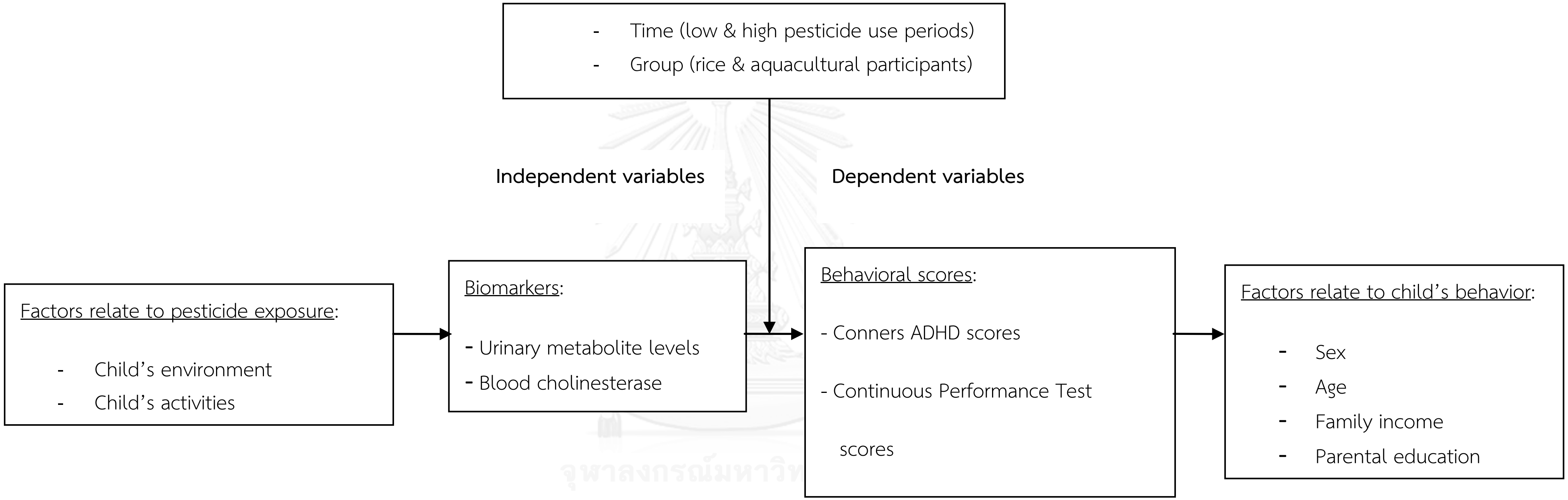

Figure 1 Conceptual framework of pesticide exposure and health effects in children living in agricultural area, Pathum Thani Province, Thailand.

1.7 Operational Definition

Terms	**Definitions**
Rice farming participants	Children participants who living on rice growing areas at Khlong Luang District, Pathum Thani Province, Thailand
Aquacultural farming participants	Children participants who living on fish/shrimp farming areas at Lum Luk Ka District, Pathum Thani Province, Thailand
Pilot session (dry season)	First sampling time in March 2011, low OPs application period on rice farming areas
High session (wet season)	Second sampling time in October 2011, high OPs application on rice farming areas
Low session (dry season)	Third sampling time in April 2012, low OPs application period on rice farming areas
Continuous performance test (CPT)	A computer program in the Behavioral Assessment and Research System (BARS) which uses to assess sustained attention.
The Conners 3-Parent (Conners 3-PS)	The short form of the Conners 3 Content questionnaire for screening ADHD symptoms including inattention, hyperactivity/impulsivity, learning problems, executive functioning, defiance/aggression, and peer relations.
The Conner 3 ADHD Index (Conner 3 AI-Parent)	The screening questionnaire that use to differentiate ADHD patient from normal children
ADHD behavior	The symptoms characterized by inattention, hyperactivity, and impulsivity
Pesticide exposure	Levels of OP and PYR in biomarkers
Biomarkers	Urinary pesticide metabolites and blood cholinesterase

1.8 Abbreviations

Abbreviations	Definitions
3-PBA	3-phenoxybenzoic acid
AChE	Erythrocyte cholinesterase
ADD	Absorbed daily dose
ADHD	Attention Deficit/Hyperactivity Disorder
BMI	Body mass index
BW	Body weight
Conners 3AI	The Conners 3 ADHD Index
Conners 3 PS	The Conners 3-Parent (short form)
CPT	Continuous performance test
DAPs	Dialkylphosphates
DCCA	*Cis*- and *trans*-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane-1-carboxylic acid
DEDTP	Diethyldithiophosphate
DEP	Diethylphosphate
DETP	Diethylthiophosphate
DMP	Dimethylphosphate
DMDTP	Dimethyldithiophosphate
DMTP	Dimethylthiophosphate
GM	Geometric mean
OPs	Organophosphate pesticides
LOD	Limit of detection
µg/L	Microgram per litre
µg/g Cr	Microgram per gram creatinine
PChE	Plasma cholinesterase
PYR	Pyrethroid insecticides
RSD	Relative standard deviation
TCPy	3, 5, 6-Trichloro-2-pyridinol

CHAPTER II

LITERATURE REVIEWS

Agriculture is a primary occupation in Thailand. In 2012, agricultural sector shared 12.2% of GDP (24). There were 14.88 million agriculturists or 38.7% of labor force in Thailand (25). Rice is a major crop that can be cultivated all year. Thailand was the top rice exporter in the world with 8 million tons (World Markets & Trade, 2011). Hence, the intensive production to increase the rice yield was enhanced by numerous agrochemicals. There were several studies about pesticide contaminations in Khlong 7, Pathum Thani Province. Organochlorine pesticides were previously reported as residues in canal and aquatic food chain. These contaminations were not only accumulated in environment but also increased the risk to the residential health (26-28). Organophosphorus and pyrethroid insecticides were heavily used in both major rice and second rice cultivations (29). Raksanam et al. (2012) reported that rice farmers in Khlong 7 were unaware of proper and safe pesticide handling. The risk behavior and contaminated environment can enhance the pesticide toxicity not only in applicators but also in residential population in rice farming community.

2.1 Pesticide information

2.1.1 Organophosphorus insecticides (OP)

Organophosphorus insecticides (OP) were discovered by English and German scientist groups led by B. C. Saunders and Gerhard Schrader in 1940. The initial aims to synthesize OP with the esterification of alcohols to phosphoric acid were used as chemical warfare agents. Later, OP was developed to broad spectrum insecticides which are also highly toxic to mammals. In 1970, over 200 OP insecticides were available in worldwide markets (33).

Most of OP are lipophobic and easily degrade in the environment. They do not persist in human tissue. The OP metabolize through oxidation, reduction, hydrolysis and conjugation reactions. Some OP metabolites are known as anticholinesterases. The bioactivation by cytochrome P450 enzymes are responsible for desulfuration and cause neurotoxicity. Other important pathway is the metabolite forms in urine. Dialkylphosphate (DAP) metabolites are mostly found in urine after OP exposure. The DAP metabolites show in Figure 2.

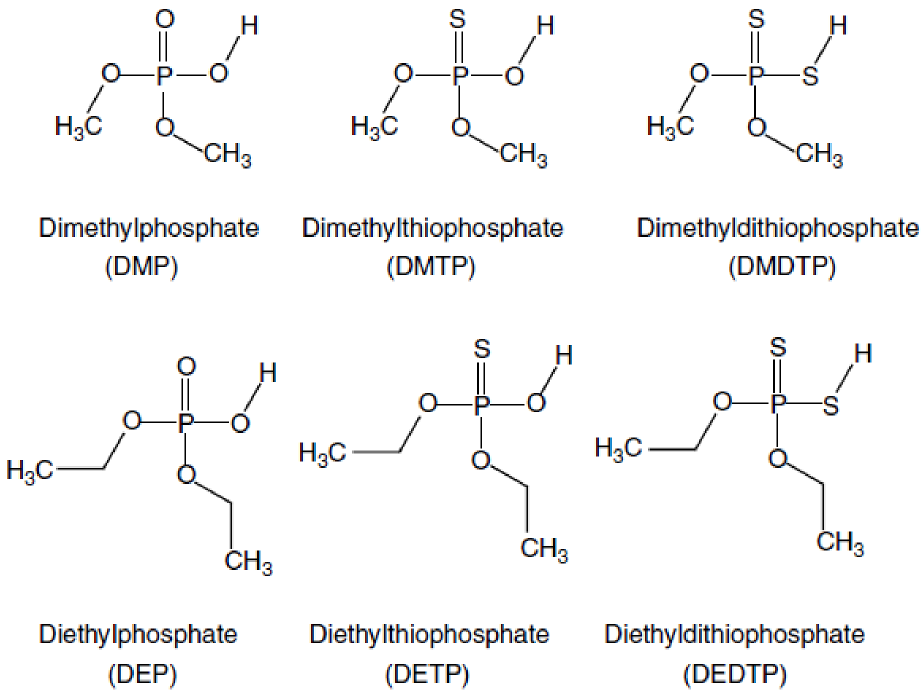

Figure 2 Chemical structures for six common urinary organophosphate pesticide metabolites (34)

Cholinesterases (ChEs) are specialized carboxylic ester hydrolases that break down esters of choline. There are two important ChE types; acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE) or pseudocholinesterase (PChE). AChE and BuChE or PChE are found in synapses and in neuron cell bodies of central nervous system (CNS). Erythrocytes (red blood cell) of mammals also found AChE. BuChE or PChE are found in serum. Blood ChE forms are common used for CNS ChEs in toxicity studies. Acetylcholine (ACh) is a preferred substrate for AChE while PChE prefers vary substrates depending on species. An important difference between AChE and PChE is their sensitivity to substrate concentration. AChE is more highly responsive to low substrate concentration compared to PChE. OP and carbamate pesticides can act as alternate substrates to Ach. The inhibition of Ach causes cholinergic poisoning symptoms including diarrhea, urination, lacrimation, and salivation. Anti-ChEs effect CNS producing hypothermia, tremors, headache, anxiety, convulsions, coma, and death at high dose levels of OPs and carbamates. The consistent low dose exposure can cause the behavioral effects such as learning and memory deficits (35). Because AChE has close structural affinity to brain ChE, it is considered as a marker of potential adverse effects on the nervous system.

In Central Thailand, there was a study reporting that the blood AChE level was significantly associated with chronic symptoms of the CNS system and blood PChE level was significantly related to acute symptoms including respiratory and visual systems (12).

2.1.2 Pyrethroid insecticides (PYR)

Pyrethroid insecticides (PYR) are extracted from the flowers of *Chrysanthemum cinerariaefolium* or *Pyrethrum cinerariaefolium*. PYR can be classified into first and second generation of pyrethroids. The first generation of PYRs are esters of chrysanthemic acid derivatives and alcohols. This PYR properties are highly sensitive to light, air, and temperature. Therefore, these PYR products have been used mainly for indoor pest control. The second generation of PYRs are 3-phenoxybenzyl alcohol derivatives in the alcohol moiety. This new synthesis PYRs increase insecticidal activity and sufficient stability in outdoor conditions. Hence, these PYR products have been used worldwide for agricultural pest control. The important PYR compounds are cypermethrin, permethrin, etofenprox, etc.

PYR are biodegradable and nonbioaccumulative compounds. These properties lead to low toxicity in mammals. In human body, the main metabolic reactions of PYR are oxidation, hydrolysis, and conjugation. There are two major forms regarding cleavage of the ester linkage; trans- and cis-isomers. The trans isomers of PYR having chrysanthemic acid derivatives in acid moiety such as permethrin. This isomer forms are more rapidly hydrolyzed than cis isomer forms. The major metabolite forms of PYR are cis/trans-3-(2,2-dichlorovinyl)-2,2-

dimethylcyclopropane carboxylic acid (DCCA), and 3-phenoxybenzyl alcohol (3-PBA). DCCA is specific for cyfluthrin, permethrin, and cypermethrin. 3-PBA is nonspecific for parental compound but it is representative of the commercially available pyrethroids (9, 36). The chemical structures of PYR metabolites are showed in Figure 3.

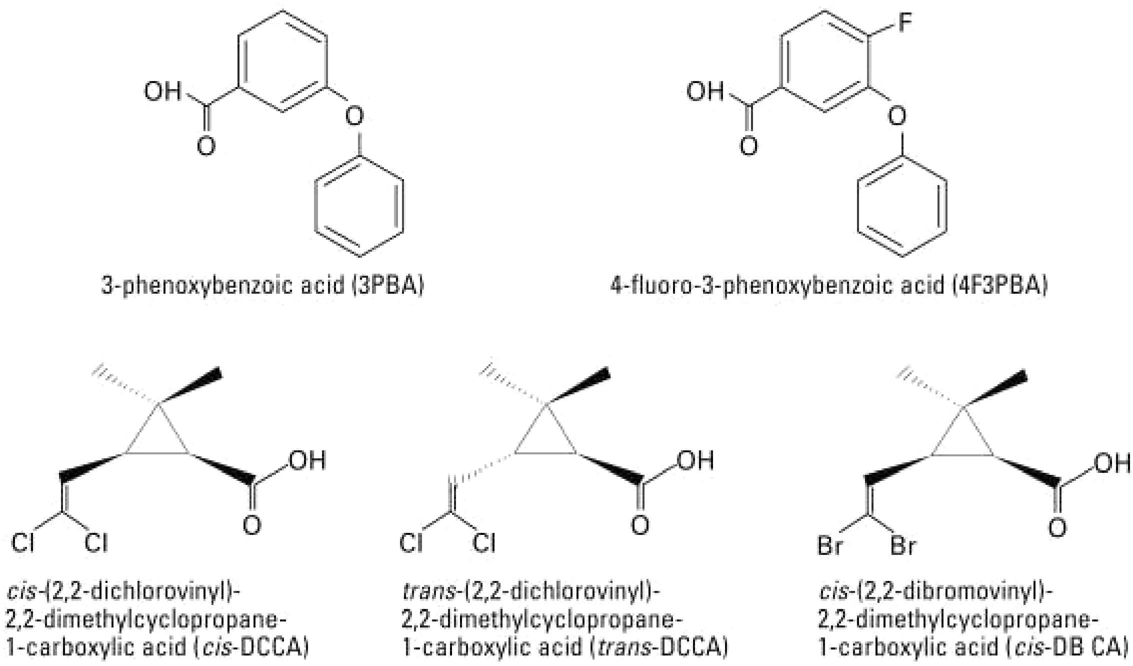

Figure 3 Chemical structures for urinary pyrethroid pesticide metabolites (37).

2.2 Behavioral Health Effects - Attention-deficit/hyperactivity disorder (ADHD)

2.2.1 ADHD background

Attention deficit and hyperactivity disorder (ADHD) is characterized by symptoms of inattention, hyperactivity, and impulsivity that can significantly impact many aspects of behavior and performance. For young children with ADHD, up to two-thirds of these children have one or more comorbid conditions including oppositional defiant and/or conduct disorder, depression, anxiety, substance abuse, and autistic spectrum disorder (38). In western countries, ADHD prevalence is 3-5% in children. The American Academy of Pediatrics reported the prevalence is higher among boys (9.2%) relative to girls (2.9%) (39). The recently published article in 2013 reported that the ADHD prevalence in Thai children was increased to 8.1% in grade 1 to grade 5 with boys (12.0%) showing higher prevalence than girls (4.2%) (40).

In childhood, boys are diagnosed with ADHD more than girls. Boys are more likely to present with disruptive behavior and conduct problems leading them to be noticed by health and educational professionals. In contrast, girls are more likely to present with attention and peer relationship problems (41).

Two-thirds of ADHD children have persistent symptoms into adulthood (42) and are more likely to be engaging in antisocial or criminal behavior (43) compared with ADHD females who have higher rates of psychiatric admissions (44).

The cause of ADHD is unclear and most likely includes genetic, environmental and psychosocial factors (45). For example, a family history of ADHD is often observed in children with ADHD (46). Environmental factors such as smoking, drinking, and substance use during pregnancy may also affect brain development and increase the risk of developing ADHD (47). Psychosocial factors such as disruption to early attachment, social adversity and deprivation may be associated with ADHD development (48).

From the previous studies, there are many factors associated with ADHD. Brain dysfunction development and genetic can cause delayed development. The abnormality of pre-frontal cortex, basal ganglia, and cerebellum together with the dysfunction of cholinergic system including dopamine and noradrenaline are found in ADHD patients (49-51). The study of Halperin and Schulz (2006) reported that ADHD is a result of sub-cortical neurological dysfunction which persists over development. However, ADHD is not always associated with brain dysfunction among children. It can also be found among children with normal brain development but who have been exposed to toxicants during prenatal development (e.g. lead, smoking, alcohol etc.) and environmental conditions i.e. parental care may lead to ADHD behavior (52-55).

2.2.2 ADHD evaluation tools

To diagnose ADHD, there is no single test due to many similar disorders such as anxiety, depression, and learning disability. The integration of each piece of evidence becomes important to the final diagnosis (56).

2.2.2.1 Behavior rating scales

The behavior rating scales are widely used to diagnose ADHD. The American Academy of Pediatrics (AAP) recommended the guideline using evidence gathered from parents and teachers to make the diagnosis of ADHD. Rating scales are viewed as one option for collecting evidence from people who are familiar or regularly observe the suspected ADHD patient in everyday life (39). The ADHD evaluation tools must be well designed to maintain diagnostic accuracy from both informant's perception and ADHD behaviors. Regarding the basis for this discrepancy among informants, it highlights the need for multi-informant assessment, particularly as DSM-IV criteria require impairment across settings (57).

ADHD is often found in children and should be observed by teachers and parents. Physician or pediatrician can diagnosis whether the child should be treated. Nowadays, the Diagnostic and Statistical Manual of Mental Disorders, 4th edition-text revision (DSM-IV-TR) has been used to diagnosis ADHD in Thailand. The detail of DSM-IV-TR shows in following paragraph (58). The behavioral disorder must be observed within 2 places (home and school) or more. The parents and teachers information are used to diagnosis ADHD in children and these 2 information sources need to be the same.

DSM-IV criteria

I. Either A or B:

A: Six or more of the following symptoms of inattention have been present for at least 6 months to a point that is inappropriate for developmental level:

Inattention:

- Often does not give close attention to details or makes careless mistakes in schoolwork, work, or other activities.
- Often has trouble keeping attention on tasks or play activities.
- Often does not seem to listen when spoken to directly.
- Often does not follow through on instructions and fails to finish schoolwork, chores, or duties in the workplace (not due to oppositional behavior or failure to understand instructions).
- Often has trouble organizing activities.

- Often avoids, dislikes, or doesn't want to do things that take a lot of mental effort for a long period of time (such as schoolwork or homework).
- Often loses things needed for tasks and activities (e.g. toys, school assignments, pencils, books, or tools).
- Is often easily distracted.
- Is often forgetful in daily activities.

B: Six or more of the following symptoms of hyperactivity-impulsivity have been present for at least 6 months to an extent that is disruptive and inappropriate for developmental level:

Hyperactivity:

- Often fidgets with hands or feet or squirms in seat when sitting still is expected.
- Often gets up from seat when remaining in seat is expected.
- Often excessively runs about or climbs when and where it is not appropriate (adolescents or adults may feel very restless).
- Often has trouble playing or doing leisure activities quietly.

- Is often "on the go" or often acts as if "driven by a motor".
- Often talks excessively

Impulsivity:

- Often blurts out answers before questions have been finished.
- Often has trouble waiting one's turn.
- Often interrupts or intrudes on others (e.g., butts into conversations or games).

II. Some symptoms that cause impairment were present before age 7 years.

III. Some impairment from the symptoms is present in two or more settings (e.g. at school/work and at home).

IV. There must be clear evidence of clinically significant impairment in social, school, or work functioning.

V. The symptoms do not happen only during the course of a Pervasive Developmental Disorder, Schizophrenia, or other Psychotic Disorder. The symptoms are not better accounted for by another mental disorder (e.g. Mood Disorder, Anxiety Disorder, Dissociative Disorder, or a Personality Disorder).

Based on these criteria, three types of ADHD are identified:

- *Combined Type*: if both criteria IA and IB are met for the past 6 months
- *Predominantly Inattentive Type*: if criterion IA is met but criterion IB is not met for the past six months
- *Predominantly Hyperactive-Impulsive Type*: if Criterion IB is met but Criterion IA is not met for the past six months.

The Conners 3 ADHD questionnaire

The Conners ADHD questionnaire is an instrument assessing ADHD symptoms. This scale is suitable for children age 3 to 17 years old. This questionnaire has acceptable reliability and overall accuracy of 70% (56). The Conners 3 ADHD Index (Conners 3AI Parent) and the short form of the Conners 3-Parent (Conners 3-P(S)) are shown in Appendix G and H. The Conners 3-P(S) content scales include inattention, hyperactivity/impulsivity, learning problems, executivefunctioning, defiance/aggression, and peer relations. The Conners also includes validity scales in both positive and negative impression. The positive impression refers to the parents attempt to rate on their child's behavior in positive direction. The negative impression refers to the parents attempt to rate their child's behavior in negative direction. Both positive and negative impression scores are the validity scales to warrant the reliable of parental responding style.

2.2.2.2 The Behavioral Assessment and Research System (BARS)

The BARS battery includes computerized tests adapted from IQ and other neuropsychological tests. It has been tested with agricultural workers exposed to pesticides (59-61). Rohlman et al. (2008) developed the program for children age 5 and above. This program is economical, requires limited language and education abilities, and has been translated into multiple languages. McCauley et al. (2006) reported BARS has been used for cross-cultural comparison of performance decrements associated with pesticide exposures. In order to administer the tests, the 9 button response system is required instead of standard computer keyboard.

Continuous performance test is one of attention and memory test in BARS. It is used to assess visual attention by pressing a key when a target stimulus appears along a series of non-target stimuli. The reaction time, number of hits, false alarms, signal detection, and errors of commission and omission are recorded by the program.

Halperin and Schulz (2006) suggested the commission errors as an outcome reflecting frontal lobe processing and the reaction time as an outcome reflecting sub-cortical processing. In addition, the commission errors should decrease over time and the reaction time should maintain (63).

2.2.3 ADHD study in Thailand

Aungudornpukdee, P. (2009) studied factors related to neurobehavioral effects in young children (6-13 years old) residing near petrochemical industrial estate: a community-based cross-sectional study in Map Ta Phut Sub-district, Rayong Province, Thailand. Subtests from the Wechsler Intelligence Scale for Children (WISC-III), a standardized tool for children age 6-16 years old and recommended by WHO (developed by Wechsler in 1949), were used. These tests did not require reading or writing skill, only oral questions. The results showed that visual-motor coordination deficit was associated with gender (adjusted OR=1.934), monthly parental income from high to low income (adjusted OR =1.997 to 2.612), age (adjusted OR=0.874), living period (adjusted OR=0.954), and household environmental tobacco smoke (adjusted OR=1.284). However, those factors had related to ADHD symptoms.

Benjasuwantep et al. (2002) found prevalence 6.5% of school-aged children in Bangkok, but the finding was contrast to previous observations which found a lower prevalence among boys vs. girls (1:1.09 ratio). However, Visanuyothin et al. (2013)

reported higher prevalence rate in boys relative to girls (3:1 ratio) in Thai children grade 1 to grade 5 evaluated by SNAP-IV rating scales and DSM-IV-TR. The highest prevalence found was in grade 1 (9.7%). The most common ADHD type observed was combined subtype (3.8%), followed by inattentive subtype (3.4%) and hyperactive/impulsive subtype (0.9%).

CHAPTER III

METHODOLOGY

In this chapter, the following topics are presented.

- Research design
- Study area
    - High pesticide use area in rice farming community
    - Low pesticide use area in aquacultural farming community
- Study population
    - Participants from rice farming area
    - Participants from aquacultural farming area
- Inclusion and exclusion criteria
- Sampling technique
- Sample and sample size
- Measurement tools
    - Exposure assessment
    - Neurobehavioral assessment
- Data collection

- Data analysis
- Ethical consideration

3.1 Research design

This analytical cross-sectional study was designed to compare children living in rice area to those living in aquaculture area at three different times.

3.2 Study area

The study area is in Pathum Thani Province which is located in the lower plain of Pasak and Chao Phraya river basins, north of Bangkok. The main product of the province comes from the paddy field which covers 70 percent of the province's total land area. Other products come from mango, coconut and tangerine groves (66). Rangsit irrigation system, situated east of Pathumthani province, composes of 14 sub-canals (Khlong). Each sub-canal is 20 km long and Khlong 7 is at the center of the irrigation system. Rangsit Prayun Sak canal, situated along the southern end of each sub-canal, is the main canal that receives water from sub-canals and transfers water to the Chao Phraya River which flows towards Bangkok.

High pesticide use area (Figure 4) is defined as the area where OP area used for agricultural purposes including Khlong 7. Khlong 7 sub-district, located in Khlong Luang district, Pathum Thani province. With area 22.886 km^2 and 2,532 households, there are 6,487 populations in which 70% of them are farmers (26). Khlong 7 is one of 14 sub-canals that have been used to irrigate Rangsit agricultural areas for more than 100 years. As a well water management, rice and other crops can be cultivated throughout the year. Therefore, this area is undoubtedly one of the most pesticide contaminated areas in the central plain of Thailand.

Figure 4 High pesticide exposure communities in Khlong Luang District, Pathum Thani Province

Figure 5 Low pesticide exposure communities in Lum Luk Ka District, Pathum Thani Province

Low pesticide use area (Figure 5) is defined as where OPs are not intensively used for agricultural purposes. In this study, Lum Sai sub-district, Lum Luk Ka district, Pathum Thani province was selected for the reference area. Aquacultures such as shrimp farms and fish ponds are the main area utilization. The chemical used in fishing and shrimp ponds are different from those used in agriculture fields in Khlong 7. Common chemicals uses in aquacultures are hormones, antibiotics, probiotics, etc (67). Therefore, OP exposure is less for the residents around these aquaculture ponds compared to Khlong 7 area.

3.3 Study population

Participants from rice farming area: Six to 8 year old healthy children in both physical and mental health who live in rice farming communities in Khlong 7, Khlong Luang District, where used OP as dominant pesticide for agricultural purpose.

Participants from aquacultural farming area: Six to 8 year old healthy children in both physical and mental health who live in shrimp and fish farming communities in Lum Luk Ka District, where OP are not used as dominant pesticide for agricultural purpose.

3.4 Inclusion and exclusion criteria

Inclusion criteria: Healthy children referred to children age between 6 to 8 years old who have no mental or physical heath diseases. These children have resided in Rangsit Khlong 7 and Lum Sai sub-district since they were born.

Exclusion criteria: Children were excluded if they have significant, diagnosed medical or psychiatric illness such as developmental delay or mental retardation, diabetes, neurological disease or significant head trauma with loss of consciousness, childhood psychosis, cancer, or significant lung, kidney, or cardiac disease.

3.5 Sampling techniques

In this study was used random sampling technique for selecting sampling unit (child) in both rice farming community and aquacultural farming community. Lists of children were obtained by the primary health care unit in Khlong 7 and hospital in Lum Luk Ka. Then, random sampling of each subject was conducted by picking number of child from each group.

3.6 Sample and sample size

3.6.1 Sampling period

The ADHD questionnaire, CPT and all samples were collected in 3 sessions; 1st time March 2011 (dry season- low exposure period), 2nd time October 2011 (wet season-high exposure period), and 3rd time March 2012 (dry season- low exposure period).

3.6.2 Environmental samples

3.6.2.1 Water samples

The polyethylene bottles were used to collect the drinking water (rain water) from participants' houses.

3.6.3 Personal samples

3.6.3.1 Hand wipe samples

The participant's hands were wiped for the presence of pesticide residues using the gauze pads moistened with 40% of isopropanol recommended by US EPA method (68). Wipe samples were immediately wrapped in aluminum foil and placed in plastic ziplock bags with given identity codes. These samples were transported back to Chulalongkorn University on ice packs, and stored at -40 °C until shipment to the standard Central Laboratory (Thailand) for analysis.

3.6.3.2 Urine samples

The parents were provided with one polyethylene urine collection bottle (already labeled an identifying code) and instructed to collect the urine samples. Urine samples were collected from participant children for the first morning voids and transferred to the screw cap polyethylene tubes. Then, they were put into the tube in zip-lock plastic bag and kept in an ice box during transportation to laboratory. The urine samples were stored at -40 °C in freezer before shipping for analysis on dry ice.

3.6.3.3 Fingerstick blood samples

The fingerstick blood samples were collected by the registered nurse and professional nurse. These blood samples were measured for erythrocyte cholinesterase (AChE) and plasma cholinesterase (PChE).

3.6.3.4 Sample size

Approximately 100 children ages 6-8 years old live in Khlong 7 sub-district. To conduct the number of subject, the difference between two means independent group were used. According to Lu's study (5), the difference between two means of urinary metabolite in children living in agricultural area and non-agricultural area was 0.03 (Δ) with standard deviation 0.03 (σ). The sample size calculation is as following equation;

Alpha = 0.05

Beta = 0.10 (statistical power = 90%)

$$n = \frac{2(Z_{\alpha/2} + Z_{\beta})^2 \sigma^2}{\Delta^2}$$

$$= \frac{2(1.96 + 1.28)^2 (0.03)^2}{(0.03)^2}$$

$$= 20.995$$

From the above equation, the number of subject should be not less than 21 per each group. In addition, the power calculation (at 90%) obtained from the Power and Sample Size Program (Figure 6) were used to calculate the appropriate sample size for each group. The results showed 23 participants from rice community (experimental subjects) and 20 participants from aquacultural community (control subjects). Hence, the final sample sizes should not less than 23 and 21 participants from rice and aquacultural farming areas, respectively.

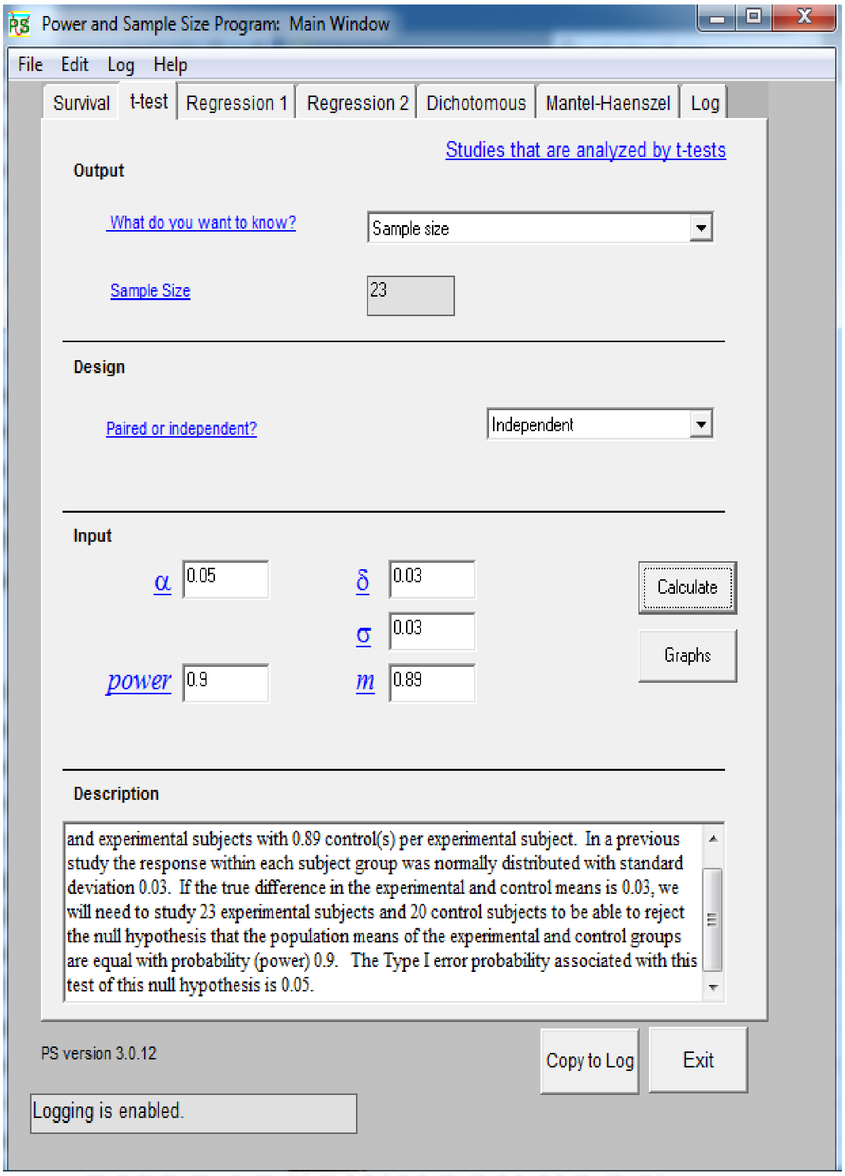

Figure 6 Power analysis of sample size calculation

3.7 Measurement tools

Part I: Exposure Assessment

3.7.1 Exposure questionnaire

Environmental conditions and activities of participant children were evaluated via a structured questionnaire (Appendix I) administered during home visits. The face-to-face interview with the child participant's parent was conducted by a trained examiner. The questionnaire (adapted from Petchuay et al., 2006) was used to collect the following information: parental occupation, proximity to rice farms, floor cleaning frequency, residential pesticide use (and type of pesticide if used), indoor and outdoor child activities, and parentally observed child behaviors (e.g. mouthing behavior, hygiene behavior, etc.). Data collected about activities and behaviors of children participants included duration, frequency, and dichotomous outcomes (yes / no).

3.7.2 Urine samples

The first morning void urine samples were separated for 2 analyses; OPs and PYR metabolites.

3.7.2.1 OP metabolites analysis

For class-specific dialkylphosphate (DAPs), the six common DAP metabolites were measured including dimethylphosphate (DMP), diethylphosphate (DEP), dimethylthiophosphate (DMTP), dimethyldithiophosphate (DMDTP), diethylthiophosphate (DETP), and diethyldithiophosphate (DEDTP). The DAPs analysis was performed at the Research Institute for Health Sciences (RIHES), Chiang Mai University, Chiang Mai, Thailand. Briefly, the urine samples were saturated with salt and acidified 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).

In order to combine all 6 DAP metabolites, the untransformed concentrations (C) were divided by molecular weight of each metabolite by following equations;

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 specific metabolite of chlorpyrifos (3, 5, 6- trichloropyridinol; TCPy) was measured. TCPy was measured using a minor modification of a method previously published (69). The TCPy analysis was performed at the Department of Environmental Health, Rollins School of Public Health (RSPH), Emory University, Atlanta, Georgia, USA. 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 (HPLC-MS/MS).

3.7.2.2 Absorbed daily dose (ADD)

For each participant, the ADD value (μg/kg/day) for chlorpyrifos was calculated using the equation below, obtained from Curwin et al., (2007).

$$ADD\ (\mu g/kg/day) = [(C)(C_n)(CF)(R_{mw})] / BW$$

C is the concentration of chlorpyrifos metabolite in urine per gram creatinine (μg/g Cr) multiplied by calculated mass of creatinine excreted per day (C_n), correction factor for children (CF =1.4) obtained from Nolan et al. (1984), and the ratio of chlorpyrifos and TCPy metabolite molecular weights (R_{mw}=1.77), then divided by the body weight (BW; kg). The ADD values were compared with the EPA acute and chronic population adjusted doses (PADs) which are reference doses (RfD) with additional safety factors included to be protective of children (72).

3.7.2.3 PYR metabolites analysis

Two common PYR metabolites including DCCA and 3-PBA were measured. The urinary metabolites were analyzed by the method developed by Angerer and Ritter (1997) at the Analytical Exposure Science and Environmental Health Laboratory at Rollins School of Public Health, Emory University, USA. The method was briefly as follow: first urine samples were hydrolyzed by concentrated sulfuric acid; second, solid-phase extraction (SPE) polypropylene cartridges were preconditioned by de-ionized water. Then the analytes were eluted by methanol and derivertized in water bath. After cooling under room temperature, the centrifugation was used for extraction by hexane. The final volumes were adjusted and analyzed by gas chromatography with mass spectrometer (GC-MS) for detecting the PYR metabolites.

3.7.2.4 Urinary creatinine measurement

The automated colorimetric method, adapted from Jaffe reaction (73), was used for the urinary creatinine measurement at Maharaj Nakorn Chiang Mai Hospital, Chiang Mai University, Thailand. The urinary creatinine levels were used to normalize the detectable metabolite concentrations based on the dilution of urine. The units of adjusted DAPs and PYR metabolite concentrations were presented in microgram per gram creatinine (μg/g Cr) and sum molar concentration of DAPs were presented in microgram mole per gram creatinine (μmol/g Cr).

3.7.3 Fingerstick blood samples

The Test-mate ChE Cholinesterase Test System (EQM Research, Inc., Cincinnati, OH) used for quantitative measurement of AChE and PChE in whole blood samples. The method was based on Ellman method (74). The participants had to wash their hands with non-contained quaternary ammonium soaps to avoid the analytical inference and remove pesticide residues on the hand. The fingerstick blood was collected for 10 μL in capillary tubes and placed in assay tubes; one for AChE and another for PChE analyses. Briefly, acetylthiocholine (AcTC) was hydrolyzed by AChE and butyrylthiocholine (BuTC) was hydrolyzed by PChE. Then, carboxylic acid and thiocholine were produced and reacted with the Ellman reagent (DTNB, dithionitrobenzoic acid) to form a yellow color which measured by the spectrophotometric analyzer at wavelength 450 nm. The absorbance was measured and calculated to final analyst concentrations. The reaction to measure cholinesterase activity is using the following equation. This instrument has been certified by the American Board of Clinical Chemistry.

thiocholine ester (AcTC/BuTC) $\xrightarrow{\text{Cholinesterase}}$ thiocholine

thiocholine + DTNB $\longrightarrow$ TNB-thiocholine + TNB (yellow)

3.7.4 Hand wipe samples

Hand wipe samples were analyzed by using in-house method (TE-CH-030) modified from previous published method (75) at Central Laboratory (Thailand) Co.,Ltd. Briefly, the wiping samples were extracted by acetone, dichloromethane, and sodiumchlorine. Then, the OP and PYR residues were analyzed by gas chromatography with flame photometric detector (GC-FPD).

3.7.5 Water samples

Water samples were analyzed by using in-house method based on standard method for the examination of water and wastewater (76), Method 6630B I, II, and Method 6410 B5, 1a) at Central Laboratory (Thailand) Co.,Ltd.. Briefly, the water samples were extracted by liquid-liquid extraction. Then, the OP and PYR residues were analyzed by gas chromatography/ mass spectrometer (GC/MS).

3.7.6 Quality control

3.7.6.1 Urinary analysis

All samples were analyzed with analytical calibration standards, blanks and quality control materials recommended by the standard method performance requirements (77). Urine samples with low DAPs and TCPy were used for blank and for spiked recovery. Limit of detections (LOD) were measured for every urinary OP metabolites. Both laboratory and methods were certified by the Clinical Laboratory Improvement Amendment of 1988.

3.7.6.2 Blood analysis

Controls were run on every testing. The intraindividual variability, the repeated measurements of AChE and PChE in same person, was conducted by a well-trained tester. In addition, the blood samples obtained from unexposed donors were used to compare the results analyzed by the Test-mate ChE photometric analyzer and by the Professional Laboratory accredited by Ministry of Public Health, Thailand.

Part II: Neurobehavioral Assessment

3.7.7 ADHD Questionnaires

The parents or caregivers who spend the most time with the child were interviewed by trained interviewers about 10 minute for screening ADHD symptoms. There were 2 versions of the Conners ADHD questionnaires used in this study. The short form of the Conners 3-Parent (Conners 3-P(S)) is a subset of items from full-length form of the Conners 3 Content scales. The words used in the Conners 3-P(S) are similar to the full-length form. There are 43 items and 2 additional questions. The symptom scales in this questionnaire include inattention (5 items), hyperactivity/impulsivity (6 items), learning problems (5 items), executive functioning (5 items), defiance/aggression (5 items), and peer relations (5 items). The validity scales in both positive impression (6 items) and negative impression (6 items) were also used in this questionnaire. The raw score ≥ 5 of positive or negative impression can be interpreted as possible positive/negative response style of interviewee. The second form of ADHD questionnaire is the Conners 3 ADHD Index (Conners 3AI Parent). This questionnaire contains 10 items that best differentiate ADHD patients from normal children. It is particularly useful for screening purposes. The raw score ≥ 2 can be interpreted as probability > 50% of a classification of ADHD. This scale is suitable for children age 3 to 17 years old. This questionnaire has acceptable reliability and overall accuracy of 70% (56). Both ADHD questionnaires were translated in Thai language and back-translated by a bilingual psychologist. The Conners 3-P(S) and The Conners 3 AI Parent are shown in Appendix.

3.7.8 The continuous performance test (CPT)

The Behavioral Assessment and Research System (BARS) was used for attention and memory test. The continuous performance test was applied for ADHD screening. It is used to assess sustained attention by pressing a key when a target stimulus appears among a series of non-target stimuli. All video and audio instructions were translated into Thai. Because the program was in development to be suitable for Thai children, there were 2 versions of CPT used in this study. The first version or original version was used in pilot session as a trial version to see how it worked with Thai children. This version showed the target stimulus along with series of stimuli for 4 minutes. The second version or alternate version was used in high and low pesticide use periods. This version extended the testing time to 7 minutes. The reliability of the prolonged testing time was provided elsewhere in Rohitrattana et al., (2014). Hence in this study, the CPT results obtained from high pesticide use period and low pesticide use period were comparable and were used to determine the behavioral health effects of pesticide exposure between high and low pesticide application periods.

The CPT program recorded the following performance parameters;

- % Hit (percent of correct response to target stimulus)
- % False alarm (percent of response to non-target stimuli)
- Hit latency (reaction time of response to target stimulus)
- False alarm latency (reaction time of response to non-target stimuli)
- D-prime (ability to discriminate targets from non-target stimuli)

All participants were given instructions and understood how to correctly respond to the CPT prior to the testing sessions. This is important to reduce practice effects as recommended by previous studies (79) where misinterpretation of the instructions led to inaccurate responses. Computerized test of BARS have the advantage in a consistent and efficient manner across participants while minimizing the impact of examiners (62).

The test administrators were doctoral and master students from the College of Public Health Sciences and Faculty of Psychology, Chulalongkorn University. All examiners were trained at least 3 times before the real testing session. At first training time, a brief introduction of the neurobehavioral tests and BARS program were given to examiners with the demonstration of appropriate test administration. The second training time, the examiners practiced the test with their colleagues and learned how to cope with troubleshooting during test administration. For example, examiners were taught what they should say and how they should react in response to participant performance during the test. They were instructed to provide encouragement such as to use phases “go on”, “keep trying”, “try more” to maintain the child’s attention to the test, but not to indicate “correct” or “wrong” response.

3.8 Data collection

There were 3 sessions for data collections in this study.

- *Pilot session* was the first sampling time in March 2011, low OPs application period on rice farming areas. The original version of CPT was used in this session as trial time.
- *High pesticide use period (wet season)* was the second sampling time in October 2011, high OPs application on rice farming areas. The alternate version of CPT was used in this session as the first real testing time.
- *Low pesticide use period (dry season)* was the third sampling time in April 2012, low OPs application period on rice farming areas. The alternate version of CPT was used in this session as the second real testing time.

3.9 Data analysis

3.9.1 Statistical analysis

SPSS for Windows (version 16) was used for statistical analysis. All data were tested for normality before appropriate statistical analyses were performed. Mean, standard deviation (SD), and frequency were reported for variables associated with participant demographics, characteristics, environments, and activities. Independent t-test was used to compare the continuous data (e.g. age and income) between participant groups. Chi-square tests (χ^2) were used for comparison of categorical data between participant groups. The urinary metabolite concentrations below the LOD were assigned a value equal to LOD/√2. Geometric means (GM) and ranges were reported for all urinary pesticide metabolite concentrations, including their molar summed concentrations.

For non-parametric statistics, Mann-Whitney U tests were used to compare the creatinine adjusted concentrations of urinary OP metabolites between participant groups. In order to determine the correlations between age and urinary pesticide metabolites, Spearman's correlation tests were used.

Multiple linear regression analyses were used to determine the association of predictor variables (independent variables) with urinary pesticide metabolite levels (dependent variables). Linear regression, adjusting for age and creatinine concentration, was used to determine the relationship between participant's environment and urinary pesticide metabolite concentrations. Linear regression, adjusting for age, parent's education, and family income, was used to determine the relationship between urinary pesticide metabolite ADHD symptoms and CPT scores.

Logarithmic transformations were used for the positive skewed concentrations of pesticide metabolites to reduce the variance in regression models (80).

3.10 Ethical consideration

This study were reviewed and approved by the Institutional Review Boards of Chulalongkorn University and UMDNJ-Robert Wood Johnson Medical School, protocol no. 078.1/53 with certificate of approval number 111/2010, 006/2011, and 008/2013.

Prior to beginning the data collection, the parents and children were informed about the study protocols. Parents signed the consent form and children were given information with an age-appropriate child assent form. The data collection from individual children was confidential. A unique numeric code was use to protect the subject privacy.

CHAPTER IV

RESULTS

In this chapter, the following topics are presented.

- Demography
- Exposure assessment
    - Hand wipe samples
    - Water samples
    - Biomarkers
        - Blood cholinesterase
        - Urinary OP metabolites
        - Urinary PYR metabolites
    - Effect of age and gender
    - Relationship between children's environmental conditions and urinary pesticide metabolites
    - Absorbed daily dose (ADD) of chlorpyrifos

- Behavioral health effects
    - Continuous performance test (CPT)
    - Conners ADHD questionnaires
    - Validity scores
- Consistency of outcomes
    - Pesticide exposure
    - Behavioral scores
- Correlation analysis
    - Correlation between concentrations found in hand wipe samples and urinary pesticide metabolites
    - Correlation between blood cholinesterase and urinary pesticide metabolites
    - Correlation between covariates and predictor variables
    - Correlation between continuous performance test (CPT) and the Conners ADHD questionnaires
- Multiple regression analysis for behavioral health effects and pesticide exposure

4.1 Demography

A total of 53 participants from 2 study areas, aged 6-8 years old, completed the study. Twenty-four were from rice area in Khlong 7 and 29 from aquaculture area in Lum Luk Ka, Pathum Thani province. The number of participants from both study areas exceeded the power calculation (Z=23). At first step of sampling, 25 participants from each study area were randomly selected from the volunteer list obtained from the Primary Health Care Unit (PCU) of rice farming areas in Khlong 7 sub-district and from Lum Luk Ka Hospital in Lum Luk Ka, Pathum Thani Province. Before the first data collection session, one participant from rice farming area was excluded because of a health problem (n=24). Four participants from aquacultural farming area were added in order to equalize the socio-demographic of participants and therefore the total participants from this area were 29 participants.

The characteristics of particpants including age, gender, body mass index (BMI), parent's education, and family income are presented in Table 1. Subject characteristics were similar between the districts, except for family income. Subjects from aquaculture area had significantly greater family income than subject from rice area.

Table 1 Characteristics of participants

Characteristics	Study area		Total (n=53) Mean (SD) or n (%)	Significance *P-value*
	Rice area (n=24) Mean (SD) or n (%)	Aquaculture area (n=29) Mean (SD) or n (%)		
Age (year)	7.3 (0.7)	7.4 (0.8)	7.3 (0.7)	0.76[a]
Gender				0.27[b]
Male	16 (66.7%)	15 (51.7%)	31 (58.5%)	
Female	8 (33.3%)	14 (48.3%)	22 (41.5%)	
BMI	16.4 (3.6)	17.7 (4.4)	17.1 (4.0)	0.27[a]
Parental education (year)	7.7 (3.3)	8.9 (4.5)	8.4 (4.0)	0.25[a]
Family income (Baht/month)	11,500 (9,124)	16,800 (10,358)	14,400 (10,090)	0.05[a]

[a] T-test, [b] Chi-square test

Note: parental education was reported as number of years each parent was educated in school.

The characteristics of participants' environment are shown in Table 2. Chi-square tests revealed no significant differences between rice and aquaculture groups with the exception of the agriculturist family the house distance from rice farm and use of OP on the farm. All participants from rice area were from rice farmers' families and their houses were less than 500 meters proximity from rice farms. Most of the parents reported that they had cleaned the floor in their home everyday with wet mop. Both parents from rice and aquaculture areas used pyrethroid insecticides in forms of sprays and/or coil sticks in their house with 62.5% of rice households using

PYR everyday, while 41.4% of aquaculture households used once a week. Most of farmers in rice area had indicated they used OP and PYR in their farms, whereas none of farmers in aquaculture area had used these insecticide groups for their agricultural purposes. The report of pesticides used in rice farming area were similar to a previous study by Pan and Siriwong (2010)(29) that chlorpyrifos, dicroptophos, triazophos which belonged to OP group were the most commonly used in rice field in Khlong 7.

The activities of participants observed by their parents are presented in Table 4.2. Chi-square tests revealed no significant differences among participants' activities with the exception of playing on farm and observable dirt on body. Most of participants washed their hands before eating a meal and spend time indoors more than outdoors. Participants from rice area played outdoor (e.g. playground, road, farm, etc.) more than participants from aquaculture area. Aquaculture parents reported that their children had significantly more hand-to-mouth behavior (29.2% of rice farming participants and 51.7% of aquacultural farming participants) while the two groups were similar in object-to-mouth behaviors (58.3% of rice farming participants and 51.7% of aquacultural farming participants). Participants from rice area (83.3%) had significantly more dirt on their body than participants from aquaculture area (55.2%), a result related to the parental report of more time spent outdoors among rice participants.

Table 2 Environmental conditions and activities of participants

Characteristics	Study area				Significance (χ^2 test)
	Rice area (n=24)		Aquaculture area (n=29)		
	n	% or Mean ± SD	n	% or Mean ± SD	
Rice farmer family	24	100	0	0	
Proximity from house to rice farm:					
≤500 m.	24	100	0	0	
>500 m.	0	0	29	100	
Frequency of floor cleaning:					
Not everyday	6	25.0	4	13.8	0.29
Everyday	18	75.0	25	86.2	
OP used on farm	23	95.8	0	0	<0.001**
Average frequency		1 time/mo		Never used	<0.001**
Hand washing	13	54.2	21	72.4	0.198
Playing duration (hr/day)					
Outdoor	24	3.5±2.2	29	2.6±1.5	0.21[a]
Indoor	24	6.5±3.5	29	6.9±3.3	0.63[a]
Sit/lay on floor (hr/day)	23	2.9±2.5	20	2.9±3.4	0.33[a]
Hand-to-mouth	7	29.2	15	51.7	0.076
Object-to-mouth	14	58.3	15	51.7	0.730
Dirt on body	20	83.3	16	55.2	0.041*
Playing on farm	12	50.0	5	17.2	0.014*

* significant level at $p < 0.05$

** significant level at $p < 0.01$

[a] Mann-Whitney U Test

4.2 Exposure assessment

4.2.1 Hand wipe samples

There was no OP pesticide group detected in hand wipe samples, only PYR pesticide group including permethrin and cypermethrin were detected. The limit of detection was 0.01 mg/kg in both permethrin and cypermethrin. Most of participants' hands had no detectable residues of OP and PYR. The concentrations of pesticide residues in hand wipe samples are presented in Table 4.3. In pilot session, permethrin and cypermethrin were detected in participants living in rice farming area 0.03 and 0.09 mg/kg, respectively. In high pesticide use period, cypermethrin was detected in both participants living in rice farming area (0.07 mg/kg) and aquacultural farming area (<0.02 mg/kg), permethrin was detected in participants living in aquacultural farming area (0.05 mg/kg). In low pesticide use period, cypermethrin was detected in participants living in rice farming area (0.07 mg/kg), while permethrin was detected in participants living in aquacultural farming area (0.07 mg/kg). In this study, a single pesticide was detected from participants who had contaminated hands. There were 2 participants who were twice detected the same pesticide at different sessions. One participant from rice farming area had detected cypermethrin in pilot and low pesticide use periods. Another participant from aquacultural area had detected permethrin in both high and low pesticide use periods. The highest concentration of permethrin was 0.07 mg/kg found in a participant from aquacultural farming area at low pesticide use period and cypermethrin was 0.09 mg/kg found in participant from rice farming area at pilot session.

There were some participants who had pesticide contaminated hands presented above median level of 3-PBA and DCCA (Table 3), suggesting that there were positively correlated between PYR residues on hands and urinary PYR metabolites.

Table 3 Concentrations of pesticide residues detected in hand wipe samples.

ID	Permethrin (mg/kg)	Cypermethrin (mg/kg)	Urinary metabolite concentration (μg/g creat.)	
			DCCA	3-PBA
1020	nd	0.09	12.44*	11.09*
1023	0.03	nd	0.60	4.02*
2013	nd	0.07	13.16*	15.26*
2148	0.05	nd	3.24*	5.98*
2150	nd	<0.02	0.64	1.24
3020	nd	0.07	0.66	3.54*
3148	0.07	nd	116.85*	68.35*

nd = no detectable

*Above the group median of each session

4.2.2 Water samples

None of pesticide residues were detected in rain and well water samples collected from participants' houses in both rice and aquaculture areas in any sessions, suggesting that the degradation of OP and PYR residues during storage in households might lead to concentrations lower than detection limits.

4.2.3 Biomarkers

4.2.3.1 Blood cholinesterase

4.2.3.1.1 Quality control of blood ChE test

The AChE and PChE results measured by the test kit were confirmed by full scale laboratory method performed by Ramathibodi hospital and the Professional Laboratory Management Corp. Co. Ltd., respectively. The results obtained from test kits and laboratories were comparable. Moreover, the intraindividual variability of both AChE and PChE were less than 5% per week reflecting the acceptable reliability of the tester.

4.2.3.1.2 Blood cholinesterase levels

Levels of AChE and PChE (Table 4-5) in fingerstick blood samples were used to determine the OP exposure. The results were categorized into safe and risky as determined by the manufacturer of the test kit (EQM Research, Inc.). Safe level refers to normal range of blood cholinesterase and has no health effect. Risky level refers to the range of blood cholinesterase which is lower than 50% of normal range and probably has health effects. Although more aquacultural participants were classified as higher risk cases than rice participants, the mean levels of AChE and PChE were not different between participant groups in all sessions. From repeated measurement, the levels of AChE and PChE in individual participants were similar among 3 sessions.

Table 4 Levels of AChE cholinesterase in blood of participants at 3 sessions

Interpretation	AChE levels (U/mL)	Rice farming participants	Aquacultural farming participants
		Number (%)	Number (%)
Pilot:			
n		24	29
Safe	2.35-5.57	24 (100%)	23 (77.8%)
Risky	< 2.35	0 (0%)	6 (22.2%)
Mean ± SD		2.95 ± 0.34 U/mL	2.72 ± 0.51 U/mL
Range		2.47 – 3.64 U/mL	1.88 – 3.90 U/mL
High:			
n		24	28
Safe	2.35-5.57	23 (95.8%)	24 (85.7%)
Risky	< 2.35	1 (4.2%)	4 (14.3%)
Mean ± SD		2.89 ± 0.43 U/mL	2.80 ± 0.43 U/mL
Range		2.32 – 3.85 U/mL	2.09 – 3.73 U/mL
Low:			
n		23	29
Safe	2.35-5.57	22 (95.7%)	24 (82.8%)
Risky	< 2.35	1 (4.3%)	5 (17.2%)
Mean ± SD		2.95 ± 0.40 U/mL	2.80 ± 0.40 U/mL
Range		2.29 – 3.82 U/mL	2.16 – 3.61 U/mL

Table 5 Levels of PChE cholinesterase in blood of participants at 3 sessions

Interpretation	PChE levels (U/mL)	Rice farming participants	Aquacultural farming participants
		Number (%)	Number (%)
Pilot:			
n		24	29
Safe	1.27-3.23	24 (100%)	29 (100%)
Risky	< 1.27	0 (0%)	0 (0%)
Mean ± SD		2.32 ± 0.54 U/mL	2.34 ± 0.42 U/mL
Range		1.57 – 3.36 U/mL	1.64 – 3.27 U/mL
High:			
n		24	28
Safe	1.27-3.23	24 (100%)	28 (100%)
Risky	< 1.27	0 (0%)	0 (0%)
Mean ± SD		2.41 ± 0.51 U/mL	2.48 ± 0.38 U/mL
Range		1.39 – 3.62 U/mL	1.71 – 3.47 U/mL
Low:			
n		23	29
Safe	1.27-3.23	23 (100%)	29 (100%)
Risky	< 1.27	0 (0%)	0 (0%)
Mean ± SD		2.40 ± 0.49 U/mL	2.53 ± 0.55 U/mL
Range		1.55 – 3.54 U/mL	1.82 – 3.58 U/mL

4.2.3.2 Urinary OP metabolites

4.2.3.2.1 Quality control for urinary OP metabolites

All samples were analyzed concurrently with analytical calibration standards, blanks and quality control materials using a previously published method (69, 81). The method has been cross-validated with a mass spectrometry based method and has achieved international certification. The six common DAPs and TCPy were measured and their limits of detection (LODs) were presented in Table 4.6. For DAPs, the relative recoveries ranged from 83-117% with relative standard deviations (RSDs) ranged from 2-9%. For TCPy, relative recovery in distinguishable from 100% and RSDs less than 10%.

Table 6 Limit of detections (LODs) and number of detects of urinary OP metabolites in participants

Urinary pesticide metabolites	LOD (μg/L)	Number of detects (%)					
		Rice farming area			Aqucultural farming area		
		Pilot	High	Low	Pilot	High	Low
TCPy	0.02	21 (100%)	24 (100%)	22 (95.6%)	27 (96.4%)	23 (82.1%)	28 (96.5%)
DMP	2.5	9 (37.5%)	13 (54.2%)	6 (26.1%)	8 (27.6%)	8 (27.6%)	7 (25.0%)
DMTP	0.2	14 (58.3%)	18 (35.0%)	16 (69.6%)	11 (37.9%)	14 (48.3%)	12 (42.9%)
DMDTP	0.2	2 (8.3%)	5 (20.8%)	5 (21.7%)	1 (3.4%)	3 (10.3%)	4 (14.3%)
DEP	0.2	22 (91.7%)	21 (87.5%)	23 (100%)	11 (37.9%)	16 (55.2%)	20 (71.4%)
DETP	0.1	23 (100%)	23 (95.8%)	23 (100%)	22 (75.9%)	19 (65.5%)	21 (75.0%)
DEDTP	0.2	6 (26.1%)	5 (23.8%)	5 (22.7%)	4 (14.3%)	2 (7.1%)	6 (21.4%)

4.2.3.2.2 Results of urinary OP pesticide metabolites

Number of detects of urinary OP metabolites are presented in Table 6. TCPy and DETP were the most common OP pesticide present in all sampling sessions. Descriptive data of urinary OP metabolites were presented in Table 7–12. The comparisons between urinary OP metabolites were showed in table 13.

Participants from rice area had significantly higher levels than participants from aquaculture area for TCPy, DEP, DETP, and DEAP in all sessions (creatinine adjusted results; Mann-Whitney test, $p < 0.05$).

In additional analysis from high pesticide use period, concentrations of non-creatinine adjusted DEP and DETP had a positively significant correlation with DAP (DEP; rho=0.92, p<0.001, DETP; rho=0.69, p<0.001, respectively), because they were the largest contributors to the summed value. Concentrations of non-creatinine adjusted DEP and DETP were found to be significantly correlated with TCPy (DEP; rho=0.49, p<0.001, DETP; rho=0.75, p<0.001, respectively), suggesting that the primary OP to which participants were exposed was chlorpyrifos.

Table 7 Descriptive data of urinary OP metabolites concentrations in participants in pilot session (nonadjusted creatinine in unit μg/L)

Participant groups	Statistics	TCPy	DMP	DMTP	DMDTP	DMAP	DEP	DETP	DEDTP	DEAP	DAP
Rice farming area	n	21	24	24	24	24	24	23	23	24	24
	Mean	8.86	2.54	0.74	0.18	26.53	3.59	1.71	0.33	244.49	271.02
	Std. Deviation	5.96	2.00	0.85	0.16	19.36	3.52	1.87	0.38	236.36	245.12
	Median	7.09	1.77	0.25	0.14	18.01	2.84	1.51	0.14	193.41	209.39
	Std. Error of Mean	1.30	0.41	0.17	0.03	3.95	0.72	0.39	0.08	48.25	50.03
	Minimum	0.67	1.61	0.08	0.14	15.36	0.03	0.20	0.14	3.99	19.36
	Maximum	22.74	10.72	2.75	0.81	101.74	12.10	9.33	1.24	837.45	853.37
Aquacultural farming area	n	28	28	28	28	28	28	28	27	28	28
	Mean	2.71	2.83	0.37	0.16	26.08	0.79	0.72	0.24	56.55	82.62
	Std. Deviation	2.55	4.28	0.63	0.11	35.00	1.27	1.33	0.25	85.25	90.87
	Median	1.86	1.77	0.14	0.14	15.92	0.14	0.20	0.14	11.02	29.50
	Std. Error of Mean	0.48	0.81	0.12	0.02	6.61	0.24	0.25	0.05	16.11	17.17
	Minimum	0.18	1.03	0.03	0.14	14.06	0.14	0.03	0.14	10.02	25.94
	Maximum	10.68	23.74	3.43	0.73	190.28	5.59	6.90	1.09	369.27	383.33

Note: DMAP, DEAP, and DAP were showed in unit nM.

Table 8 Descriptive data of urinary OP metabolites concentrations in participants in pilot session (adjusted creatinine in unit µg/g creatinine)

Participant groups	Statistics	TCPy	DMP	DMTP	DMDTP	DMAP	DEP	DETP	DEDTP	DEAP	DAP
Rice farming area	n	21	24	24	24	24	24	24	24	24	24
	Mean	11.25	3.06	0.85	0.25	31.86	4.47	2.35	0.38	306.04	337.91
	Std. Deviation	8.17	1.58	0.94	0.21	15.35	5.08	3.69	0.36	348.92	352.77
	Median	7.94	2.66	0.47	0.19	27.53	2.96	1.38	0.22	205.26	224.57
	Std. Error of Mean	1.78	0.32	0.19	0.04	3.13	1.04	0.75	0.07	71.22	72.01
	Minimum	1.81	1.43	0.11	0.10	12.83	0.06	0.00	0.00	8.07	22.71
	Maximum	30.51	7.82	3.69	1.01	74.21	24.03	18.62	1.26	1671.56	1703.34
Aquacultural farming area	n	28	28	28	28	28	28	28	28	28	28
	Mean	4.64	8.23	0.85	0.43	74.04	1.44	1.54	0.52	105.13	179.16
	Std. Deviation	2.60	15.68	1.63	0.38	127.28	2.05	3.21	0.53	137.28	181.17
	Median	4.17	4.22	0.43	0.34	38.78	0.46	0.54	0.36	37.23	88.24
	Std. Error of Mean	0.49	2.96	0.31	0.07	24.05	0.39	0.61	0.10	25.94	34.24
	Minimum	0.50	1.56	0.07	0.13	16.98	0.17	0.07	0.00	12.58	34.03
	Maximum	11.30	85.09	9.00	1.82	682.02	8.46	17.16	2.86	558.65	718.78

Note: DMAP, DEAP, and DAP were showed in unit µmol/g creatinine

Table 9 Descriptive data of urinary OP metabolites concentrations in participants in high pesticide use period (nonadjusted creatinine in unit µg/L**)**

Participant groups	Statistics	TCPy	DMP	DMTP	DMDTP	DMAP	DEP	DETP	DEDTP	DEAP	DAP
Rice farming area	n	24	24	24	24	24	24	24	21	24	22
	Mean	6.74	7.89	9.69	0.28	132.66	4.29	4.97	0.24	9.47	461.55
	Std. Deviation	7.26	14.51	43.65	0.39	399.00	5.83	14.34	0.19	18.23	655.59
	Median	4.84	1.77	0.46	0.14	20.25	1.60	1.33	0.14	4.57	196.50
	Std. Error of Mean	1.48	2.96	8.91	0.08	81.45	1.19	2.93	0.04	3.72	139.77
	Minimum	0.87	1.15	0.02	0.14	12.20	0.14	0.07	0.14	0.38	26.35
	Maximum	35.91	55.68	214.53	1.99	1965.27	21.67	71.65	0.71	90.90	2383.71
Aquacultural farming area	n	28	28	28	28	28	28	28	27	28	28
	Mean	2.44	2.67	1.27	0.22	31.54	1.05	1.09	0.20	2.34	107.48
	Std. Deviation	1.87	3.02	3.25	0.27	34.34	1.54	2.86	0.22	3.76	117.53
	Median	2.07	1.77	0.14	0.14	16.56	0.14	0.18	0.14	0.79	34.87
	Std. Error of Mean	0.35	0.57	0.61	0.05	6.49	0.29	0.54	0.04	0.71	22.21
	Minimum	0.18	0.95	0.14	0.14	9.41	0.03	0.07	0.14	0.21	20.80
	Maximum	6.93	13.92	17.22	1.32	146.69	5.29	14.82	1.21	18.50	375.55

Note: DMAP, DEAP, and DAP were showed in unit nM.

Table 10 Descriptive data of urinary OP metabolites concentrations in participants in high pesticide use period (adjusted creatinine in unit µg/g creatinine)

Participant groups	Statistics	TCPy	DMP	DMTP	DMDTP	DMAP	DEP	DETP	DEDTP	DEAP	DAP
Rice farming area	n	24	24	24	24	24	24	24	24	24	24
	Mean	9.00	12.57	12.75	0.40	192.05	6.47	5.57	0.30	12.34	646.31
	Std. Deviation	11.17	24.81	57.98	0.59	541.95	9.64	14.80	0.38	20.20	912.74
	Median	5.62	2.66	0.57	0.21	26.58	2.33	1.62	0.20	4.65	222.53
	Std. Error of Mean	2.28	5.06	11.84	0.12	110.62	1.97	3.02	0.08	4.12	186.31
	Minimum	1.90	1.50	0.06	0.10	13.99	0.14	0.09	0.00	0.74	40.01
	Maximum	55.24	82.22	284.90	2.64	2609.93	33.34	74.10	1.86	94.00	3165.62
Aquacultural farming area	n	28	28	28	28	28	28	28	28	28	28
	Mean	4.17	4.81	1.85	0.41	53.76	1.96	1.62	0.29	3.87	191.93
	Std. Deviation	4.28	4.91	3.73	0.49	48.99	3.72	4.12	0.22	6.31	267.63
	Median	2.99	2.68	0.44	0.23	36.94	0.44	0.32	0.21	1.31	82.62
	Std. Error of Mean	0.81	0.93	0.71	0.09	9.26	0.70	0.78	0.04	1.19	50.58
	Minimum	0.15	0.80	0.08	0.08	7.97	0.03	0.04	0.00	0.20	15.05
	Maximum	18.64	21.39	19.22	2.14	177.77	18.18	21.60	0.95	26.97	1290.57

Note: DMAP, DEAP, and DAP were showed in unit µmol/g creatinine

Table 11 Descriptive data of urinary OP metabolites concentrations in participants in low pesticide use period (nonadjusted creatinine in unit µg/L)

Participant groups	Statistics	TCPy	DMP	DMTP	DMDTP	DMAP	DEP	DETP	DEDTP	DEAP	DAP
Rice farming area	n	23	23	23	23	23	23	23	22	23	23
	Mean	11.93	10.25	10.94	0.50	161.50	3.23	2.38	0.24	386.34	386.34
	Std. Deviation	10.18	22.28	27.78	0.81	371.89	3.08	3.30	0.20	440.58	440.58
	Median	8.84	1.77	0.41	0.14	19.02	2.12	1.39	0.14	201.14	201.14
	Std. Error of Mean	2.12	4.65	5.79	0.17	77.54	0.64	0.69	0.04	91.87	91.87
	Minimum	0.18	1.77	0.12	0.14	15.78	0.05	0.18	0.14	21.69	21.69
	Maximum	42.62	91.18	105.65	3.04	1359.93	10.14	15.61	0.84	1608.38	1608.38
Aquacultural farming area	n	29	28	28	28	28	28	28	28	28	28
	Mean	4.78	3.66	0.88	0.22	36.61	1.74	0.69	0.28	118.72	155.33
	Std. Deviation	3.34	5.91	2.18	0.23	60.77	2.98	0.92	0.31	198.15	249.42
	Median	4.22	1.77	0.14	0.14	15.92	0.77	0.40	0.14	60.37	84.47
	Std. Error of Mean	0.62	1.12	0.41	0.04	11.49	0.56	0.17	0.06	37.45	47.14
	Minimum	0.18	1.14	0.01	0.14	10.92	0.10	0.07	0.14	8.36	24.28
	Maximum	16.80	30.32	10.43	1.19	321.62	14.50	3.67	1.29	963.90	1285.52

Note: DMAP, DEAP, and DAP were showed in unit nM.

Table 12 Descriptive data of urinary OP metabolites concentrations in participants in low pesticide use period (adjusted creatinine in unit μg/g creatinine)

Participant groups	Statistics	TCPy	DMP	DMTP	DMDTP	DMAP	DEP	DETP	DEDTP	DEAP	DAP
Rice farming area	n	24	23	23	23	23	23	23	23	23	23
	Mean	13.60	9.70	9.60	0.50	147.74	3.42	2.38	0.30	385.48	385.48
	Std. Deviation	12.52	17.83	21.80	0.69	292.34	3.61	2.75	0.34	374.48	374.48
	Median	9.37	2.38	0.50	0.18	26.69	2.33	1.79	0.17	199.50	199.50
	Std. Error of Mean	2.56	3.72	4.54	0.14	60.96	0.75	0.57	0.07	78.08	78.08
	Minimum	0.32	0.94	0.12	0.10	11.91	0.09	0.15	0.00	36.33	36.33
	Maximum	51.79	72.25	80.22	2.41	1077.60	13.63	13.43	1.43	1221.25	1221.25
Aquacultural farming area	n	28	28	28	28	28	28	28	28	28	28
	Mean	5.10	3.82	0.85	0.27	38.01	1.57	0.70	0.34	107.92	145.93
	Std. Deviation	2.83	4.13	1.61	0.23	42.09	2.15	0.78	0.34	141.97	168.82
	Median	4.20	2.24	0.20	0.18	22.36	0.96	0.50	0.18	69.97	96.47
	Std. Error of Mean	0.53	0.78	0.30	0.04	7.95	0.41	0.15	0.06	26.83	31.90
	Minimum	1.29	0.88	0.01	0.07	7.94	0.13	0.03	0.07	10.45	23.83
	Maximum	10.93	19.73	6.79	1.01	209.25	9.43	2.96	1.16	627.13	836.38

Note: DMAP, DEAP, and DAP were showed in unit μmol/g creatinine.

Table 13 Concentrations of urinary OP metabolites in participants from rice and aquacultural farming areas, Pathum Thani Province, Thailand

Urinary pesticide metabolites	Rice farming area				Aquacultural farming area				Significance
	Creatinine unadjusted (μg/L)		Creatinine adjusted (μg/g creatinine)		Creatinine unadjusted (μg/L)		Creatinine adjusted (μg/g creatinine)		
	Median	GM	Median	GM	Median	GM	Median	GM	
Pilot:									
TCPy	7.09	6.77	7.94	8.76	1.85	1.83	4.16	3.91	<0.001**
DMP	1.77	2.19	2.66	2.74	1.77	2.07	4.22	4.75	0.006**
DMTP	0.14	0.38	0.46	0.48	0.14	0.22	0.43	0.49	0.91
DMDTP	0.14	0.16	0.18	0.2	0.14	0.15	0.33	0.34	0.002**
DEP	2.83	1.76	2.96	2.21	0.14	0.32	0.46	0.73	0.004**
DETP	1.51	1.15	1.38	0	0.2	0.27	0.54	0.61	0.03*
DEDTP	0.14	0.22	0.21	0	0.14	0.18	0.36	0	0.04*
High:									
TCPy	4.83	4.63	5.62	6.07	2.06	1.58	2.98	2.65	0.007**
DMP	1.77	3.31	2.65	4.34	1.77	2.09	2.68	3.37	0.88
DMTP	0.46	0.49	0.57	0.64	0.14	0.4	0.44	0.64	0.87
DMDTP	0.14	0.19	0.21	0.26	0.14	0.17	0.23	0.27	0.73
DEP	1.6	1.71	2.33	2.35	0.14	0.36	0.44	0.57	0.003**
DETP	1.33	1.29	1.61	1.7	0.18	0.28	0.31	0.45	0.002**
DEDTP	0.14	0.19	0.19	0	0.14	0.16	0.21	0	0.55
Low:									
TCPy	8.84	8.19	9.56	9.32	4.22	3.73	4.02	4.35	<0.001**
DMP	1.77	3.3	2.38	3.75	1.77	2.34	2.23	2.71	0.51
DMTP	0.41	0.85	0.5	0.98	0.14	0.24	0.19	0.28	0.06
DMDTP	0.14	0.24	0.18	0.27	0.14	0.17	0.18	0.2	0.51
DEP	2.12	1.55	2.33	1.77	0.76	0.67	0.95	0.78	0.01*
DETP	1.39	1.36	1.79	1.55	0.39	0.34	0.49	0.4	<0.001**
DEDTP	0.14	0.19	0.17	0	0.14	0.2	0.18	0.23	0.79

Note: Significance was tested by Mann-Whitney U test

* $p < 0.05$

** $p < 0.01$

Table 14 Concentrations of sums of OP metabolites in participants from rice and aquacultural farming areas, Pathum Thani Province, Thailand

Urinary pesticide metabolites	Rice farming area				Aquacultural farming area				Significance
	Creatinine unadjusted (nM)		Creatinine adjusted (μmol/g creatinine)		Creatinine unadjusted (nM)		Creatinine adjusted (μmol/g creatinine)		
	Median	GM	Median	GM	Median	GM	Median	GM	
Pilot:									
DMAP	18.00	22.80	27.53	28.64	16.24	19.74	38.78	48.22	0.03*
DEAP	193.40	129.99	205.26	163.26	11.02	24.63	37.22	56.43	0.003**
DAP	209.39	172.42	224.56	216.52	29.50	52.44	88.24	120.17	0.02*
High:									
DMAP	20.25	35.57	26.57	46.65	17.10	23.27	36.93	37.51	0.94
DEAP	4.57	4.16	4.65	5.65	0.78	1.05	1.31	1.70	0.001**
DAP	196.50	210.26	222.53	270.61	34.86	62.78	82.61	101.21	0.008**
Low:									
DMAP	19.02	40.34	26.69	45.87	16.55	22.87	22.35	26.57	0.29
DEAP	201.14	207.06	199.50	235.46	60.36	49.38	69.96	57.35	<0.001**
DAP	201.14	207.06	199.50	235.46	84.46	82.98	96.46	96.38	0.002**

Note: Significance was tested by Mann-Whitney U test

* $p < 0.05$

** $p < 0.01$

4.2.3.3 Urinary pyrethroid pesticide metabolites

4.2.3.3.1 Quality control for urinary PYR metabolites

All samples were analyzed concurrently with analytical calibration standards, blanks and quality control materials using a previously published method (69). The LOD of urinary PYR metabolites are showed in Table 15.

4.3.2.1 Results of urinary PYR pesticide metabolites

The percent of detection of urinary PYR metabolites are presented in Table 15. 3-PBA was the most common PYR pesticide present in all sampling sessions. Descriptive data of urinary PYR metabolites are presented in Table 16–17. The comparisons between urinary PYR metabolites were showed in table 18. There were no differences between participant groups in every session.

The correlation between 3-PBA and DCCA were positively significant in high pesticide use period (wet season) (rho = 0.37, p = 0.007) and low pesticide use period (dry seasons) (rho = 0.38, p = 0.006), suggesting that pyrethroid and cypermethrin were the primary PYR which participants were exposed in both seasons.

Table 15 LODs and number of detects of urinary PYR metabolites in participants

Urinary pesticide metabolites	LOD (μg/L)	Number of detects (%)					
		Rice farming area			Aqucultural farming area		
		Pilot	High	Low	Pilot	High	Low
DCCA	1.00	5 (23.8%)	5 (20.8%)	4 (17.4%)	2 (8.3%)	1 (3.6%)	6 (20.7%)
3-PBA	0.25	18 (85.7%)	21 (87.5%)	22 (95.6%)	17 (60.7%)	24 (85.7%)	25 (86.2%)

Table 16 Descriptive data of urinary PYR metabolite concentrations in participants (nonadjusted creatinine in unit μg/L)

Participant groups	Statistics	Pilot		High		Low	
		DCCA	3-PBA	DCCA	3-PBA	DCCA	3-PBA
Rice farming area	n	21	21	24	24	23	23
	Mean	4.24	4.29	1.74	2.15	1.71	2.74
	Std. Deviation	11.35	6.87	1.98	2.73	3.36	2.15
	Median	0.71	1.50	0.71	1.25	0.71	2.23
	Std. Error of Mean	2.48	1.50	0.40	0.56	0.70	0.45
	Minimum	0.71	0.18	0.71	0.18	0.71	0.18
	Maximum	52.26	29.99	7.14	13.53	16.58	8.16
Aquacultural farming area	n	28	28	28	28	29	29
	Mean	1.53	1.28	1.00	1.58	14.45	9.59
	Std. Deviation	3.49	2.13	1.56	1.09	38.97	23.98
	Median	0.71	0.61	0.71	1.37	0.71	1.86
	Std. Error of Mean	0.66	0.40	0.29	0.21	7.24	4.45
	Minimum	0.71	0.18	0.71	0.18	0.71	0.18
	Maximum	18.65	10.76	8.95	4.34	153.77	104.70

Table 17 Descriptive data of urinary PYR metabolite concentrations in participants (adjusted creatinine in unit μg/g creatinine)

Participant groups	Statistics	Pilot		High		Low	
		DCCA	3-PBA	DCCA	3-PBA	DCCA	3-PBA
Rice farming area	n	21	21	24	24	23	23
	Mean	7.33	6.24	2.66	3.02	1.97	2.92
	Std. Deviation	21.51	12.67	3.58	4.33	3.21	2.09
	Median	0.95	2.53	0.97	1.66	0.84	2.57
	Std. Error of Mean	4.69	2.76	0.73	0.88	0.67	0.44
	Minimum	0.52	0.24	0.51	0.30	0.38	0.32
	Maximum	99.35	57.02	13.16	17.96	14.73	8.44
Aquacultural farming area	n	28	28	28	28	29	29
	Mean	4.54	2.99	1.83	2.85	15.91	10.83
	Std. Deviation	12.53	7.21	2.31	2.79	46.72	30.58
	Median	1.65	1.28	1.16	1.63	0.91	1.94
	Std. Error of Mean	2.37	1.36	0.44	0.53	8.68	5.68
	Minimum	0.64	0.32	0.41	0.26	0.35	0.18
	Maximum	66.85	38.55	12.75	13.11	228.14	155.34

Table 18 Concentrations of urinary PYR metabolites in participants from rice and aquacultural farming areas, Pathum Thani Province, Thailand

Urinary pesticide metabolites	Rice farming area				Aquacultural farming area				Significance
	Creatinine unadjusted (µg/L)		Creatinine adjusted (µg/g creatinine)		Creatinine unadjusted (µg/L)		Creatinine adjusted (µg/g creatinine)		
	Median	GM	Median	GM	Median	GM	Median	GM	
Pilot:									
DCCA	0.71	1.27	0.95	1.65	0.71	0.86	1.65	1.28	0.10
3-PBA	1.50	1.78	2.53	2.31	0.60	0.61	1.83	1.30	0.07
High:									
DCCA	0.71	1.13	0.96	1.48	0.71	0.78	1.16	1.30	0.96
3-PBA	1.24	1.33	1.65	1.74	1.37	1.16	1.63	1.94	0.65
Low:									
DCCA	0.71	0.98	0.84	1.11	0.71	1.61	0.91	1.87	0.58
3-PBA	2.23	1.97	2.57	2.24	1.86	2.06	1.94	2.40	0.58

Note: Significance was tested by Mann-Whitney U test

4.2.4 Effect of age and gender (analyzed by high pesticide use period)

Creatinine adjusted and non-creatinine adjusted values were used to determine the association between age and urinary pesticide metabolites because the creatinine levels had a positive significant correlation with age (Pearson's correlation; r = 0.35, p = 0.01).

From Spearman's correlation, the result found negatively significant association between age of children and urinary TCPy (rho = -0.29, p = 0.03) and DAP (rho = -0.31, p = 0.02) creatinine adjusted concentrations, but not with non-creatinine adjusted concentrations. However, there were no significant differences for gender of the children and urinary TCPy and DAP concentrations (creatinine adjusted, Mann-Whitney U test, p>0.05).

Spearman's correlation analysis revealed no significant association between age of children and urinary 3-PBA (r = -0.067, p>0.05) creatinine adjusted concentrations, but not with non-creatinine adjusted concentrations. There were also no significant difference for gender of the children and urinary 3-PBA concentrations (creatinine adjusted, Mann-Whitney U test, p>0.05).

4.2.5 Relationships between children's environmental conditions and urinary metabolites

The concentrations of urinary OP metabolites in high pesticide use period were used in regression analyses because it represented the highest OP exposure in participants. The results found significant associations between log-transformed, non-creatinine adjusted DAP concentrations and rice farmer family (p=0.009), and frequency of OP use on farms (p=0.001). Significant associations were found between log-transformed, non-creatinine adjusted TCPy concentrations and being a member of a rice farming family (p<0.001), proximity to rice farm (p=0.03), parentally observed dirt on the body (p=0.02), being with a parent on the rice farm (p=0.02), playing on rice farms (p=0.03), and frequency of OP application (p=0.001) (Table 19). Analysis of some variables, such as "proximity to rice farm," returned results indistinguishable from, "rice farming family member," because all rice farming participants lived close to the fields. This analysis was published elsewhere in Rohitrattana et al. (2014).

Table 19 Results of linear regression analysis of levels of OP exposure

(log-transformed creatinine unadjusted concentrations, controlled for age and creatinine).

Metabolites	Predictor variables	Slope	t	P-value
∑DAP	Frequency of OPs used on farm	0.444	3.591	0.001^{**}
	Being a member of a rice farming family	0.361	2.734	0.009^{**}
TCPy	Frequency of OPs used on farm	0.416	3.824	0.001^{**}
	Being a member of a rice farming family	0.451	3.805	$<0.001^{**}$
	Proximity to rice farm	0.274	2.150	0.037^{*}
	Being with parent on rice farm	0.304	2.403	0.020^{*}
	Playing on rice farm	0.273	2.127	0.039^{*}
	Parentally observed dirt on body	0.287	2.291	0.026^{*}

*significant level at $p < 0.05$

**significant level at $p < 0.01$

The concentrations of urinary PYR metabolites in low pesticide use period were used in regression analyses because it represented the highest PYR exposure in participants. Although the result (Table 20) revealed that environmental conditions and activities were not significant predictors of log-transformed, creatinine adjusted urinary concentrations of PYR, some factors might be used to predict trends of PYR exposure. Frequency of PYR use on farms and households were likely related to increased concentrations of PYR metabolite. Proximity to rice farm was possibly associated to increased PYR exposure. Participants who had ever playing on rice farms and put object-to-mouth (non-edible materials) tended to be elevated PYR exposure by their activities. This analysis was published elsewhere in Rohitrattana et al. (2014).

Table 20 Results of linear regression analysis of levels of PYR exposure

(log-transformed creatinine adjusted concentrations, controlled for age).

Factors	Intercept	Slope	R^2	95% CI
Frequency of floor cleaning	1.80 (p=0.02)	0.06 (p=0.74)	0.08	-0.31, 0.44
Frequency of PYR use in house	1.81 (p=0.01)	0.07 (p=0.25)	0.10	-0.05, 0.20
Frequency of PYR use on farm	1.89 (p=0.01)	0.004 (p=0.94)	0.08	-0.10, 0.11
Proximity to farm	1.81 (p=0.02)	0.09 (p=0.52)	0.09	-0.20, 0.38
Wash hand	1.87 (p=0.01)	0.01 (p=0.90)	0.08	-0.28, 0.31
Play on farm	1.88 (p=0.01)	0.11 (p=0.46)	0.09	-0.19, 0.42
Object-to-mouth	1.80 (p=0.02)	0.08 (p=0.58)	0.09	-0.21, 0.38
Observable dirt on body	2.04 (p=0.01)	-0.17 (p=0.23)	0.10	-0.47, 0.12

4.2.6 Absorbed daily dose (ADD) of chlorpyrifos

The GM of the TCPy ADD (range 0.07-1.78 μg/kg/day; GM = 0.23 μg/kg/day) was significantly higher (Mann-Whitney test, *p* = 0.004) in rice farming participants than the participants from aquacultural farming areas (range 0.01-0.61 μg/kg/day; GM = 0.10 μg/kg/day). All of the ADD estimates for rice farming participants and 82% of the aquacultural farming participants exceeded the US EPA's chronic PAD (0.03 μg/kg/day), but none of the participants had an ADD value exceeding the acute PAD (0.5 μg/kg/day) recommended by EPA. Younger participants tended to have higher doses than older participants (Spearman's correlation, *rho* = -0.246, *p* = 0.07), but this difference was only nominally significant.

4.3 Behavioral health effects

4.3.1 Continuous performance test (CPT)

The descriptive results of continuous performance test among children from rice and aquaculture areas are shown in Table 21 – 23. Rice and aquaculture groups did not differ significantly in performance during each session, with the exception of false alarm latency in low pesticide use period. Contrary to our hypothesis, aquaculture subjects spent longer time to respond to incorrect stimuli than rice farm subjects did (independent t-test, $p = 0.01$). However, the CPT version used in pilot session was different from the CPT version used in high and low pesticide use periods. Therefore, the results from high and low pesticide use periods were used to compare the consistency of performance.

Table 21 Outcomes of continuous performance test (CPT) in participants in pilot session

Participant groups		% Hit	% False alarm	Hit latency (ms)	False alarm latency (ms)
Rice farming area	n	24	24	24	24
	Mean	76.4	18.2	481	447
	SD	15.8	14.2	135	156
	Median	81.2	12.7	449	396
	SE	3.2	2.9	28	32
	Minimum	35.0	3.3	317	277
	Maximum	97.5	56.7	842	837
Aquacultural farming area	n	29	29	29	29
	Mean	79.5	12.7	499	464
	SD	17.6	10.3	139	223
	Median	85.0	10.0	462	372
	SE	3.3	1.9	26	41
	Minimum	47.5	1.7	338	286
	Maximum	100.0	40.7	994	1266
Significant		0.45	0.10	0.63	0.75

Note: Significance was tested by independent t-test

Table 22 Outcomes of continuous performance test (CPT) in participants in high pesticide use period

Participant groups		% Hit	% False alarm	Hit latency (ms)	False alarm latency (ms)	Dprime
Rice farming area	n	24	24	24	24	24
	Mean	75.2	9.6	505	453	2.3
	SD	22.3	7.9	114	197	1.2
	Median	82.4	7.1	502	448	2.4
	SE	4.6	1.6	23	40	0.2
	Minimum	30.9	0.0	310	0	0.1
	Maximum	100.0	33.8	765	988	5.3
Aquacultural farming area	n	28	28	28	28	28
	Mean	80.4	12.2	521	459	2.5
	SD	23.0	12.7	99	161	1.2
	Median	93.2	6.8	523	398	2.6
	SE	4.3	2.4	19	30	0.2
	Minimum	16.7	2.1	360	254	0.2
	Maximum	100.0	56.7	810	821	4.4
Significant		0.35	0.39	0.60	0.90	0.70

Note: Significance was tested by independent t-test

Table 23 Outcomes of continuous performance test (CPT) in participants in low pesticide use period

Participant groups		% Hit	% False alarm	Hit latency (ms)	False alarm latency (ms)	Dprime
Rice farming area	n	23	23	23	23	23
	Mean	81.8	11.9	417	470	2.4
	SD	20.2	8.0	103	166	1.0
	Median	88.6	9.8	425	436	2.6
	SE	4.2	1.7	21	35	0.2
	Minimum	28.4	0.8	249	279	0.4
	Maximum	100.0	33.8	647	866	3.9
Aquacultural farming area	n	28	28	28	28	28
	Mean	88.2	14.4	472	553	2.6
	SD	13.7	13.3	108	270	1.1
	Median	91.5	11.3	469	443	2.8
	SE	2.6	2.5	20	51	0.2
	Minimum	39.5	1.5	294	261	0.3
	Maximum	100.0	52.7	702	1305	4.5
Significant		0.13	0.43	0.07	0.19	0.46

Note: Significance was tested by independent t-test

Participants from rice and aquacultural communities had similar performance of correct response to target stimulus (% hits) in every session. Rice participants in high pesticide use period performed slightly lower % hits than they did in low pesticide use period, the performances from both sessions were the same. Participants from rice and aquacultural communities had similar performance of incorrect response to non-target stimuli (false alarm) in every session. Both participant groups had lowest percent false alarm in high pesticide use period.

Participants from rice and aquacultural communities had similar performance of response time to target stimulus (hit latency) in each session. Both participant groups had faster response time to target stimulus in low pesticide use period. Both participant groups in high pesticide use period performed significantly longer hit latencies than they did in low pesticide use period (paired t-test; $p < 0.05$). Thus, it appears that subjects from both groups benefited from practice by improving their speed of performance on the test.

Participants from rice and aquacultural communities had similar performance of response time to non-target stimuli (false alarm latency) in pilot session. For within subject performance, only aquacultural participants in low pesticide use period showed significantly greater false alarm latency than they did in high pesticide use period (paired t-test; $p < 0.05$).

Participants from rice and aquacultural communities had similar performance of ability to discriminate targets stimulus from non-target stimuli in high and low pesticide use periods. The DPrime of pilot session was not shown because the calculation from the program was error. The within subject performances from high and low pesticide use periods were consistent in both participant groups.

4.3.2 Conners ADHD questionnaires

Overall, scores of rice and aquaculture participants (Table 24 – 26) were similar in all sessions. Only learning problems in pilot session were significantly different. Parents of rice farm subjects reported that their children had significantly more learning problems (Mann-Whitney U test, $p = 0.01$). In high pesticide use period, aquaculture subjects were reported to have significantly higher scores than rice farm subjects for the Conner 3AI (independent t-test, $p = 0.04$) and peer relations (Mann-Whitney U test, $p = 0.01$). In low pesticide use period, rice farm subjects were reported to have significantly more aggression/defiance (Mann-Whitney U test, $p = 0.01$).

Table 24 Scores of ADHD symptoms in participants in pilot session

Participant groups		ADHD index	Inattention	Hyperactivity/Impulsivity	Learning problems	Executive functioning	Aggressive/Defiance	Peer relation
Rice farming area	n	24	24	24	17	24	24	24
	Mean	4.5	4.1	9.0	2.8	5.1	2.5	0.9
	SD	4.9	2.4	4.0	2.2	3.4	3.1	1.2
	Median	2.0	5.0	8.5	3.0	5.0	2.0	0.5
	SE	1.0	0.5	0.8	0.5	0.7	0.6	0.2
	Minimum	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	Maximum	18.0	12.0	18.0	7.0	13.0	15.0	3.0
Aquacultural farming area	n	29	29	29	28	29	29	29
	Mean	4.4	4.2	8.4	0.9	4.2	1.4	1.2
	SD	4.0	3.0	4.4	1.4	2.7	2.4	2.7
	Median	4.0	4.0	10.0	0.0	4.0	1.0	0.0
	SE	0.7	0.6	0.8	0.3	0.5	0.4	0.5
	Minimum	0.0	0.0	0.0	0.0	1.0	0.0	0.0
	Maximum	12.0	11.0	15.0	5.0	10.0	11.0	13.0
Significant		0.75[a]	0.99[a]	0.59[a]	0.01[b,*]	0.29[a]	0.05[b]	0.66[b]

Note:

[a] Significance was tested by independent t-test

[b] Significance was tested by Mann-Whitney U test

[*] $p < 0.05$

[**] $p < 0.01$

Table 25 Scores of ADHD symptoms in participants in high pesticide use period

Participant groups		ADHD index	Inattention	Hyperactivity/Impulsivity	Learning problems	Executive functioning	Aggressive/Defiance	Peer relation
Rice farming area	n	22	23	23	23	23	21	22
	Mean	4.5	5.0	8.4	4.0	5.8	2.2	0.2
	SD	4.4	2.5	3.3	1.8	2.5	1.9	0.4
	Median	4.0	5.0	8.0	4.0	6.0	2.0	0.0
	SE	0.9	0.5	0.7	0.4	0.5	0.4	0.1
	Minimum	0.0	0.0	1.0	1.0	1.0	0.0	0.0
	Maximum	13.0	10.0	13.0	8.0	10.0	7.0	1.0
Aquacultural farming area	n	29	29	29	28	29	29	28
	Mean	8.4	6.6	9.3	5.0	5.8	2.2	1.4
	SD	6.5	3.7	4.9	3.6	3.0	2.4	1.6
	Median	8.0	6.0	9.0	4.0	5.0	1.0	1.0
	SE	1.2	0.7	0.9	0.7	0.6	0.4	0.3
	Minimum	0.0	1.0	3.0	0.0	0.0	0.0	0.0
	Maximum	20.0	15.0	18.0	13.0	11.0	9.0	5.0
Significant		0.04[a,*]	0.11[a]	0.5[a]	0.2[a]	0.83[a]	1.00[a]	0.01[b,*]

Note:

[a] Significance was tested by independent t-test

[b] Significance was tested by Mann-Whitney U test

[*] $p < 0.05$

Table 26 Scores of ADHD symptoms in participants in low pesticide use period

Participant groups		ADHD index	Inattention	Hyperactivity/Impulsivity	Learning problems	Executive functioning	Aggressive/Defiance	Peer relation
Rice farming area	n	23	23	23	23	23	23	23
	Mean	3.5	5.1	8.3	4.0	5.2	2.5	1.3
	SD	3.6	2.2	3.9	1.7	2.3	1.9	1.8
	Median	3.0	5.0	9.0	4.0	5.0	3.0	0.0
	SE	0.8	0.5	0.8	0.4	0.5	0.4	0.4
	Minimum	0.0	0.0	1.0	1.0	1.0	0.0	0.0
	Maximum	12.0	8.0	17.0	7.0	10.0	8.0	6.0
Aquacultural farming area	n	29	29	29	29	29	29	29
	Mean	4.1	4.8	8.0	3.5	5.2	1.2	0.6
	SD	3.7	3.2	3.5	2.4	2.1	1.3	1.1
	Median	4.0	4.0	8.0	4.0	6.0	1.0	0.0
	SE	0.7	0.6	0.7	0.4	0.4	0.3	0.2
	Minimum	0.0	0.0	3.0	0.0	0.0	0.0	0.0
	Maximum	12.0	15.0	15.0	10.0	8.0	5.0	4.0
Significant		0.88[a]	0.60[a]	0.54[a]	0.56[a]	0.96[a]	0.01[a,*]	0.10[b]

Note:

[a] Significance was tested by independent t-test

[b] Significance was tested by Mann-Whitney U test

[*] $p < 0.05$

4.3.3 Validity scores

The validity score are presented in Table 27. The positive impression of interviewed parents in both rice and aquacultural participants were not different. Parents of both participant groups expressed more positive feeling to their child's behavior in pilot session which associated with lowest negative impression (expressed more negative feeling to their child' behavior) found in same session. The positive impression of parents of aquacultural participants seems to decrease across sessions from pilot session to low pesticide use period.

Table 27 Validity scores of positive and negative impression

Participant groups		Pilot		High		Low	
		Positive	Negative	Positive	Negative	Positive	Negative
Rice farming areas	n	22	23	21	23	23	23
	Mean	1.82	0.43	1.00	0.43	1.09	0.96
	SD	1.74	0.66	1.14	0.73	1.56	1.07
	Median	2.00	0.00	1.00	0.00	1.00	1.00
	SE	0.37	0.14	0.25	0.15	0.33	0.22
	Minimum	0	0	0	0	0	0
	Maximum	5	2	4	2	5	3
Aquacultural farming area	n	28	29	29	29	28	28
	Mean	1.96	0.45	1.45	0.83	1.21	0.57
	SD	1.48	0.95	1.15	1.20	1.26	1.00
	Median	2.00	0.00	1.00	0.00	1.00	0.00
	SE	0.28	0.18	0.21	0.22	0.24	0.19
	Minimum	0	0	0	0	0	0
	Maximum	5	4	4	5	4	4
Significant		0.74	0.95	0.17	0.17	0.74	0.18

Note: Significance was tested by Mann-Whitney U test

4.4 Consistency of outcomes

The repeated ANOVA analysis was used to analyze the consistency of outcomes obtained from within-subject outcomes at 3 sessions.

4.4.1 Pesticide exposure

Both participants from rice and aquacultural areas had similar blood cholinesterase levels (both AChE and PChE) and urinary pesticide metabolite levels (both OPs and PYR) across the 3 sessions.

4.4.2 Behavioral scores

For participants from rice area, the CPT and ADHD scores measured from individual participant at 3 different sessions were mostly similar, with the exception of % false alarm (p=0.01), hit latency (p=0.03) and peer relations (p=0.02). The change scores were clarified by using Post Hoc Multiple Comparisons. Percent of false alarms in pilot session was significantly greater (p=0.03) than high pesticide use period low pesticide use period. Although the longer testing time was used in high pesticide use period, the percent of false alarm was significantly lower (p=0.02) than when used shorter version in pilot session. When using the same test version in high pesticide use period low pesticide use period, the hit latency in high pesticide use period was significantly longer (p=0.03) than in low pesticide use period. The peer relation problems in high pesticide use period were significantly lower (p=0.02) than in low pesticide use period.

For participants from aquacultural area, the CPT and ADHD scores measured from individual participant at 3 different sessions were mostly similar, with the exception of overall ADHD scores (Conner 3 AI; p=0.001), inattention (p=0.01), and learning problems (p<0.001). The change scores were clarified by using Post Hoc Multiple Comparisons. The overall ADHD scores in high pesticide use period were significantly greater than in pilot session (p=0.003) and low pesticide use period (p=0.007). The inattention scores in high pesticide use period were significantly greater (p=0.02) than pilot session. The learning problems in high pesticide use period were significantly greater than in pilot session (p<0.001) and in low pesticide use period (p=0.001).

4.5 Correlation analysis

4.5.1 Correlation between concentrations found in hand wipes and urinary pesticide metabolites.

Pearson's correlation was used to determine the association between concentrations found in hand wipes and urinary pesticide metabolites. Permethrin had positive correlation with DCCA (r = 0.73, p = 0.06) and 3-PBA (r = 0.72, p = 0.06). No association was found for cypermethrin.

4.5.2 Correlation between blood ChE levels and urinary pesticide metabolites

In pilot and low pesticide use periods, the metabolite of chlorpyrifos (TCPy) concentrations were higher than in another session and were slightly negative correlation with PChE levels of rice participants (r = -0.20 and -0.14, respectively). This can be presuming that the high level of chlorpyrifos exposure may associate with PChE depression. In contrast, this association was not observed in high pesticide use period when the lowest chlorpyrifos exposure was detected.

4.5.3 Correlation between covariates and predictor variables.

In order to select the covariate for regression analysis to determine the relationship between pesticide exposure and behavioral health effects, the correlation of covariates and biomarkers were performed. Age had negatively significant correlation with DAP concentration (Pearson correlation, r = -0.293, p = 0.03). Body mass index (BMI) had positively significant correlation with PChE level (Pearson correlation, r = 0.319, p = 0.02). Parent education had positively significant correlation with 3-PBA concentration.

Table 28 Correlation between covariates and biomarkers.

Covariates	DAP		3PBA		AChE		PChE	
	r	p-value	r	p-value	r	p-value	r	p-value
Age	-0.293*	0.035	-0.104	0.464	-0.044	0.756	-0.151	0.290
Birth weight	-0.230	0.107	0.002	0.987	0.109	0.455	0.071	0.631
Body mass index (BMI)	-0.012	0.935	0.152	0.298	0.173	0.230	0.319*	0.025
Parent education	0.001	0.994	0.281*	0.044	0.001	0.997	0.070	0.628
Family income	-0.168	0.234	0.224	0.110	0.077	0.589	-0.107	0.457

In order to select the covariate for regression analysis to determine the relationship between pesticide exposure and behavioral health effects, the correlation of covariates and behavioral health effects were performed.

Age had negatively significant correlation with hit latency (Pearson correlation, r = -0.45, p = 0.001). Family income had significant correlation with false alarm latency (Spearman correlation, r = -0.30, p = 0.03).

Table 29 Correlation between covariates and CPT outcomes.

Covariates	% Hit		% False alarm		Hit latency		False alarm latency		DPrime	
	r	p-value	r	p-value	r	p-value	r	p-value	r	p-value
Age	0.220	0.116	0.174	0.218	-0.446**	0.001	-0.095	0.504	0.113	0.423
Birth weight	-0.073	0.614	0.166	0.147	-0.147	0.308	0.139	0.334	-0.193	0.179
Body mass index (BMI)	-0.085	0.558	0.222	0.122	-0.021	0.883	0.140	0.333	-0.170	0.237
Parent education	0.127	0.370	-0.065	0.647	-0.047	0.740	0.059	0.678	0.126	0.373
Parent vocabulary	0.085	0.551	0.012	0.931	-0.108	0.444	0.038	0.790	0.069	0.626
Family income	0.158	0.262	-0.193	0.169	0.083	0.559	-0.294*	0.034	0.169	0.230

*Correlation is significant at 0.05 level (2-tailed)

** Correlation is significant at 0.01 level (2-tailed)

Table 30 Correlation between covariates and the Conners ADHD scores.

Covariates	Inattention		Hyperactivity		Learning problem		Executive		Aggression		Peer relation		Conner 3AI	
	r	p-value	r	p-value	r	p-value	r	p-value	r	p-value	r	p-value	r	p-value
Age	0.112	0.431	0.167	0.236	-0.045	0.753	0.19	0.177	-0.099	0.496	-0.017	0.906	0.178	0.207
Birth weight	-0.183	0.208	-0.075	0.608	-0.339	0.019	-0.184	0.205	-0.153	0.304	-0.075	0.618	-0.148	0.312
Body mass index (BMI)	0.137	0.348	0.145	0.319	0.17	0.249	0.188	0.197	0.225	0.129	0.327*	0.025	0.196	0.177
Parent education	-0.05	0.723	0.051	0.717	-0.077	0.59	-0.089	0.529	0.014	0.921	0.165	0.252	0.042	0.768
Parent vocabulary	-0.037	0.795	-0.023	0.87	0.005	0.975	-0.12	0.399	-0.184	0.201	-0.048	0.743	-0.149	0.301
Family income	-0.178	0.208	-0.082	0.564	-0.233	0.099	-0.225	0.109	-0.418**	0.003	0.114	0.429	-0.068	0.633

* Correlation is significant at 0.05 level (2-tailed)

** Correlation is significant at 0.01 level (2-tailed)

Finally, the selected coviariates for the regression analysis were age, parent education, and family income because these covariates showed significant association with both CPT outcomes and ADHD symptoms in participants.

4.5.4 Correlation between continuous performance test (CPT) and Conners ADHD questionnaires.

The correct response to target stimulus (% hit) were positively associated with hyperactivity/impulsivity scores ($r = 0.28$, $p = 0.04$). The incorrect response to non-target stimuli (% false alarm) were positively correlated with inattention ($r = 0.29$, $p = 0.03$) and hyperactivity/impulsivity scores ($r = 0.28$, $p = 0.04$).

4.6 Multiple regression analysis for behavioral health effects and pesticide exposure.

Although there were no significant associations between CPT and ADHD outcomes and pesticide exposure (both OP and PYR) in children participants (Table 31–34), the regression analysis might be showed some association (selected interpretation by $p < 0.30$) as following;

- For 10-fold increase in DAP level we predict 0.4 milliseconds decrease in false alarm latency, controlling for age, parental education, and family income. This means participants who had higher OP exposure tended to have faster response to non-target stimuli than who had lower OP exposure.
- For 10-fold increase in DAP we predict 0.02 scores decrease in Conner 3 AI, controlling for age, parental education, and family income. This means

participants who had higher OP exposure tended to have lower overall ADHD index scores than who had lower OP exposure.

Table 31 Result of multiple regression analysis of DAP levels and neurobehavioral outcomes

(adjusted for age, parent education, and family income)

						95% Confidence interval	
Outcomes	R^2	Intercept	Slope	t	p-value	Lower bound	Upper bound
% Hit	0.100	0.13	0.00001	0.32	0.74	0.00	0.00
% False alarm	0.034	-0.17	-0.00006	-0.84	0.40	0.00	0.00
Hit latency	0.210	963.44	0.01	0.49	0.62	-0.03	0.05
False alarm latency	0.075	727.73	-0.04	-1.99	0.27	-0.12	0.03
Dprime	0.063	0.16	0.00	0.85	0.39	0.00	0.001
Conner 3 AI	0.068	-1.31	-0.002	-1.25	0.21	-0.004	0.001
Inattention	0.024	4.43	0.00	-0.92	0.36	-0.002	0.001
Hyperactivity/Impulsivity	0.056	2.00	0.00	-0.63	0.53	-0.002	0.001
Learning problem	0.037	8.19	0.00	-0.25	0.80	-0.001	0.001
Executive functioning	0.084	1.62	0.00	0.34	0.73	0.00	0.001
Aggression	0.149	4.95	0.00	0.27	0.78	0.00	0.001
Peer relation	0.044	0.85	0.00	-0.72	0.47	0.00	0.00

- For 10-fold increase in PBA level we predict 50.9 milliseconds decrease in hit latency, controlling for age, parental education, and family income. This means participants who had higher PYR exposure tended to have faster response to target stimulus than who had lower PYR exposure.
- For 10-fold increase in PBA level we predict 0.9 units increase in aggressive score, controlling for age, parental education, and family income. This means participants who had higher PYR exposure tended to have higher aggressive symptoms than who had lower PYR exposure.

Table 32 Result of multiple regression analysis of 3-PBA levels and neurobehavioral outcomes

(adjusted for age, parent education, and family income)

						95% Confidence interval	
Outcomes	R^2	Intercept	Slope	t	p-value	Lower bound	Upper bound
% Hit	0.105	0.19	-0.006	-0.60	0.54	-0.02	0.01
% False alarm	0.022	0.13	0.001	0.23	0.82	-0.01	0.01
Hit latency	0.230	1015.72	-5.09	-1.24	0.21	-13.30	3.12
False alarm latency	0.070	608.76	7.32	0.97	0.33	-7.83	22.47
Dprime	0.056	0.72	-0.03	-0.60	0.55	-0.13	0.07
Conner 3 AI	0.036	-3.82	0.001	0.01	0.99	-0.501	0.50
Inattention	0.007	3.66	-0.03	-0.24	0.81	-0.30	0.24
Hyperactivity/Impulsivity	0.048	0.97	0.02	0.09	0.92	-0.34	0.37
Learning problem	0.036	7.79	0.02	0.17	0.86	-0.22	0.26
Executive functioning	0.092	1.35	0.09	0.75	0.45	-0.15	0.32
Aggression	0.170	4.51	0.09	1.07	0.28	-0.08	0.27
Peer relation	0.039	0.72	-0.03	-0.55	0.58	-0.16	0.09

- For 10-fold decrease in AChE level we predict 0.6 units increase in % false alarm, controlling for age, parental education, and family income. This means participants who had higher AChE depression tended to have higher incorrect response to non-target stimuli than who had lower AChE depression.
- For 10-fold decrease in AChE level we predict 412.2 milliseconds increase in hit latency, controlling for age, parental education, and family income. This means participants who had higher AChE depression tended to have slower response to correct stimulus than who had lower AChE depression.
- For 10 unit decrease in AChE level we predict 882.9 milliseconds increase in false alarm latency, controlling for age, parental education, and family income. This means participants who had higher AChE depression tended to have slower response to incorrect stimuli than who had lower AChE depression.

- For 10-fold decrease in AChE level we predict 7.3 units decrease in aggressive scores, controlling for age, parental education, and family income. This means participants who had higher AChE depression tended to have lower aggressive symptoms than who had lower AChE depression.

Table 33 Result of multiple regression analysis of AChE levels and neurobehavioral outcomes

(adjusted for age, parent education, and family income)

						95% Confidence interval	
Outcomes	R^2	Intercept	Slope	t	p-value	Lower bound	Upper bound
% Hit	0.116	0.37	-0.07	-0.98	0.32	-0.22	0.07
% False alarm	0.085	0.33	-0.06	-1.82	0.07	-0.13	0.01
Hit latency	0.233	1108.54	-41.22	-1.31	0.19	-104.68	22.22
False alarm latency	0.094	920.49	-88.29	-1.48	0.14	-201.05	30.47
Dprime	0.049	0.58	-0.02	-0.06	0.95	-0.80	0.76
Conner 3 AI	0.039	-1.55	-0.75	-0.38	0.70	-4.67	3.17
Inattention	0.009	4.49	-0.35	-0.33	0.74	-2.47	1.76
Hyperactivity/Impulsivity	0.068	5.25	-1.38	-0.99	0.32	-4.17	1.41
Learning problem	0.051	10.34	-0.78	-0.84	0.40	-2.66	1.09
Executive functioning	0.080	2.26	-0.10	-0.10	0.91	-1.97	1.77
Aggression	0.169	2.91	0.73	1.06	0.29	-0.65	2.11
Peer relation	0.034	0.20	0.11	0.22	0.82	-0.87	1.08

- For 10-fold decrease in PChE level we predict 0.7 units increase in % hit, controlling for age, parental education, and family income. This means participants who had higher PChE depression tended to have increase correct response to target stimulus than who had lower PChE depression.
- For 10-fold decrease in PChE level we predict 0.4 units decrease in % false alarm, controlling for age, parental education, and family income. This means participants who had higher PChE depression tended to have decrease incorrect response to non-target stimuli than who had lower PChE depression.
- For 10-fold decrease in PChE level we predict 437.4 milliseconds increase in hit latency, controlling for age, parental education, and family income. This means participants who had higher PChE depression tended to have slower response to target stimulus than who had lower PChE depression.
- For 10-fold decrease in PChE level we predict 6.1 units increase in DPrime, controlling for age, parental education, and family income. This means participants who had higher PChE depression tended to have increase ability to discriminate target from non-target stimuli than who had lower PChE depression.

- For 10-fold decrease in PChE level we predict 11 units decrease in inattention scores, controlling for age, parental education, and family income. This means participants who had higher PChE depression tended to have lower inattention symptoms than who had lower PChE depression.
- For 10-fold decrease in PChE level we predict 20 units decrease in hyperactivity/impulsivity scores, controlling for age, parental education, and family income. This means participants who had higher PChE depression tended to have lower hyperactivity/impulsivity symptoms than who had lower PChE depression.
- For 10-fold decrease in PChE level we predict 10 units decrease in aggressive scores, controlling for age, parental education, and family income. This means participants who had higher PChE depression tended to have lower aggressive symptoms than who had lower PChE depression.

Table 34 Result of multiple regression analysis of PChE levels and neurobehavioral outcomes

(adjusted for age, parent education, and family income)

						95% Confidence interval	
Outcomes	R^2	Intercept	Slope	t	p-value	Lower bound	Upper bound
% Hit	0.120	0.42	-0.07	-1.03	0.30	-0.22	0.07
% False alarm	0.042	0.01	0.04	1.11	0.27	-0.03	0.11
Hit latency	0.221	1100.09	-43.74	-1.38	0.17	-107.53	20.04
False alarm latency	0.058	765.32	-34.60	-0.58	0.56	-154.75	85.53
Dprime	0.098	2.43	-0.61	-1.59	0.11	-1.39	0.16
Conner 3 AI	0.058	-9.85	1.55	0.80	0.42	-2.32	5.42
Inattention	0.032	-0.16	1.10	1.05	0.29	-1.00	3.21
Hyperactivity/Impulsivity	0.304	-5.36	2.05	1.48	0.14	-0.73	4.84
Learning problem	0.045	8.34	-0.28	-0.30	0.76	-2.17	1.61
Executive functioning	0.123	-0.38	0.50	0.55	0.58	-1.32	2.33
Aggression	0.189	2.31	1.02	1.47	0.14	-0.37	2.42
Peer relation	0.032	-0.22	0.20	0.43	0.66	-0.74	1.15

CHAPTER V
DISCUSSION

Most of behavioral health effects were not significant different between participant groups and the findings were less consistently among pesticide use periods (or seasons). Pesticide exposure and ADHD behavior are unlikely to be causal, perhaps resulting from cross-sectional research design as a pilot study and limited study population.

Similar to a cross-sectional study of Lu et al. (2009), there were no associations between OP and PYR metabolite levels and the cognitive performance also measured by BARS in children aged 4-10 years in coffee plantation communities in Costa Rica. In addition the study of Bouchard et al. (2011), reported that there was no significant relationship between IQ score and OP exposure in school-aged children. However, other cross-sectional studies reported significantly lower neurobehavioral performance among pre-school children living in agricultural areas compared to those not living in agricultural areas (17, 86). However, only location was used as an indicator of exposure which may account for the discrepancy in their findings from the present study. It is also possible that 6-8 year old children may not be as sensitive to the effects of concurrent pesticide exposure on cognitive performance. This is consistent with birth cohort studies in which prenatal but not child pesticide exposure measurements showed significant association with neurobehavioral deficits (87).

Most of ADHD symptoms in both participant groups were similar and showed consistent at low pesticide use periods but changed at high pesticide use period. The findings associated with inconsistent neurobehavioral performance in postnatal OP exposure but consistent in prenatal OP exposure (87). The levels of pesticide exposure might have different effect to neurobehavioral outcomes in each developmental stage or it may be that ADHD symptoms are not predicted by concurrent exposure.

Our study found no significant relationship between OP exposure and ADHD symptoms. The finding agreed with Rodríguez's study (2012) that OP exposure among Nicaraguan children of agricultural workers were not related to ADHD symptoms evaluated by the Conners' Teacher Rating Scale-Revised (short form). The Conners ADHD questionnaires may need to be adjusted to increase their sensitivity for ADHD behaviors among Thai children.

In this study, participants who had low AChE levels (higher OP exposure) tended to have slower response to target stimulus. This findings were similar to Grandjean et al. (2006) that OP exposed children (7 years old) performed slower response to the Catsys equipment (comparable to CPT).

Interestingly, participants with high 3-PBA (higher PYR exposure) were likely to have more aggressive behavior than who had lower levels. This was a new finding but need to be explored more in larger study population. Previously, the study of Rodríguez (2012) found the PYR exposure during the first year of life related to ADHD symptom in Nicaraguan girl of agricultural workers aged 7-9 years by using the Conners' Teacher Rating Scale-Revised (short form). PYR exposure during childhood was also associated with behavioral problems as assessed by the Strengths and Difficulties Questionnaire (SDQ). However, this questionnaire is not specific to ADHD symptoms (89). Those studies did not report the specific kind of behavioral problem but our study was the first study to report the relationship between PYR exposure and aggressive behavior in children. In addition, there was an animal experiment which reported impulsive-like behavior in the offspring of PYR exposed pregnant mice (90).

However, there were some negative findings in our study compared to other studies. Bouchard et al. (2010) reports that US children with high DAP and low AChE levels tended to show more hyperactivity/impulsivity symptoms. This study had much more sample sizes than our study and therefore more possibly for significant finding. Moreover, the neurobehavioral deficits which related to AChE depression were found in children who worked as pesticide applicators (91). From this finding, it is possible to suggest that the relationship between pesticide exposure and behavioral health effects were clearly found with amount of pesticide exposure that high enough to impact the biomarker of effect (e.g blood cholinesterase level), probably from direct exposure in pesticide applicators rather than indirect exposure in children living in agricultural areas.

The performance from CPT was positively associated to ADHD behavior. Participants with high correct and incorrect responses on CPT were associated with high inattention and hyperactivity/impulsivity on ADHD questionnaires reported by their parents. This finding can be explained by the impulsivity that reflected the child to respond to every appeared stimulus without attention to right or wrong reaction. The findings were supported by previous studies that children with poor performance on CPT were related to ADHD symptoms (63, 92).

By using the same CPT version in high pesticide use period and low pesticide use period, our participants mostly performed consistent on CPT over time with exception of % false alarm and hit latency which decreased from high pesticide use period to low pesticide use period. The improvement of CPT performance from high pesticide use period to low pesticide use period might be explained by the practice effects that the child learned how to cope with similar test and performed better than the previous session (93).

The metabolites of chlorpyrifos (TCPy, DEP, and DETP) were the only metabolites differing among rice and aquacultural farming participants, suggesting that chlorpyrifos is widely used in rice farming. This result was related to the previous study that reported chlorpyrifos was the most popularly used pesticides in rice fields in Khlong 7 (29).

Despite previously reported observations in Northern Thailand demonstrating otherwise (9), parental occupation as it relates to proximity to farms and child behaviors tended to have a large impact on pesticide exposures. Children of rice farmers lived in closer proximity to farms, tended to have more dirt on their bodies and often played while parents worked on the farm. Conversely, children whose parents were aquacultural farmers spent less time outdoors, lived further from rice farms, and had less dirt on their bodies. All of these factors likely interplay to increase exposures in rice farmer children as compared to those whose parents worked in aquacultural farming.

Previous research revealed that the mouthing behavior in young children is a potential activity leading to non-dietary ingestion (94). Hand-to-mouth and object-to-mouth activities can lead to intake of OPs from contaminated soil or from surfaces that the child is playing around (95). We hypothesized that younger participants in our study would have higher levels of OP metabolites than older participants. Although they had more opportunity to be exposed to OPs from contaminated environments than older children because they had been frequently observed with soil or dirt attached to their bodies after outdoor playing and they spent more time on the farm while their parents were working, we did not find an association between age and creatinine-corrected urinary metabolite levels.

Although participants from aquacultural farming areas had significantly lower OP metabolite concentrations than participants from rice areas, they still had measureable concentrations suggesting exposure through a different pathway. Consumption of OP contaminated foods can be another potential pathway of exposure to OPs among children irrespective of their proximity to farms using OPs

(14). In 2011, the Thai Food and Drug Administration reported that 5.3% of fresh food samples available in local markets were contaminated with pesticide residues and exceeded the safety threshold (96). In addition, OPs are commonly used for pest control in home gardens (97).

Petchuay et al. (2006) reported DAP concentrations in children living near vegetable and rubber farms in Songkla Province, southern Thailand. In the wet season, DAP concentrations of vegetable farm children were lower than concentrations detected in our participants from rice farms, except for DMTP. The DAP concentrations found in our study are also higher than the concentrations found in children living in vegetable and fruit farming communities in Nakhon-ratchasima Province, north-eastern Thailand (98). Concentrations of TCPy in school-aged children residing on vegetable, fruit, and ornamental farms in Chiang Mai, Northern Thailand (9) were lower than the concentrations detected in our rice and aquacultural farming participants even though the same methods were used for both studies.

Regarding to chlorpyrifos, a widely used chemical in rice farming areas, it is a weak AChE inhibitor (99) and therefore AChE levels in rice farming children were not depressed. Participants from aquacultural area had more risky cases of AChE depressions than participants from rice area suggesting that the participants from aquacultural area may have possibility of exposure to pesticides that used in fish or shrimp farming e.g. carbamates (100) which also have mode of action in cholinesterase inhibition. In this study, there were no risky PChE levels in both participant groups. This is probably because the level of OP exposure among children living in agricultural areas was not high enough to depressed AChE levels. Our sample is rice farmers who directly apply and are exposed to OP resulting in significantly depressed PChE levels and acute signs of toxicity (12, 101).

Six month intervals among sampling sessions were appropriate because both urinary pesticide metabolites and blood cholinesterase levels were fully recovery (102). The multiple measurements of behavioral assessment can reveal how the behavioral symptoms persist over time. The baseline of cholinesterase levels were suitable to compare with other sampling session. This can reduce the biological variability among the population (103).

Our data can also be compared with other countries to understand better the exposure situation in Thailand relative to more developed countries. The DAP concentrations in our study were lower than concentrations in German children aged 6-11 years in the GerES IV Pilot Study 2001-2002 and lower than reference value for German population when chlorpyrifos was still actively used in residential pest control (104, 105). Concentrations of metabolites of chlorpyrifos (TCPy, DEP, and DETP) were higher among our participants from rice farms than children of applicator families in Washington State, USA (10) and children aged 6-11 years in the US general population as measured in the US National Health and Nutrition Examination Survey (NHANES 1999-2004) (106-108).

In order to extrapolate the population risk of participant children living in rice areas in our population, we calculated the ADD of all study participants (109). All ADDs calculated in the rice farming participants exceeded the US EPA chronic PAD (0.03 μg/kg/day). While we are aware that using a single sample, even a more concentrated first morning void urine sample which integrates urine for 8 hours or more, can bias the estimate of ADD, this comparative result is quite alarming. Based on the pharmacokinetics of chlorpyrifos/TCPy, the first 24 hours after dermal exposure to chlorpyrifos are expected to be the highest peak of TCPy excretion (110). The sample we collected may not represent peak exposure or even daily average exposure. Nonetheless, these ADD levels may provide a reasonable estimate of daily chronic exposures, since the urinary concentrations appear to be related to dwelling location and behaviors that are relatively consistent over a season.

Although PYR products had been used in both rice farms and residential areas, the concentrations of PYR metabolites were not significant different between participant groups. This finding can be suggesting that PYR use on rice farms was not a primary source of PYR exposure, but PYR used in households might probably consider as a main exposure source. Based on type and frequency of PYR products use in households, the levels of PYR exposure were slightly different between participant groups. Participants living in aquacultural area, where had more frequency used of insecticide spray products, were slightly higher PYR metabolite concentrations than participants living in rice farming area. This finding confirmed that PYR contained in insecticide products used in households had more relevant to level of PYR metabolites in participants than products used on rice farms.

Environmental conditions and children's activities were somewhat associated to PYR exposure. PYR residues on rice farms might increase chance to contact PYR to the body when children were playing around. Children behavior such as object-to-mouth could also increase possibility to intake PYR via contact the contaminated objects. However, there were negative findings from this study which were not associated with PYR exposure i.e. floor cleaning, personal hygiene, etc. Floor wipes and dust samples should be collected to investigate PYR residues on floors. The quantitative data including feet and body wipe should be used together with hand wipe samples to examine dirt on body which might related to PYR exposure via dermal contact.

Panuwet et al. (2009) studied 3-PBA metabolite concentrations in school-aged children in north Thailand. The metabolite concentrations were lower than the concentration detected in our rice and aquacultural farming participants for every session. The 3-PBA concentrations found in our study had higher concentration than US children aged 6-11 years in the study of NHANES 1999-2002 (37) for every session. In addition, both DCCA and 3-PBA concentrations detected in our study were above the reference values of German population (105), suggesting that Thai had higher PYR used for residential pest controls.

In participants from rice farming area, the patterns of urinary PYR metabolite concentrations were increased in low pesticide use periods but decreased in high pesticide use period suggesting that during high pesticide application on rice farms the pests in households located around rice farms were also decreased. Therefore, only few PYR use in household pest control but increase to use when the pesticide use on farms were decreased. Unlike the patterns of urinary PYR metabolite concentrations in participants from aquacultural farming area, the increased levels from high pesticide use period to low pesticide use period were from the increasing PYR use for household pest control after the flooding in this area. Previously, there were plenty of fish ponds and fish play a role as natural enemy to kill mosquitos and insects. Unfortunately, the ponds were damaged and loss of fish by heavy flooding during October to December 2011. When the natural enemy was decreased, the PYR use was increased to control the pest in households.

Interestingly, the AChE levels were positively correlated to PChE levels in all sampling sessions (pilot; $r = 0.33$, $p = 0.03$, high; $r = 0.23$, $p = 0.09$, low; $r = 0.26$, $p = 0.05$), suggesting that participants who had low AChE levels were also likely to detect low PChE levels. The modes of action of pesticides belonging to OP group are different. Some compounds inhibit AChE, while other may inhibit PChE. Therefore, the measurement of both AChE and PChE are necessary to cover the toxicity that the varieties of OPs might affect.

CHAPTER VI
CONCLUSION AND RECOMMENDATION

The exact causes of ADHD symptoms are unclear, but the evidence has shown it likely to include OP and PYR exposure. However, pesticide metabolites and blood cholinesterase could not reflect long-term exposure because they have short half-lives. The biomarker of chronic health effects such as DNA damage may associate to neurobehavioral deficits (111). Possible positive association between pesticide exposure and ADHD require continued investigation in longitudinal study design and larger sample size.

This study had strength and weakness. The strength of this study was a new evidence of behavioral health effects and pesticide exposure and demonstrated the new psychometric test development for Thai children. Only few studies had been determined association between behavioral health effects and OP and PYR exposure.

Participants from rice farming areas had higher OP exposure than participants from aquacultural area. Although levels of OP exposure were significant different between participant groups, most of behavioral health effects were similar over time and did not differ between groups. We have to bear in mind that the sample size calculation in this study were derived from the difference between levels of urinary OP metabolites in children living in agricultural area and non-agricultural area (5). Hence, the power analysis of sample size was sufficient to show the difference between participant groups in OP metabolite concentrations but not for the behavioral health effect. In further study, the difference of behavioral outcomes should be used to determine an appropriate sample size for detection of neurobehavioral health effects.

The development of psychometric tests including the CPT and the Conners ADHD questionnaires in this study are in stage of cross-cultural development to be suitable for Thai children. Most of CPT and Conners ADHD scores were consistent across testing sessions. Therefore, the translated Thai version of CPT and Conners ADHD in Thai were reliable over a 6 month period. Furthermore, some items in Conners ADHD questionnaires were needed to be adjusted to be more suitable for Thai culture.

This study showed the reliability of the neurobehavioral tests because there were evaluated from both particpants' performance (CPT) and parents' observation (ADHD questionnaires). The significant correlation between the CPT as an indicator of behavior associated with ADHD and the Conners questionnaire support the validity of the Conners as a reflection of ADHD behaviors.

The levels of PYR exposure were similar between participant living in rice farming area and participants living in aquacultural farming area. PYR is commonly used in household pest control but also used on rice farming. Concerning higher OP application on rice farming than other types of pesticides, sources of PYR exposure were mainly considered in household usage. Therefore, the levels of PYR exposure were similar between participant groups.

The biomarkers used in this study were biomarkers of exposure (urinary pesticide metabolites) and biomarkers of effect (blood cholinesterase). These biomarkers had advantage in both qualitative and quantitative data. The biomarkers of exposure gave the information on what kinds and amount of pesticides that the participants had been exposed to and the biomarkers of effect gave the information on the toxicity levels of the pesticide. In fact, the blood cholinesterase depression is related to exposure of OP and carbamate pesticides. To be confirmed that the toxicity is from OP exposure, the urinary pesticide metabolites are needed to be analyzed. However, the biological sampling should consider the pharmacokinetics of those biomarkers. According to DAP half-lives range 1 to 15.5 hour (112) after oral and dermal exposure, the urine samples should be collected within 2-24 hours after high and medium OP exposure (102). For blood cholinesterase measurements, the half-lives are 1 month and a few weeks after exposure for AChE and PChE, respectively (113, 114). Nevertheless, there are no half-lives for low OP exposure among non-occupational and residential population (115), the single void urine samples may be sufficient to estimate the daily absorbed doses even though it may miss variations during 24 hours and lead to error of exposure estimation. First morning voids with creatinine adjusted concentrations may overestimate concentrations compared to 24-hr urine samples (107). Using creatinine correction for child populations may not be an appropriate way of correcting for urine dilution because of their endogenously lower creatinine concentrations. The full 24-hr urine sample may be preferable to estimate the daily dose but this study was unavoidable because of the financial limitation. The DAP metabolites may be derived from exposure to the parent chemical or the preformed metabolites so we may have overestimated exposure to the biological active pesticides (116).

Other limitations were the limited number of published studies of association between pesticide exposure and behavioral health effects made difficult to compare the result with other studies (117).

This study used the cut points (safe/risky) of cholinesterase levels from the adult population in the US and therefore it might not appropriate for Thai children. The cut points should be obtained from the local population with same age group. To my knowledge, none of the cut points of children are available in Thailand.

In future study, the blood cholinesterase analysis should be considered both OPs and carbamates exposure because both have an effect to cholinesterase depression. Therefore, the researcher should collect urinary OP and urinary carbamate metabolites to confirm the exposure.

Finally, the participants in this study had higher OP exposure than those reported in children residing in other areas in Thailand. Although the daily exposure was not at risk level but it might cause cognitive deficits as reported in previous study (16). The risk of pesticide exposure among participant children living near rice farming area in Pathum Thani province is undoubtedly a concern that requires public health attention, with an emphasis on potential neurobehavioral deficits among children with long-term pesticide exposure. The future investigations should consider a longitudinal design and a larger study population.

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APPENDIX

CONNERS 3 - Parent Short

Instructions: Here are some things parent might say about their children. Please tell us about *your* child and what he/she has been like in the **past month**. Read each item carefully, then mark how well it describes your child or how frequently it has happened in the **past month**.

0 = In the past month, this was **not true at all** about my child. It never (seldom) happened.
1 = In the past month, this was **just a little true** about my child. It happened occasionally.
2 = In the past month, this was **pretty much true** about my child. It happened often (or quite a bit).
3 = In the past month, this was **very much true** about my child. It happened very often (very frequently).

Please circle only one answer for each item. It is very important to respond to every item.
For items that you find difficult to answer, please give your best guess.

	Rating: In the past month, this was…	0 = Not true at all (Never, Seldom) 1 = Just a little true (Occasionally)	2 = Pretty much true (Often, Quite a bit) 3 = Very much true (Very often, Very frequently)			
1	Forgets to turn in completed work.		0	1	2	3
2	Is perfect in every way.		0	1	2	3
3	Fidgets or squirms in seat.		0	1	2	3
4	Is one of the last to be picked for teams or games.		0	1	2	3
5	Restless or overactive.		0	1	2	3
6	Does not know how to make friends.		0	1	2	3
7	Runs or climbs when he/she is not supposed to.		0	1	2	3
8	Cannot grasp arithmetic.		0	1	2	3
9	Is difficult to please or amuse.		0	1	2	3
10	Needs extra explanation of instructions.		0	1	2	3
11	Is hard to motivate (even with rewards like candy or money).		0	1	2	3
12	Make mistakes.		0	1	2	3
13	Acts as if driven by a motor.		0	1	2	3
14	Starts fights with others on purpose.		0	1	2	3
15	Has trouble getting started on tasks or projects.		0	1	2	3
16	Is happy, cheerful, and has a positive attitude.		0	1	2	3
17	Doesn't pay attention to detail; makes careless mistakes.		0	1	2	3
18	Has trouble keeping friends.		0	1	2	3
19	Bullies, threatens, or scares others.		0	1	2	3
20	Loses things (for example, schoolwork, pencils, books, tools, or toys).		0	1	2	3
21	Tell lies to hurt other people.		0	1	2	3
22	I cannot figure out what makes him/her happy.		0	1	2	3
23	Threatens to hurt others.		0	1	2	3
24	Is constantly moving.		0	1	2	3
25	Has trouble with reading.		0	1	2	3
26	Is angry and resentful.		0	1	2	3
27	Has a short attention span.		0	1	2	3
28	Excitable, impulsive.		0	1	2	3
29	Cannot do things right.		0	1	2	3
30	Has trouble concentrating.		0	1	2	3
31	Tell the truth; doesn't even tell "little white lies."		0	1	2	3
32	Has trouble organizing tasks or activities.		0	1	2	3
33	Is fun to be around.		0	1	2	3
34	Inattentive, easily distracted.		0	1	2	3
35	Is messy or disorganized.		0	1	2	3
36	Spelling is poor.		0	1	2	3
37	Is patient and content, even when waiting in a long line.		0	1	2	3
38	Has no friends.		0	1	2	3
39	Does not understand what he/she reads.		0	1	2	3
40	Behaves like an angel.		0	1	2	3
41	Has trouble keeping his/her mind on work or on play for long.		0	1	2	3
42	Has to struggle to complete hard tasks.		0	1	2	3
43	Does not get invited to play or go out with others.		0	1	2	3

Additional Questions:

44 Do you have any other concerns about your child?____

45 What strengths or skills does your child have?____

CONNERS 3 Parent Short

ข้อความต่อไปนี้ เป็นพฤติกรรมโดยทั่วไปของลูก ๆ ที่พ่อแม่มักจะเล่าให้ฟัง

กรุณาอ่านข้อความแต่ละข้อให้เข้าใจ และลองนึกดูว่า **ในช่วง 1 เดือนที่ผ่านมา** **ลูกของท่านทำพฤติกรรมตรงตามนี้บ้างหรือไม่ และทำบ่อยเพียงใด**

กรุณาตอบคำถามให้ครบทุกข้อ

ในการตอบคำถามแต่ละข้อ กรุณาทำเครื่องหมาย X เพื่อเลือกเพียงคำตอบเดียวที่**ตรง**กับลูกของท่าน**มากที่สุด**

ถ้าท่านไม่แน่ใจในบางคำตอบ ขอให้พยายามตอบให้ดีที่สุด ตามที่ท่านรู้จักลูกของท่าน

ในช่วง 1 เดือนที่ผ่านมา ลูกทำพฤติกรรมตรงตามนี้บ้างหรือไม่ และบ่อยเพียงใด	ไม่ตรงเลย ลูกไม่เคยทำแบบนี้เลย	ไม่ค่อยตรง ลูกทำบ้างแต่ไม่บ่อย	ค่อนข้างตรง ลูกทำค่อนข้างบ่อย	ตรงที่สุด ลูกทำเป็นประจำ
1 ลืมส่งการบ้าน ทั้งที่ทำเสร็จแล้ว Forgets to turn in completed work.	0	1	2	3
2 เป็นเด็กดีในทุกเรื่อง ไม่มีปัญหาอะไรเลย Is perfect in every way.	0	1	2	3
3 นั่งนิ่งๆ อยู่กับที่ไม่ค่อยได้ ต้องหยุกหยิกไปมาเวลานั่ง Fidgets or squirms in seat.	0	1	2	3
4 ไม่ค่อยมีใครอยากเลือกเข้ากลุ่ม มักเป็นคนสุดท้ายที่เพื่อน ๆ เลือก หรือต้องเป็นตัวแถม Is one of the last to be picked for teams or games.	0	1	2	3
5 ซนมาก หรืออยู่นิ่งๆ ไม่เป็น Restless or overactive.	0	1	2	3
6 ไม่รู้ว่าจะเข้ากับเด็กคนอื่นอย่างไร Does not know how to make friends.	0	1	2	3
7 วิ่งซน หรือปีนป่ายอย่างไม่รู้กาละเทศะ Runs or climbs when he/she is not supposed to.	0	1	2	3
8 ไม่เข้าใจเรื่องการคิดเลข Cannot grasp arithmetic.	0	1	2	3
9 เอาใจยาก ไม่รู้จะเอาใจยังไงถูก Is difficult to please or amuse.	0	1	2	3
10 เวลาจะให้ทำอะไร ต้องอธิบายหลายครั้ง หรืออธิบายอยู่นานถึงจะเข้าใจ Needs extra explanation of instructions.	0	1	2	3
11 กระตุ้นให้ทำอะไรได้ยาก ถึงแม้จะมีขนมหรือเงินมาล่อใจเป็นรางวัล Is hard to motivate (even with rewards like candy or money).	0	1	2	3
12 ทำอะไรผิดพลาด Make mistakes.	0	1	2	3
13 ทำตัวเหมือนติดเทอร์โบ มีพลังเยอะ Acts as if driven by a motor.	0	1	2	3
14 ชอบหาเรื่องคนอื่น ทำร้ายคนอื่นก่อนโดยตั้งใจ Starts fights with others on purpose.	0	1	2	3
15 อิดออด ไม่ยอมเริ่มทำงานที่ต้องทำ Has trouble getting started on tasks or projects.	0	1	2	3
16 มีความสุข ร่าเริงแจ่มใส และมองโลกในแง่ดี Is happy, cheerful, and has a positive attitude.	0	1	2	3
17 ไม่ค่อยใส่ใจในรายละเอียด ทำอะไรผิดพลาดเพราะความสะเพร่าไม่เอาใจใส่ Doesn't pay attention to detail; makes careless mistakes.	0	1	2	3
18 เพื่อนไม่ค่อยชอบ เป็นเพื่อนกับใครได้ไม่นาน Has trouble keeping friends.	0	1	2	3
19 ทำตัวเป็นนักเลง รังแก แกล้งคนอื่น Bullies, threatens, or scares others.	0	1	2	3
20 ทำของหาย เช่น สมุดการบ้าน ดินสอ ยางลบ หนังสือ ของเล่น เป็นต้น Loses things (for example, schoolwork, pencils, books, tools, or toys).	0	1	2	3
21 พูดโกหก เพื่อแกล้งคนอื่นให้เสียใจ Tell lies to hurt other people.	0	1	2	3
22 พ่อแม่ไม่รู้เลยว่าควรทำอะไรให้ลูก ลูกถึงจะชอบ I cannot figure out what makes him/her happy.	0	1	2	3
23 ขู่ว่าจะทำร้ายคนอื่น Threatens to hurt others.	0	1	2	3
24 อยู่ไม่นิ่ง เดินหรือวิ่งไปมาอยู่ตลอดเวลา Is constantly moving.	0	1	2	3
25 มีปัญหาในการอ่านหนังสือ Has trouble with reading.	0	1	2	3
26 โกรธ เจ้าคิดเจ้าแค้น Is angry and resentful.	0	1	2	3
27 สนใจอะไรไม่นาน ทำได้แค่ช่วงสั้น ๆ Has a short attention span.	0	1	2	3

ในช่วง 1 เดือนที่ผ่านมา ลูกทำพฤติกรรมตรงตามนี้บ้างหรือไม่ และบ่อยเพียงใด	ไม่ตรงเลย ลูกไม่เคยทำแบบนี้เลย	ไม่ค่อยตรง ลูกทำบ้างแต่ไม่บ่อย	ค่อนข้างตรง ลูกทำค่อนข้างบ่อย	ตรงที่สุด ลูกทำเป็นประจำ
28 ขี้ตื่น ผลีผลาม ตื่นเต้นง่าย ทำอะไรเร่งรีบโดยไม่คิด Excitable, impulsive.	0	1	2	3
29 ทำอะไรก็มีแต่จะผิดพลาด ไม่เคยทำอะไรถูกต้อง Cannot do things right.	0	1	2	3
30 ไม่มีสมาธิ Has trouble concentrating.	0	1	2	3
31 พูดความจริง ไม่เคยโกหกเลย แม้จะเป็นการโกหกเพื่อเอาใจผู้อื่น Tell the truth; doesn't even tell "little white lies."	0	1	2	3
32 ทำสิ่งต่าง ๆ อย่างไม่เป็นระบบ ไม่มีระเบียบ Has trouble organizing tasks or activities.	0	1	2	3
33 ใครๆ ก็ชอบ อยากเล่นด้วย อยากอยู่ด้วย Is fun to be around.	0	1	2	3
34 เหม่อ ใจลอย วอกแวกง่าย Inattentive, easily distracted.	0	1	2	3
35 ทำรก เลอะเทอะ ไม่เก็บของเข้าที่ ไม่มีระเบียบ Is messy or disorganized.	0	1	2	3
36 สะกดคำต่าง ๆ ไม่ค่อยถูก Spelling is poor.	0	1	2	3
37 มีความอดทนใจเย็น แม้ต้องรอต่อคิวยาว ๆ Is patient and content, even when waiting in a long line.	0	1	2	3
38 ไม่มีเพื่อน Has no friends.	0	1	2	3
39 ไม่เข้าใจสิ่งที่อ่าน Does not understand what he/she reads.	0	1	2	3
40 น่ารัก แสนดี เหมือนเทวดาตัวน้อย ๆ Behaves like an angel.	0	1	2	3
41 ทำหรือเล่นอะไรได้แค่เวลาสั้นๆ เล่นนานๆไม่ค่อยได้ Has trouble keeping his/her mind on work or on play for long.	0	1	2	3
42 ต้องใช้ความพยายามอย่างมาก ถึงจะทำงานยากๆให้เสร็จได้ Has to struggle to complete hard tasks.	0	1	2	3
43 ไม่ค่อยมีใครอยากชวนเล่นด้วย หรือชวนไปไหนด้วย Does not get invited to play or go out with others.	0	1	2	3
Additional Questions				
44 คุณมีเรื่องอะไรบ้าง ที่รู้สึกเป็นห่วงเกี่ยวกับลูกของคุณคนนี้ Do you have any other concerns about your child?				
45 ลูกเก่ง หรือมีความสามารถในเรื่องอะไรบ้าง What strengths or skills does your child have?				

CONNERS 3 AI - Parent

Instructions: Here are some things parent might say about their children. Please tell us about *your* child and what he/she has been like in the **past month**. Read each item carefully, then mark how well it describes your child or how frequently it has happened in the **past month**.

0 = In the past month, this was **not true at all** about my child. It never (seldom) happened.
1 = In the past month, this was **just a little true** about my child. It happened occasionally.
2 = In the past month, this was **pretty much true** about my child. It happened often (or quite a bit).
3 = In the past month, this was **very much true** about my child. It happened very often (very frequently).

Please circle only one answer for each item. It is very important to respond to every item.
For items that you find difficult to answer, please give your best guess.

	Rating: In the past month, this was…	0 = Not true at all (Never, Seldom) 1 = Just a little true (Occasionally)	2 = Pretty much true (Often, Quite a bit) 3 = Very much true (Very often, Very frequently)			
1	Fidgeting.		0	1	2	3
2	Does not seem to listen to what is being said to him/her.		0	1	2	3
3	Doesn't pay attention to details; makes careless mistakes.		0	1	2	3
4	Inattentive, easily distracted.		0	1	2	3
5	Has trouble organizing tasks or activities.		0	1	2	3
6	Give up easily on difficulty tasks.		0	1	2	3
7	Fidgets or squirms in seat.		0	1	2	3
8	Restless or overactive.		0	1	2	3
9	Is easily distracted by sights or sounds.		0	1	2	3
10	Interupts others (for example, butts into conversations or games).		0	1	2	3

CONNERS 3 AI - Parent

ข้อความต่อไปนี้ เป็นพฤติกรรมโดยทั่วไปของลูก ๆ ที่พ่อแม่มักจะเล่าให้ฟัง

กรุณาอ่านข้อความแต่ละข้อให้เข้าใจ และลองนึกดูว่า **ในช่วง 1 เดือนที่ผ่านมา** **ลูกของท่านทำพฤติกรรมตรงตามนี้บ้างหรือไม่ และทำบ่อยเพียงใด**

กรุณาตอบคำถามให้ครบทุกข้อ

ในการตอบคำถามแต่ละข้อ กรุณาทำเครื่องหมาย X เพื่อเลือกเพียงคำตอบเดียวที่**ตรง**กับลูกของท่าน**มากที่สุด**

ถ้าท่านไม่แน่ใจในบางคำตอบ ขอให้พยายามตอบให้ดีที่สุด ตามที่ท่านรู้จักลูกของท่าน

	ในช่วง 1 เดือนที่ผ่านมา ลูกทำพฤติกรรมตรงตามนี้บ้างหรือไม่ และบ่อยเพียงใด	ไม่ตรงเลย ลูกไม่เคยทำแบบนี้เลย	ไม่ค่อยตรง ลูกทำบ้างแต่ไม่บ่อย	ค่อนข้างตรง ลูกทำค่อนข้างบ่อย	ตรงที่สุด ลูกทำเป็นประจำ
1	หลุกหลิก อยู่ไม่สุข Fidgeting.	0	1	2	3
2	ไม่ค่อยสนใจฟัง เวลาใครพูดด้วย Does not seem to listen to what is being said to him/her.	0	1	2	3
3	ไม่ค่อยใส่ใจในรายละเอียด ทำอะไรผิดพลาดเพราะความสะเพร่าไม่เอาใจใส่ Doesn't pay attention to details; makes careless mistakes.	0	1	2	3
4	เหม่อ ใจลอย วอกแวกง่าย Inattentive, easily distracted.	0	1	2	3
5	ทำสิ่งต่าง ๆ อย่างไม่เป็นระบบ ไม่มีระเบียบ Has trouble organizing tasks or activities.	0	1	2	3
6	เวลาเจอเรื่องยาก ๆ จะหมดความพยายาม เลิกทำกลางคัน Give up easily on difficulty tasks.	0	1	2	3
7	นั่งนิ่งๆ อยู่กับที่ไม่ค่อยได้ ต้องหยุกหยิกไปมาเวลานั่ง Fidgets or squirms in seat.	0	1	2	3
8	ซนมาก หรืออยู่นิ่ง ๆ ไม่เป็น Restless or overactive.	0	1	2	3
9	วอกแวกได้ง่าย จากสิ่งรอบตัวที่มองเห็นหรือได้ยิน Is easily distracted by sights or sounds.	0	1	2	3
10	ขัดจังหวะคนอื่น (เช่น พูดแทรกขณะที่คนอื่นคุยกัน หรือเข้าไปขัดจังหวะขณะที่คนอื่นกำลังเล่นกันอยู่) Interupts others (for example, butts into conversations or games).	0	1	2	3

Exposure Questionnaire

Pesticide Exposure for Children

☐ Rice Farm Children ☐ Aquacultural Farm Children

Part I General Information *Date:* _____________ *Session*__________

Officer

1. Name_________________ Surname___________________

ID___________________________

Age_______ Relationship to child ___________Child's age_______

2. Address____________________________ Village Number________

3. What is your main job?

( ) 1. Vegetable/fruit farmer ( ) 2. Rice farmer

( ) 3. Sale ( ) 4. Government official

( ) 5. Labour________ ( ) 6. Others____________

4. How much is the average income of the family each month? _________

5. What's the highest level of your studies?

( ) 1. None ( ) 2. Primary school

( ) 3. Grade 7-9 ( ) 4. Grade 10-12

( ) 5. Undergraduate________ ( ) 6. Others____________

6. How many children in your family aged less than 6 years old? _____

	Name	Gender	Age	Weight/Height (kg/cm)	living time (yr/mo)
(a)	__________________	_____	______	___/___	____/____
(b)	__________________	_____	______	___/___	____/____
(c)	__________________	_____	______	___/___	____/____

7. How many family members work as vegetable farmer? ____ person (s)

8. Is your family of Rangsit origin?

( ) 1. Yes (Next to #11) ( ) 2. No (next to #10)

9. Where is your family originally from? ________________________

10. How long has your family lived in the Rangsit region? _____ (days/ months/ years)

11. Where is your residence located?

( ) 1. Inside the farm area

( ) 2. Next to the farm area

( ) 3. Outside the farm area

12. What structure of your home? How many floors?

( ) 1. Contemporary structure ____floor(s)

( ) 2. Permanent structure ____floor(s)

13. What the main type of floor does your home have?

( ) 1. Cement floor ( ) 2. Wood floor

( ) 3. Porcelain floor ( ) 4. Dirt floor

14. How frequently do you clean the house floor?

( ) 1. Everyday ( ) 2. 2-3 day/time ( ) 3. 4-5 day/time
( ) 4. Once a week ( ) 5. Never ( ) Others________

15. How is the floor cleaned? (you can select more than one choice)

( ) 1. Wet mop ( ) 2. Sweep ( ) 3. Others _____________
16. Do you use household pesticides in your home?
( ) 1. Yes ( ) 2. No
If Yes then,
What kind of pesticides (e.g. insecticide, termiticide, rodenticide, etc.) have been used in your home?
____

How frequently do you use pesticides in your home? ______ times/year

FOR ONLY FARM FAMILY

17. Please indicate the names of pesticide you've used in your farm in last 6 months?

Pesticide Name	Frequency
___________________________________	_________
___________________________________	_________
___________________________________	_________
___________________________________	_________
___________________________________	_________

18. At the present, do you do any plantation?

( ) 1. Yes, Please indicate crops :____

( ) 2. No
19. At the present, do you use any kind of pesticides in agricultural purposes?

( ) 1. Yes, Please indicate pesticide name :____________________________________

( ) 2. No

Part II Children Exposure Information

1. How long does your child spend traveling outside the home in the day? ______ (min/hrs)
2. How long does your child approximately spend laying down or sitting on the house floor in the day? ______ (min/hrs)
3. How many time does your child wash his/her hands in the day? ______ time
4. How many time does your child take a shower or bath in the day? _____ time

5. Where does your child spend time for playing in the day? (you can select more than one choice)

( ) 1. Floor indoor ( ) 2. Outside at home

( ) 3. Inside the farm ( ) 4. School

( ) 5. Playground ( ) 6. Others_________

6. How frequently does your child put hands or suck fingers into mouth in the day?
( ) 1. often ( ) 2. sometimes ( ) 3. almost never ( ) 4. Never

7. How frequently does your child place non-food items (such as toy or contaminated soil) in his/her mouth?
( ) 1. often ( ) 2. sometimes ( ) 3. almost never ( ) 4. Never

8. Does your child has soil or dirt from your yard/farm in contact with the skin in the day?
( ) 1. Yes ( ) 2. No
9. How frequently does your child has an illness in the past sixth months?
( ) 1. often ( ) 2. sometimes ( ) 3. almost never ( ) 4. Never
10. How frequently does your child play in the farm area in the past sixth months?
( ) 1. often ( ) 2. sometimes ( ) 3. almost never ( ) 4. Never
11. Does your child pass the vegetable farm on the way to school?
( ) 1. Yes ( ) 2. No

FOR ONLY RICE FARM FAMILY

12. How frequently do you take your child together with you while you are working in the farm?
( ) 1. often ( ) 2. sometimes ( ) 3. almost never ( ) 4. never

13. How frequently does your child play in the farm during pesticide spraying?
( ) 1. often ( ) 2. sometimes ( ) 3. almost never ( ) 4. never
How do you prevent your child away from the sprayed pesticide?
____
____
14. Has your child ever directly come into contact with contaminated pesticide containers?
( ) 1. Yes ( ) 2. No
15. Do your contaminated clothes separate from family clothes?
( ) 1. Yes ( ) 2. No

Child Activity Diary

Time Period # 1 (Time Child Woke Up-Lunch Time)

1. Your child woke up at:___:___ (AM)
2. Your child finished breakfast at:___:___(AM)
3. Your child finished lunch at:___:___ (AM/PM)
4. How long did your child stay indoors during this period of time?
   _____ hours/minutes.

   How long did your child stay outdoors during this period of time?

   _____ hours/minutes.

5. Did your child do any of the following things during this period of time?
   - ☐ a. Put hand in the mouth (______ times)
   - ☐ b. Put objects in the mouth (______ times)
   - ☐ d. Play dirt or soil
   - ☐ e. Eat outside the house
   - ☐ f. Eat on the floor inside the house
   - ☐ g. Wash hands before eating
   - ☐ h. Walk barefeet inside the house
   - ☐ i. Walk barefeet outside at home
   - ☐ j. Walk barefeet in the farm
   - ☐ k. Take a bath
   - ☐ l. Go somewhere away from home

If your child went somewhere away from home, please answer 5a & 5b

5a. Where away from home?
____

5b. Total time away from home _______hours______minutes

6. Where did your child spend most time outdoors at home?

   ____

7. What's the most common toys handled by your child?

   Indoor____

   Outdoor____

Time Period # 2 (Lunch Time –Time Child Went To Sleep)

1. Your child finished dinner at:___:___(PM)
2. Your child went to sleep at:___:___ (PM)
3. How long did your child stay indoors during this period of time?
   _____ hours/minutes.

   How long did your child stay outdoors during this period of time?

   _____ hours/minutes.

4. Did your child do any of the following things during this period of time?
   - ☐ a. Put hands in the mouth (______ times)
   - ☐ b. Put objects in the mouth (______ times)
   - ☐ d. Play dirt or soil
   - ☐ e. Eat outside the house
   - ☐ f. Eat on the floor inside the house
   - ☐ g. Wash hands before eating
   - ☐ h. Walk barefeet inside the house
   - ☐ i. Walk barefeet outside at home
   - ☐ j. Walk barefeet in the farm
   - ☐ k. Take a bath
   - ☐ l. Go somewhere away from home

If your child went somewhere away from home, please answer 4a & 4b

4a. Where away from home? ____

4b. Total time away from home _______hours/minutes

5. Where did your child spend most time outdoors at home?
   ____

6. What's the most common toys handled by your child?

Indoor____

Outdoor____

แบบสอบถามเบื้องต้นเกี่ยวกับการรับสัมผัสสารกำจัดศัตรูพืชในเด็ก

☐ เด็กที่เติบโตในพื้นที่เกษตรกรรม ☐ เด็กที่เติบโตนอกพื้นที่เกษตรกรรม

ส่วนที่ 1 ข้อมูลทั่วไป **วันที่: __________**

สำหรับผู้วิจัย

1. ชื่อ-สกุล____

หมายเลขบัตรประจำตัวประชาชน______________________________

อายุ______ ความสัมพันธ์กับเด็ก __________อายุบุตร___________

2. ที่อยู่____

หมู่บ้าน________

3. อาชีพหลักของท่านคืออะไร

( ) 1. ทำสวน ( ) 2. ทำนา

( ) 3. ค้าขาย ( ) 4. รับราชการ

( ) 5. รับจ้าง______ ( ) 6. อื่นๆ_______

4. ท่านมีรายได้เฉลี่ยต่อเดือนเท่าไหร่_________

5. ท่านได้รับการศึกษาสูงสุดระดับใด

( ) 1. ไม่ได้เรียน ( ) 2. ประถม

( ) 3. มัธยมต้น ( ) 4. มัธยมปลาย

( ) 5. ปริญญาตรี ( ) 6. อื่นๆ_______

6. ในครอบครัวของท่านมีสมาชิกที่อายุต่ำกว่า 6 ปีกี่คน _____

ชื่อ	เพศ	อายุ	น้ำหนัก/ส่วนสูง (กก./ซม.)	ช่วงเวลาที่อยู่ในพื้นที่ (ปี/เดือน)
(ก) ________________	_____	____	___/___	____/____
(ข) ________________	_____	____	___/___	____/____
(ค) ________________	_____	____	___/___	____/____

8. ครอบครัวของท่านมีภูมิลำเนาอยู่ในพื้นที่รังสิตหรือไม่

( ) 1. ใช่ (ข้ามไปยังข้อ11)　　( ) 2. ไม่ใช่ (ข้ามไปยังข้อ10)

9. ครอบครัวของท่านมีภูมิลำเนาดั้งเดิมอยู่ที่ใด ______________________

10. ครอบครัวของท่านได้พำนักอยู่ในพื้นที่รังสิตนานเท่าใด _____________ (วัน/ เดือน/ ปี)

11. บ้านของท่านตั้งอยู่ที่บริเวณใด

( ) 1. ในพื้นที่เกษตรกรรม

( ) 2. ใกล้พื้นที่เกษตรกรรม

( ) 3. นอกพื้นที่เกษตรกรรม

12. ลักษณะโครงสร้างของบ้านท่านเป็นอย่างไร

( ) 1. สิ่งปลูกสร้างชั่วคราว___ชั้น　　( ) 2. สิ่งปลูกสร้างถาวร___ชั้น

สำหรับผู้วิจัย

13. ลักษณะพื้นบ้านของท่านส่วนใหญ่เป็นอย่างไร

( ) 1. พื้นปูน　　( ) 2. พื้นไม้

( ) 3. พื้นกระเบื้อง　　( ) 4. พื้นดิน

14. ท่านทำความสะอาดพื้นบ้านบ่อยครั้งแค่ไหน

( ) 1. ทุกวัน　　( ) 2. 2-3 วัน/ครั้ง ( ) 3. 4-5 วัน/ครั้ง

( ) 4. สัปดาห์/ครั้ง　　( ) 5. ไม่เคยทำ　　( ) 6. อื่นๆ________

15. ท่านได้ใช้อุปกรณ์ใดทำความสะอาดพื้นบ้าน (สามารถเลือกได้มากกว่าหนึ่งข้อ)

( ) 1. ผ้าเปียก ( ) 2. ไม้กวาด ( ) 3. อื่นๆ ____________

16. ท่านได้ใช้สารกำจัดศัตรูพืชภายในบ้านหรือไม่ (เช่น ยากันยุง, ยาฆ่าแมลง, ยาฆ่าปลวก, ยาเบื่อหนู ฯลฯ)

( ) 1. ใช้　　( ) 2. ไม่ใช้

ถ้าใช้ โปรดระบุชนิด ____

ท่านได้มีการใช้สารกำจัดศัตรูพืชภายในบ้านบ่อยครั้งแค่ไหน ______ ครั้ง/เดือน

สำหรับครอบครัวเกษตรกรเท่านั้น

17. โปรดระบุชื่อของสารกำจัดศัตรูพืชที่ท่านใช้ในเกษตรกรรมในช่วง 6 เดือนที่ผ่านมา

ชื่อสารกำจัดศัตรูพืช	ความถี่ในการใช้
________________________________	_________
________________________________	_________
________________________________	_________

ส่วนที่ 2 ข้อมูลการได้รับสัมผัสสารกำจัดศัตรูพืชในเด็ก

สำหรับผู้วิจัย

1. ในแต่ละวันบุตรของท่านใช้เวลาในการอยู่นอกบ้านนานเท่าใด ______ (ชั่วโมง/นาที)

2. ในแต่ละวันบุตรของท่านใช้เวลาในการนอนหรือนั่งบนพื้นบ้านโดยประมาณนานเท่าใด ______ (ชั่วโมง/นาที)

3. บุตรของท่านล้างมือกี่ครั้งต่อวัน ______ ครั้ง

4. บุตรของท่านอาบน้ำกี่ครั้งต่อวัน _____ ครั้ง

5. ในแต่ละวันพื้นที่ใดที่บุตรของท่านใช้เวลาในการเล่น (สามารถเลือกได้มากกว่าหนึ่งข้อ)

( ) 1. บนพื้นในบ้าน ( ) 2. บนพื้นนอกบ้าน

( ) 3. ในพื้นที่เกษตรกรรม ( ) 4. ที่โรงเรียน

( ) 5. สนามเด็กเล่น ( ) 6. อื่นๆ_________

6. ในแต่ละวันบุตรของท่านมีการใช้มือหรือนิ้วเข้าปากหรือดูดนิ้วบ่อยครั้งแค่ไหน

( ) 1. บ่อยครั้ง ( ) 2. บางครั้ง ( ) 3. นานๆครั้ง ( ) 4. ไม่เคย

7. ในแต่ละวันบุตรของท่านมีการใช้มือหยิบสิ่งของที่ไม่ใช่อาหาร (เช่น ของเล่น, ดิน สกปรก)เข้าปากบ่อยครั้งแค่ไหน

( ) 1. บ่อยครั้ง ( ) 2. บางครั้ง ( ) 3. นานๆครั้ง ( ) 4. ไม่เคย

8. ในแต่ละวันบุตรของท่านมีดินหรือสิ่งสกปรกจากสวนหรือจากพื้นที่เกษตรกรรมติดอยู่บนผิวหนังหรือไม่

( ) 1. มี ( ) 2. ไม่มี

9. ในช่วง 6 เดือนที่ผ่านมาบุตรของท่านมีการเจ็บป่วยบ่อยครั้งแค่ไหน

( ) 1. บ่อยครั้ง ( ) 2. บางครั้ง ( ) 3. นานๆครั้ง ( ) 4. ไม่เคย

10. ในช่วง 6 เดือนที่ผ่านมาบุตรของท่านมีการเล่นในพื้นที่เกษตรกรรมบ่อยครั้งเพียงใด

( ) 1. บ่อยครั้ง ( ) 2. บางครั้ง ( ) 3. นานๆครั้ง ( ) 4. ไม่เคย

11. ในระหว่างเดินทางไปโรงเรียนบุตรของท่านต้องเดินผ่านพื้นที่เกษตรกรรมหรือไม่

( ) 1. ผ่าน ( ) 2. ไม่ผ่าน

สำหรับครอบครัวเกษตรกรเท่านั้น

12. ในระหว่างที่ท่านทำงานในพื้นที่เกษตรกรรมท่านนำบุตรไปด้วยบ่อยครั้งเพียงใด

( ) 1. บ่อยครั้ง ( ) 2. บางครั้ง ( ) 3. นานๆครั้ง ( ) 4. ไม่เคย

13. ในระหว่างที่ท่านฉีดพ่นสารกำจัดศัตรูพืชบุตรของท่านได้เล่นอยู่ในพื้นที่นั้นด้วยบ่อยครั้งแค่ไหน

( ) 1. บ่อยครั้ง ( ) 2. บางครั้ง ( ) 3. นานๆครั้ง ( ) 4. ไม่เคย

ท่านได้ป้องกันบุตรจากการฉีดพ่นสารกำจัดศัตรูพืชอย่างไร

____

____

14. บุตรของท่านเคยได้รับสัมผัสโดยตรงจากภาชนะบรรจุสารกำจัดศัตรูพืชหรือไม่

( ) 1. เคย ( ) 2. ไม่เคย

15. เสื้อผ้าที่ปนเปื้อนสารกำจัดศัตรูพืชของท่านถูกแยกทำความสะอาดจากเสื้อผ้าของคนในครอบครัวหรือไม่

( ) 1. ใช่ ( ) 2. ไม่ใช่

กิจวัตรประจำวันของเด็ก

ช่วงเวลาที่ 1 (เวลาเด็กตื่นนอนถึงเวลาอาหารกลางวัน)

1. บุตรของท่านตื่นนอนในเวลาเท่าไหร่ :___:___ (เช้า)
2. บุตรของท่านรับประทานอาหารกลางวันเสร็จเมื่อไหร่:___:___ (เช้า)
3. บุตรของท่านรับประทานอาหารกลางวันเสร็จเมื่อไหร่:___:___ (เช้า/บ่าย)
4. ในช่วงเวลาตื่นนอนถึงเวลาอาหารกลางวันบุตรของท่านใช้เวลาอยู่ในบ้านนานเท่าไหร่ _____ ชั่วโมง/นาที
   ในช่วงเวลาตื่นนอนถึงเวลาอาหารกลางวันบุตรของท่านใช้เวลาอยู่นอกบ้านนานเท่าไหร่ _____ ชั่วโมง/นาที
5. ในช่วงเวลาหลังตื่นนอนถึงเวลาอาหารกลางวันบุตรของท่านได้ทำสิ่งใดบ้างต่อไปนี้

   - ☐ ก. เอามือเข้าปาก (______ ครั้ง)
   - ☐ ข. เอาสิ่งของเข้าปาก (______ ครั้ง)
   - ☐ ค. เล่นสิ่งสกปรกหรือเล่นดิน
   - ☐ ง. รับประทานอาหารนอกบ้าน
   - ☐ จ. รับประทานอาหารบนพื้นในบ้าน
   - ☐ ฉ. ล้างมือก่อนรับประทานอาหาร
   - ☐ ช. เดินในบ้านด้วยเท้าเปล่า
   - ☐ ซ. เดินนอกบ้านด้วยเท้าเปล่า
   - ☐ ฌ. เดินในพื้นที่เกษตรกรรมด้วยเท้าเปล่า
   - ☐ ญ. อาบน้ำ, เล่นน้ำ
   - ☐ ฎ. ออกไปนอกบ้าน

ถ้าบุตรของท่านได้ออกไปนอกบ้าน โปรดตอบคำถามข้อ 5ก และ 5ข

5ก. บุตรของท่านได้ไปที่ไหน ____

5ข. รวมเวลาทั้งหมดที่ได้ออกจากบ้าน _______ชั่วโมง______นาที

6. สถานที่นอกบ้านที่ไหนที่บุตรของท่านใช้เวลาอยู่นานที่สุด

   ____

ช่วงเวลาที่ 2 (เวลาอาหารกลางวันถึงเวลาเข้านอนของเด็ก)

1. บุตรของท่านรับประทานอาหารเย็นเสร็จเมื่อไหร่:___:___ (บ่าย)

2. บุตรของท่านเข้านอนในเวลาเท่าไหร่:___:___ (บ่าย)

3. ในช่วงเวลาอาหารกลางวันถึงเวลาเข้านอนบุตรของท่านใช้เวลาอยู่ในบ้านนานเท่าไหร่ _____ ชั่วโมง/นาที

ในช่วงเวลาอาหารกลางวันถึงเวลาเข้านอนบุตรของท่านใช้เวลาอยู่นอกบ้านนานเท่าไหร่ _____ ชั่วโมง/นาที

4. ในช่วงเวลาอาหารกลางวันถึงเวลาเข้านอนบุตรของท่านได้ทำสิ่งใดบ้างต่อไปนี้

☐ ก. เอามือเข้าปาก (______ ครั้ง)

☐ ข. เอาสิ่งของเข้าปาก (______ ครั้ง)

☐ ค. เล่นสิ่งสกปรกหรือเล่นดิน

☐ ง. รับประทานอาหารนอกบ้าน

☐ จ. รับประทานอาหารบนพื้นในบ้าน

☐ ฉ. ล้างมือก่อนรับประทานอาหาร

☐ ช. เดินในบ้านด้วยเท้าเปล่า

☐ ซ. เดินนอกบ้านด้วยเท้าเปล่า

☐ ฌ. เดินในพื้นที่เกษตรกรรมด้วยเท้าเปล่า

☐ ญ. อาบน้ำ, เล่นน้ำ

☐ ฎ. ออกไปนอกบ้าน

ถ้าบุตรของท่านได้ออกไปนอกบ้าน โปรดตอบคำถามข้อ 4ก และ 4ข

4ก. บุตรของท่านได้ไปที่ไหน ____

4ข. รวมเวลาทั้งหมดที่ได้ออกจากบ้าน _______ชั่วโมง______นาที

5.สถานที่นอกบ้านที่ใดที่บุตรของท่านใช้เวลาอยู่นานที่สุด

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VITA

Ms.Juthasiri Rohitrattana was born in Bangkok, Thailand. She received her Bachelor of Science in General Science in 2001 and Master of Science in Environmental Management in 2005 from Chulalongkorn University, Thailand. She finished another Master of Science in Agricultural Science (Organic Food Chain Management) in 2009 from University of Hohenheim, Germany. Now, she is a Ph.D. student in College of Public Health Sciences, Chulalongkorn University. She is a research assistant for a project "Neurobehavioral Effects of Pesticide Exposure Among Children in Rural Thailand", granted by NIH (NIEHS R21ES18722).

In 2011, she got the research fellowships in psychometrics, questionnaire design, and data analysis at Environmental and Occupational Health Science Institute (EOHSI), University of Medicine and Dentistry of New Jersey (UMDNJ) and in urinary pesticide metabolite analysis at Rollins School of Public Health, Emory University, Atlanta, United States. After her Ph.D. study is completed, she interests to explore more health researches relating to environmental toxicants exposure further.