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Household pyrethroid insecticides exposure in relation to pyrethroid metabolite and GABA level effects of young children in urban area, Bangkok Thailand

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Household Pyrethroid Insecticides Exposure in Relation to Pyrethroid Metabolite and GABA Level Effects of Young Children in Urban Area, Bangkok Thailand

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การสัมผัสสารกำจัดแมลงกลุ่มไพรีทรอยด์ที่ใช้ในบ้านเรือนต่อระดับไพรีทรอยด์เมตาโบไลท์และระดับกาบ้าของเด็กเล็กในเขตเมือง กรุงเทพมหานคร ประเทศไทย

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เจษฎา คุณโน : การสัมผัสสารกำจัดแมลงกลุ่มไพรีทรอยด์ที่ใช้ในบ้านเรือนต่อระดับไพรีทรอยด์เมตาโบไลท์และระดับกาบ้าของเด็กเล็กในเขตเมือง กรุงเทพมหานคร ประเทศไทย. (Household Pyrethroid Insecticides Exposure in Relation to Pyrethroid Metabolite and GABA Level Effects of Young Children in Urban Area, Bangkok Thailand) อ.ที่ปรึกษาหลัก : รศ. ดร. วัฒน์สิทธิ์ ศิริวงศ์

การสัมผัสสารกำจัดแมลงกลุ่มไพรีทรอยด์ที่ใช้ในบ้านเรือนมีผลต่อระบบประสาทของเด็กเล็ก ซึ่งข้อมูลการสัมผัสสารกำจัดแมลงกลุ่มไพรีทรอยด์ต่อระดับกาบ้าในเด็กเล็กยังมีน้อย การวิจัยครั้งนี้มีจุดประสงค์เพื่อหาความสัมพันธ์ระหว่างการสัมผัสสารกำจัดแมลงกลุ่มไพรีทรอยด์ที่ใช้ในบ้านเรือนต่อระดับไพรีทรอยด์เมตาโบไลท์และระดับกาบ้า และปัจจัยเสี่ยงที่ส่งผลต่อการสัมผัสสารกำจัดแมลงกลุ่มไพรีทรอยด์ของเด็กเล็กในเขตเมือง การศึกษาภาคตัดขวางแบบซ้ำ ได้ดำเนินการศึกษาในช่วงฤดูฝน (พฤศจิกายน – ธันวาคม 2561) และฤดูร้อน (เมษายน – พฤษภาคม 2562) ในแต่ละช่วงฤดูกาลศึกษาได้เลือกเด็กเล็กจำนวน 80 คน (อายุ 2 -3 ปี) โดยเลือกกลุ่มเด็กเล็กที่อาศัยอยู่ในบ้านเรือนตลอดเวลาและผู้ปกครองถูกสัมภาษณ์ เก็บปัสสาวะเด็กเล็กเพื่อวิเคราะห์ระดับไพรีทรอยด์เมตาโบไลท์โดยใช้วิธีแก๊สโครมาโตกราฟฟี (GC/MS) และระดับกาบ้าโดยใช้วิธีเอนไซม์ตึงเฉพาะเจาะจง (ELISA) และเก็บสารกำจัดแมลงกลุ่มไพรีทรอยด์ที่ตกค้างบนมือเด็กเล็กโดยวิเคราะห์ด้วยวิธีแก๊สโครมาโตกราฟฟี (GC/MS) จากการศึกษาพบว่าทั้งสองช่วงฤดูกาล การเพิ่มขึ้นของความเข้นข้นของสารไพรีทรอยด์เมตาโบไลท์ในปัสสาวะเด็กเล็กมีความสัมพันธ์กับการลดลงของความเข้มข้นของระดับกาบ้าในปัสสาวะเด็กเล็ก (r_s = -0.230, p-value 0.04) นอกจากนี้ การเพิ่มขึ้นของความเข้นข้นของสารไพรีทรอยด์เมตาโบไลท์ในปัสสาวะเด็กเล็กมีความสัมพันธ์กับการเพิ่มขึ้นของความเข้นข้นของสารกำจัดแมลงกลุ่มไพรีทรอยด์ที่ตกค้างบนมืดเด็กเล็กในช่วงฤดูฝน (r_s = 0.226 – 0.274, p-value < 0.05) ในขณะที่ไม่มีความสัมพันธ์ในช่วงฤดูร้อน (r_s = 0.160, p-value > 0.05) นอกจากนั้น งานวิจัยนี้ยังพบว่า ในช่วงฤดูฝนการไม่ค่อยทำความสะอาดพื้นบ้านมีความสัมพันธ์กับการเพิ่มขึ้นของความเข้นข้นของสารไพรีทรอยด์เมตาโบไลท์ในปัสสาวะเด็กเล็ก และในช่วงฤดูร้อนพบว่า เพศ (p-value = 0.041, OR = 0.333, 95%CI 0.116 – 0.956) และการเดินเท้าเปล่าภายในบ้านเรือน (p-value = 0.009, OR = 6.789, 95%CI 1.597 – 28.854) มีความสัมพันธ์กับการเพิ่มขึ้นของความเข้นข้นของสารไพรีทรอยด์เมตาโบไลท์ในปัสสาวะเด็กเล็ก ดังนั้นงานวิจัยนี้จึงสรุปได้ว่า การสัมผัสสารกำจัดแมลงกลุ่มไพรีทรอยด์ที่ใช้ในบ้านเรือนมีผลต่อระดับกาบ้าในเด็กเล็ก แนะนำควรให้ความรู้วิธีการป้องกันการสัมผัสสารกำจัดแมลงกลุ่มไพรีทรอยด์ที่ใช้ในบ้านเรือนกับผู้ปกครอง เพื่อลดความเสี่ยงหรือลดการสัมผัสของสารกลุ่มนี้ในเด็กเล็กที่อาศัยอยู่ในบ้านเรือนเขตเมือง

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Pyrethroids are commonly used in households and affect to nervous system in children. Data on pyrethroids exposure related to neurotransmitter gamma-aminobutyric acid (GABA) is unknown. This study investigated the relationship between 3-PBA metabolites and GABA concentrations, and also determined the factors influencing pyrethroids metabolites among young children in urban areas. A repeated cross-sectional design was designed to collect the information on PYR insecticide exposure between wet (November – December 2018) and dry (April – May 2019) seasons. To collect the data, urine samples were collected from children age 2–3 years(n = 80), and the questionnaires were used with parents. The study focused on children living in full time households where PYR insect products have been applied daily. Urine samples were analyzed for the 3-PBA metabolite using gas chromatography (GC/MS) and GABA concentrations using an enzyme-linked immunosorbent assay kit (ELISA). Spearman correlations was used to determine the relationship between 3-PBA metabolite and GABA concentrations in each season, and binary logistic regression was applied to determine an association between 3-BPA metabolite and the exposure variables in each season. Mann–Whitney U test was used to evaluate difference in continuous data between wet and dry seasons. Chi-square test was applied to present difference in categorical data between wet and dry seasons. The results revealed a negative association between an increase in 3-PBA metabolite concentrations and low GABA concentrations in urine in both seasons (r_s = -0.230, p-value 0.004). This study also found the increase in 3-PBA metabolite was significantly associated with the increase in cypermethrin (r_s = 0.226, p-value < 0.05), and allethrin (r_s = 0.274, p-value < 0.05) in dry season, but not in wet season (r_s = 0.160, p-value > 0.05). Moreover, the association between exposure factors and 3-PBA metabolite concentrations were observed. seldom cleaned the floor was significantly associated with increased 3-PBA metabolite concentration in wet season (p-value = 0.038, OR = 0.089, 95%CI 0.009 – 0.870). In addition, genders and always walk bare feet inside household in a day were significantly associated with increased 3-PBA metabolite concentration in dry season (p-value = 0.041, OR = 0.333, 95%CI 0.116 – 0.956) and (p-value = 0.009, OR = 6.789, 95%CI 1.597 – 28.854), respectively. Hence, these factors could affect the levels of the neurotransmitter GABA in pyrethroids-exposed children. The study suggests that health education, raising awareness and strategies to reduce the risks from long-term PYR insecticide exposure of children living in households should be implemented.

Field of Study: Public Health Student's Signature ....

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CHAPTER I
INTRODUCTION

1.1 Background and rationale

Pyrethroids (PYRs) are insecticide that common use in agriculture farms and in households. In tropical country have been using for control of mosquito, fleas in kitchens and bedrooms and insect repellent products (Bradberry et al. 2005a). PYRs were divided into two types based on the toxicology and physical properties, Type I includes allethrin, bioallethrin, bifenthrin, permethrin, *d*-phenothrin, prallethrin, resmethrin, bioresmethrin, tefluthrin, tetramethrin and phenothrin, Type II includes cyfluthrin, cyhalothrin, **λ**-cyhalothrin, cypermethrin, **α**-cypermethrin, deltamethrin, fenpropathrin, fenvalerate, esfenvalerate, flucythrinate, flumethrin, *tau*-fluvalinate and tralomethrin (Bradberry et al. 2005a; Thatheyus and Gnana 2013).

PYRs are exposed to humans by inhaling and skin contact causingwinding, shortness of breath, runny nose, and chest pain (Bradberry et al. 2005a). The effect and toxicity of PYRs affected to sodium and chloride channel occurred in nerve and muscle, PYRs affected to neurotoxicity at high doses (Bradberry et al. 2005a; Thatheyus and Gnana 2013; Fiedler et al. 2015; Wang et al. 2016). Effect to melatonin (Asghari et al. 2017; Sarabia, Maurer, and Bustos 2009; Sharma et al. 2013). and effect to myopia (Migneron et al. 2017). PYRs affected within 4-48 hours after exposure, half-life of PYR lower than 24 hour are rapidly metabolize once absorbed to polar metabolites, eliminated primarily in urine and normal management to reduce PYR exposure by *dl*-αtocopherol acetate (vitamin E) (Bradberry et al. 2005b; Glorennec, Serrano, Fravallo, Warembourg, Monfort, Cordier, Viel, Le Gléau, et al. 2017). The sysptoms caused by skin contact nclude, continuous tingling, pricking leading tomore severe burning. Treating the condition within 24 hours with the use of locally available vitamin *E* proved to effective. Acute neonatal respiratory syndrome was

reported in the air room after sterilization with permethrin. The effect of PYRs (particularly Type II PYRs containing the cyano group) frequently leads to paresthesia (abnormal cutaneous sensations such as tingling, burning, numbness, and itching). Type II PYRs has been shown to inhibit localization of or near the site of picrotoxin GABA receptors in mice brains (Crofton, Reiter, and Mailman 1987).

PYRs have lipophilic substances, but it was not kept to a significantly extented in mammals as it has a faster metabolic rate for fat and lipophilic digestion. In mammals, the most important process in the metabolism of PYRs occurs in liver microsomes is cleavage of the central ester linkage, Ester cleavage produces a cyclopropane acid and an alcohol moiety (Fig 2.) the latter is then hydroxylated to produce 3-phenoxybenzyl alcohol. Most of study were used 3 phenoxybenzoic acid(3-PBA) as biomarker to analyze PYRs metabolites, 3-PBA metabolites were presented in all PYRs components, other PYRs metabolite such as cis-3-(2,2-dichlorovinyl)-methylcyclopropane carboxylic acid (cis-DCCA), trans-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane carboxylic acid (trans-DCCA), 4-fluoro-3-phenoxybenzoic acid (F-PBA) and cis-3-(2,2-dibromovinyl)-2,2-dimethylcyclopropane carboxylic acid (cis-DBCA).

PYRs significantly affect young children because children brains are not fully developed and sensitive to neurotoxins, while the relative amounts of inhaled air, weight ratio calculations, food intake and water intake for children is several times greater than adults, suggesting that children have more contact with environmental contaminants, thus, children may be more exposed than adults to PYRs (Oulhote and Bouchard 2013b; Wang et al. 2016). Moreover, PYRs affected with young children in household, may concerts to protect and management, while children can be exposed to PYRs and other hazardous chemicals through multiple pathways and by multiple routes. Diet was considered the primary exposure pathway for most PYR contamination and drinking water and residential contact contributing to aggregate

exposure in some cases. Children in households may have increased risk from PYRs exposure.

PYRs were blinded and inhibited GABA channels (Fig 4), although it was higher concentrations that affect sodium channels (10^{-7} M - 10^{-10} M). This effect was believed to result in seizures with severe PYRs type II poisoning, which other reported targets for PYRs include calcium ATPase and voltage-gated calcium channels, which are, however, affected at higher concentrations (10–5 M and 10–6 M, respectively). The latter reaction may be interesting since PYRs has been shown to increase calcium-dependent neurotransmitter release. As (Fig 4), that Adjustment of calcium ions through the synaptosome cell membrane may cause neurotransmitter release at the synaptic junction, due to the direct agonistic effects of deltamethrin on the calcium ion channel selection. However, knowledge of PYRs affects the calcium channel as well as the toxicity of GABA (neurotransmitters toxicity) directly upon exposure to PYRs and time of exposure (Soderlund et al. 2002; Ray and Fry 2006; Hossain et al. 2008; Soderlund 2012).

Young children are vulnerable to environmental toxins including pesticides. Children's organs are not fully developed until later in life. They are constantly experiencing significant development moments. Adverse exposures can cause permanent damage, particularly in utero. The behavior of children and their ability to interact with their physical environment changes at each stage of growth and development and can put them at greater risk of exposure. Children may crawl on the floor, explore verbal objects and play with objects found in the environment, these substances may block the absorption of important nutrients in children's diets, which affect health outcomes. Routes that come into contact with pesticides may occur in the womb or through breastfeeding, eating contaminated food, pesticides, and skin contact through the skin. However, the relationship between the

concentration of pesticides at low levels over time and the neurological and behavioral development will be less (Antwi and Reddy 2015).

This work focuses on PYRs insecticide exposure effect to PYRs metabolized and GABA level. A few study, PYRs exposure were affected to PYRs metabolized and GABA level for children age 2-3 years at home in Thailand. PYRs exposure were significantly associated with young children because their brain was not fully developed, susceptible to neurotoxins, working memory and verbal comprehension. We aim to assess the association between household PYRs exposure with PYRs metabolize and GABA level among children living in urban area.

1.2 Research objectives

1.2.1 Main objective

- To assess the relationship between household PYR exposure with PYRs metabolite concentration and GABA concentration among children living in urban area.

1.2.2 Specific objective

- To determine factors association with PYRs metabolites concentration in children household.
- To determine correlation between PYRs metabolites concentration and GABA concentration in urine sample of children.
- To find correlation between wipe sample concentration and PYRs metabolites concentration in children household.
- To find differences of wipe sample concentration, PYRs metabolites concentration and GABA concentration between wet and dry seasons.

1.3 Research hypothesis

1 Ho There is no association between factors association with PYRs metabolites concentration in children household

Ha There is association between factors association with PYRs metabolites concentration in children household

2 Ho There is no correlation between PYRs metabolites concentration and GABA concentration in urine sample of children

Ha There is correlation between PYRs metabolites concentration and GABA concentration in urine sample of children

3 Ho There is no correlation between wipe sample concentration and PYRs metabolites concentration in children household

Ha There is correlation between wipe sample concentration and PYRs metabolites concentration in children household

4 Ho There are no differences of wipe sample concentration, PYRs metabolites concentration and GABA concentration between wet and dry seasons

Ha There are differences of wipe sample concentration, PYRs metabolites concentration and GABA concentration between wet and dry seasons

1.4 Scope of this study

- The effect of PYRs exposure in this study was referred to PYRs metabolite and GABA concentration in children at household because PYRs exposure were significantly associated with young children.
- The concentration of PYRs insecticide exposure were interested in this study due to their high intensity use in households for control of mosquito, fleas in kitchens and bedrooms.

1.5 Conceptual framework

Independent

Socio-demographic Characteristics:

- Age (2-3 years old)
- Gender
- Weight
- Current address
- Duration to live in this region
- Location of residence

Factors related to children:

- Father/Mather education level
- Main source of Father/Mather incomes
- Father/Mather income/year

Factors related to PYR exposure:

- Type insecticide products use household
- Name of insecticide products use household
- Store for keep insecticide products
- Frequency of insecticide use in household
- Frequency of floor-cleaning
- Type floor cleaning
- Frequency of open window or door
- Frequency of insecticide control operators by officer

Personal hygiene

- Frequency of travel outside
- Frequency of travel outside in the time/day
- Location of travel outside
- Frequency of play at household floor
- Location of play at household floor
- Frequency of play dirt or soil
- Frequency of wash hand
- Frequency of wash foot
- Frequency of take a shower
- Walk bare feet inside household
- Walk bare feet outside household
- Frequency of illness

Contact variable behavior:

- Furniture
- Bottle/dish
- Object to mouth
- Hand to mouth
- Fingers to mouth
- Hand floor
- Toy to mouth
- Hair / Skin / Clothes
- Grass/vegetation
- Crawling across the floor

Home Characteristics:

- Type of household
- How many floors
- Main type floor
- Direction of front household
- Number of windows
- Reported insecticide sighting in the home
- Reported pesticide use

Dietary habit:

- Eat outside the household
- Eat on the floor inside the household
- Wash hands before eating
- Vegetable washing soaking time
- Fruits washing soaking time
- Fruits peeled

Hands wipe sample

Dependent

→ Urinary metabolite levels: (*3*-PBA)

↓

GABA concentration

1.6 Operational definition

1.6.1 Children socio-demographic characteristics

It specifies to age, current address, gender, height and weight

- Age: it means children age 2 and 3 years old refer to PYRs exposure. Reasons for children's increased vulnerability to pesticide exposure including higher basal metabolic rate, higher respiratory rate, higher skin permeability, Developmental stages (crawling, hand-to-mouth behavior) and Developing organ systems, especially the central nervous system. Children's organs are not fully developed until later in life. They continually experience critical periods in development; adverse exposures can cause permanent damage, particularly in utero.
- Gender: it means classified into male and female. Gender differences might be show boys spent time in outdoor more than girls which girls spent time in indoor more than boys.
- Weight: it means classified weight of children age 2 and 3 years old. Additional if high level of insecticides might affect to adverse birth outcomes including physical birth defects and low birth weight.
- Current address: it means the current address or residence in Bangkok.
- Duration to live in this region: it refers to days, months and years.
- Location of residence: it refers to possible areas that contaminated with pyrethroid insecticide.
- Household: It refers to the residential factors. Residential factors that influence chronic exposure include the use of insecticides and rodenticides in the home, and herbicide and fungicide use on lawns, as well. Indoors, broadcast applications including sprays, "flea bombs," and foggers can leave

lingering residues in the air, carpet, toys, and house dust. Typical exploratory behavior, including playing on and crawling across the floor, increases the risk of dermal, inhalation, and oral exposure to residues on surfaces or the air as it settles.

1.6.2 Factors related to children

It refers to the factor that relate to children including, father/mother education level, main source of father/mother incomes, father/mother income/year, father/mother smoking, number of household members and number of children in household members.

- Father/Mother education level: it is categorized into: (1) illiterate, (2) can read and write, (3) primary school, (4) secondary school, (5) high school, (6) university and gradate.
- Main source of Father/Mother incomes: it means both current work and previous work.
- Father/Mother income/year: it means an average monthly income of the Father/Mother and family income relative to federal poverty level. Poor housing conditions in low-income homes, such as overcrowding and housing disrepair, are associated with pest infestations and increased home pesticide use in both urban and agricultural communities, potentially increasing pesticide residues indoors.
- Number of household members: it means the total of people that live in household.
- Number of children in household members: it means the total children 2 and 3 years old.

1.6.3 Factors related to PYR exposure

It means the history of possible sources that refer to the factor relate to PYRs exposure to children including, type insecticide products use household, household insecticide aerosol, frequency of insecticide use in household, frequency of floor-cleaning, wash hand before a meal and observable dirt on body.

- Type insecticide products use household: it means PYR products used in your household. It includes (1) Mosquito coil, (2) Mosquito repellent spray, (3) Traps, (4) Gels, (5) Smoke bombs and (6) Others.
- Name of insecticide products use household: it refers to brand or name of insecticide products, which difference brand, might be difference concentration of insecticide products.
- Store for keep pyrethroids insecticide products: if refer to location for store pyrethroids insecticide products might be contaminating to children.
- Frequency of insecticide use in household: it means frequently do you use pesticides in your home (times/week).
- Frequency of floor-cleaning: it includes (1) every day, (2) 2-3 day/time, (3) 4-5 day/time, (4) once a week, (5) never and (6) others.
- Type floor cleaning: it includes (1) Wet mop (2) Sweep and (3) Others.
- Frequency of open window or door: it refers to sunlight exposure and possible of pyrethroids insecticide products distribution.
- Frequency of insecticide control operators by officer: it refers to confounder of pyrethroids insecticide products distribution by control operators indulging, (1) everyone month, (2) every 2 month and (3) others

1.6.4 Personal hygiene

Personal hygiene may be described as the principle of maintain cleanliness and grooming of the external body including: frequency of travel outside, frequency of travel outside in the time/day, location of travel outside, frequency of play at household floor, location of play at household floor, frequency of play dirt or soil, frequency of wash hand, frequency of wash foot, frequency of take a shower, walk barefoot inside household, walk barefoot outside household and frequency of illness.

- Frequency of travel outside: It includes, (1) every week, (2) every month and (3) Others.
- Frequency of travel outside in the time/day: It includes, (1) ≤ 1 hr., (2) 2 -3 hrs. and (3) ≥ 4 hrs.
- Location of travel outside: if refer to location for children travel outside might be contaminate with pyrethroids insecticide products.
- Frequency of play at household floor: It includes, (1) ≤ 1 hr., (2) 2 -3 hrs. and (3) ≥ 4 hrs.
- Location of play at household floor: it includes (1) Floor indoor, (2) Bedroom, (3) Living room, (4) Garden at home, (5) Outside at home and (6) Others.
- Frequency of play dirt or soil: It includes (1) 1 – 2 times, (2) 3 – 5 times, and (3) ≥ 6 times.
- Frequency of wash hand: It includes (1) 1 – 2 times, (2) 3 – 5 times, and (3) ≥ 6 times.
- Frequency of wash foot: It includes (1) 1 – 2 times, (2) 3 – 5 times, and (3) ≥ 6 times.
- Frequency of take a shower: It includes (1) 1 – 2 times, (2) 3 – 5 times, and (3) ≥ 6 times.

- Walk barefoot inside household: it includes (1) Often, (2) Sometimes, (3) Almost never and (4) Never.
- Walk barefeet outside household: it includes (1) Often, (2) Sometimes, (3) Almost never and (4) Never.
- Frequency of illness: it includes (1) Often, (2) Sometimes, (3) Almost never and (4) Never.

1.6.5 Contact variable behavior

It means the history of possible sources that refer to contact variable behavior might be relate to PYRs exposure to children including upholstered furniture, bottle/dish, object to mouth, hand to mouth, hand floor and crawling across the floor.

The behavioral activities of young children put them at increased risk for exposure to these residues as they crawl and play on the floor and put toys and other objects into their mouths, similar to the patterns of behavior that put children at increased risk for toxic lead exposure. Pesticides can be measured in indoor air samples and persist in dust vacuumed from carpeted areas, upholstered objects, and children's toys, such as stuffed animals, and can also be brought home from the workplace.

- Furniture: it includes (1) often, (2) sometimes, (3) almost never and (4) never.
- Bottle/dish: it includes (1) often, (2) sometimes, (3) almost never and (4) never.
- Object to mouth: it includes (1) often, (2) sometimes, (3) almost never and (4) never.
- Hand to mouth: it includes (1) often, (2) sometimes, (3) almost never and (4) never.

- Fingers to mouth: it includes (1) often, (2) sometimes, (3) almost never and (4) never.
- Hand floor: it includes (1) often, (2) sometimes, (3) almost never and (4) never.
- Toy to mouth: it includes (1) often, (2) sometimes, (3) almost never and (4) never.
- Hair: it includes (1) often, (2) sometimes, (3) almost never and (4) never.
- Skin: it includes (1) often, (2) sometimes, (3) almost never and (4) never.
- Clothes: it includes (1) often, (2) sometimes, (3) almost never and (4) never.
- Grass/vegetation: it includes (1) often, (2) sometimes, (3) almost never and (4) never.
- Crawling across the floor: it includes (1) often, (2) sometimes, (3) almost never and (4) never.

1.6.6 Home Characteristics

It means the history of possible sources of exposure to PYRs exposure in home characteristics including type of household, how many floors, reported mosquito sighting in the home, reported cockroach sighting in the home and reported pesticide application in the last 3 months.

- Type of household: it means the status refer to contemporary structure and permanent structure
- How many floors: it means the total floors of household.
- Main type floor: it includes (1) Cement floor, (2) Laminate floor, (3) Tile floor and (4) Dirt floor.
- Direction of front household: If refer to sunlight exposure and possible of pyrethroids insecticide products distribution and air flow. It included (1) West, (2) East, (3) South, (4) North and (5) Other.

- Number of windows: If refer to sunlight exposure and possible of pyrethroids insecticide products distribution and air flow. It included (1) ≥ 2, (2) ≥ 6, (3) ≥ 10 and (4) ≥ 16.
- Reported insecticide sighting in the home: It included (1) mosquito, (2) cockroach, (3) Ant, (4) Termite and (5) Others
- Reported pesticide use: It included (1) Yes and (2) No.

1.6.7 Dietary habit

It refers to the factor that relate dietary habit might be refer PYR exposure including vegetable washing soaking time, Fruits washing soaking time and fruits peeled.

For pyrethroids, both food residues and household pest control products are important sources.

- Eat outside the household: it includes (1) often, (2) sometimes, (3) almost never and (4) never.
- Eat on the floor inside the household: it includes (1) often, (2) sometimes, (3) almost never and (4) never.
- Wash hands before eating it includes (1) often, (2) sometimes, (3) almost never and (4) never.
- Vegetable washing soaking time: it includes (1) often, (2) sometimes, (3) almost never and (4) never.
- Fruits washing soaking time: it includes (1) often, (2) sometimes, (3) almost never and (4) never.
- Fruits peeled: it includes (1) often, (2) sometimes, (3) almost never and (4) never.

1.6.8 Environmental pesticide measures

It means the history of possible sources of exposure to PYR exposure in home that refer to environmental pesticide in household including living room floor wipes, bedroom floor wipes, fabric toy and furniture wipes.

Residentially related sources may be relevant in other settings where children spend time, depending on indoor and outdoor pesticide use patterns and proximity to pesticide use.

- Wipes sample from living room floor: it means children spend time at this area.
- Wipes sample from bedroom floor: it means children spend time at this area.
- Fabric toy: it includes (1) often, (2) sometimes, (3) almost never and (4) never.
- Furniture wipes: it means children spend time at this area.

CHAPTER II
LITERATURE REVIEW

2.1 Pyrethroids

Pyrethroids (PYRs) are several active substances and used for insecticide properties. The first insecticide chemical presented pyrethrum that isolated from Chrysanthemum lowers, it was used to kill ticks and insects such as fleas and mosquitoes. Six chemicals are used in pyrethrum extracts and these compounds were called pyrethrins. In addition, pyrethrins can be dissolved in water but not soluble in organic solvents like alcohol, chlorinated hydrocarbons and kerosence. They are used in household insecticides and products to control insects in livestock, it can quickly break down in the environment when exposed to sunlight in nature (Thatheyus and Gnana Selvam 2013; Bradberry et al. 2005b; Ye et al. 2017; Hyland and Laribi 2017).

PYRs structure has similarity with pyrethrins and toxin to insects, mammals and remain in environment for a longtime. Commercial processes use PYR multi-molecular, the same chemical formula with atoms in the same order but there is some sequence of atoms of space together, some compounds are called stereoisomers. If stereoisomers are not reflecting each other's image, will called diastereomers and have different boiling point, melting point and solubility of physical properties (George 2002; Roy, Goswami, and Pal 2017; Pinto, Cerqueira-Coutinho, et al. 2017; Wang et al. 2016; Diaz 2016; Bouzid et al. 2016). However, both diastereomers and enantiomers can have different insecticidal properties and different toxicities. Some PYRs were composed of as many as eight different stereoisomers (Thatheyus and Gnana Selvam 2013).

PYRs insecticide were commonly used in agriculture farms and in households. In tropical country have been using for control of mosquito, fleas in kitchens and

bedrooms and insect repellent products (Bradberry et al. 2005b; Carvalho 2017; Wang et al. 2016; Charbotel, Fervers, and Droz 2014). If the organism were exposed to PYRs should be harmed. These factors were included how much (how long) the duration (how long) and how to contact them. Consider other chemicals that were related and age, gender, age, food, family, lifestyle, and health (George 2002; Thatheyus and Gnana 2013).

2.2 Pyrethroids chemical properties

PYRs were isolated from pyrethrins and representative of more than 1,000 diverse species of insecticidal effective. Although the chemical structure and biological activity of PYRs were presented by synthetic PYRs, extensive chemical modifications have made the compounds toxic and non-biodegradable in the environment. A list of PYRs were presented in **Table 1.** (Thatheyus and Gnana Selvam 2013; Bradberry et al. 2005b). The individual PYRs were typically grouped into two general classes, called Type I and Type II, based on a combination of toxicological and physical properties. In addition, personal PYRs, because of complex chemical structures often consist of two, four or eight isomers, and commercially produced products often contain these isomers. Therefore, the production of individual PYRs with a slightly different isomer ratio may be responsible for differences in the toxicity profile of the same compound (Thatheyus and Gnana 2013).

Table 1. Pyrethroids discussed in the profile

Type I	Type II
allethrin, bioallethrin, bifenthrin, permethrin, *d*-phenothrin, prallethrin, resmethrin, bioresmethrin, tefluthrin, tetramethrin and phenothrin,	cyfluthrin, cyhalothrin, λ-cyhalothrin, cypermethrin, α-cypermethrin, deltamethrin, fenpropathrin, fenvalerate, esfenvalerate, flucythrinate, flumethrin, *tau*-fluvalinate and tralomethrin

(Thatheyus and Gnana Selvam 2013; Bradberry et al. 2005b)

PYRs were presented fast-paced, low-toxicity insecticide and rapid degradation rate in the environment. They are often used as insecticides for home and commercial use. PYRs products were classified as generic pesticides. Most pesticides (such as pesticides) also contain other ingredients (sometimes called "inert"), many of which may be toxic. Federal law requires that a percentage of the ingredients be "inert" and not necessarily chemicals that make the "inert" ingredient listed in the pesticide label (Tyler et al. 2000).

All PYRs insecticides were contained an acid moiety, a central ester bond, and an alcohol moiety **(Fig 1).** The acid moiety was contained two chiral carbons, thus PYRs were typically existed as stereoisomeric compounds (trans and cis). Additionally, some PYRs have a chiral carbon on the alcohol moiety, allowing for a total of eight different stereoenantiomers. Chemical considerations were relevant, as PYRs effects on sodium channels, their insecticidal activity, and their mammalian toxicity, are stereospecific. The cis-isomers were generally more toxic than the corresponding trans-isomers. A key structural difference between type I and type II PYRs were presence only in the latter of a cyano group at the carbon of the alcohol moiety of the compound (Costa 2015).

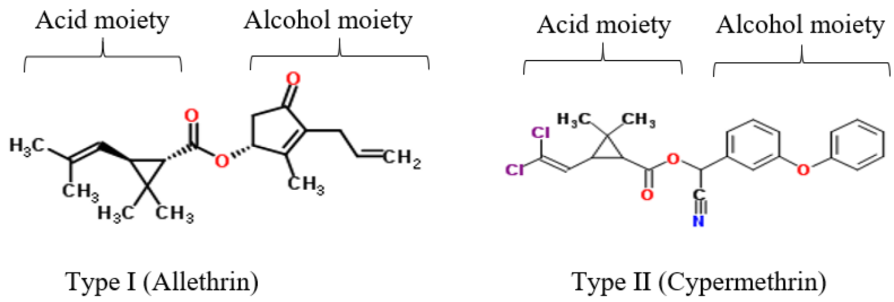


Figure 1 Structure of type I and type II PYRs insecticides.

(Costa 2015).

PYRs were released into the environment as they are used as insecticides. They were applied to plants from sprayers in the air and ground or used in buildings by commercial spraying or aerosol spraying. These compounds can be easily degraded in the atmosphere by natural sunlight and typically take a few days to a couple of weeks. In addition, some of the PYRs, such as permethrin and cyhalothrin, which iso-butenyl attached to cyclopropane moiety, stable natural sunlight (Liu et al. 2010; Cycon and Piotrowska-Seget 2016).

2.3 Pyrethroids exposure and metabolite

The general population is exposed to PYRs primarily from eating foods, especially fruits and vegetables. Indoor air ingestion may be possible after use of these substances. In addition, PYRs are used in many pet shampoos, including aerosols, which can be used in or around the home and the use of these products can be taken (Antwi and Reddy 2015). Skin contact and inhalation. Exposure to agricultural workers using these substances in crops may be important, as skin contact was the most important pathway (Angerer 2001). The average daily intake (AVDI) of permethrin for eight different age / sex groups was estimated by the Food and Drug Administration (FDA) (Bradberry et al. 2005b). Based on market basket

surveyed, the AVDI of permethrin ranges from about 36 to 71 ng / kg / day. Since permethrin is the most commonly used PYRs in the United States, the data from these surveys may represent a reasonable first approximation for the total intake of all PYR (Wang et al. 2016; Bradberry et al. 2005b).

PYRs are absorbed into the body within minutes causing sore throat, nausea, vomiting and abdominal pain. They may cause ulcers in the mouth, increased secretion and dysphagia. Side effects that occur in the body start 4-48 hours after exposure to PYRs. Less than 24 hours were rapidly absorbed when absorbed into the polar carcinogen and most urine was excreted from the urine. Regularly to reduce the exposure of PYRs by *dl*- α tocopherol acetate (vitamin E) (Bradberry et al. 2005b; Glorennec, Serrano, Fravallo, Warembourg, Monfort, Cordier, Viel, Le Gléau, et al. 2017).

2.3.1 Pyrethroids metabolize

PYRs have low toxicity in mammals. It may be highly toxic in other animals, such as fish that more sensitive than mammals to PYRs. PYRs are a very fatty substance. However, it is not stored in mammals significantly because of the fast metabolism of digested foods that are less fatty and easier to digest. In mammals, the most important metabolic process of PYRs that occurs in liver microsomes was the cleavage of the central ester linkage; the split ester produces cyclopropane acids and alcohol **(Fig 1.)**. After that, the hydroxide will produce 3-phenoxybenzyl alcohol, it was a 3-phenoxyben-zoic acid (3-PBA) that oxidation catalyzed by PYR in mammals are show in **(Fig 2)**. The cyclopropane acid was further oxidized to the 2-hydroxy derivative, and the 3-phenoxyben-zoic acid was oxidized to 2- and 4-hydroxy derivatives. Primary alcohols that result from this hydroxylation may further oxidized via aldehydes to carboxylic acids. The final metabolic pathway was the conjugated which causes the glucoronides of the carboxylic acid or sulfates of phenols, which

are excreted in the urine (Tyler et al. 2000; Sharma 2001; Bradberry et al. 2005a; Wang et al. 2016; Islam et al. 2017; Misni, Nor, and Ahmad 2017)

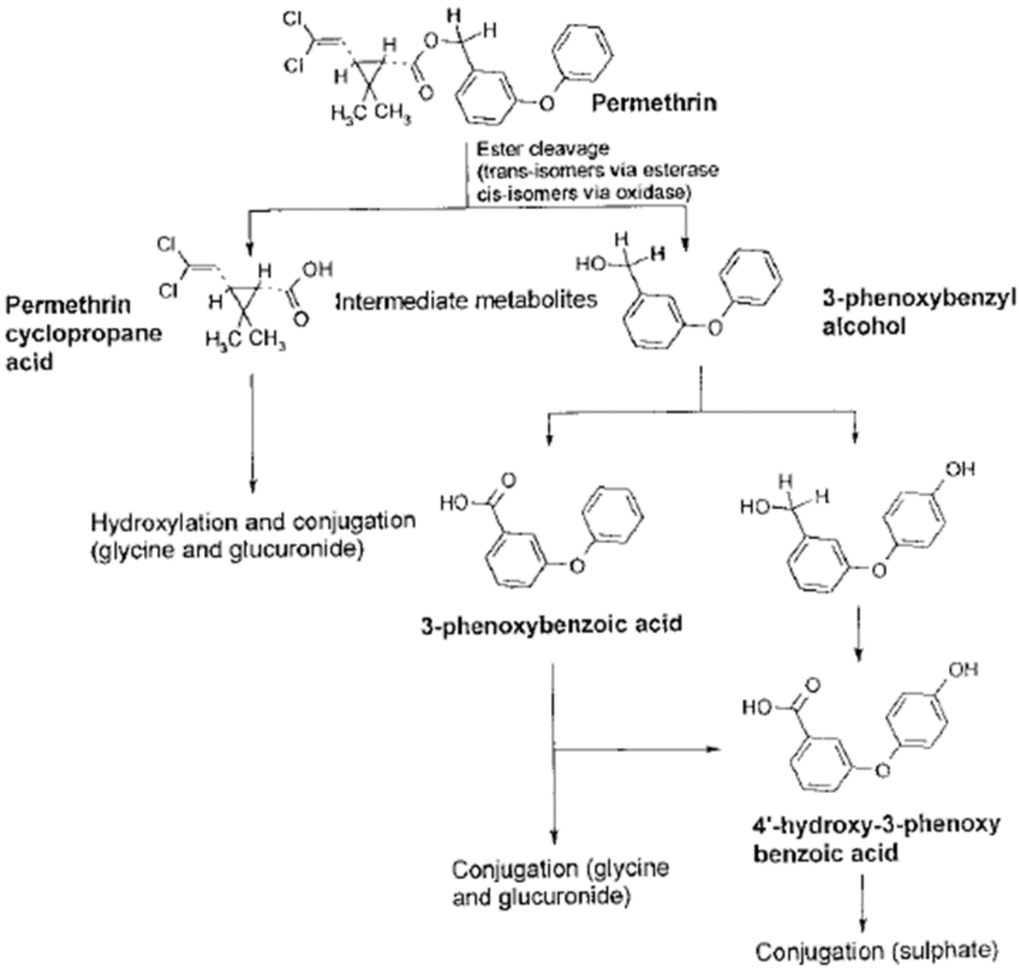


Figure 2. The modify PYRs (permethrin) metabolize in mammals

(Tyler, Beresford et al. 2000)

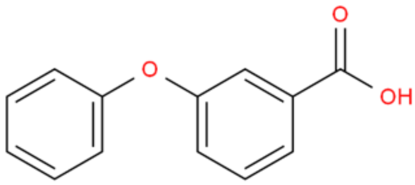
In the PYRs environment, it was reported that it rapidly decomposed to non-toxic products. However, the rate of degradation depends on the type and condition of the soil. The half-life report ranges from 1-16 weeks, the environmental degradation of cyclopropane-based PYRs, as with their metabolism, ester cleavage was a major processed that result in the production of cyclopropane acid and 3-phenoxybenzyl alcohol. The 3-phenoxybenzyl alcohol fragment was oxidized to 3-phenoxybenzoic acid, 3-phenoxybenzyl alcohol also often is an intermediate in the photocatabolism of PYR, which can then undergo oxidation to form the corresponding carboxylic acid (Tyler et al. 2000; Bradberry et al. 2005a; Diaz 2016; Wang et al. 2016; Fujiwara et al. 2017; Pinto, Cerqueira, et al. 2017).

After getting PYRs into the body, the concentration of the metabolite in the urine reflects the last touch. The five typical urine nutrients may reflect different patterns of PYRs exposure, as shown in **(Table 2)**, and the metabolism of PYRs shown in **(Fig 3)** (Tyler et al. 2000; Thatheyus and Gnana 2013; Glorennec, Serrano, Fravallo, Warembourg, Monfort, Cordier, Viel, Le Gleau, et al. 2017).

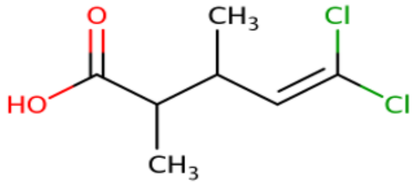
Table 2. The five urinary metabolites of PYRs

Type	Metabolites	Full name	Types of Pyrethroid
1	3-PBA	3-phenoxybenzoic acid	cypermethrin, deltamethrin, permethrin, lambda-cyhalothrin, cyphenothrin, fenpropathrin, fenvalerate, fluvalinate-tau, phenothrin, and tralomethrin.
2	*cis*-DCCA	cis-3-(2,2-dichlorovinyl)-methylcyclopropane carboxylic acid	cyfluthrin, cypermethrin, or permethrin
3	*trans*-DCCA	trans-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane carboxylic acid	cyfluthrin, cypermethrin, or permethrin
4	*F*-PBA	4-fluoro-3-phenoxybenzoic acid	cyfluthrin and flumethrin
5	*cis*-DBCA	cis-3-(2,2-dibromovinyl)-2,2-dimethylcyclopropane carboxylic acid	deltamethrin

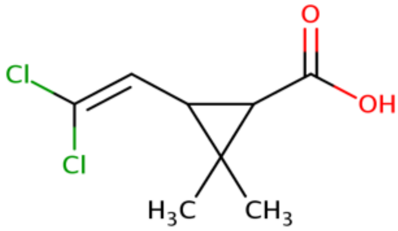
(Thatheyus and Gnana 2013)



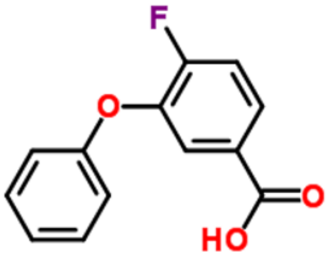
3-PBA



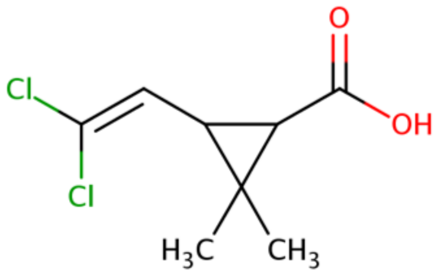
cis-DCCA



trans-DCCA



F-PBA



cis-DBCA

Figure 3. Structure of pyrethroids metabolites from urine sample.

(Thatheyus and Gnana 2013)

2.4 Effect of pyrethroid to human

PYRs in genera that toxic less than the organochlorine, organophosphate and carbamate pesticides with the exeption of esfenvalerate, deltamethrin, bifenthrin, tefluthrin, flucythrinate, cyhalothrin and fenpropathrin which show the highest acute oral toxicity (Thatheyus and Gnana 2013). Humans expose to PYRs by inhaling, winded, shortness of breath, runny or stuffy nose, chest pain and skin contact (Bradberry et al. 2005a). The effects and toxicity of PYRs were on sodium and chloride channels as a results in nerve and muscle, effect to neurotoxicity at high doses (Bradberry et al. 2005a; Thatheyus and Gnana 2013; Fiedler et al. 2015; Wang et al. 2016). effect to melatonin (Asghari et al. 2017; Sarabia, Maurer, and Bustos 2009; Sharma et al. 2013). However, effect of PYRs on human health and environment depend on how much PYRs were presented and length and frequency of exposure.

PYRs commonly presented ingredient in household pesticides and animal control products containing ectoparasite. Inactivity in the home environment increases the risk of exposure and side effects in the general population. The actions of PYRs depend on their ability to bind to and disrupt voltage-gated sodium channels of insect nerves. Sodium channels are also important targets for the neurotoxin effects of PYRs in mammals but other targets, particularly voltage-gated calcium and chloride channels, have been implicated as alternative or secondary sites of action for a subset of PYRs. Understanding PYRs neurotoxicity were complicated by the presence of two different intoxicating groups in mammals that related to different structural subgroups of this pesticide class (Louis, Lerro, Friesen, Andreotti, Koutros, Sandler, Blair, Robson, and Beane Freeman 2017; Lebov et al. 2015).

There were two types of PYRs depending on the difference in infrastructure, presence or absence of cyanotic groups in alpha and toxic symptoms, Type I, PYRs

do not include a cyano group; its effects in rodents usually include the onset of aggressive behavior and increased sensitivity to external stimuli, followed by fine tremor, the term T-syndrome (from tremor) was applied to Type I responses. Type II, PYRs include a cyano group that effects in rodents are usually characterized by pawing and burrowing behavior, followed by profuse salivation, increased startle response, abnormal hind limb movements, and coarse whole-body tremor that progresses to sinuous writhing (choreoathetosis). The term CS-syndrome (from choreoathetosis and salivation) has been applied to Type II responses. The cyano-PYR, fenpropathrin and cyphenothrin were showed to trigger responses intermediate to those of T-syndrome and CS-syndrome, characterized by both tremors and salivation. The profile type I and II of PYRs were showed in **(Table 1).**

The effects caused by skin contact with PYRs include falling, symptoms include constant tingling or stabbing, or when more severe burns occur, recurrence within 24 hours and the use of locally available vitamin *E* has proven to be an effective treatment. Due to abnormal PYRs-induced repetitive activity in skin nerve terminals and is more pronounced with type II compounds. Cases of mild acute illness, with neurologic and respiratory symptoms, have been reported in flight attendants following aircraft disinfection with permethrin. Occupational exposure to PYRs (particularly Type II PYRs containing the cyano group) frequently leads to paresthesia (abnormal cutaneous sensations such as tingling, burning, numbness, and itching). Type II PYRs have been shown to inhibit specific binding at or near the picrotoxin site of GABA receptors in mouse brain (Crofton, Reiter, and Mailman 1987).

2.4.1 The main effect of PYRs to sodium and calcium channel in human

The action of PYRs depends on the ability to bond and destroy the sodium channel with insect nerve. Sodium channel was an important target for neurological effects of PYRs in mammals but the other goal, especially, is the channel of calcium

and chloride that enters the voltage of the PYRs site. Type I PYRs, which linked to the T-syndrome and Type II PYRs, which linked with the CS syndrome, in order to be able to decrease in vitro and insect systems, this model shows that the sodium channel has significant pressure. In different mammals, different isoforms of sodium are different in tissue distribution and physical and pharmacological properties, as well as some or all PYRs sensitivity. In addition, PYRs has been shown to interact with isoforms of the calcium channel with a voltage that contributes to the release of neurotransmitter and to PYRs toxicity (Clark and Symington 2007; Cao, Shafer, and Murray 2011; Soderlund 2012; Meijer et al. 2014; Li, Ma, and Liu 2018).

PYRs were presented by bind and inhibits GABA channels **(Fig 4),** although it has higher concentrations that affect sodium channels (10^{-7} M - 10^{-10} M) (Heudorf, Angerer, and Drexler 2004). This effect was believed to result in seizures with severe PYRs type II poisoning, which other reported targets for PYRs include calcium ATPase and voltage-gated calcium channels, which are, however, affected at higher concentrations (10^{-5} M and 10^{-6} M, respectively) (Gammon and Casida 1983; Soderlund et al. 2002; Heudorf, Angerer, and Drexler 2004). The latter reaction may be interesting since PYRs have been showed to increase calcium-dependent neurotransmitter release. As **(Fig 4),** that Adjustment of calcium ions through the synaptosome cell membrane may cause neurotransmitter release at the synaptic junction, due to the direct agonistic effects of deltamethrin on the calcium ion channel selection. However, knowledge of PYRs affects the calcium channel as well as the toxicity of GABA (neurotransmitters toxicity) directly upon exposure to PYRs and time of exposure (Gammon and Casida 1983; Clark and Symington 2007; Breckenridge et al. 2009).

PYRs have been shown to react with isoforms of calcium channels with voltage that contribute to the release of neurotransmitter and hence PYRs toxicity

which PYRs pressure sensitive channel blockage was associated with the occurrence of salivation, a hallmark of the CS intoxication syndrome, it may also increase excitement in the central nervous system. Effects on peripheral-type benzodiazepine receptors associated with the GABA receptor are unlikely to be directly responsible for PYR toxicity, but may contribute to, or enhance convulsions resulting from effects at other target sites (Gammon and Casida 1983; Barlow, Sullivan, and Lines 2001; Soderlund et al. 2002; Heudorf, Angerer, and Drexler 2004; Ray and Fry 2006; Soderlund 2012).

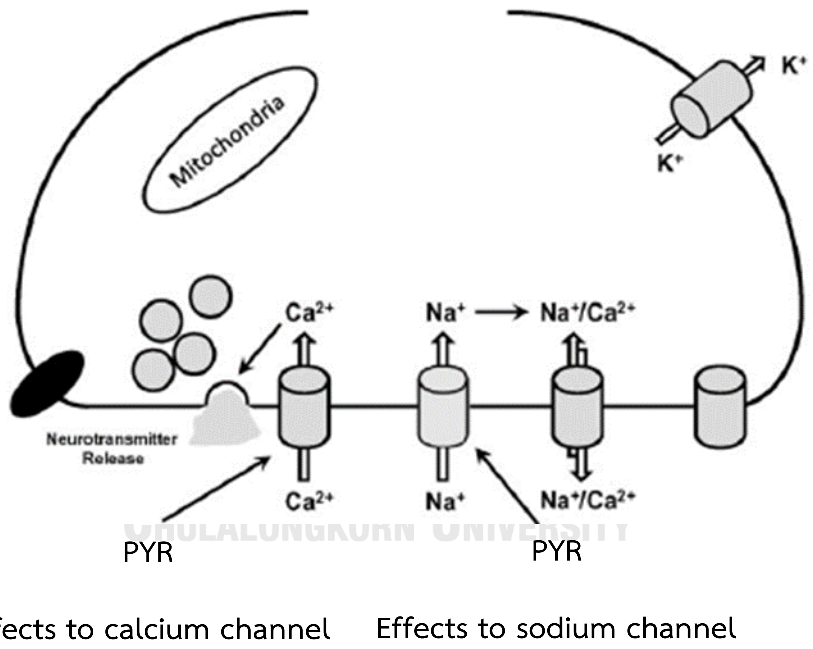


Figure 4. Proposed mechanism of PYRs type I and II PYR son calcium and sodium (Breckenridge, Holden et al. 2009)

Possible target sites associated with PYRs specific action on sodium and calcium and potassium are only susceptible to change, with relatively low PYRs concentrations. Two main classes 1) PYRs that induce a tremor response are T-syndrome PYR 2) PYRs that induce a choreoathetosis with salivation response are CS-syndrome PYRs. However, the neurotransmitter was released from the critical voltage to calcium during decoding. The neurotransmitter was released quantitatively by the fusion of synaptic vesicles with the active release zone (Clark and Symington 2007).

The main symptom of PYRs were presented toxicity the characteristic of the stomach. (Abdominal pain, nausea, vomiting), and dizziness, headache, fatigue, confusion, unconsciousness and seizures, PYRs were presented bind to and inhibit GABA-gated chloride channels, albeit at higher concentrations than those sufficient to affect sodium channels (10^{-7} M - 10^{-10} M). This effect was believed to contribute to severe seizures PYRs type II poisoning. However, drugs that enhance GABA transmission (e.g., diazepam), have modest effects toward deltamethrin-induced choreoathetosis or seizures, type II PYRs such as deltamethrin, also inhibit at low concentration (10^{-10} M) voltage dependent chloride channels (Costa 2015).

The PYRs were divided into Type I (T syndrome tremors) and Type II (CS syndrome choreoathetosis with salivation). There were evidenced that the pressure-sensitive sodium channel of PYRs insecticide were an important target in mammals. Mammals differ from the insects which different isoforms of sodium channels differ in their tissue distribution and biophysical and pharmacological properties, including some or all of the PYRs sensitivity, this demonstrates the interaction with the isoform of the calcium channel that sensitive to voltage which helps to release the neurotransmitter and PYR toxicity, the blocking of voltage-sensitive chloride channels by some PYRs were associated with the occurrence of salivation, a hallmark of the CS intoxication syndrome it may also increase excitement in the central nervous

system, effects on peripheral-type benzodiazepine receptors associated with the GABA receptor are unlikely to be directly responsible for PYRs toxicity but may contribute to or enhance convulsions resulting from effects at other targets sit, although other target sites for PYRs action have been identified in vitro (e.g. nicotinic acetylcholine receptors), these targets do not seem to play a role in the PYRs toxicity studies, the study of neuronal structure and function of PYRs extensively over the last 25 years has identified clear biological targets, including sodium which were calcium and chloride channel (Breckenridge et al. 2009). Sodium channel was an important target for neurological effects of PYRs in mammals and other targets including calcium and chloride channels have been implicated as alternative found that PYRs were effected to Sodium and chloride channels directly after than effected to neurotransmitter release (Soderlund 2012).

2.4.2 Neurotransmitters

Nerve cells in the human brain communicate with each other by releasing chemicals called neurotransmitters, this a substance produced by neurons stored in synapses that emits synaptic cracks to stimulate the postsynaptic membrane and they bind to special receptors and affect their activity. The task neurotransmission it was the transmission of signals from the neurons sent to the neurons through a gap called the synapse are show in (Fig 5).

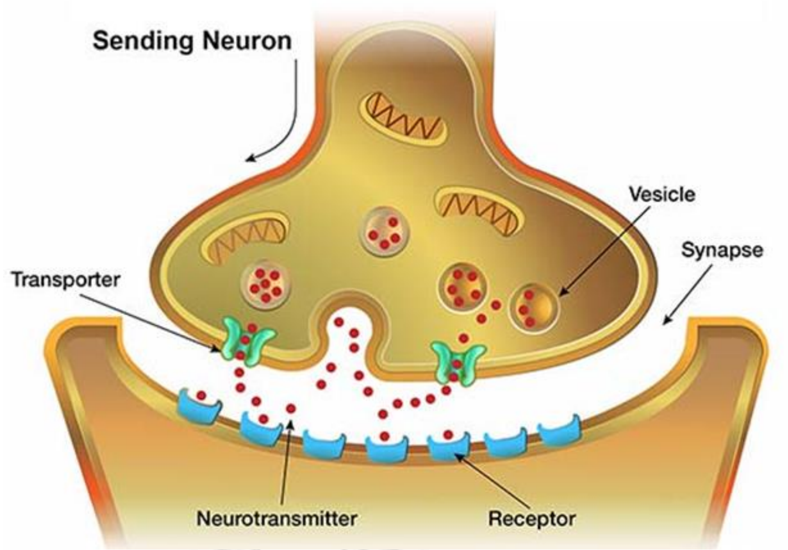


Figure 5. The neurotransmission from a sending neuron (Sumner, Edden et al. 2010, Cellot and Cherubini 2014)

When a neurotransmitter interacting with receptors of neurons that receive neurons which neural communication was completed. Neurotransmitter molecules release receptors in a synapse, they encounter one of the three fates attached to another receiver, some find the chemical enzymes that separate them apart and some reenter the neurons through a special structure that covers the membrane of the neuron. Once inside the neurons, they will be able to release them when they are genetically engineered in the future (Lydiard 2003; Bhagwagar et al. 2004; Sumner et al. 2010; Cellot and Cherubini 2014).

A lot of neurotransmitters were now known and continue to be discovered, neurotransmitters postynaptic electrical responses are bound to members of a variety of protein groups called receptors. There were two classes of receptors, which were receptors for molecules into ion channels and receptors and ion channels as separate molecules, previously called ligand gated ion channels and quickly responded to postsynaptic responses, it typically takes only a few milliseconds. There are over 100 different agents that are known to act as neurotransmitters, many transmitters allow for very high levels of chemical signaling

between neurons. Individual amino acids, such as glutamate and GABA, as well as the transmitters acetylcholine, serotonin, and histamine, are much smaller than neuropeptides and have therefore come to be called small-molecule neurotransmitters. Within the category of small-molecule neurotransmitters, the biogenic amines (dopamine, norepinephrine, epinephrine, serotonin, and histamine) are often discussed separately because of their similar chemical properties and postsynaptic actions (Boy et al. 2010; Sigel and Steinmann 2012; Aufhaus et al. 2013). The particulars of synthesis, packaging, release, and removal differ for each neurotransmitter **(Fig 6).** This study was used GABA as neurotransmitter for biomarker. Moreover, functional features of the major neurotransmitters are show in **(Table 3).**

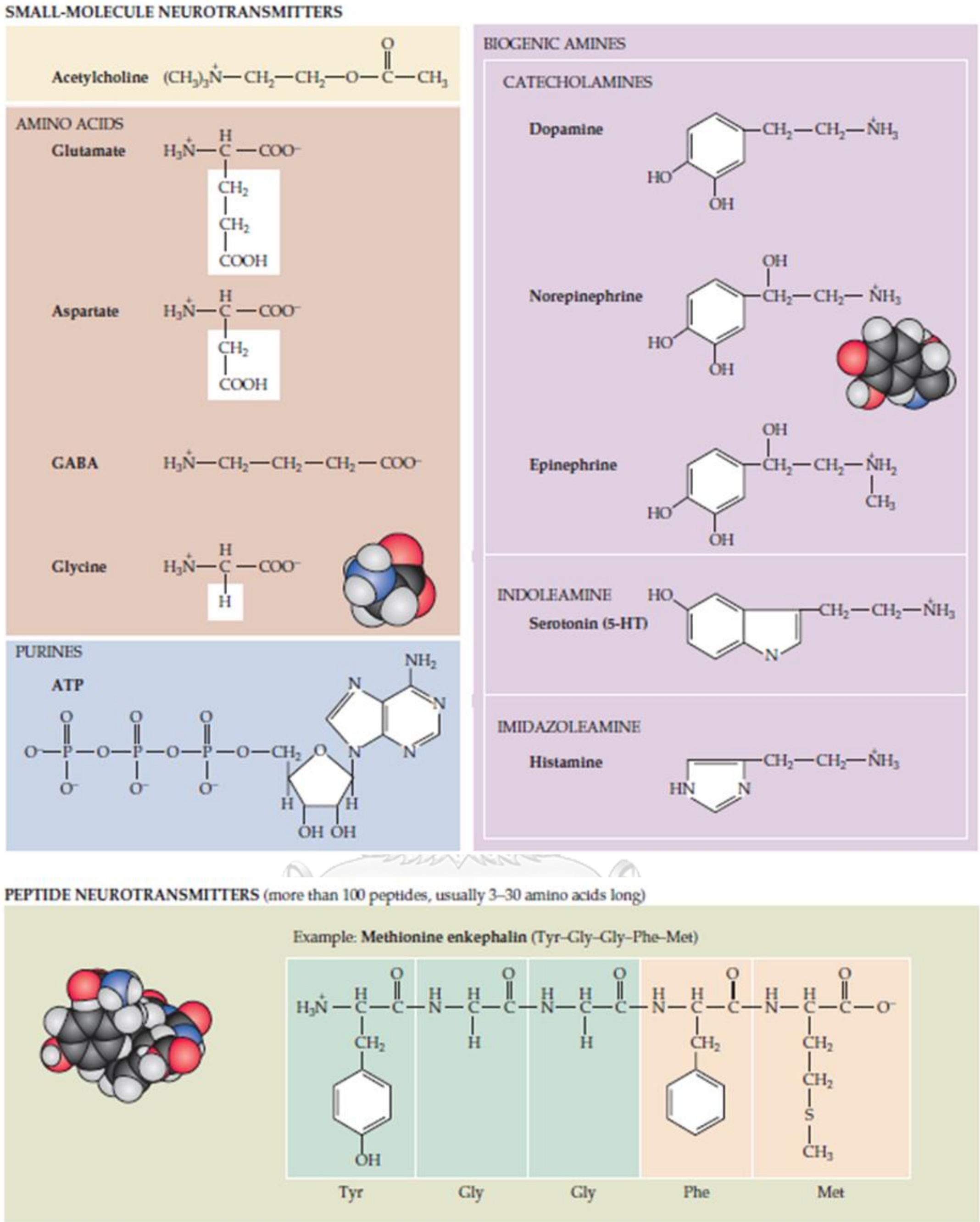


Figure 6. The neurotransmitter.

(Boy, Evans et al. 2010, Sigel and Steinmann 2012, Aufhaus, Weber-Fahr et al. 2013)

Table 3. Functional features of the major neurotransmitters

Neurotransmitter	*Postsynaptic effect*[a]	*Precursor(s)*	*Rate-limiting step in synthesis*	*Removal mechanism*	*Type of vesicle*
ACh	Excitatory	Choline + acetyl CoA	CAT	AChEase	Small, clear
Glutamate	Excitatory	Glutamine	Glutaminase	Transporters	Small, clear
GABA	Inhibitory	Glutamate	GAD	Transporters	Small, clear
Glycine	Inhibitory	Serine	Phosphoserine	Transporters	Small, clear
Catecholamines (epinephrine, norepinephrine, dopamine)	Excitatory	Tyrosine	Tyrosine hydroxylase	Transporters, MAO, COMT	Small dense-core, or large irregular dense-core
Serotonin (5-HT)	Excitatory	Tryptophan	Tryptophan hydroxylase	Transporters, MAO	Large, dense-core
Histamine	Excitatory	Histidine	Histidine decarboxylase	Transporters	Large, dense-core
ATP	Excitatory	ADP	Mitochondrial oxidative phosphor-ylation; glycolysis	Hydrolysis to AMP and adenosine	Small, clear
Neuropeptides	Excitatory and inhibitory	Amino acids (protein synthesis)	Synthesis and transport	Proteases	Large, dense-core
Endocannabinoids	Inhibits inhibition	Membrane lipids	Enzymatic modification of lipids	Hydrolasis by FAAH	None
Nitric oxide	Excitatory and inhibitory	Arginine	Nitric oxide synthase	Spontaneous oxidation	None

(Boy, Evans et al. 2010, Sigel and Steinmann 2012, Aufhaus, Weber-Fahr et al. 2013)

However, the knowledge of PYRs were affected to calcium channels, as well as directly to GABA (neurotransmitters toxicity) base on dose exposure of PYRs concentration and duration time exposure. In addition, PYRs also bind to and inhibit GABA-gated channels at higher concentrations than those sufficient to affect sodium channels (10^{-7} M - 10^{-10} M) (Sumner et al. 2010; Edden et al. 2012; Cellot and Cherubini 2014). This effect is believed to contribute to the seizures that accompany severe type II PYRs poisoning. However, drugs that enhance GABA transmission (e.g., diazepam), have modest effects toward deltamethrin-induced choreoatetosis or seizures (Boy et al. 2010; Sigel and Steinmann 2012; Aufhaus et al. 2013).

However, the results of biochemical studies and electrophysiologic Calcium channels are always inconsistent due to stimulation and inhibition. Therefore, the relevance of such interactions in PYRs body toxicity remains unclear. A large body of biochemical evidence documents the ability of PYRs bind and block GABA receptors

in mammalian brain preparations. Blockade of GABA receptors is an indirect neuroexcitatory effect, involving the removal of inhibitory neuronal input and is the established mode of action for convulsants such as picrotoxinin (Bhagwagar et al. 2004; Shafer and Meyer 2004; Breckenridge et al. 2009). The action of PYR on GABA receptors is somewhat stereoselective for neurotoxic isomers of alpha-cyano compounds but does not exhibit the absolute stereospecificity predicted by structure–toxicity relationships (Sigel and Steinmann 2012). Experimental systems that affect GABA receptors and sodium can be monitored in the same preparation (Cellot and Cherubini 2014). GABA receptor closure was not observed at PYRs concentrations that interfere with sodium channel activity (Lydiard 2003; Sumner et al. 2010; Costa 2015).

2.4.3 Gamma-aminobutyrate acid (GABA)

Gamma-aminobutyrate acid (GABA) was an amino acid classified as a neurotransmitter, it was messengers that communicate between neighboring brain cells (Forshaw, Lister, and Ray 2000). The particulars of synthesis, packaging, release, and removal differ for each neurotransmitter **(Table 6).** Most synapse inhibitors in the brain and spinal cord use GABA or glycine as neurotransmitters such as glutamate (Bhagwagar et al. 2004). GABA was identified in brain tissue in the 1950s. GABA is synthesized from glutamate by enzyme glutamate carboxylase which requires pyridoxal phosphate are show in **(Fig 7).**

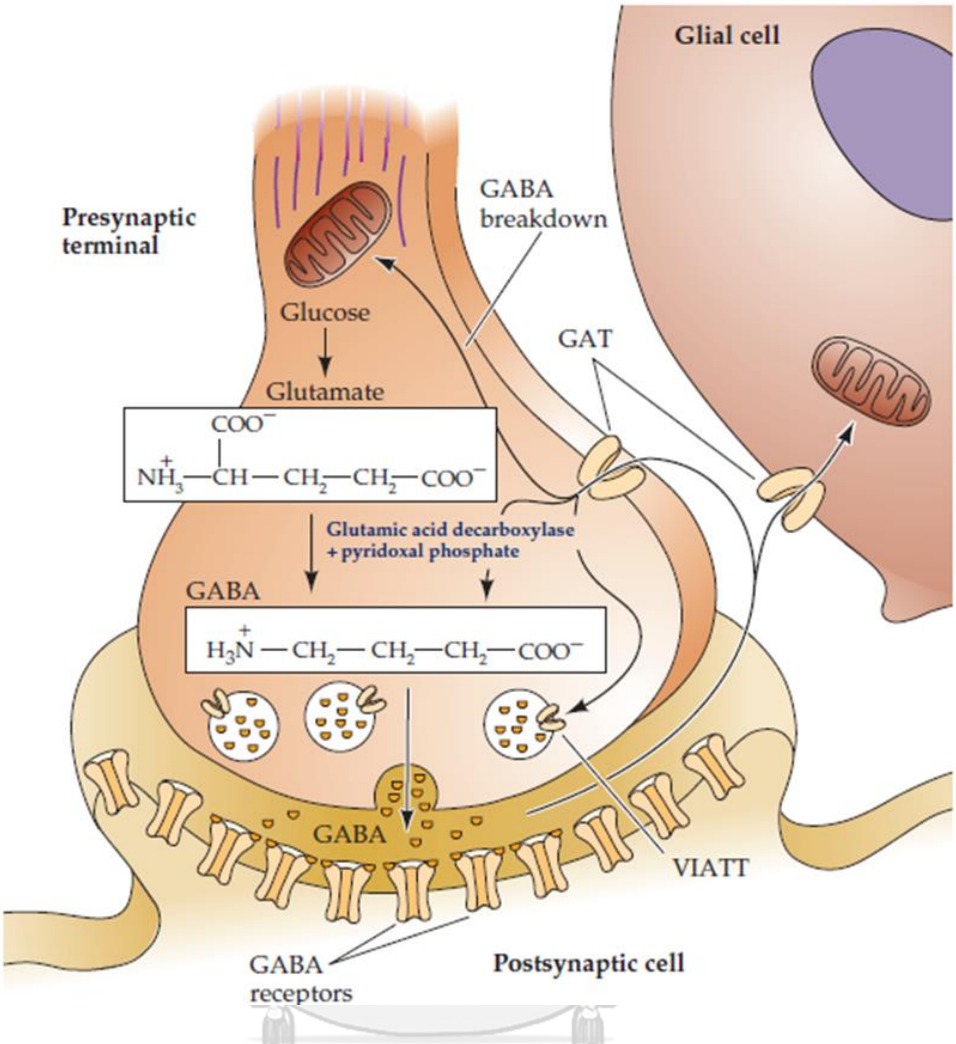


Figure 7. Synthesis, release, of the inhibitory neurotransmitter's GABA (Bhagwagar, Wylezinska et al. 2004)

GABA was a common neurotransmitter that affects many aspects of health, emotional and physical stability. The first reported strong indicate a novel mechanism of PYRs insecticide action causing the type II syndrome antagonize the action of GABA in neuromuscular transmission (Gammon and Casida 1983). In addition, PYRs can also bind and block GABA receptors in mammalian brain preparations, GABA blockade is an indirect side effect associated with the elimination of nerve input inhibition and is an actionable form of stimulation, such as pesticides,

picrotoxinin (Muthukumaraswamy et al. 2009; Sumner et al. 2010; Sigel and Steinmann 2012). From a functional perspective, GABA receptor activity is consistent with neurological symptoms that induce intoxication of PYRs in the body, in experimental systems that affect gabapentin and sodium receptors, they can be analyzed in the same formulation, Blockade of GABA receptors was not observed at PYRs concentrations that blocked sodium channels. Symptoms of low GABA levels such as anxiety, depression, ADD/ADHD (attention deficit hyperactivity disorder occur from GABA), insomnia, fatigue, Weight problems, compulsive disorder, and migraine, which can trigger the effects by neurobehavioral (Forshaw, Lister, and Ray 2000; Shafer and Meyer 2004; Breckenridge et al. 2009; Edden et al. 2012; Cellot and Cherubini 2014).

In 1983, this study was clearly demonstrated the new mechanism of pesticide use by PYRs, which prompts psychotic syndrome, GABA acts primarily on neurotransmitters and precise muscle mechanisms, various classes of GABA antibiotics interfere with the (open) or non-stimulating (off) ionophore depending on the GABA (Gammon and Casida 1983). After that in 1985, found PYRs that produced both T syndrome (tremors syndrome) and CS syndrome (choreoathetosis with salivation) (Saillenfait, Ndiaye, and Sabate 2015). Specific specificity of stereo channel on sodium channel in mammalian brain preparation. The PYRs compounds that induce CS syndrome in the sodium channel as compared to the receptor efficiency show that the Interaction between PYRs and sodium channels in the body may contribute to the production of CS intoxication syndrome, finding implication the voltage dependent sodium channel as a site of PYR action in mammals, the causal relationship between function and production of T and CS poisoning syndrome (Ghiasuddin and Soderlund 1985). The widespread impact of PYR's disruptive actions on sodium channel activity plus other sites of action, the main effected of PYRs 1) the primary ion channel effects of the PYRs 2) To control the secondary effects of

specific chemical neurotransmitters, both types I and II pyrethroids (permethrin, fenvalerate, cypermethrin, or deltamethrin) can act as proconvulsants via GABA and glutamatergic systems (Ray and Fry 2006).

2.5 Pyrethroids exposure to children

PYRs insecticides were normally used to control insects in household and agriculture area and worldwide. Moreover, children can be potentially exposed to PYRs component from food and some product that contain of PYR at home (Morgan 2012; Oulhote and Bouchard 2013b). Nowadays there are many research showing observational exposure measurement studies were presented that examined children on 5 months to 17 years of age that exposures to PYR in media including floor dust, food, floor wipes, air and urine were collected at household, while permethrin was normally detected (>50%) of PYRs, followed by cypermethrin (wipes, dust, and food), as the same time 3-phenoxybenzoic acid (3-PBA), a urinary metabolite of several PYRs, was the most frequently (≥67%) detected PYRs biomarker, indicate that children were exposed to several PYRs, but primarily to permethrin and cypermethrin, from several sources including food, dust, and surfaces at household, while dietary ingestion followed by nondietary ingestion were the dominate exposure routes for these children, except in homes with frequent pesticide applications (dermal followed by dietary ingestion), urinary 3-PBA concentration data confirm that the majority of the children sampled were exposed to one or more PYRs (Lu et al. 2000; Barr et al. 2005; Heudorf et al. 2006; Abdel Rasoul et al. 2008; Morgan 2012; Oulhote and Bouchard 2013b; Antwi and Reddy 2015).

Moreover, many studies about PYRs observation and exposure that collected the sample from environmental at children household in US, they showed that the media collected including outdoor air and indoor air at children household age between 0-2 years old, while sampling frequency by 24-h integrated sample at 2.5

L/min, analytical method by gas chromatograph/mass spectrometer in the selected ion monitoring mode, they found tetramethrin, cyfluthrin, cypermethrin, esfenvalerate, *cis*- and *trans*-permethrin, bifenthrin, *cis*-and *trans*-Allethrin, λ-cyhalothrin, deltamethrin, phenothrin and resmethrin were presented in their children household (Heudorf et al. 2006; Morgan 2012). Other researches collected the sample from dust (carpets, sofas) and hard floor surface wipes at children household age between 4-17 years old, while sampling frequency by 9 A Eureka Mighty-mite vacuum cleaner sampled 'all accessible surfaces' in living rooms and SOF-WICK pad wetted with 5 mL of 2-propanol; sample collected each in a 929 cm^2 area in kitchen and living room, analytical method by gas chromatograph/mass spectrometer in the selected ion monitoring mode, they found tetramethrin, phenothrin, esfenvalerate, cypermethrin, cyfluthrin, bifenthrin, λ-cyhalothrin, deltamethrin, permethrin and resmethrin were presented in their children household (Morgan 2012). In a few year, some literature review showed that collected the sample from house dust, while in 13 urban homes from CHAMACOS cohort and Okaland in CA from children 3-6 years, they analyzed 22 insecticides (OP, PYRs, phthalate, dicarboximide fungicide, herbicide, and pesticide synergist) found that *cis*-Permethrin (concentrations 568 ng/g and 291 ng/g in farm and non-farm homes, respectively) and *trans*-permethrinin (concentrations 952 ng/g and 504 ng/g in farm and non-farm homes, respectively) (Tyler et al. 2000; Barr et al. 2005; Abdel Rasoul et al. 2008; Morgan 2012; Oulhote and Bouchard 2013b; Antwi and Reddy 2015; Hyland and Laribi 2017).

For PYRs observation and exposure that collected the sample from urine at children household, they showed the collection method by spot sample, frequency of collection including one morning void and one bedtime void each day for15 consecutive days; each sample analyzed separately, analytical method by gas chromatograph/mass spectrometer in the selected ion monitoring mode and high

performance liquid chromatograph- electrospray chemical ionization/tandem mass spectrometer, they found 4-F-3-PBA, 3-PBA, *cis*-DBCA, *cis*-DCCA and *trans*-DCCA were presented in their urine children at household (Morgan 2012). 97% children age between 6-11 years in Canada were exposure from PYRs, they analyzed urinary metabolites found that *cis*-DCCA and *trans*-DCCA were presented in their urine children, while they suggested that longitudinal studies should be conducted on the potential risks of PYRs and help for behavioral problems in children (Oulhote and Bouchard 2013b).

2.5.1 Children's metabolic pathways

The metabolic pathways of children, especially in the fetus and in the first month after birth are underage. The ability of children to metabolize toxins and excrete allergens were different from that of adults. In some cases, children can outperform adults in dealing with environmental agents because they cannot create toxic substances for toxicity. In other cases, children are less likely to cope with toxic chemicals and risk the metabolic differences between prenatal and postnatal life and may vary over time the course of pregnancy. Additional sources of additional vulnerabilities in the fetus and young children are barriers in the blood and brain that are not fully developed, so it may be easier to use drugs in the central nervous system, developmental processes that cause disruptions during growth and rapid development before and after birth, growth and rapid development occur between embryos and fetal life as well as in the first years after birth (Landrigan et al. 2004). In the brain, like a billion cells, it must be moved to a designated location and create a precise connection with other cells, development of the endocrine organs and reproductive system is guided by complex chemical message sequences, many diseases caused by toxins in the environment take decades to develop (Landrigan et al. 2004).

Risk in each age range. Children exposed to toxins breathe in the air, drink the water they drink, eat the medication they eat, and a variety of environments, including homes, childcare centers, schools and cars. Children have a unique path of non-parallel exposure among adults, and the pathways of exposure and risk to health vary in different stages of childhood and contact with the toxins may occur in the uterus by transferring the substance from the mother to the fetus which it can happen through the milk in the newborn; and it can happen in childhood by transferring poisonous chemicals from the hands, analysis of the patterns and routes of exposure of children's allergens and the health effects that occur at various stages of development are essential to establishing a child protection approach to risk assessment (Landrigan et al. 2004).

Mechanisms of pesticides are effected to human, including organophosphates compounds, especially the enzyme-mediated neurotransmitter, such as acetylcholine, since many children may receive pesticides at various stages of their lives, it is difficult to assess the neurological effects of specific insecticides, age and genetics may play a role in how pesticide toxins affect individuals, the results of the study on children 2 years and older indicate poorer outcomes and higher levels of exposure, psychomotor or mental development is inconsistently affected, various pesticides have been linked to neurobehavioral outcomes, including verbal, memory, response-speed, and possibly autism in preschool aged children, numerous studies are limited to small sample sizes, emphasizing the need for future research to be used in conjunction with similar methods and measures (Liu and Schelar 2012b).

2.5.2 Significance of the problem in children

Young children are vulnerable to environmental toxins including pesticides. Children’s organs were not fully developed until later in life. They were constantly experiencing significant development moments; adverse exposures can cause

permanent damage, particularly in utero, the behavior of children and their ability to interact with their physical environment changes at each stage of growth and development and can put them at greater risk of exposure, children may crawl on the floor, explore verbal objects and play with objects found in the environment, these substances may block the absorption of important nutrients in children's diets, which affect health outcomes. Routes that come into contact with pesticides may occur in the womb or through breastfeeding by eating contaminated food, pesticides, and skin contact through the skin. However, the relationship between the concentration of pesticides at low levels over time and the neurological and behavioral development will be less (Liu and Schelar 2012b).

Risk assessment of children can made at home by the employment of parents (e.g., parents are agricultural workers), in the community, or at school and day care. For example, children living in agricultural areas have higher levels of PYRs urinary metabolites than urban children. A major route of pesticide exposure in young children is dietary ingestion. However, the use of pesticides in habitat means greater risks and changes in age risk as children become more involved with their environment.

2.5.3 Pyrethroid effect to neurobehavioral in children

Exposure to PYRs were an important risk for children because the brain development is more sensitive to neurotoxins, while the proportion of air, breathing, eating and water intake is higher than that of adults. Say that children are more connected. Therefore, children may experience more exposure than adults in exposure to PYR (Fiedler et al. 2015; Wang et al. 2016).

Studies on urinary metabolic levels and their relation to their exposure and metabolism, neurobehavioral of the children while they analyzed 406 children's morning urine samples, aged 3–6 years from Nanjing in China, they found that

detection of 3,5,6-trichloropyridinol (TCP) and 3-phenoxybenzoic acid (3-PBA) in urine, it is possible to involve living areas adjacent to agricultural fields and the use of mosquito repellents in the indoor, two metabolites were negative related with the soaking time of vegetables and fruits, when treated as dichotomous variables, TCP was significantly related with arithmetic test scores in adjusted models and 3-PBA was significantly related with the scores on the Chinese Binet and arithmetic tests, when treated as a continuous variable, higher urinary 3-PBA levels were significantly related with lower cancellation test scores, they point out that PYR's exposure to organophosphate and PYR may have a significant impact on children's memory and verbal understanding (Wang et al. 2016).

Recent research shows that low levels of pesticide exposure may affect neurological development and the behavior of young children a link between pesticides and neonatal reflexes, psychomotor and mental development, and attention-deficit hyperactivity disorder, their action targets the nervous system by altering the effect of an enzyme that regulates select neurotransmitters, PYR and carbamate also target the nervous system, making children especially vulnerable to the effects of pesticides. In contrast, in a longitudinal study of Latin American farmer families in California, there is no link between preterm or childhood with organophosphate in the first 2 years of life and mental development. However, researchers found a negative correlation between prenatal exposure to prenatal developmental disorders and developmental disorders at the age of 24 months. In addition to receiving pesticides in the environment, then children living in the area Agriculture may be at risk for increased exposure - the risk of parental occupation and the location of their home, recent research has also linked ADHD with organophosphates in children 8 to 15 years old (Liu and Schelar 2012a).

2.6 Children behavior

Children were received pesticides in their homes and assess the activities of their children's activities and activities in their children and the survey respondents indicated that the youngest children are likely to Show behavior that promotes environmental contamination, hand-to-mouth and object-to-mouth activities are most common among the youngest children, the youngest is usually barefoot inside and outside, gender differences were found in out-of-home behaviors and the proportion of time spent in outdoor observation with reported behavior (observed activity/hours) showed that object to mouth, touch clothing and touch smooth surface, risk factor of gender differences were found boys spent time in outdoor more than girls which girls spent time in indoor more than boys (Freeman et al. 2001; Freeman et al. 2005; Paulson and Barnett 2010; Liu and Schelar 2012b). Children are particularly vulnerable to the environment agents, children drink more water, eat more food, and breathe more air pound-for-pound of body weight compared with adults, for example, children in the first six months of life drink up to seven times more water, while children 1-5 years of age eat 3-4 times more weight than adults (Landrigan et al. 2004).

The role of children's activities leading to pesticide exposure was assessed by comparing the amount of pesticides in the hands of children with the same activity observed in the last 4 hours, children between 2-5 years of age show child behaviors that are quantified using virtual tracking devices and frequency and duration of work, the hourly rate of behaviors, and the place where the behavior occurs when compared to hand-picked pesticides, the amount of pesticides received at the time of filming was correlated with the levels of pesticides in the surface and toys, no airborne concentrations of pesticides received during the video recording period were correlated with the frequency, duration and hourly rate of contact with the bottle and the behavior of the oral organ, the duration of contact with the surface of the

work load is also related to the number of places where children behave in hand-to-mouth (Freeman et al. 2001; Landrigan et al. 2004; Freeman et al. 2005). The impact of children's activities on exposure to environmental contaminants is evident from studies of lead exposure where hand-to-mouth activities and hygiene practices are associated with higher blood lead levels, lead levels in the hands, and lead levels in the diet and the same researcher in year 2005 were showed children behavior including upholstered furniture, bottle/dish and object to mouth were associated with pesticide exposure (Freeman et al. 2005).

The reason that children were more vulnerable to pesticides include higher metabolic rates, higher respiratory rates, higher permeability of the skin, developmental stage (crawling, hand-to-mouth behavior) and the development of organ systems, especially the central nervous system, which can be caused by exposure to pesticides in children, children of agricultural workers must be exposed to residues in the home, clothing, shoes and body of family members after work in the field, children's behavioral activities place them at risk for exposure to these residues as they crawl and play on the floor and put toys and other objects into their mouths, Similar to the pattern of behavior that puts children at increased risk of getting lead poisoning (Lucas and Allen 2009). In addition, the behavior of children and their ability to interact with their physical environment changes over time, of growth and development, and can increase the risk of exposure: children may crawl on the floor, explore objects orally, and play with items they find in the environment (Lucas and Allen 2009).

However, children have different activity patterns (e.g., crawling), they are closer to the ground and eat more food and water per kilogram than adults. In addition, children may be at greater risk of exposure to pollution due to higher

ventilation and metabolic rate, rapid physical development, greater surface-to-volume ratios, and immature organ systems (Freeman et al. 2005).

2.7 Residential Factors

The use of pesticide residues prevalent throughout the world. However, there were little information available and variations in the indoor environment. Some studies have shown that insecticides are at home with less variability in the last 2 months and contribute to chronic maternal inhalation during pregnancy (Whyatt et al. 2007; Morgan et al. 2008). Many insecticides are volatile and can detect. Found in the air inside the building after using the housing. Although modern insecticides tend to fall sharply in the outdoor environment due to the availability of water and ultraviolet light, which leads to biodegradation and photolytic, half-life seems to be within the longer indoors (Whyatt et al. 2007).

In addition, the living factors that influence chronic exposure include the use of insecticides and mice in the home, and the use of herbicides and fungicides in lawns as well. In-house broadcasting applications, including "flea bombs" and fogs, can leave residues in the air, rugs, toys and dust from homes, increased risk of skin inhalation. Inhalation and contact of residues on the surface or air as it melts, pesticides can be measured in indoor air samples and remain in dusty dust from carpeted areas and children's toys such as stuffed animals and can be brought home from work, the herbicides used in lawns or gardens can be tracked into homes with residues created over time which the application of diazinon to lawns has been proven to be carried out in the home by paws of pet dogs, residual pesticide residues of residential pesticides also vary geographically according to the specific requirements of pesticides in the area in which the source relates, residentially it may involve other settings that children spend time including school, child care,

relative home, etc, depending on the pesticide both inside and outside, use patterns and proximity to using pesticides (Julien et al. 2008; Roberts and Karr 2012)

For PYRs, both pesticide residues and household pest control products are important sources among locally produced samples, 49% of fruit, 29% of vegetables, 26% of grain products, 24% of fish/shellfish, and 0% of milk/dairy tested had detected pesticides but legitimate. In general, the presence of residues in fruit and vegetable samples was low but legal errors are more than (5–7% of imported fruits/vegetables sampled), organic diets may reduce pesticide intake as well as the study that children eat organic food for 5 consecutive days, rapid and dramatic decline in their urinary excretion from the insecticides of malathion and chlorpyrifos, OP during the organic eating phase (Roberts and Karr 2012). The first report on the concentration of 10 PYR and two organophosphates in an urban setting, and more specifically, In the multiunit housing setting, the results show the range of pesticide levels with PYRs (permethrin) and organophosphates (chlorpyrifos) detected in every home, cyfluthrin detected in most homes which significant relationship between vacuum and floor sampling methods for some widespread analysts indicates that the kitchen floor can be wiped and probably a proxy at a price, these can be useful in challenging sampling environments and choose the characteristics of the household, including the ethnic composition: (Hispanic, Black, Caucasian), the use of pesticides in the past year.: (Ever used pesticides, take weekly or more often and use it every month or less, and the type of pesticide.: (Traps, Gels, Sprays, Smoke bombs) the prevalence of pesticide use, such as kitchen wipes, living room wipes and vacuum cleaners, has been found to be contaminated in such areas (Julien et al. 2008).

Some studies have shown that participants and their pet dogs are more likely to get diazinon from a variety of sources (i.e., air, dust, and soil), through many paths and paths after using these lawn homes, pet-like dogs are an important pathway for

the transfer and movement of diazinon residues within homes and residents that are exposed by personal contact (i.e., petting) (Morgan et al. 2008). Strong relationships between termite treatment and treatment with chlordane and flea or tick and permethrin, most of the major associations are consistent with the use of well-known pesticides, the association is unlikely to be consistent between questionnaire data and pesticide residues in dusts, adding to the reliability of both methods for assessing pesticide exposure, pesticides exposure into the home from indoor use drift or drifting from the outdoors, intrusion of vapors from foundation treatments or take-home contamination from occupational use (Colt et al. 2004; Morgan et al. 2005).

The use of pesticide residues in low-income homes is common due to poor housing conditions and pest outbreaks; however, pesticide exposure information in low income households is limited. In children aged 3-6, low income children may experience a combination of pesticides as a result of lower quality housing, pesticides are measured in the living environment and the most prominent are indoor dust, poor housing conditions in low-income homes, overcrowding and deterioration of habitat are associated with outbreaks of pests and increased use of pesticides in urban and agricultural communities, the increase in pesticide residues in the home and the presence of housewives in the home or nearby neighborhoods in farming communities are associated with higher concentrations of indoor pesticides (Quiros et al. 2011b).

2.8 Pyrethroids degradation in environment

In a PYRs environment it is biodegradable by one or more biotic or abiotic processes, metabolic degradation by plants, animals and microorganisms, and photo degradation. Although PYRs is one of the oldest active insecticides in use (Rafique and Tariq 2015; Cycon and Piotrowska-Seget 2016). But there are limits to the environment. Many fate environment variables are evaluated using the chemical properties estimation method, in contrast to laboratory or field studies (Liu et al. 2010). PYR is a particularly effective insecticide that demonstrates its potency, low toxicity to mammals. Rapid degradation in the environment due to the reaction between light and air and is metabolized rapidly (Miyamoto 1976). For example, the degradation of PYRs light in the sunlight is rapid and results in the oxidation of the side chain, the oxidation reaction with the carboxylic acids, and the catalytic action and isomerization of the cyclopropane acid isomer. Water digestion data indicate that PYRs slows down in water. However, in the presence of microbial communities, degradation is expected to accelerate with oxidative metabolism (Tyler et al. 2000). This compound is toxic to fish and marine invertebrates, so use in close proximity to water habitat can be a problem (Tyler et al. 2000; Liu et al. 2010).

2.8.1 Pyrethroids degradation by photodegradation

PYRs drops sharply when exposed to natural sunlight and does not stay in the environment for more than a few weeks (Tyler et al. 2000; Liu et al. 2010). Degradation of PYRs under light and dark environments, clearly evident in **(Fig 8)** is that under dark conditions, there is little PYR degradation over a period of time, but in light it will rapidly degrade from 100% to less than 1% within 5 hours (Thatheyus and Gnana 2013; Rafique and Tariq 2015; Cycon and Piotrowska-Seget 2016). The photochemical degradation of PYR acid components has been investigated found that the carbon 1 and 3 **(Fig 1).** of the main cyclopropane group gets cleaved and subsequently. Then, the compound will be dispersed into a new age that is

transformed into lactone or become a primary material by regeneration bond between the carbon 1 and 3 **(Fig 1** and **Fig 2).** However, the importance of ester degradation is the decomposition of PYRs photochemical occurs rapidly with oxygen and sunlight, some studies on chemical oxidation in PYRs found that PYRs I was stable for 24 hours under nitrogen and oxygen conditions in the dark, but not very stable in the presence of oxygen and light **(Fig 8)** (George 2002; Thatheyus and Gnana 2013; Rafique and Tariq 2015; Cycon and Piotrowska-Seget 2016).

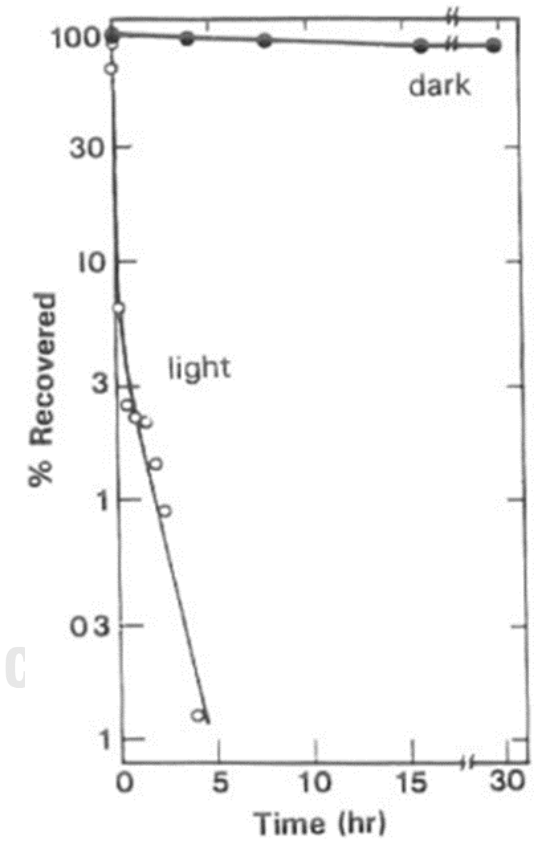


Figure 8. Photodegradation of PYRs (cypermethrin) in the light and dark (Thatheyus and Gnana 2013)

2.9 Situation of pyrethroids using in Thailand

Synthetic insecticides of various chemical classes have been used in the national vector health control program, most of which are PYRs. In Thailand, the combination of PYRs were often used by homeowners to control mosquitoes and other insect pests at relatively low doses, PYRs were a powerful and relatively safe compound for controlling common mosquitoes in the home, especially *Aedes aegypti* and *Culex quinquefasciatus*, since 1994, deltamethrin has been widely used in public health projects in Thailand in an effort to halt the spread of dengue fever, according to an outbreak of permethrin and deltamethrin (e.g., aerosols, coils, and gels) commercial service to the general public, but less for lambda-cyhalothrin, the selection of PYRs resistance by mosquitoes is largely due to exposure to commonly used chemicals and often to the management and prevention of vector-induced diseases, most published reports on PYRs resistance in *Aedes aegypti* and *Aedes albopictus* populations in Thailand have been restricted in geographical scope, dengue hemorrhagic fever detection and dengue vaccine validation in PYRs synthesis should be an essential component of resistance management policies and disease control activities (Chuaycharoensuk et al. 2011; Nkya et al. 2013; Amelia et al. 2018).

Thailand relies on the use of pesticides to protect crops and increase productivity, over the past decade, the Kingdom of Thailand has been increasing its use of pesticides by about four times, the increased use of pesticides is due to a number of factors, such as insect resistance and pest recovery, plants and plant varieties from one season to the next, to meet the needs of the market despite the changing environment (Panuwet et al. 2012). The spread of major human diseases, such as influenza, dengue and mosquito nematodes, is a serious threat to the global public health and poses a major economic burden for Africa and tropical development, most vector control programmers rely on the application, impregnated nets (ITNs) or indoor residual spraying (IRS) are mostly based on synthetic PYRs, which

is only recommended by WHO for pesticides. However, the efficacy of PYRs were threatened by an increase in resistance in the target population, this phenomenon occurs worldwide in all major mosquitoes of the mosquito species and spreads at a rapid rate, PYRs resistance is believed to be mainly caused by high ITNs and IRS coverage, other studies related to the acceptance of PYRs mosquitoes and the presence of allergens in urban, industrial, agricultural or industrial areas (Nkya et al. 2013).

Thus, PYRs resistance in dengue vector *Aedes aegypti* in Southeast Asia: Current situation and management trends. Pesticide resistance is one of the most serious problems in mosquito control. Loss of efficacy of PYR may result in high *Aedes aegypti* is ability to control disease and may contribute to the spread of the disease. Thailand is a country where most of the PYRs insecticide resistance assessments of *Aedes aegypti* in Southeast Asia reflect significant geographical bias, as this species is widespread in many countries in the region, dengue epidemic in Thailand in the first in year 1958, there was an outbreak of the disease and use of synthetic PYRs drugs has been a major player in the market since 1992, with 12 clearly-available ingredients are commercially available to all households nationwide to control mosquitoes in response to their low prices, the knockdown effect is fast and safe for human contact because of toxicity (Chuaycharoensuk et al. 2011; Panuwet et al. 2012; Amelia et al. 2018).

Reliable data on vector resistance to pesticides in Thailand is low due to the lack of systematic education. Information on current knowledge about pest vector resistance in Thailand and provide possible management guidelines when severe pesticide resistance occurs (Chuaycharoensuk et al. 2011; Panuwet et al. 2012; Nkya et al. 2013; Amelia et al. 2018).

However, in this study was interested to understanding children's exposures and potential health risks to PYRs at home, including identification of sources that important and routes of exposure.

CHAPTER III
METHODOLOGY

3.1 Research design

This study was a repeated cross-sectional study. it was designed to compare data regarding PYRs insecticide exposure between wet (November – December 2018) and dry (April – May 2019) seasons. It also investigated a relationship between pyrethroids metabolite and GABA level effects of young children (2-3 years) in urban area, Bangkok Thailand. Urine samples were collected from the same participants who met the inclusion two times in order to analyze both pyrethroids metabolize and GABA levels.

The first step, the researcher selected the participants using the inclusion. Those who met the criteria were recruited in the study. For second step, the researcher observed and used the questionnaires to assess the exposure sources at children's home. The final step was to collect urine samples from the subjects. PYRs metabolites were classified into two groups, as high and low level and were used to GABA levels that effect to children health.

3.2 Study area

The study was conducted in the two areas in Bangkok Thailand including Khlong Toei and Pathum wan district. This study focused on children in low-income families because of the uses of PYRs insecticide products daily among their parents. In addition, parents probably lack of awareness or knowledge on how to protect their children from the exposures. people in the areas used PYRs products such as repellent products to control mosquitos and fleas in kitchens and bedrooms.

Khlong Toei district

Pathum wan district

(Thailand: Bangkok Metropolitan Region / กรุงเทพมหานครและปริมณฑล". City Population. Retrieved 27 November 2015)

3.3 Study population

2 - 3-year-old healthy children living in households in both two districts where PYRs have been applied were purposively selected. In addition, their parents must consent them to participate in this study.

To collect data, the researchers prepared the device in a zip lock bag to put napkins that were contained urine. The napkins were collected two time in the morning and evening at the same day and put in the bags. Eventually, the researcher analyzed the concentration of PYRs metabolite from the urines.

3.4 Inclusion and Exclusion criteria

Inclusion criteria:

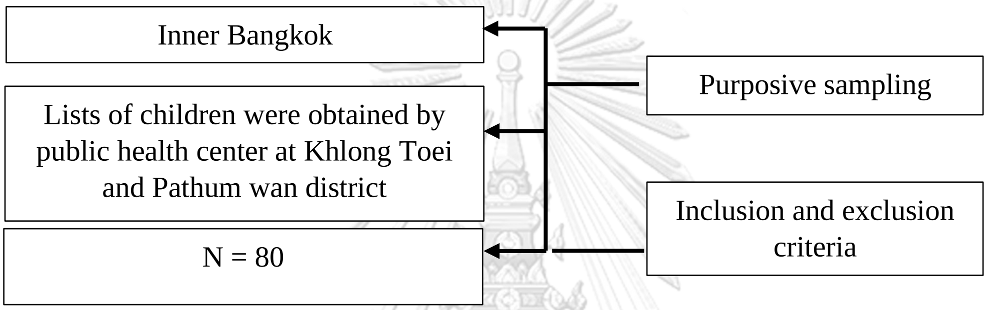
1. Children age between 2 – 3 years old.
2. Children with both male and female
3. Children who are consented by their parents or caregivers
4. Children who have live and were born in BKK.
5. Healthy children (identify by researchers)

Exclusion criteria:

1. Children who have significant psychiatric illness such as developmental delay, mental retardation, neurological disease. (researchers asked this information from parent or caregiver)
2. Children who have fever or other disease identifying by the researcher.

3.5 Sampling techniques

In Thailand, at congested community in Bangkok was purposively selected. This study used purposive sampling technique to select a sampling unit (child) in Bangkok. Lists of children were provided by a Public Health Center. Children who met the inclusion criteria were included in this study. The researcher then applied random sampling technique to select the participants.



3.6 Sample and sample size

Sampling period

Participants who met the inclusion criteria were included in this study. They were then collected urine samples fro0m the same participants in each season to analyze PYRs metabolites and GABA concentration.

Sample size

Eighty children ages 2-3 years old who have lived in BKK were recruited in the study. To compute the number of participants, the difference between two means independent group was used. According to (Rohitrattana et al. 2014a), the difference between two means of urinary metabolite in children living in BKK area was 0.03 (Δ) with standard deviation 0.03 (σ). The sample size calculation by G*Power program.

Effect size d = 0.70

α err prob = 0.05

Power (1-β err prob) = 0.8

Sample size group 1 = 40 (PYR exposure)

Sample size group 2 = 40 (Low exposure)

Total sample size = 80

3.7 Sample collection

3.7.1 Living environmental samples

Wipes sample

Wipes sample was applied with children's hands and the samples were frozen at -18 °C unit. This method was used to ensure preservation of the compounds (Glorennec, Serrano, Fravallo, Warembourg, Monfort, Cordier, Viel, Le Gleau, et al. 2017).

3.7.2 Personal samples

Urine samples for PYR metabolite and GABA level

The parents were received one polyethylene urine collection bottle (already labeled an identifying code) and instructed how to collect the urine samples. The samples were collected from the participants in the morning, 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 on dry ice before shipping for analyzing (Rohitrattana et al. 2014a; Glorennec, Serrano, Fravallo, Warembourg, Monfort, Cordier, Viel, Le Gleau, et al. 2017).

3.8 Measurement tools

the questionnaires were used to interview the participants' parents at homes.

Part I: Exposure Questionnaire

Socio-demographic Characteristics:

- Age (2-3 years old)
- Gender
- Weight
- Current address

- Duration to live in this region
- Location of residence

Factors related to children:

- Father/Mother educational attainments
- Main source of Father/Mother incomes
- Father/Mother income/year

Factors related to PYR exposure:

- Type insecticide products that are used in a home
- Name of insecticide products that are used in a home
- Store for keep insecticide products
- Frequency of insecticide use in household
- Frequency of floor-cleaning
- Type of cleaning floor
- Frequency of open window or door
- Frequency of insecticide control operators by officer

Personal hygiene

- Frequency of traveling outside
- Frequency of traveling outside during day time
- Location of traveling outside
- Frequency of playing on the floor in a home
- Location of playing on the floor in a home
- Frequency of playing dirt or soil
- Frequency of washing hand
- Frequency of washing foot
- Frequency of taking a shower
- Walking bare feet inside a home

- Walking bare feet outside a home Frequency of illness

Contact variable behavior:

- Furniture
- Bottle/dish
- Object to mouth
- Hand to mouth
- Fingers to mouth
- Hand floor
- Toy to mouth
- Hair
- Skin
- Clothes
- Grass/vegetation
- Crawling across the floor

Home Characteristics:

- Type of a home How many floors
- Main type floor
- Direction of front household
- Number of windows
- Reported insecticide sighting in the home
- Reported pesticide use

Dietary habit:

- Eating outside a home
- Eating on the floor inside a home
- Washing hands before eating

- ➢ Washing and soaking vegetable long time Washing and soaking fruits long time
- ➢ Fruits peeled

Part II: Exposure analysis

3.8.1 Urine samples

The first morning void urine samples analysis for PYRs metabolites using gas chromatography with mass spectrometer (GC-MS) (Rohitrattana et al. 2014b).

3.8.2 PYR metabolites analysis.

Five common PYRs metabolites including *3*-PBA was measured. The urinary metabolites were analyzed using the method developed by (Glorennec, Serrano, Fravallo, Warembourg, Monfort, Cordier, Viel, Le Gleau, et al. 2017), The method was briefly elucidated as follows: first urine samples was hydrolyzed by concentrate sulfuric acid; second, solid-phase extraction (SPE) polypropylene cartridges were preconditioned by de-ionized water. Then the analysts eluted methanol and derivatized in water bath. After cooling at the room temperature, the centrifugation was used to extract hexane. The final volumes were adjusted and analyzed by gas chromatography with mass spectrometer (GC-MS) for detecting the PYRs metabolites (Colume et al. 2001; Heudorf and Angerer 2001; Schettgen et al. 2002; Olsson et al. 2004; Leng and Gries 2005; Leon et al. 2005; Barr et al. 2007; Rohitrattana et al. 2014a).

- Internal standard solution (IS1)
- Preparation IS1, add 10 mg 2-PBA and 10 methanol, dissolve by H_2O.
- Preparation IS2, add 50 µL IS1 and dissolve by H_2O. The standard solution IS2 is used for the sample preparation.

- Sample preparation
- Hydrolysis extraction

Hydrolysis of conjugate carboxylic acid.

- Prepare urine 10 mL
- add 100 µL IS2
- add 1 mL hydrochloric acid (37%)
- heat at 90 °C for 1 hr.
- cool at room temperature

Acidic extraction

- add 5 mL n-hexane
- shaking for 10 min
- centrifuge at 1,500 rpm for 5 min
- select organic phase to add 2 mL NaOH (0.1 N)
- shaking for 10 min, centrifuge at 1,500 rpm for 5 min
- select aqueous phase to add 100 µL (hydrochloric acid 37 °C)
- Re-extraction to make high concentration to add 2 ml hexane
- shaking for 10 min, centrifuge at 1,500 rpm for 5 min
- select aqueous phase

Evaporation

- Evaporation to dryness using dissolve in 50 µL toluene and 10 µL N-tert.-butyldimethylsilyl-N-methyltrifluoroacetamid (MTBSTFA)

Analysis

Before analysis by GC-MS, heat at 70°C for 45 min

Analysis condition

3-PBA Metabolite detection and identification were achieved through a sensitive and selective capillary gas chromatographic procedure coupled to mass spectrometric detection (GC/MS), using conditions modified from (Colume et al. 2001; Schettgen et al. 2002). Briefly, the following GC/MS conditions used: an operating temperature of the injector set to 280°C; chromatographic separation through a HP-5 capillary column (30 m, 0.25 mm I.D., 0.25-μm film thickness) purchased from Hewlett- Packard (Waldbronn, Germany); helium 5.0 as the carrier gas at a constant flow of 2.2 ml/min; an initial column temperature of 90°C held for 1 min, then raised at a rate of 25°C/min to 120°C; then raised at a rate of 2.1°C/min to 240°C, and held at this temperature for 1.5 minutes; and finally raised at 25°C/min to 310°C, where it remained for 7 minutes. An injection volume of 1 ml was used for the retention times of the derivatized analytes. For each of the analytes, quantitative analysis of the PYR metabolites and selected ion monitoring (SIM) were applied, with two or three fragment ions scanned (3-PBA M/e: 121, 214, 215).

Calibration was carried out using a pooled urine sample to which known amounts of 3-PBA were added; this was then processed and analyzed in the same manner as the above samples. Calibrations curves were linear between 0.2 and 100 μg/mL of each of the 3-PBA metabolite. The determination of recovery for the target 3-PBA analytes was 92% at a concentration of 10 μg/mL in the urine samples, and its corresponding method detection limits (MDLs) were 0.01–0.1 μg/mL of urine.

3.8.3 Hand wipe sample

Hand wipe sample was collected and frozen at -18 °C unit to ensure preservation of the compounds (Glorennec, Serrano, Fravallo, Warembourg, Monfort, Cordier, Viel, Le Gleau, et al. 2017), including cypermethrin, and allethrin. They then were extracted using pressurize liquid extraction with dichloromethane, followed by gas chromatography coupled to mass spectrometry (Glorennec, Serrano, Fravallo, Warembourg, Monfort, Cordier, Viel, Le Gleau, et al. 2017).

Part III: GABA level analysis

GABA level sample was analyzed using immunoassay enzyme-linked immunosorbent (ELISA) for quantitative detection. Gamma-aminobutyric acid (GABA) in urine samples (Crookes et al. 2004; Kheirandish-Gozal et al. 2013; Lee et al. 2017). Calibration was done using a standard concentration from the GABA ELISA kit, for which known amounts of GABA concentration were added and then processed and analyzed in the same manner as the samples above. The resulting curves appeared linear between 0 and 7500 ng/mL of each of the GABA concentration, for which the MDL was < 49 ng/mL in urine. The determination of recovery for the GABA concentration analytes were 99% in a urine sample at the standard concentration provided in the GABA ELISA kit.

Urine sample preparation

Take urine or urine 24 hours. And store in a 10 -15 ml HCl 6 M bottle. Use urine for 24 hours, please record the total amount of urine collected. Storage: For prolonged periods (not over 6 months) at -20 ° C. Avoid freezing and thawing ice. Avoid direct sunlight.

Preparation of sample

For standard samples in the range of 25 to 2500 ng / mL, the standard and control should be diluted with 1: 3 (100 μl standard + 200 μl) (deionized, distilled or ultra-pure) water. Calculation of results the standard was diluted to ensure that the sample falls within the straight line of the standard curve.

Assay Procedure

- Procedure (25–2500 ng/mL):

Note: Extraction, Derivatization and will be following GABA ELISA kit protocol.

GABA ELISA:

1. Pipette 25 µL of the derivatized standards, controls and samples into the appropriate wells of the GABA. microtiter strip.
2. Pipette 50 µL of the GABA Antiserum into all wells and mix shortly.
3. Cover plate with Adhesive Foil and incubate for 15-20 hours (overnight) at 2- 8 °C. Alternatively incubate 2 hours at RT (20-25°C) on a shaker (appro x.600rpm).
4. Remove the foil. Discard or aspirate the contents of the wells. Wash the plate 3x by adding 300 µL of Wash Buffer, discarding the content and blotting dry each time by tapping the inverted plate on absorbent material.
5. Pipette 100 µL of the Enzyme Conjugate into all wells.
6. Cover plate with Adhesive Foil and incubate for 30 min at RT (20-25°C) on a shaker (approx. 600 rpm)
7. Remove the foil. Discard or aspirate the contents of the wells. Wash the plate 3x by adding 300 µL of Wash Buffer, discarding the content and blotting dry each time by tapping the inverted plate on absorbent material.

8. Pipette 100 µL of the Substrate into all wells and incubate for 20-30 min at RT (20-25°C) on a shake (approx. 600 rpm). Avoid exposure to direct sun light.
9. Add 100 µL of the Stop Solution to each well and shake the microtiter plate to ensure a homogeneous distribution of the solution.
10. Read the absorbance of the solution in the wells within 10 minutes, using a microplate reader set to 450 nm (if available a reference wavelength between 620 nm and 650 nm is recommended). GABA Data Analysis:

Urine samples and sample concentration control and control are read directly from the standard curve. The total number of GABA excreted in the urine over a 24-hour period is calculated as follows:

$$\mu g/24h = \mu g/L \times L/24h$$

- Conversion, GABA (ng/mL) x 9.7 = GABA (nmol/L).

- It is recommended that each laboratory set its own reference values for urine: 230-1290 µg / g creatinine.

- Quality control shows the confidence limit of the control unit QC-Report.

3.9 Data collection

The first steps:

Children were selected using the inclusion criteria such as children age between 2 – 3 years old who live in a home in BKK. They were born and have continued to live in the current areas. They also have lived in a home where pesticides have been applied such as using insecticide products in a house/ ≥ 4 time/month). information regarding the use of insecticides was collected using the questionnaire.

The second step:

This step, the researcher collected data by interviewing parents or caregivers regarding children information at home

The final step:

Researchers collected and analyzed urine samples from children. After analysis of PYRs metabolites, the results were categorized into two levels as high and low level, and were also used to analyze GABA levels. This step the researcher and parents collected the urine samples from children. At the same time, hand wipe samples were collected and observed PYRs contamination in children's hands.

Survey

Interview parent

Collection of urine and hand wipe sample

Photo by Kunno, J.

3.10 Data analysis

SPSS for Windows (version 22) was used for a statistical analysis. Descriptive statistics were presented as mean ± standard deviation. Categorical variables were showed frequency and percentages. All data were tested for normality before appropriate statistical analyses are performed. Mean, standard deviation (SD), and frequency were reported for variables associated with participant demographics, characteristics, environments, and activities.

Descriptive statistics:

1. Data was presented as mean ± standard deviation. Categorical variables were showed as frequency and percentages.
2. Kolmogorov-Smirnov test
    - The date was presented non-normal distributions.
3. Spearman correlations test:
    - To test the association between 3-PBA metabolite and GABA concentrations.
    - To test the association between 3-PBA metabolite and wipes hands/foots.
    - The data presented by continuous data.
4. Binary logistic regression test:
    - To test an association between 3-PBA metabolite and exposure factors such as children personal exposure and exposure characteristics. A bivariate analysis of each variable was first done, and then variables with value < 0.25 were included in the multivariate analysis.
    - The data was presented using the categorical data.

5. Mann–Whitney U test:

    - Mann–Whitney U test was used to evaluate differences in continuous data between wet and dry seasons because of a non-normal distribution.

6. Chi-square tests:

    - Chi-square test was used to present difference in categorical data between wet and dry seasons

3.11 Ethical consideration

This study was approved by the Ethics Review Committee for Research Involving Human Research Subjects, Health Science Group, Chulalongkorn University, Thailand (COA No. 290/2561).

CHAPTER IV
RESULTS

This repeated cross-sectional was designed to collect information on PYRs insecticide exposure between wet (November – December 2018) and dry (April – May 2019) seasons. The participants presented in different person between wet and dry seasons. The study areas were in Khlong Toei and Pathum wan district, Bangkok Thailand. Young children were included in this study, if they were 1) children age between 2 – 3 years old, 2) both male and female children, 3) children who were born and have lived in Bangkok. This study focused on children in low-income families because their parents used a PYRs insecticide products daily and also probably lacked awareness or knowledge on how to protect a child from the exposures. One parent of a child was asked to complete demographic and children's personal exposure questionnaires, while, their children were collected urine samples in their homes to analyze PYRs metabolite determination and GABA analyses. The purposive sampling technique was used to select the study areas. The number of participants in district were randomized using the proportional to size from the family caregiver who willing to participate. The youngest child from each household was purposively selected. The total of 80 young children in each season, but different person between wet and dry seasons were included. Data was collected after children and family caregiver obtained the information about this study. The purpose of this study were to 1) to determine factors association with PYRs metabolites concentration in children household, 2) to determine correlation between PYRs metabolites concentration and GABA concentration in urine sample of children, 3) to find correlation between wipe sample concentration and PYRs metabolites concentration in children household, and 4) to find differences of wipe sample

concentration, PYRs metabolites concentration and GABA concentration between wet and dry seasons.

Topic of results are following:

4.1 Characteristics and factors exposure related to 3-PBA metabolites in dry and wet seasons

4.1.1 Socio-demographic characteristics and parent information

4.1.2 Factors related to PYR exposure

4.1.3 Personal hygiene characteristics

4.1.4 Contact variable behavior

4.1.5 Home Characteristics

4.1.6 Dietary habit

4.2 Concentration of 3-PBA metabolite, GABA and wipe samples in dry and wet seasons

4.2.1 The 3-PBA metabolite concentration detected in urine sample between wet and dry seasons

4.2.2 The GABA concentration detected in urine sample between wet and dry seasons

4.2.3 The hand wipes concentration detected in urine sample between wet and dry seasons

4.3 Correlation among 3-PBA metabolite, GABA and wipe sample concentration in dry and wet seasons

4.3.1 Correlation between 3-PBA metabolite and GABA concentration in urine children

4.3.2 Correlation between 3-PBA metabolite and hand wipes sample children

4.4 Factors exposure associated with 3-PBA metabolite between dry and wet seasons

4.4.1 Factors associated with 3-PBA metabolite in wet season (November – December 2018)

4.4.1.1 Factors associated with 3-PBA metabolite in wet season by bivariate analysis

4.4.1.2 Factors associated with 3-PBA metabolite in wet season by multivariate analysis

4.4.2 Factors associated with 3-PBA metabolite in dry season (April – May 2019)

4.4.2.1 Factors associated with 3-PBA metabolite in dry season by bivariate analysis

4.4.2.2 Factors associated with 3-PBA metabolite in dry season by multivariate analysis

4.1 Characteristics and factors exposure related to 3-PBA metabolites in dry and wet seasons

Parent in each season provided key information about child socio-demographic characteristics, parent information, factors related to PYRs exposure, personal hygiene, contact variable behavior, home characteristics and dietary habit (**Table 4)**.

4.1.1 Socio-demographic characteristics and parent information

Descriptive analysis was presented regarding children and parent characteristics. In wet season, participants were represented children age between 2-3 years. Average age was 27 months with 36 females (45%) and 44 males (55%). Median of weight and high were showed 13.3 (kg) and 87.5 (cm), respectively. Current address was showed in community 1 (Khlong Toei district) representing 32 persons (40%) and community 2 (Pathum wan district) revealing 48 persons (60%). Most parents graduated at primary school at 68 persons (85%) and illiterate 12 persons (15%). Most parents main source income received from work 51 persons (63.7%) and non-work 29 persons (36.3%). Caregiver of children was reported 33 mothers (41.2%) and others (including father, grandmother) 47 persons (58.8%). In dry season, participants were showed children age between 2-3 years, average age was 30 months with 28, females (35%) and 52 males (65%). Median of weight and high were 14 (kg) and 91 (cm), respectively. Current address was showed in community 1 (Khlong Toei district) with 32 persons (40%) and community 2 (Pathum wan district) with 48 persons (60%). Most parents graduated at primary school 72 persons (90%) and illiterate 8 persons (10%). Most parent main source income received from work 42 persons (52.5%) and non-work 38 persons (47.5%). Caregiver of children was showed as 30 mothers 37.5%) and others (including father, grandmother) 50 persons (62.5%).

There were significant differences of age, weight and duration to live in both region between dry and wet seasons (p-value <0.05).

4.1.2 Factors related to PYRs exposure

Parents in each season provided the key information about factors related to PYRs exposure. In wet season, most type of PYRs insecticide products used in household was used 48 (60%) insecticide spraying and 32 (40%) coil insecticide. Name of PYR insecticide product used in household showed spraying brand 48 (60%) and coil brand 32 (40%). Fifty-two households (65%) stored PYRs insecticide products outside, while, 28 families (35%). stored them inside homes. 50 (62.5%) of households used PYRs products in their houses sometimes, whereas, 30 families (37.5%) used them every day. Most families 70 or (87.5%) cleaned the floors once a week, while, 10 households (12.5%) never cleaned their floors. Type of floor cleaned was showed non-wet broom 10 (12.5%) and wet broom 70 (87.5%). Frequency of opened window and door were showed never opened 49 (61.3%) and opened all day 31 (38.7%). Both communities presented insecticide control by an officer every 2 months. In dry season, type PYRs insecticide product used in household was showed 13 (16.3%) other insecticide (including spray insecticide) and 67 (83.7%) for coil insecticide. Name of PYRs insecticide product used in household was showed 13 (16.3%) other brand (including spray brand) and 67 (83.7%) for coil brand. Store for keep PYR insecticide product used in household was showed outside household 13 (16.3%) and inside household 67 (83.7%). Frequency of PYR product use in household (time/month) was showed used some time 17 (21.3%) and used every day 63 (78.7%). Frequency of floor cleaned was showed never cleaned 28 (35%) and cleaned once a week 52 (65%). Type of floor cleaned was showed non-wet broom 28 (35%) and wet broom 52 (65%). Frequency of opened window and door were showed never opened 13 (16.3%) and opened all day 67 (83.7%). Both communities were presented insecticide control operators by officer every 2 months.

There were significant differences of type PYRs insecticide used, name of PYRs insecticide product used, store for keep PYRs insecticide product used, frequency of

PYR product use in household (time/month), frequency of floor cleaned, type of floor cleaned frequency of opened window and door between dry and wet seasons (p-value <0.05).

4.1.3 Personal hygiene characteristics

Parents in each season provided the key information regarding personal hygiene characteristics. In wet season, most children did not spend time outside everyday accounted for 49 children (61.3%), while, 31 children (38.2%). Spent time outside household every day. half of children did not play on the floor in their houses during day time, whereas, one-third of them played on the floors around 2 – 3 hours. The majority of children (72.5%) played on the floors, while, 27.5% of them did not play on the floors. Children walked bare feet inside/outside household in the day approximately 40.0% and non- bare feet walk accounted for 60.0%. 42 (52.5% of Children (42) washed hands/foots daily, whereas, 47.5% did not wash their hands/foots 39 Children (48.8%) took a shower more than 2 times a day , whereas, 41 children (51.12%) took a shower 1-2 times per day. Most frequency of non-illness within 6 months were 65 persons (81.3%), while, illness within 6 months were 5 persons (18.2%). In dry season, most children spend time outside household not every day was showed 52 persons (65.0%) and spend time outside household every day was showed 28 persons (35.0%). Children non-play on the floor at household in the day was showed 17 persons (21.3%) and play on the floor at 2 -3 hrs. was showed 63 persons (78.7%). Children walk bare feet inside household in the day was showed 62 persons (77.5%) and non- walk bare feet was showed 18 persons (22.5%). Children walk bare feet outside household in the day was showed 48 persons (60.0%) and non- walk bare feet was showed 32 persons (40.0%). Children wash hands/foots in the day was showed 47 persons (58.7%) and non- hands/foots in the day was showed 33 persons (41.3%). Children take a shower in the day more than 2 times was showed 38 persons (47.5%) and take a shower 1-2 times in the day was

showed 42 persons (52.5%). Most of frequency non-illness within 6 months were showed 38 persons (47.5%) and illness within 6 months were showed 42 persons (52.5%).

There were significant differences of children play on the floor at household in the day, children walk bare feet inside/outside household in the day, frequency of illness within 6 months between dry and wet seasons (p-value <0.05).

4.1.4 Contact variable behavior

Parents in each season provided the key information about contact variable behavior. In wet season, 61 children (76.2%) touched the furniture or bottle/dish-to-mouth, while, 19 children (23.8%) did not touch them. 32 children (40.0%) touched the objects or hand -to-mouth, while, 48 children (60.0%) did not touch them Children put their fingers, playing with toys, or touch hair-to-mouth accounted for 68 persons (85.0%), whereas, 12 children (15.0%) did not. Children touched skin, clothes, grass/vegetation-to-mouth or crawling across on the floor at 78.7%), whereas, 17 children (21.3%) did not. In dry season, children touch furniture, bottle/dish, object or put toy-to-mouth were showed 66 persons (82.5%) and non-touch furniture, bottle/dish, object or put toy-to-mouth were showed 14 persons (17.5%). Children put hand or grass/vegetation-to-mouth were showed 68 persons (85.0%) and non- put hand or grass/vegetation-to-mouth 12 persons (15.0%). Children put fingers-to-mouth was showed 62 persons (77.5%) and non- put fingers-to-mouth 15 persons (22.5%). Children touch hair, skin, clothes-to-mouth or crawling across on the floor were showed 62 persons (77.5%) and non- touch hair, skin, clothes-to-mouth or crawling across on the floor were showed 18 persons (22.5%).

There were significant differences of children touch object and hand-to-mouth between dry and wet seasons (p-value <0.05).

4.1.5 Home Characteristics

Parents in each season provided the key information regarding contact variable behavior. In wet season, permanency house accounted for 52.5%, while, 47.5% of houses were non-permanency.) Main type floor was showed cement floor 42 (52.5%) and non- cement floor 38 (47.5%). The air flow in front of the house showed at 52.5%, while, 47.5% showed no air flow. Reported insect sighting in the home and pesticide use were presented 32 (40.0%) and non-reported insecticide sighting in the home and reported pesticide use were presented 48 (60.0%). In dry season, most of type of household in both communities were showed permanency 52 (65%) and non-permanency 28 (35%). Main type floor was showed cement floor 52 (65.0%) and non- cement floor 28 (35.0%). Direction of front household was showed as air flow 52 (65.0%) and non-air flow 28 (35.0%). Reported insecticide sighting in the home and reported pesticide use were presented 67 (83.7%) and non-reported insecticide sighting in the home and reported pesticide use were presented 13 (16.3%).

There were significant differences of reported insecticide sighting in the home and reported pesticide used between dry and wet seasons (p-value <0.05).

4.1.6 Dietary habit

Parents in each season provided the key information regarding contact variable behavior. In wet season, children ate outside their homes at 46.3%, while, 53.8% of them did not. Children ate on the floor inside the household accounted for 40.0%, whereas, 60.0% of children did not, 42 of Children (52.5%) washed hands before eating, while, 38 of them (47.5%) did not. Vegetable washing soaking time was showed 34 (42.5%) and non- washing soaking time was showed 46 (57.5%). Fruits washing soaking time was showed 34 (42.5%) and non- washing soaking time was showed 46 (57.5%). Fruits peeled was showed 39 (48.8%) and non- peeled was showed 41 (51.2%). In dry season, children eat outside the household, eat on the

floor inside the household and fruits peeled were showed 66 (82.5%) and non- eat outside the household, eat on the floor inside the household and fruits peeled were showed 14 (17.5%). Children wash hands before eating, vegetable washing and fruits washing were showed 52 (65.0%) and non- wash hands before eating, vegetable washing and fruits washing were showed 28 (35.0%).

There were significant differences of eat outside the household, eat on the floor inside the household, vegetable washing, fruits washing, and fruits peeled between dry and wet seasons (p-value <0.05).

Table 4 Characteristics and factors exposure related to 3-PBA metabolites

Characteristics / Association factors exposure	Wet season November – December 2018 n (%) or (Mean ± SD) / (Median)	Dry season April – May 2019 n (%) or (Mean ± SD) / (Median)	Compare two seasons (*P*-value)
Socio-demographic Characteristics:			
Aged 2-3 year (months)			0.011[a]
Mean ± SD / median	26.92 ± 9.41 / 29.50	30.02 ± 11.15 / 36.00	
Weight (kg)			< 0.01[a]
Mean ± SD / median	13.4.67 ± 0.52 / 13.10	14.20 ± 4.75 / 13.65	
High (cm)			0.095[a]
Mean ± SD / median	87.55 ± 13.27 / 90.00	91.22 ± 13.49 / 91.25	
Duration to live in this region			0.011[a]
Mean ± SD / median	26.92 ± 9.41/ 29.50	30.02 ± 11.15 / 36.00	
	n (%)	**n (%)**	
Gender			0.259[b]
Female	44 (55.0%)	52 (65.0%)	
Male	36 (45.0%)	28 (35.0%)	
Current address / Location of residence			>0.05[b]
Community 1	32 (40.0%)	32 (40.0%)	
Community 2	48 (60.0%)	48 (60.0%)	
Parent information:			
Parents education level			0.474[b]
Illiterate	12 (15.0%)	8 (10.0%)	
≥ Primary school	68 (85.0%)	72 (90.0%)	
Parent main source income			0.200[b]
No work	29 (36.3%)	38 (47.5%)	
Work	51 (63.7%)	42 (52.5%)	
Care giver of children			0.746[b]
Others	47 (58.8%)	50 (62.5%)	
Mother	33 (41.2%)	30 (37.5%)	
Children breast milk			0.735[b]
No	27 (33.8%)	24 (30.0%)	
Yes	53 (66.2%)	56 (70.0%)	

[a]Mann–Whitney U test, [b]Chi-square tests.

Table 4 Characteristics and factors exposure related to 3-PBA metabolites **(Conti.)**

Characteristics / Association factors exposure	Wet season November – December 2018 n (%)	Dry season April – May 2019 n (%)	Compare two seasons (*P*-value)
Factors related to PYR exposure:			
Type insecticide products use household			< 0.01[b]
Others insecticide	48 (60.0%)	13 (16.3%)	
Coil insecticide	32 (40.0%)	67 (83.7%)	
Name of insecticide products use household			< 0.01[b]
Others brand	48 (60%)	13 (16.3%)	
Coil brand	32 (40%)	67 (83.7%)	
Store for keep insecticide products			< 0.01[b]
Outside household	52 (65.0%)	13 (16.3%)	
Inside household	28 (35.0%)	67 (83.7%)	
Frequency of PYR insecticide use in household (times/month)			< 0.01[b]
Some time	50 (62.5%)	17 (21.3%)	
Every day	30 (37.5%)	63 (78.7%)	
Frequency of floor-cleaning			< 0.01[b]
Never	10 (12.5%)	28 (35%)	
Once a week	70 (87.5%)	52 (65%)	
Type of floor cleaning			< 0.01[b]
Not wet broom	10 (12.5%)	28 (35%)	
Wet broom	70 (87.5%)	52 (65%)	
Frequency of open window or door			< 0.01[b]
Never	49 (61.3%)	13 (16.3%)	
All day	31 (38.7%)	67 (83.7%)	

[a]Mann–Whitney U test, [b]Chi-square tests.

Table 4. Characteristics and factors exposure related to 3-PBA metabolites **(Conti.)**

Characteristics / Association factors exposure	Wet season November – December 2018 n (%)	Dry season April – May 2019 n (%)	Compare two seasons (*P*-value)
Personal hygiene			
Children spend time outside household			0.743[b]
Not every day	49 (61.3%)	52 (65.0%)	
Every day	31 (38.2%)	28 (35.0%)	
Children spend time outside household in the time/day			0.462[b]
Never	22 (27.5%)	17 (21.3%)	
2 -3 hr.	58 (72.5%)	63 (78.7%)	
Location for spend time outside household			0.462[b]
Non on the floor	22 (27.5%)	17 (21.3%)	
On the floor	58 (72.5%)	63 (78.7%)	
Children play on the floor at household in the day			<0.01[b]
Never	50 (62.5%)	17 (21.3%)	
2 -3 hrs.	30 (37.5%)	63 (78.7%)	
Location of play at household floor			0.462[b]
Non on the floor	22 (27.5%)	17 (21.3%)	
On the floor	58 (72.5%)	63 (78.7%)	
Children play soil at household in the day			0.515[b]
No	47 (58.7%)	52 (65.0%)	
Yes	33 (41.3%)	28 (35.0%)	
Children walk bare feet inside household in the day			<0.01[b]
No	48 (60.0%)	18 (22.5%)	
Yes	32 (40.0%)	62 (77.5%)	
Children walk bare feet outside household in the day			0.011[b]
No	48 (60.0%)	32 (40.0%)	
Yes	32 (40.0%)	48 (60.0%)	
Children wash hands/foots in the day			0.525[b]
No	38 (47.5%)	33 (41.3%)	
Yes	42 (52.5%)	47 (58.7%)	
Children take a shower in the day			0.752[b]
1 – 2 times	41 (51.2%)	38 (47.5%)	
> 2 times	39 (48.8%)	42 (52.5%)	
Frequency of illness within 6 months			<0.01[b]
No	65 (81.3%)	38 (47.5%)	
Yes	15 (18.2%)	42 (52.5%)	

[a]Mann–Whitney U test, [b]Chi-square tests.

Table 4. Characteristics and factors exposure related to 3-PBA metabolites **(Conti.)**

Characteristics / Association factors exposure	Wet season November – December 2018 n (%)	Dry season April – May 2019 n (%)	Compare two seasons (*P*-value)
Contact variable behavior:			
Children touch furniture-to-mouth			0.435[b]
No	19 (23.8%)	14 (17.5%)	
Yes	61 (76.2%)	66 (82.5%)	
Children touch bottle/dish-to-mouth			0.435[b]
No	19 (23.8%)	14 (17.5%)	
Yes	61 (76.2%)	66 (82.5%)	
Children touch object-to-mouth			<0.01[b]
No	48 (60.0%)	14 (17.5%)	
Yes	32 (40.0%)	66 (82.5%)	
Children put hand-to-mouth			<0.01[b]
No	48 (60.0%)	12 (15.0%)	
Yes	32 (40.0%)	68 (85.0%)	
Children put fingers-to-mouth			0.311[b]
No	12 (15.0%)	15 (22.5%)	
Yes	68 (85.0%)	62 (77.5%)	
Children put hand on the floor			0.640[b]
No	9 (11.3%)	12 (15.0%)	
Yes	71 (88.7%)	68 (85.0%)	
Children put toy-to-mouth (football etc.)			0.831[b]
No	12 (15%)	14 (17.5%)	
Yes	68 (85%)	66 (82.5%)	
Children touch hair-to-mouth			>0.05[b]
No	12 (15.0%)	18 (22.5%)	
Yes	68 (85.0%)	62 (77.5%)	
Children touch skin-to-mouth			0.412[b]
No	17 (21.3%)	18 (22.5%)	
Yes	63 (78.7%)	62 (77.5%)	
Children touch clothes-to-mouth			>0.05[b]
No	17 (21.3%)	18 (22.5%)	
Yes	63 (78.7%)	62 (77.5%)	
Children touch grass/vegetation-to-mouth			0.412[b]
No	17 (21.3%)	12 (15.0%)	
Yes	63 (78.7%)	68 (85.0%)	
Children crawling across on the floor			>0.05[b]
No	17 (21.3%)	18 (22.5%)	
Yes	63 (78.7%)	62 (77.5%)	

[a]Mann–Whitney U test, [b]Chi-square tests.

Table 4. Characteristics and factors exposure related to 3-PBA metabolites **(Conti.)**

Characteristics / Association factors exposure	Wet season November – December 2018 n (%)	Dry season April – May 2019 n (%)	Compare two seasons (*P*-value)
Home Characteristics:			
Type of household			0.148[b]
Non permanency	38 (47.5%)	28 (35%)	
Permanency	42 (52.5%)	52 (65%)	
Main type floor			0.148[b]
Non cement floor	38 (47.5%)	28 (35.0%)	
Cement floor	42 (52.5%)	52 (65.0%)	
Direction of front household			0.148[b]
Non air flow	38 (47.5%)	28 (35.0%)	
Air flow	42 (52.5%)	52 (65.0%)	
Reported insecticide sighting in the home			<0.01[b]
No	48 (60.0%)	13 (16.3%)	
Yes	32 (40.0%)	67 (83.7%)	
Reported pesticide use			<0.01[b]
No	48 (60.0%)	13 (16.3%)	
Yes	32 (40.0%)	67 (83.7%)	

[a]Mann–Whitney U test, [b]Chi-square tests.

Table 4. Characteristics and factors exposure related to 3-PBA metabolites **(Conti.)**

Characteristics / Association factors exposure	Wet season November – December 2018 n (%)	Dry season April – May 2019 n (%)	Compare two seasons (*P-value*)
Dietary habit:			
Eat outside the household			<0.01[b]
No	43 (53.8%)	14 (17.5%)	
Yes	37 (46.3%)	66 (82.5%)	
Eat on the floor inside the household			<0.01[b]
No	48 (60.0%)	14 (17.5%)	
Yes	32 (40.0%)	66 (82.5%)	
Wash hands before eating			0.148[b]
No	38 (47.5%)	28 (35.0%)	
Yes	42 (52.5%)	52 (65.0%)	
Vegetable washing soaking time			0.007[b]
No	46 (57.5%)	28 (35.0%)	
Yes	34 (42.5%)	52 (65.0%)	
Fruits peeled			<0.01[b]
No	41 (51.2%)	14 (17.5%)	
Yes	39 (48.8%)	66 (82.5%)	

[a]Mann–Whitney U test, [b]Chi-square tests.

4.2 Concentration of 3-PBA metabolite, GABA and wipe samples in dry and wet seasons

PYRs metabolites were presented by 3-PBA metabolite, which measured in urine of children. GABA concentration was also measured in urine. Hand wipes sample were measured of cypermethrin and allethrin. The exposure of PYRs of young children in wet and dry seasons are show in **(Table 5)**.

Table 5. Detections and concentrations of 3-PBA metabolite, GABA and wipe samples in children 2-3 years at households (n = 80).

Biomarkers	Wet season (November – December 2018)				Dry season (April – May 2019)				Compare two seasons
	Concentration				Concentration				
	Median	Mean ± SD	Range	% Detection	Median	Mean ± SD	Range	% Detection	p- value
3-PBA (µg/mL)[a]	0.53	0.73 ± 0.57	0.23 – 2.02	92	1.46	1.47 ± 0.77	0.23 – 2.53	92	< 0.001
GABA (ng/mL)[b]	355.11	554.08 ± 1124.22	<0.01 - 7490.75	99	450.00	534.07 ± 331.68	94.24 - 1300	99	0.003
Wipe hands/foots[c] (µg/mL)									
Cypermethrin	0.012	0.012 ± 0.002	0.014 – 0.009	92	0.013	0.016 ± 0.004	0.02 -0.005	92	<0.001
Allethrin	0.002	0.002 ± 0.0003	0.003 – 0.009	92	0.002	0.002 ± 0.0004	0.003 – 0.009	92	< 0.001

Mann–Whitney U test, p-value < 0.001. [a]Method detection limits (MDLs) = 0.01 µg/mL. [b]Method detection limits (MDLs) = less than 49 ng/mL, [c]Method detection limits (MDLs) = 0.001 µg/mL.

4.2.1 The 3-PBA metabolite concentration detected in urine sample between wet and dry seasons

In wet season, 3-PBA metabolite concentration was detected (92%) and measured range 0.23 – 2.02 µg/mL which the median showed 0.53 µg/mL and mean ± SD showed 0.73 ± 0.57 µg/mL. In dry season, 3-PBA metabolite concentration was detected (92%) and measured range 0.23 – 2.53 µg/mL which the median showed 1.46 µg/mL and the mean ± SD showed 1.47 ± 0.77 µg/mL. There were significant differences of 3-PBA metabolite concentration between dry and wet seasons (p-value <0.01).

4.2.2 The GABA concentration detected in urine sample between wet and dry seasons

In wet season, GABA concentration was detected (99%) and measured range <0.01 - 7490.75 ng/mL which the median showed 355.11 ng/mL and the mean ± SD showed 554.08 ± 1,124.22 ng/mL. In dry season, GABA concentration was detected (99%) and measured range 94.24 – 1300 ng/mL which median showed 450.00 ng/mL and mean ± SD showed 534.07 ± 331.68 ng/mL. There were significant differences of GABA concentration between dry and wet seasons (p-value 0.003). Thus, in dry season was presented GABA concentration higher than wet season.

4.2.3 The hand wipes concentration detected in urine sample between wet and dry seasons

Hand wipes sample concentration were presented by cypermethrin and allethrin in wet and dry seasons. In both seasons, cypermethrin and allethrin were detected (92%). In wet season, cypermethrin insecticide were highest concentration than allethrin follow by 0.012 ± 0.002 µg/mL and 0.002 ± 0.0003 µg/mL, respectively. In dry season, cypermethrin insecticide were highest concentration than allethrin also

follow by 0.016 ± 0.004 µg/mL and 0.002 ± 0.0004 µg/mL, respectively. There were significant differences of hand wipes sample concentration between dry and wet seasons (p-value <0.001). Thus, in dry season was presented hand wipes concentration higher than wet season.

4.3 Correlation among 3-PBA metabolite, GABA and wipe sample concentration in dry and wet seasons

The Kolmogorov-Smirnov test was indicated the data non-normal distribution. Each season was collected of 80 children. The date in wet and dry seasons were presented p<0.005 indicated to non-normal distribution **(Table 6).**

Table 6. The Kolmogorov-Smirnov test

Test of Normality		
	Kolmogorov-Smirnov	
	Wet season	Dry season
Exposure factors	Sig	Sig
Socio-demographic Characteristics:		
Aged 2-3 year (months)	<0.001	<0.001
Weight (kg)	<0.001	0.034
High (cm)	0.013	0.043
Gender	<0.001	<0.001
Current address / Location of residence	<0.001	<0.001
Duration to live in this region	<0.001	<0.001
Children breast milk	<0.001	<0.001
Parent information:		
Parents education level	<0.001	<0.001
Parent main source income		
Care giver of children		
Factors related to PYR exposure:		
Type insecticide products use household	<0.001	<0.001
Name of insecticide products use household		
Store for keep insecticide products		
Frequency of PYR insecticide use in household (times/month)		
Frequency of floor-cleaning		
Type of floor cleaning		
Frequency of open window or door		

Personal hygiene		
Children spend time outside household	<0.001	<0.001
Children spend time outside household in the time/day		
Location for spend time outside household		
Children play on the floor at household in the day		
Location of play at household floor		
Children play soil at household in the day		
Children walk bare feet inside household in the day		
Children walk bare feet outside household in the day		
Children wash hands/foots in the day		
Children take a shower in the day		
Frequency of illness within 6 months		
Contact variable behavior:		
Children touch furniture-to-mouth	<0.001	<0.001
Children touch bottle/dish-to-mouth		
Children touch object-to-mouth		
Children put hand-to-mouth		
Children put fingers-to-mouth		
Children put hand on the floor		
Children put toy-to-mouth (football etc.)		
Children touch hair-to-mouth		
Children touch skin-to-mouth		
Children touch clothes-to-mouth		
Children touch grass/vegetation-to-mouth		
Children crawling across on the floor		
Home Characteristics:		
Type of household	<0.001	<0.001
Main type floor		
Reported insecticide sighting in the home		
Reported pesticide use		
Dietary habit:		
Eat outside the household	<0.001	<0.001
Eat on the floor inside the household		
Wash hands before eating		
Vegetable washing soaking time		
Fruits washing soaking time		
Fruits peeled		
Biomarkers:		
3-PBA metabolites (mg/mL)	<0.001	<0.001
GABA concentration (ng/mL)		
Hand wipe sample (µg/mL)		
Cypermethrin	<0.001	<0.001
Allethrin		

4.3.1 Correlation between 3-PBA metabolite and GABA concentration in urine children

In both season between wet and dry, 3-PBA metabolite concentration on urine children was presented moderately negative significant relation with GABA concentration in children (r_s = -0.230, p-value 0.004) **(Table 7).**

4.3.2 Correlation between 3-PBA metabolite and hand wipes sample children

In wet season, 3-PBA metabolite concentration on urine children was presented low positive relationship between with hand wipes sample including cypermethrin and allethrin (r_s = 0.160, p-value >0.05). In dry season, 3-PBA metabolite concentration on urine children was presented significant relationship between with hand wipes sample including cypermethrin (r_s = 0.226, p-value < 0.05) and allethrin (r_s = 0.276, p-value < 0.05). **(Table 7).**

Table 7. Spearman analyses for predictors association between 3-PBA metabolite and GABA concentration

Predictor	Spearman's coefficient (r_s)			
	3-PBA	GABA	Wipe hands/foots	
			Cypermethrin	Allethrin
Wet season (November – December 2018)				
3-PBA (μg/mL)	1.000			
GABA (ng/mL)	**-0.230***	1.000		
Wipe hands/foots (μg/mL):				
Cypermethrin (μg/mL)	0.160	0.013	1.000	
Allethrin (μg/mL)	0.160	0.013	1.000	1.000
Dry season (April – May 2019)				
3-PBA (μg/mL)	1.000			
GABA (ng/mL)	**-0.230***	1.000		
Wipe hands/foots (μg/mL):				
Cypermethrin (μg/mL)	**0.226***	0.106	1.000	
Allethrin (μg/mL)	**0.276***	-0.067	0.306	1.000

* Correlation is significant at p-value < 0.05 level

4.4 Factors exposure associated with 3-PBA metabolite between dry and wet seasons

4.4.1 Factors associated with 3-PBA metabolite in wet season (November – December 2018)

The factors related to the exposures (socio-demographic characteristics, parent information, factor related to PYR exposure, personal hygiene, contact variable behavior, home characteristics and dietary habit) from questionnaire were used to analyze the factors association with 3-PBA metabolites concentrations in urine of children. A bivariate analysis of each variable was first done, and then variable with p-value < 0.25 were included in the multivariate analysis. The associations between factors exposure and 3-PBA metabolites concentrations were presented by bivariate analysis in **(Table 8)**, and multivariate analysis **(Table 9)**. The results are following:

4.4.1.1 Factors associated with 3-PBA metabolite in wet season by bivariate analysis

Socio-demographic characteristics including aged 2-3 years (months), genders, current address and duration live in this region were possible associated to 3-PBA metabolites on urine children (p-value = 0.131, 0.253, 0.003 and 0.131, respectively).

Parent information including parent main source income and care giver of children were possible associated to 3-PBA metabolites on urine children (p-value = 0.185 and 0.188, respectively).

Factors related to PYR exposure including type insecticide products used, name of insecticide products used, store for keep insecticide products, frequency of PYR insecticide use in household (times/month), frequency of floor-cleaning, type of

floor cleaning and frequency of open window or door were possible associated to 3-PBA metabolites on urine children (p-value < 0.2).

Personal hygiene including children play on the floor at household in the day, children walk bare feet inside household in the day, children walk bare feet outside household in the day, children wash hands/foots in the day, children take a shower in the day and frequency of illness within 6 months were possible associated to 3-PBA metabolites on urine children (p-value < 0.2).

Contact variable behavior including children touch object-to-mouth, children put hand-to-mouth, children put fingers-to-mouth, children put hand on the floor, children put toy-to-mouth (football etc.) and children touch hair-to-mouth were possible associated to 3-PBA metabolites on urine children (p-value < 0.2).

Home characteristics including type of household, main type floor, direction of front household, reported insecticide sighting in the home and reported pesticide use were possible associated to 3-PBA metabolites on urine children (p-value < 0.2).

Dietary habit including eat outside the household, eat on the floor inside the household, wash hands before eating, vegetable washing soaking time, fruits washing soaking time and fruits peeled were possible associated to 3-PBA metabolites on urine children (p-value < 0.2).

Table 8. Bivariate analysis of each factor's exposure with 3-PBA metabolites concentration as wet season (November – December 2018).

Factor exposure	B	S.E.	Wald	p-value	OR (95% CI)
Socio-demographic Characteristics:					
Aged 2-3 year (months)	0.038	0.025	2.278	0.131	1.038 (0.989 – 1.090)
Weight (kg)	0.045	0.050	0.786	0.375	1.046 (0.947 – 1.154)
High (cm)	0.018	0.018	1.102	0.294	1.019 (0.984 – 1.054)
Gender	-0.519	0.454	1.307	0.253	0.595 (0.245 – 1.449)
Current address / Location of residence	1.449	0.493	8.624	0.003	4.259 (1.619 – 11.203)
Duration to live in this region	0.038	0.025	2.278	0.131	1.038 (0.989 – 1.090)
Children breast milk	0.036	0.473	0.006	0.939	1.037 (0.410 – 2.621)
Parent information:					
Parents education level	1.216	0.710	2.939	0.086	3.375 (0.840 – 13.560)
Parent main source income	-0.625	0.471	1.756	0.185	0.535 (0.213 – 1.349)
Care giver of children	0.605	0.459	1.737	0.188	1.832 (0.745 – 4.509)
Factors related to PYR exposure:					
Type insecticide products use household	1.025	0.466	4.844	0.028	2.787 (1.119 – 6.944)
Name of insecticide products use household	1.157	0.477	5.891	0.015	3.182 (1.250 – 8.102)
Store for keep insecticide products	0.745	0.478	2.432	0.119	2.107 (0.826 – 5.379)
Frequency of PYR insecticide use in household (times/month)	0.952	0.476	3.995	0.046	2.591 (1.019 – 6.590)
Frequency of floor-cleaning	-2.485	1.081	5.280	0.022	0.083 (0.010 – 0.694)
Type of floor cleaning	-2.485	1.081	5.280	0.022	0.083 (0.010 – 0.694)
Frequency of open window or door	0.613	0.465	1.742	0.187	1.846 (0.743 – 4.589)
Personal hygiene					
Children spend time outside household	0.399	0.461	0.748	0.387	1.490 (0.603 – 3.680)
Children spend time outside household in the time/day	0.182	0.502	0.132	0.717	1.200 (0.448 – 3.212)
Location for spend time outside household	0.182	0.502	0.132	0.717	1.200 (0.448 – 3.212)
Children play on the floor at household in the day	0.952	0.476	3.995	0.046	2.591 (1.019 – 6.590)
Location of play at household floor	0.182	0.502	0.132	0.717	1.200 (0.448 – 3.212)
Children play soil at household in the day	0.396	0.456	0.752	0.386	1.486 (0.607 – 3.634)
Children walk bare feet inside household in the day	1.157	0.477	5.891	0.015	3.182 (1.250 – 8.102)
Children walk bare feet outside household in the day	1.157	0.477	5.891	0.015	3.182 (1.250 – 8.102)
Children wash hands/foots in the day	-1.127	0.466	5.856	0.016	0.324 (0.130 – 0.807)
Children take a shower in the day	-0.815	0.457	3.180	0.075	0.443 (0.181 – 1.084)
Frequency of illness within 6 months	-1.127	0.466	5.856	0.160	0.324 (0.130 – 0.807)

3-PBA metabolite concentration in urine (1 = ≤ 0.53 µg/mL, 2 = ≥ 0.53 µg/mL). B, regression coefficient; S.E., standard error; OR, odds ratio; CI, confidence interval. Reference category: first. A bivariate analysis of each variable was first done, and then variable with p-value < 0.25 were included in the multivariate analysis.

Table 8. Bivariate analysis of each factor's exposure with 3-PBA metabolites concentration as wet season (November – December 2018). **(Conti.)**

Factor exposure	B	S.E.	Wald	p-value	OR (95% CI)
Contact variable behavior:					
Children touch furniture-to-mouth	0.073	0.526	0.019	0.890	1.075 (0.383 – 3.015)
Children touch bottle/dish-to-mouth	0.073	0.526	0.019	0.890	1.075 (0.383 – 3.015)
Children touch object-to-mouth	1.157	0.477	5.891	0.015	3.182 (1.250 – 8.102)
Children put hand-to-mouth	1.157	0.477	5.891	0.015	3.182 (1.250 – 8.102)
Children put fingers-to-mouth	1.216	0.710	2.939	0.086	3.375 (0.840 – 13.560)
Children put hand on the floor	1.337	0.836	2.557	0.110	3.809 (0.740 – 19.165)
Children put toy-to-mouth (football etc.)	1.216	0.710	2.939	0.086	3.809 (0.740 – 19.165)
Children touch hair-to-mouth	1.216	0.710	2.939	0.086	3.809 (0.740 – 19.165)
Children touch skin-to-mouth	0.086	0.547	0.025	0.875	1.090 (0.373 – 3.186)
Children touch clothes-to-mouth	0.086	0.547	0.025	0.875	1.090 (0.373 – 3.186)
Children touch grass/vegetation-to-mouth	0.086	0.547	0.025	0.875	1.090 (0.373 – 3.186)
Children crawling across on the floor	0.086	0.547	0.025	0.875	1.090 (0.373 – 3.186)
Home Characteristics:					
Type of household	-1.127	0.466	5.857	0.016	0.324 (0.130 – 0.807)
Main type floor	-1.127	0.466	5.857	0.016	0.324 (0.130 – 0.807)
Direction of front household	-1.127	0.466	5.857	0.016	0.324 (0.130 – 0.807)
Reported insecticide sighting in the home	1.157	0.477	5.856	0.015	3.182 (0.130 – 0.807)
Reported pesticide use	1.157	0.477	5.856	0.015	3.182 (0.130 – 0.807)
Dietary habit:					
Eat outside the household	0.808	0.458	3.117	0.077	2.243 (0.915 – 5.501)
Eat on the floor inside the household	1.157	0.477	5.891	0.015	3.182 (0.130 – 0.807)
Wash hands before eating	-1.127	0.466	5.857	0.016	0.324 (0.130 – 0.807)
Vegetable washing soaking time	-0.531	0.457	1.349	0.245	0.588 (0.240 – 1.441)
Fruits washing soaking time	-0.531	0.457	1.349	0.245	0.588 (0.240 – 1.441)
Fruits peeled	0.603	0.453	1.774	0.183	1.827 (0.753 – 4.435)

3-PBA metabolite concentration in urine (1 = ≤ 0.53 μg/mL, 2 = ≥ 0.53 μg/mL). B, regression coefficient; S.E., standard error; OR, odds ratio; CI, confidence interval. Reference category: first. A bivariate analysis of each variable was first done, and then variable with p-value < 0.25 were included in the multivariate analysis.

4.4.1.2 Factors associated with 3-PBA metabolite in wet season by Multivariate analysis

In multivariate analysis, frequency of floor-cleaning in household in a day was statistically significant with 3-PBA metabolites (p-value = 0.038). non-floor-cleaning in a day was showed an increase in 0.089-fold odds of 3-PBA metabolites than floor-cleaning in a day, but only presented in low risk exposure (OR = 0.089, 95%CI 0.009 – 0.870). However, aged of children was likely significant association with 3-PBA metabolites (p-value = 0.080), while age increased/month was showed increases 1.050-fold odds of 3-PBA metabolites (OR = 1.050, 95% 0.994 – 1.110). however, genders were not significantly associated with 3-PBA metabolites. female was show increased 1.853-fold odds of 3-PBA metabolites than male but presented in high risk exposure (OR = 1.853, 95%CI 0.697 – 4.924). frequency of walk bare foot inside household was also not significantly associated with 3-PBA metabolites. Children spent time on the floor everyday by walk bare foot inside household was show increased 1.962-fold odds of 3-PBA metabolites than non-walk bare foot inside household, which presented in high risk exposure (OR = 1.962, 95%CI 0.691 – 5.570) **(Table 9)**.

Table 9. Multivariate analysis of factor's exposure with 3-PBA metabolites concentration as wet season (November – December 2018).

Factor exposure	B	S.E.	Wald	p-value	OR (95% CI)
Aged 2-3 year (months)	0.049	0.028	3.056	0.080	1.050 (0.994 – 1.110)
Gender	0.617	0.499	1.531	0.216	1.853 (0.697 – 4.924)
Children walk bare feet inside household in the day	0.674	0.532	1.601	0.206	1.962 (0.691 – 5.570)
Frequency of floor-cleaning	-2.414	1.160	4.327	0.038*	0.089 (0.009 – 0.870)

3-PBA metabolite concentration in urine (1 = ≤ 0.53 µg/mL, 2 = ≥ 0.53 µg/mL). B, regression coefficient; S.E., standard error; OR, odds ratio; CI, confidence interval. Adjusted for: age (months). Reference category: first. * Significant at p-value < 0.05.

4.4.2 Factors associated with 3-PBA metabolite in dry season (April – May 2019)

The factors related to the exposures (socio-demographic characteristics, parent information, factor related to PYR exposure, personal hygiene, contact variable behavior, home characteristics and dietary habit) from questionnaire were used to analyze the association with 3-PBA metabolites concentrations in urine of children. A bivariate analysis of each variable was first done, and then variables with p-value < 0.25 were included in the multivariate analysis. The associations between the exposure factors and 3-PBA metabolites concentrations were presented by bivariate analysis in **(Table 10)**, and multivariate analysis **(Table 11).** The results are following:

4.4.2.1 Factors associated with 3-PBA metabolite in dry season by bivariate analysis

Socio-demographic characteristics, gender was only one possible factor that was associated with 3-PBA metabolites in urine of children (p-value = 0.079).

Parent information was not associated with 3-PBA metabolites in urine of children.

Factors related to PYR exposure including type insecticide products use household, name of insecticide products use household, store for keep insecticide products, frequency of PYR insecticide use in household (times/month), frequency of floor-cleaning, type of floor cleaning, frequency of open window or door and frequency of insecticide control operators by officer were found to be possible associated with 3-PBA metabolites in urine of children (p-value < 0.2).

Personal hygiene including children spend time outside household, children spend time outside household in the time/day, location for spend time outside

household, children play on the floor at household in the day, location of play at household floor, children play soil at household in the day, children walk bare feet inside household in the day, children wash hands/foots in the day, children take a shower in the day and frequency of illness within 6 months were possible associated with 3-PBA metabolites in urine of children (p-value < 0.2).

Contact variable behavior including children touch furniture-to-mouth, children touch bottle/dish-to-mouth, children touch object-to-mouth, children put hand-to-mouth, children put fingers-to-mouth, children put hand on the floor , children put toy-to-mouth (football etc.), children touch hair-to-mouth, children touch skin-to-mouth, children touch clothes-to-mouth, children touch grass/vegetation-to-mouth and children crawling across on the floor were reported possible factors that were associated with 3-PBA metabolites in urine of children (p-value < 0.2).

Home characteristics including type of household, main type floor, direction of front household, reported insecticide sighting in the home and reported pesticide use were possible associated with 3-PBA metabolites in urine of children (p-value < 0.2).

Dietary habit including eat outside the household, eat on the floor inside the household, wash hands before eating, vegetable washing soaking time, fruits washing soaking time and fruits peeled were found to be possible factors that were associated with 3-PBA metabolites in urine of children (p-value < 0.2).

Table 10. Bivariate analysis of each factor's exposure with 3-PBA metabolites concentration as dry season (April – May 2019).

Factor exposure	B	S.E.	Wald	p-value	OR (95% CI)
Socio-demographic Characteristics:					
Aged 2-3 year (months)	-0.002	0.020	0.014	0.905	0.998 (0.958 – 1.038)
Weight (kg)	-0.030	0.049	0.386	0.543	0.970 (0.882 – 1.067)
High (cm)	-0.018	0.017	1.068	0.301	0.983 (0.950 – 1.016)
Gender	0.843	0.478	3.083	0.079	2.316 (0.907 – 5.913)
Current address / Location of residence	0.000	0.460	0.000	1.000	1.000 (0.406 – 2.464)
Duration to live in this region	-0.002	0.020	0.014	0.905	0.998 (0.958 – 1.038)
Children breast milk	-0.121	0.494	0.060	0.806	0.886 (0.337 – 2.331)
Parent information:					
Parents education level	0.288	0.768	0.140	0.708	1.333 (0.296 – 6.005)
Parent main source income	-0.076	0.451	0.029	0.866	0.926 (0.412 – 2.560)
Care giver of children	0.027	0.466	0.003	0.954	1.027 (0.412 – 2.560)
Factors related to PYR exposure:					
Type insecticide products use household	1.675	0.807	4.312	0.038	5.338 (1.099 – 25.941)
Name of insecticide products use household	1.675	0.807	4.312	0.038	5.338 (1.099 – 25.941)
Store for keep insecticide products	1.675	0.807	4.312	0.038	5.338 (1.099 – 25.941)
Frequency of PYR insecticide use in household (times/month)	2.110	0.790	7.065	0.008	8.250 (1.740 – 39.105)
Frequency of floor-cleaning	-0.840	0.478	3.083	0.079	0.432 (0.169 – 1.103)
Type of floor cleaning	-0.840	0.478	3.083	0.079	0.432 (0.169 – 1.103)
Frequency of open window or door	1.675	0.807	4.312	0.038	8.250 (1.740 – 39.105)
Personal hygiene					
Children spend time outside household	0.840	0.478	3.083	0.079	2.316 (0.907 – 5.913)
Children spend time outside household in the time/day	2.110	0.794	7.065	0.008	8.250 (1.740 – 39.105)
Location for spend time outside household	2.110	0.794	7.065	0.008	8.250 (1.740 – 39.105)
Children play on the floor at household in the day	2.110	0.794	7.065	0.008	8.250 (1.740 – 39.105)
Location of play at household floor	2.110	0.794	7.065	0.008	8.250 (1.740 – 39.105)
Children play soil at household in the day	0.840	0.478	3.083	0.079	2.316 (0.907 – 5.913)
Children walk bare feet inside household in the day	1.675	0.682	6.032	0.014	5.333 (1.402 – 20.285)
Children walk bare feet outside household in the day	-0.211	0.460	0.211	0.646	0.810 (0.329 – 1.993)
Children wash hands/foots in the day	-0.750	0.463	2.626	0.105	0.472 (0.191 – 1.170)
Children take a shower in the day	-0.693	0.457	2.296	0.130	0.500 (0.204 – 1.226)
Frequency of illness within 6 months	-1.356	0.692	3.842	0.050	0.258 (0.066 – 1.000)

3-PBA metabolite concentration in urine (1 = ≤ 1.46 µg/mL, 2 = ≥ 1.46 µg/mL). B, regression coefficient; S.E., standard error; OR, odds ratio; CI, confidence interval. Reference category: first. A bivariate analysis of each variable was first done, and then variable with p-value < 0.25 were included in the multivariate analysis.

Table 10. Bivariate analysis of each factor's exposure with 3-PBA metabolites concentration as dry season (April – May 2019). **(Conti.)**

Factor exposure	B	S.E.	Wald	p-value	OR (95% CI)
Contact variable behavior:					
Children touch furniture-to-mouth	1.792	0.802	4.986	0.026	6.000 (1.245 – 28.920)
Children touch bottle/dish-to-mouth	1.792	0.802	4.986	0.026	6.000 (1.245 – 28.920)
Children touch object-to-mouth	1.792	0.802	4.986	0.026	6.000 (1.245 – 28.920)
Children put hand-to-mouth	1.551	0.812	3.649	0.056	4.714 (0.960 – 23.139)
Children put fingers-to-mouth	1.674	0.682	6.032	0.014	5.333 (1.402 – 20.285)
Children put hand on the floor	1.551	0.812	3.649	0.056	4.714 (0.960 – 23.139)
Children put toy-to-mouth (football etc.)	1.792	0.802	4.986	0.026	6.000 (1.245 – 28.920)
Children touch hair-to-mouth	1.551	0.812	3.649	0.056	4.714 (0.960 – 23.139)
Children touch skin-to-mouth	1.551	0.812	3.649	0.056	4.714 (0.960 – 23.139)
Children touch clothes-to-mouth	1.674	0.682	6.032	0.014	5.333 (1.402 – 20.285)
Children touch grass/vegetation-to-mouth	1.551	0.812	3.649	0.056	4.714 (0.960 – 23.139)
Children crawling across on the floor	1.674	0.682	6.032	0.014	5.333 (1.402 – 20.285)
Home Characteristics:					
Type of household	-0.840	0.478	3.083	0.079	0.432 (0.169 – 1.103)
Main type floor	-0.840	0.478	3.083	0.079	0.432 (0.169 – 1.103)
Direction of front household	-0.840	0.478	3.083	0.079	0.432 (0.169 – 1.103)
Reported insecticide sighting in the home	1.674	0.682	6.032	0.014	5.333 (1.402 – 20.285)
Reported pesticide use	1.674	0.682	6.032	0.014	5.333 (1.402 – 20.285)
Dietary habit:					
Eat outside the household	1.792	0.802	4.986	0.026	6.000 (1.245 – 28.920)
Eat on the floor inside the household	1.792	0.802	4.986	0.026	6.000 (1.245 – 28.920)
Wash hands before eating	-0.840	0.478	3.083	0.079	0.432 (0.169 – 1.103)
Vegetable washing soaking time	-0.840	0.478	3.083	0.079	0.432 (0.169 – 1.103)
Fruits washing soaking time	-0.840	0.478	3.083	0.079	0.432 (0.169 – 1.103)
Fruits peeled	1.792	0.802	4.986	0.026	6.000 (1.245 – 28.920)

3-PBA metabolite concentration in urine (1 = ≤ 1.46 μg/mL, 2 = ≥ 1.46 μg/mL). B, regression coefficient; S.E., standard error; OR, odds ratio; CI, confidence interval. Reference category: first. A bivariate analysis of each variable was first done, and then variable with p-value < 0.25 were included in the multivariate analysis.

4.4.2.2 Factors associated with 3-PBA metabolite in dry season by multivariate analysis

In multivariate analysis, frequency of walk bare foot inside household was significantly associated with 3-PBA metabolites (p-value = 0.009). Children spent time on the floor everyday by walk bare foot inside household was showed an increase in 6.789-fold odds (OR = 6.789, 95%CI 1.597 – 28.854). of 3-PBA metabolites than non-walk bare foot inside household. Moreover, genders were significantly associated with 3-PBA metabolites (0.041) reporting an increase in 0.333-fold odds (OR = 0.333, 95%CI 0.116 – 0.956). of 3-PBA metabolites for female Although, frequency of floor-cleaning in household in a day was not significantly associated with 3-PBA metabolites (p-value = 0.157), non-floor-cleaning in a day was showed an increase in 0.480-fold odds (OR = 0.480, 95%CI 0.175 – 1.324). of 3-PBA metabolites than floor-cleaning in a day. aged of children was not also significantly associated with 3-PBA metabolites (p-value > 0.05), however, age decreased/month was showed 0.987-fold odds of 3-PBA metabolites (OR = 0.987, 95% 0.944 – 1.033) **(Table 11)**.

Table 11. Multivariate analysis of factor's exposure with 3-PBA metabolites concentration as dry season (April – May 2019).

Factor exposure	B	S.E.	Wald	p-value	OR (95% CI)
Aged 2-3 year (months)	-0.013	0.023	0.304	0.581	0.987 (0.944 – 1.033)
Gender	-1.101	0.539	4.175	0.041	0.333 (0.116 – 0.956)
Children walk bare feet inside household in the day	1.915	0.738	6.730	0.009	6.789 (1.597 – 28.854)
Frequency of floor-cleaning	-0.730	1.715	2.004	0.157	0.480 (0.175 – 1.324)

3-PBA metabolite concentration in urine (1 = ≤ 1.46 μg/mL, 2 = ≥ 1.46 μg/mL). B, regression coefficient; S.E., standard error; OR, odds ratio; CI, confidence interval. Adjusted for: age (months). Reference category: first. * Significant at p-value < 0.05.

CHAPTER V
DISCUSSION

This study focused on children age 2 -3 years old in Bangkok Thailand which was similar to the previous study regarding insecticide importation into Thailand (Siriwat et al. 2019) Children in families low-income were targeted because their parents have used PYR insecticide products daily and they lack of awareness or knowledge on how to protect their children from the exposures (Quiros et al. 2011a).

This study demonstrated young children exposure to PYRs insecticides in households between wet and dry seasons. The results revealed that both seasons showed the same study area and criterial, but different participants because we design by repeated cross sectional study.

This study reported an increase in 3-PBA metabolite concentration was significantly correlated with low GABA concentration in urine samples in both seasons. Moreover, the increase of 3-PBA metabolite was significantly associated with hand wipes samples (including cypermethrin and allethrin) in dry season, but not in wet season. The association between the exposure factors with 3-PBA metabolite concentration were observed. Hardly ever floor-cleaning was significantly associated with increased 3-PBA metabolite concentration in wet season. While genders and always walk bare feet inside household in a day were significantly associated with increased 3-PBA metabolite concentration in dry season.

Topic of discussions are as followings:

5.1 Concentration of 3-PBA metabolite, GABA and wipe samples in dry and wet seasons

5.1.1 The 3-PBA metabolite concentration were detected in urine samples between wet and dry seasons

5.1.2 The GABA concentration were found in urine samples between wet and dry seasons

5.1.3 The hand wipes concentration was detected between wet and dry seasons

5.2 Correlation among 3-PBA metabolite, GABA and wipe sample concentration in dry and wet seasons

5.2.1 Correlation between 3-PBA metabolite and GABA concentration in urine children

5.2.2 Correlation between 3-PBA metabolite and hand wipes sample children

5.3 Exposure factors that were associated with 3-PBA metabolite between dry and wet seasons

5.3.1 Exposure factors that were associated with 3-PBA metabolite between dry and wet seasons

5.3.2 Each exposure factor that was associated with 3-PBA metabolite 1between dry and wet seasons

5.3.2.1 Socio-demographic characteristics and parent information

5.3.2.2 Factors related to PYR exposure

5.3.2.3 Personal hygiene characteristics

5.3.2.4 Contact variable behavior

5.3.2.5 Home Characteristics

5.3.2.6 Dietary habit

5.1 Concentration of 3-PBA metabolite, GABA and wipe samples in dry and wet seasons

5.1.1 The 3-PBA metabolite concentration detected in urine sample between wet and dry seasons

The 3-PBA metabolite concentrations were detected in both seasons because people widely use PYRs insecticides daily such as coil and insecticide spraying products to control insects in their houses In wet season, most people used insecticide spraying sometime/month and kept it outside their households. In dry season, most families used coil insecticides every day and kept them inside their homes In both seasons, floors were cleaned once a week by wet and broom. Most family never opened windows or doors to increase in air flow inside their households. From the observation, this study area reported insecticide control provided by an officer every 2 months. In addition, people used PYRs insecticides in dry season more than wet season. This is due to the fact that it is based on household setting in community area and based on quantity of insects such as mosquito, cockroach and ant. This information was reported by observed and the questionnaires.

The 3-PBA metabolite concentrations were found in wet and dry seasons presenting 0.53 µg/mL and 1.46 µg/mL, respectively. The finding showed that 3-PBA metabolite concentrations in dry season were higher than wet season because most families used coil insecticides every day (79%), but in wet season was

showed (37%), and in dry season was kept it inside household (84%), but wet season was showed (35%). This situation is dangerous to young children because they spend time in households all day and they can exposure to PYRs by inhalation. Moreover, children have potentially exposed to PYRs component from food and some product that contain of PYR at home (Morgan 2012; Oulhote and Bouchard 2013b).

In addition, the findings in both seasons found 3-PBA metabolite concentrations higher than others study. For example, 3-PBA metabolite concentrations in infants living in an agricultural area of the province of Jiangsu in China (median = 0.39 μg/mL, children aged 1 years) (Wu et al. 2013). 3-PBA metabolites concentration in young children (6–11 years) from spread across Canada (median = 0.20 μg/mL) (Oulhote and Bouchard 2013a). Urinary PYR metabolites in school students from northern Thailand (median = 0.07 μg/mL, children aged 12-13 years) (Panuwet et al. 2009). Moreover, PYRs and chlorpyrifos metabolite concentrations in Northern California families and their relationship to indoor residential insecticide levels, part of the study of use of products and exposure related behavior (median = 0.75 μg/mL, children aged 2-8 years) (Trunnelle et al. 2014).

Based on the evidence found in this study, the strategies including health education and risk management regarding reducing PYRs exposure should be implemented for children at this area. This is because the study found high 3-PBA metabolite in urine of children living in households. this was consistent with the finding with (Barr et al. 2010). that 3-PBA metabolites were derived primarily from exposure to permethrin, cypermethrin, and children may have higher exposures than adolescents and adults,

5.1.2 The GABA concentration detected in urine sample between wet and dry seasons

GABA concentrations in urine children age 2 -3 years old living in households were reported in both wet and dry seasons representing 355.11 ng/mL and 450.00 ng/mL, respectively. In addition, some study revealed that age of children was not significantly correlated with GABA concentration, because this might be occurring by neurotransmitter concentration in specific brain regions and basic behavioral variation in humans (Sumner et al. 2010). From this evident is lead to ours finding why in both seasons GABA concentration of young children aged 2 -3 years old were presented different concentration.

Moreover, this study found GABA concentrations in urine of children age 2-3 years (n=80), Bangkok, Thailand higher than other studies. For example, forty-five healthy subjects (21–52 years) showed median = 1.7 ng/mL) (Aufhaus et al. 2013). Normal children 6-12 years were detected GABA and median was 332 ng/mL (Gammon and Casida 1983). Which Normal children 5-6 years showed median of GABA concentrations at 391 ng/mL (Ghiasuddin and Soderlund 1985). Currently, there is no standard of GABA concentrations of human, therefore, this result was compared GABA concentrations with other study.

Currently, there is no report how much concentration of PYRs insecticide that can affect GABA levels. There is no evidence reporting increased/decreased PYRs insecticide exposure was significantly correlated with increased/decreased GABA concentration in urine human samples.

5.1.3 The hand wipes concentration between wet and dry seasons

This finding of hand wipes concentration found PYR insecticide in children in both seasons. In wet season reported cypermethrin 0.012 µg/mL, allethrin 0.002 µg/mL. In dry season presented cypermethrin 0.016 µg/mL, allethrin 0.002 µg/mL. From observation, the study found that behaviors such as put objects to mouth or put hand to mouth etc., and household environment or frequency of PYRs used are the factors that can increase PYR insecticide exposure. This was consistent with the previous. hand wipes sample, presented 0.096 – 0.503 µg/hand, confirm that children hand, foot and toy may accumulate pesticide residues (Siriwat et al. 2019).

5.2 Correlation among 3-PBA metabolite, GABA and wipe sample concentration in dry and wet seasons

5.2.1 Correlation between 3-PBA metabolite and GABA concentration in urine children

In both seasons, 3-PBA metabolite concentrations in urine of children were found moderately significant related to GABA concentration in children (rs = -0.230, p-value 0.004). the result reported that an increase in 3-PBA metabolite concentrations was significantly correlated with low GABA concentration in urine of children for both seasons. In addition, there is no report presenting increased/decreased PYRs insecticide exposure was significantly correlated with increased/decreased GABA concentration in urine human sample.

Hence, finding new association between 3-PBA metabolite and GABA concentration in urine children samples. This evident revealed that in household where PYRs insecticide have been used every day and children spend time in

household all day. While behaviors of children are the main factors enhancing PYRs insecticide exposure to them directly. In addition, health risk assessment of residential exposure to cypermethrin among 58 children (1-3 years) in agricultural communities in northeastern Thailand, these findings shows that young children might not risk for cypermethrin exposure through the dermal route, residential exposure among young children may be reduced by improved hygiene (Siriwat et al. 2019). Recent research suggested that even low levels of pesticide exposure affects young children's neurological and behavioral development, evidence shows a link between pesticides and neonatal reflexes, psychomotor and mental development and attention-deficit hyperactivity disorder, action targets the nervous system by altering the effect of an enzyme that regulates select neurotransmitters, pyrethroids and carbamates also target the nervous system because these toxins have been developed to affect living creatures, these chemicals may affect human adversely as well (Liu and Schelar 2012b).

A few studies presented that mechanism of PYRs insecticide affects GABA as neurotransmitter, and they effort to understand in that mechanism, this finding agrees with (Gammon and Casida 1983; Ghiasuddin and Soderlund 1985; Barlow, Sullivan, and Lines 2001; Gaetz et al. 2010; Gaetz et al. 2011; Muthukumaraswamy et al. 2013; Rojas et al. 2014).

The mode of action of pyrethroids is known to be via interactions with the voltage-gated sodium channel and understanding how binding to the channel is affected by amino acid substitutions that give rise to resistance has helped to elucidate the mode of action of the compounds and the molecular basis of their selectivity for insects and mammals and between insects and other arthropods (Field et al. 2017). In addition, GABA receptors were blocked by PYRs in mammalian brain preparations, GABA blockaded an indirect side effect associated with the elimination

of nerve input inhibition and actionable form of stimulation, such as pesticides, picrotoxinin (Muthukumaraswamy et al. 2009; Sumner et al. 2010; Sigel and Steinmann 2012).

The widespread impact of PYR's disruptive actions on sodium channel activity plus other sites of action, the main effected of PYRs 1) the primary ion channel effects of the PYRs 2) To control the secondary effects of specific chemical neurotransmitters, both types I and II pyrethroids (permethrin, fenvalerate, cypermethrin, or deltamethrin) can act as proconvulsants via GABA and glutamatergic systems (Ray and Fry 2006).

In 1983, this study clearly demonstrated the new mechanism of pesticide use by PYRs, which prompts psychotic syndrome, GABA acts primarily on neurotransmitters and precise muscle mechanisms, various classes of GABA antibiotics interfere with the (open) or non-stimulating (off) ionophore depending on the GABA (Gammon and Casida 1983). After that in 1985, found PYRs that produced both T syndrome (tremors syndrome) and CS syndrome (choreoathetosis with salivation) (Saillenfait, Ndiaye, and Sabate 2015).

5.2.2 Correlation between 3-PBA metabolite and hand wipes sample children

In wet season, 3-PBA metabolite concentrations in urine of children were not significant associated with the results of hand wipes sample (rs = 0.160, p-value >0.05). In dry season, 3-PBA metabolite concentrations in urine of children were found to be related to hand wipes samples (rs = 0.226 – 0.274, p-value < 0.05). Moreover, the increase of 3-PBA metabolite was significantly associated with increase hand wipes sample (including cypermethrin and allethrin) in dry season, but not associated with hand wipes sample in wet season. This study finding is similar to (Siriwat et al. 2019).

Exposure estimation suggests that hand-to-mouth contact is another important pathway creating dust ingestion and that children are subjected to higher pesticide exposure than adults (Tan et al. 2018). Pesticide exposures are highly prevalent, and data provided herein further substantiate hand-to-mouth contact and dermal absorption as important pathways of pesticide exposure, especially for young children (Hoffman et al. 2015; Phillips et al. 2018).

5.3 Exposure factors that are associated with 3-PBA metabolite between dry and wet seasons

5.3.1 Factors exposure associated with 3-PBA metabolite between dry and wet seasons

This study applied Binary logistic regression test to investigate the association between the exposure factors and 3-PBA metabolite concentrations. observed an association between exposure factors and 3-PBA metabolite concentrations were observed. While, seldom cleaning floor was significantly associated with an increase in 3-PBA metabolite concentration in wet season. Genders and always walk bare feet inside household in a day were significantly associated with an increase in 3-PBA metabolite concentration in dry season.

In wet season, seldom cleaning floor was significantly associated with increased 3-PBA metabolite concentration. This study focused on children family low-incomes and spend time in household all day. From observation, the study found that parent used PYRs insecticide products daily and seldom cleaned the floors because they were busy and their houses are small Some studies suggested that residential dust was selected to explore the impact of PYRs metabolites on potential eliminated biomarker concentrations, and metabolic products in important

exposure media should be quantified to reduce possible overestimation of exposure when urinary biomarkers are used to reconstruct dose (Starr et al. 2008). Moreover, pesticides deposit onto or are transported to floors and other surfaces, to dinnerware and food stored in closed cabinets, and to children's toys, and are picked up on children's hands (Lewis et al. 2011). This study suggests that floor should be cleaned every day because it can reduce PYRs insecticide on the household floor.

In dry season, genders and always walk bare feet inside household in a day were significantly associated with increased 3-PBA metabolite concentration. As same as, (Freeman et al. 2001a) reported that different gender aged 3–13 years were related to PYRs exposure, because girls spent more time indoors than boys . The relationship between age of children and PYR insecticide exposure were remained unclear, which age dependent differences in PYR insecticide exposure may be occur from dose PYRs exposure (Shafer, Meyer, and Crofton 2005). This study suggests that children need to wear shoe or socks inside their homes because this protect them from exposure to PYRs insecticides via dermal contact.

5.3.2 Each factors exposure associated with 3-PBA metabolite between dry and wet seasons

5.3.2.1 Socio-demographic characteristics and parent information

This study found that children aged 2-3 years were not association with 3-PBA metabolite in urine children' sample in difference seasons. Therefore, the association between age of children and PYRs insecticide exposure was uncleared, which age dependent differences in PYRs insecticide exposure may be occurred from dose PYRs exposure (Shafer, Meyer, and Crofton 2005).

This study also found parents' main source income was not associated with 3-PBA metabolite in children's urine. Which, in other work focused

on children aged 3–6 years, assessing indoor dust concentrations of PYRs in low-income homes in an urban setting, which along with other studies indicate that low-income children are potentially exposed to a mixture of pesticides; indeed, the high detection of several insecticides in household dust suggests there is a need to educate families on the potential health impacts of insecticide use and to implement effective integrated pest management strategies to control pests and reduce insecticide-exposure to household occupants (Quiras-Alcal et al. 2011).

In addition, parents' educational level was not associated with 3-PBA metabolite in children's urine, yet most of the parents either worked or not, were likely unaware of PYRs insecticide exposure to children in households. The study suggests that these households could benefit from education, awareness, and management help to parents to reduce the risk of long-term PYRs insecticide exposure to their children.

5.3.2.2 Factors related to PYRs exposure

The results presented that type PYRs insecticide used, name of PYR insecticide product used, store for keep PYR insecticide product used, frequency of PYR product use in household (time/month), frequency of floor cleaned, type of floor cleaned and frequency of opened window and door were associated with 3-PBA metabolite between dry and wet seasons (p-value <0.05).

Now a day the observational exposure measurement studies examined children on 5 months to 17 years of age who exposure to PYRs in media including floor dust, food, floor wipes, and air. the studies revealed that permethrin was normally detected (>50%) of PYR, followed by cypermethrin (wipes, dust, and food), as the same time 3-phenoxybenzoic acid (3-PBA), a urinary metabolite of several PYRs, was the most frequently (≥67%) detected PYRs biomarker, indicate that children were exposed to several PYRs, but primarily to permethrin and cypermethrin

(Lu et al. 2000; Barr et al. 2005; Heudorf et al. 2006; Abdel Rasoul et al. 2008; Morgan 2012; Oulhote and Bouchard 2013b; Antwi and Reddy 2015).

5.3.2.3. Personal hygiene characteristics

The results presented that children play on the floor at household in the day, children walk bare feet inside/outside household in the day, frequency of illness within 6 months were associated with 3-PBA metabolite between dry and wet seasons (p-value <0.05).

Children are possibility exposure to PYRs in households than adults because of their behaviors such as crawl on the floor or put object-to-mouth. The objects were contaminated PYR from household's environment. Children are at higher risk to exposure to pesticides than adults because there are primary stage of physical development and kinetic of toxins including absorption, distribution, metabolism and elimination of chemical difference between children and adults, some function excretion systems are still immature (Zhou, Mainelis, and Weisel 2019; Kwong et al. 2019). Moreover, PYRs insecticide exposure to children affects to neurobehavior because the organs of children are not fully developed until later in life, prior to which they are constantly experiencing significant developmental changes; during this vulnerable period, adverse exposure to CNS can cause permanent damage, particularly in utero, in the behavior of children (Liu and Schelar 2012c).

5.3.2.4. Contact variable behavior

The results presented that children touch object, hair, clothes and put hand-to-mouth or crawling across on the floor were associated with 3-PBA metabolite between dry and wet seasons (p-value <0.05).

It shows that children receive pesticides in their homes and assess the activities of their children's activities and activities in their children and the survey respondents indicated that the youngest children are likely to show behavior that promotes environmental contamination. Hand-to-mouth and object-to-mouth are most common activities that lead to exposure to insecticides among the youngest children. Children are usually walk barefoot inside and outside their homes. Gender differences were found in out-of-home behaviors and the proportion of time spent in outdoor observation with reported behavior (observed activity/hours) showed that object to mouth, touch clothing and touch smooth surface, risk factor of gender differences were found boys spent time in outdoor more than girls which girls spent time in indoor more than boys (Freeman et al. 2001; Freeman et al. 2005; Paulson and Barnett 2010; Liu and Schelar 2012b). However, children have different activity patterns (e.g., crawling), they are closer to the ground and eat more food and water per kilogram than adults. In addition, children may be at greater risk of exposure to pollution due to higher ventilation and metabolic rate, rapid physical development, greater surface-to-volume ratios, and immature organ systems (Freeman et al. 2005).

5.3.2.5. Home Characteristics

The results presented that reported insecticide sighting in the home and reported pesticide used were associated with 3-PBA metabolite between dry and wet seasons (p-value <0.05).

Moreover, people with low income commonly use PYRs products to control insects and increase crops, therefore, it is possible that children who live in these homes may have a chance to exposure to insecticide (Kwong et al. 2020). Nevertheless, a few studies focused on children's exposure to PYRs insecticide in households via products of mosquito coils and sprays. The studies revealed that an adequate protection of children against toxic chemical agents in the environment require fundamental and far-reaching revisions of current approaches to toxicity testing and risk assessment (Landrigan et al. 2004).

PYRs were commonly used ingredient in household pesticides and animal control products containing ectoparasite. Inactivity in the home environment increases the risk of exposure and side effects in the general population. The action of PYRs depends on their ability to bind to and disrupt voltage-gated sodium channels of insect nerves. Sodium channels are also important targets for the neurotoxic effects of PYR in mammals but other targets, particularly voltage-gated calcium and chloride channels, have been implicated as alternative or secondary sites of action for a subset of PYRs. Understanding PYRs neurotoxicity is complicated by the presence of two different intoxicating groups in mammals that are related to different structural subgroups of this pesticide class (Lebov et al. 2015; Louis, Lerro, Friesen, Andreotti, Koutros, Sandler, Blair, Robson, and Beane 2017).

5.3.2.6. Dietary habit

The results presented that eat outside the household, eat on the floor inside the household, vegetable washing, fruits washing, and fruits peeled were associated with 3-PBA metabolite between dry and wet seasons (p-value <0.05).

Children exposed to toxins buy breathing in the air, drinking water, eating the medication, and a variety of environments including homes, childcare centers, schools and cars. Children have a unique path of non-parallel exposure among adults, and the pathways of exposure and risk to health vary in different stages of childhood and contact with the toxins may occur in the uterus by transferring the substance from the mother to the fetus which it can happen through the milk in the newborn; and it can happen in childhood by transferring poisonous chemicals from the hands, analysis of the patterns and routes of exposure of children's allergens and the health effects that occur at various stages of development are essential to establishing a child protection approach to risk assessment (Landrigan et al. 2004).

Finally, young children could not protect themselves from PYRs insecticides in households and parent represent by family low-income. Which, they are not aware of health risks and toxic effects of PYR insecticide exposure. Moreover, parents should realize and protect their children from contaminate with PYR insecticides in their homes.

CHAPTER VI
CONCLUSION

6.1 Conclusion

This study was a repeated cross-sectional design and focused on young children 2 – 3 years old. It was designed to collect information about PYR insecticide exposures between wet (November – December 2018) and dry (April – May 2019) seasons. The total of 80 young children in each season, but different person between wet and dry seasons, were recruited. The study areas are in Khlong Toei and Pathum wan district, Bangkok, Thailand. The study focused on children in low-income families because their parents have used a PYRs insecticide products daily and probably lacked awareness or knowledge how to protect their children from the exposures Data was collected from children and family caregivers using the questionnaires.

In wet season, participants were children age between 2-3 years. Average age was 27 months with female 36 persons (45%) and male 44 persons (55%). In dry season, participants were children age between 2-3 years. Average age was 30 months with female 28 persons (35%) and male 52 persons (65%). In both seasons, the results revealed that households often used coils and insecticides spraying and keep them inside their homes. In addition, people seldom cleaned the floors.

Due to 3-PBA metabolite concentration, in wet season, 3-PBA metabolite concentrations were detected (92%) and measured range 0.23 – 2.02 µg/mL. which median showed 0.53 µg/mL and mean ± SE showed 0.73 ± 0.06 µg/mL. In dry season, 3-PBA metabolite concentrations were detected (92%) and measured range 0.23 – 2.53 µg/mL. which median showed 1.46 µg/mL and mean ±

SE showed 1.47 ± 0.08 µg/mL. There were significant differences of 3-PBA metabolite concentration between dry and wet seasons (p-value <0.01).

With respect to GABA concentration, in wet season, GABA concentrations was detected (99%) and measured range <0.01 - 7490.75 ng/mL. which median showed 355.11 ng/mL and mean ± SE showed 554.08 ± 125.69 ng/mL. In dry season, GABA concentration were detected (99%) and measured range 94.24 – 1300 ng/mL. which median showed 450.00 ng/mL and mean ± SE showed 534.07 ± 37.08 ng/mL. There were significant differences of GABA concentrations between dry and wet seasons (p-value 0.003).

Hand wipes sample concentrations were presented by cypermethrin and allethrin in wet and dry seasons. In both seasons, cypermethrin and allethrin were detected (92%). In wet season, cypermethrin insecticide concentration were higher than allethrin at 0.012 ± 0.001 µg/mL and 0.002 ± 0.0002 µg/mL, respectively. In dry season, cypermethrin insecticide concentrations were found most at 0.016 ± <0.01 µg/mL, followed by allethrin at 0.002 ± 0.002 µg/mL. There were significant differences of hand wipes sample concentrations between dry and wet seasons (p-value <0.001).

The new finding in this study, in both wet and dry seasons, 3-PBA metabolite concentrations in children’s urine were found moderately significant association with GABA concentrations (r_s = -0.230, p-value 0.004). Hence, the results reveal that an increase in 3-PBA metabolite concentration was significantly correlated with low GABA concentration in children’s urine samples in both seasons.

In addition wet season, there was no association between 3-PBA metabolite concentration in urine and hand wipes sample (r_s = 0.160, p-value >0.05). In dry season, 3-PBA metabolite concentrations in urine were significant related to hand wipes sample (r_s = 0.226 – 0.274, p-value < 0.05). Hence, the increase in 3-PBA

metabolite was significantly associated with increase hand wipes sample (including cypermethrin and allethrin) in dry season, but there was no association with hand wipes sample in wet season.

Regarding multivariate analysis in wet season, frequency of floor-cleaning in household in a day was significant associated with 3-PBA metabolites (p-value = 0.038), and non-floor-cleaning in a day was showed increases a 0.089-fold odds (OR = 0.089, 95%CI 0.009 – 0.870) of 3-PBA metabolites than floor-cleaning in a day, but only presented in low risk exposure. However, age of children was likely to be associated with 3-PBA metabolites (p-value = 0.080), while age increased/month revealed an increase in 1.050-fold odds of 3-PBA metabolites (OR = 1.050, 95% 0.994 – 1.110). Moreover, genders were not significantly associated with 3-PBA metabolites. Female showed an increase in1.853-fold odds (OR = 1.853, 95%CI 0.697 – 4.924) of 3-PBA metabolites but presented in high risk exposure. Which, frequency of walking bare foot inside household was not significantly associated with 3-PBA metabolites. Children spent time on the floor everyday by walking bare foot inside household was showed increased 1.962-fold odds of 3-PBA metabolites than non-walk bare foot inside household, which presented in high risk exposure.

With respect to multivariate analysis in dry season, frequency of walk bare foot inside household was significantly associated with 3-PBA metabolites (p-value = 0.009), and if children spent time on the floor everyday by walk bare foot inside household was show increased 6.789-fold odds of 3-PBA metabolites than non-walk bare foot inside household, which presented in high risk exposure (OR = 6.789, 95%CI 1.597 – 28.854). Moreover, genders were significantly associated with 3-PBA metabolites (0.041), presented that female was show increased 0.333-fold odds of 3-PBA metabolites than male, but presented in low risk exposure (OR = 0.333, 95%CI 0.116 – 0.956). Although, frequency of floor-cleaning in household in a day

was not significantly associated with 3-PBA metabolites (p-value = 0.157), and if non-floor-cleaning in a day was showed increases a 0.480-fold odds of 3-PBA metabolites than floor-cleaning in a day, but only presented in low risk exposure (OR = 0.480, 95%CI 0.175 – 1.324). While, However, aged of children was not significantly associated with 3-PBA metabolites (p-value > 0.05), presented that age decreased/month was showed 0.987-fold odds of 3-PBA metabolites (OR = 0.987, 95% 0.944 – 1.033).

Recent studies have provided compelling evidence that PYRs insecticides have created adverse health effects on children development. In contrast, using PYRs insecticides daily may become main pathway for PYRs exposure to children who play on the floor, do not wash hands/foots, and craw on the floor. Those factors may affect to neurotransmitter GABA concentrations. These findings should be further investigated with a longitudinal design and a larger study population. Hence, this study suggests that health education, raising awareness and strategies to reduce the risks from long-term PYR insecticide exposure of children living in households should be implemented to overcome the problems.

6.2 Limitations of this study

1. Parents do not have knowledge about PYR insecticide products. Finally, parents have knowledge about PYR insecticide product after interview.

2. This study area has mosquito prevented control by Bangkok Metropolitan Administration (BMA) every 2 months. It may interfere to 3-PBA metabolites and hand wipes sample analysis.

3. The concentration of GABA in children (age 2-3 years) is not stable. Because its base on children behavior and grow hormone.

4. This study does not have GABA concentration standard. Thus, this result was compared with others study from normal human.

6.3 Recommendations of this study

1. Further study should be conducted by applying a longitudinal design with a larger population.

2. This study will suggest parents that: 1) Provide education (such as specify the type of pesticide to be used). 2) increase awareness (such as PYR insecticides were associated with GABA concentration, may be affected to children). 3) Provide management (such as should keep away from children or keep it in box).

3. This study will suggest parents that: Do not allow children to touch the mosquito repellent areas.

4. This study will suggest parents to clean the floor every day.

5. Parents should wash children's hands more often.

6.4 Benefit of this study

1. This study found that 3-PBA metabolites associated with GABA concentration. It may affect to neurobehavior (such as ADHD).

2. This study found that 3-PBA metabolites associated with hand wipe sample. It may affect to children health (such as irritation).

3. This study are useful for more understanding of the PYR exposure pathway of children living in households.

- Ingestion (dietary habit such as food and drinking water)
- Dermal (contact variable such as put toy/object/hand to mouth)
- Inhalation (inhalation of smoke of coil insecticide product), Presented by 3-PBA metabolites from urine sample

APPENDIX

APPENDIX A

The ethical approval documents

AF 01-12

คณะกรรมการพิจารณาจริยธรรมการวิจัยในคน กลุ่มสหสถาบัน ชุดที่ 1 จุฬาลงกรณ์มหาวิทยาลัย
254 อาคารจามจุรี 1 ชั้น 2 ถนนพญาไท เขตปทุมวัน กรุงเทพฯ 10330
โทรศัพท์/โทรสาร: 0-2218-3202 E-mail: eccu@chula.ac.th

COA No. 290/2561

ใบรับรองโครงการวิจัย

โครงการวิจัยที่ 253.2/61	:	การสัมผัสสารกำจัดแมลงกลุ่มไพรีทรอยด์ที่ใช้ในบ้านเรือนต่อระดับไพรีทรอยด์เมตาโบไลท์และระดับกาบ้าของเด็กเล็กในเขตเมือง กรุงเทพมหานคร ประเทศไทย
ผู้วิจัยหลัก	:	นายเจษฎา คุณโน
หน่วยงาน	:	วิทยาลัยวิทยาศาสตร์สาธารณสุข จุฬาลงกรณ์มหาวิทยาลัย

คณะกรรมการพิจารณาจริยธรรมการวิจัยในคน กลุ่มสหสถาบัน ชุดที่ 1 จุฬาลงกรณ์มหาวิทยาลัย ได้พิจารณา โดยใช้หลัก ของ The International Conference on Harmonization – Good Clinical Practice (ICH-GCP) อนุมัติให้ดำเนินการศึกษาวิจัยเรื่องดังกล่าวได้

ลงนาม..	ลงนาม..
(รองศาสตราจารย์ นายแพทย์ปรีดา ทัศนประดิษฐ)	(ผู้ช่วยศาสตราจารย์ ดร.นันทรี ชัยชนะวงศาโรจน์)
ประธาน	กรรมการและเลขานุการ

วันที่รับรอง : 21 ธันวาคม 2561 **วันหมดอายุ** : 20 ธันวาคม 2562

เอกสารที่คณะกรรมการรับรอง

1) โครงการวิจัย
2) ข้อมูลสำหรับกลุ่มประชากรหรือผู้มีส่วนร่วมในการวิจัยและใบยินยอมของกลุ่มประชากรหรือผู้มีส่วนร่วมในการวิจัย
3) ผู้วิจัย
4) แบบสอบถาม

เลขที่โครงการวิจัย 253.2/61
วันที่รับรอง 21 ธ.ค. 2561
วันหมดอายุ 20 ธ.ค. 2562

<u>เงื่อนไข</u>

1. ข้าพเจ้ารับทราบว่าเป็นการผิดจริยธรรม หากดำเนินการเก็บข้อมูลการวิจัยก่อนได้รับการอนุมัติจากคณะกรรมการพิจารณาจริยธรรมการวิจัยฯ
2. หากใบรับรองโครงการวิจัยหมดอายุ การดำเนินการวิจัยต้องยุติ เมื่อต้องการต่ออายุต้องขออนุมัติใหม่ล่วงหน้าไม่ต่ำกว่า 1 เดือน พร้อมส่งรายงานความก้าวหน้าการวิจัย
3. ต้องดำเนินการวิจัยตามที่ระบุไว้ในโครงการวิจัยอย่างเคร่งครัด
4. ใช้เอกสารข้อมูลสำหรับกลุ่มประชากรหรือผู้มีส่วนร่วมในการวิจัย ใบยินยอมของกลุ่มประชากรหรือผู้มีส่วนร่วมในการวิจัย และเอกสารเชิญเข้าร่วมวิจัย (ถ้ามี) ***เฉพาะที่ประทับตราคณะกรรมการเท่านั้น***
5. หากเกิดเหตุการณ์ไม่พึงประสงค์ร้ายแรงในสถานที่เก็บข้อมูลที่ขออนุมัติจากคณะกรรมการ ต้องรายงานคณะกรรมการภายใน 5 วันทำการ
6. หากมีการเปลี่ยนแปลงการดำเนินการวิจัย ให้ส่งคณะกรรมการพิจารณารับรองก่อนดำเนินการ
7. โครงการวิจัยไม่เกิน 1 ปี ส่งแบบรายงานสิ้นสุดโครงการวิจัย (AF 03-12) และบทคัดย่อผลการวิจัยภายใน 30 วัน เมื่อโครงการวิจัยเสร็จสิ้น สำหรับโครงการวิจัยที่เป็นวิทยานิพนธ์ให้ส่งบทคัดย่อผลการวิจัย ภายใน 30 วัน เมื่อโครงการวิจัยเสร็จสิ้น

APPENDIX B

Questionnaire (English version)

Code ………………..

Date: ....

Exposure Questionnaire

General information

Relationship to child Surname .... Age ....

Part I Socio-demographic Characteristics

1. Age

   ( ) 2 years old ....month ( ) 3 years old .... month

2. Gender

   ( ) Male ( ) Female

3. Weight (kg) .... High (cm) ....

4. Current address: .... District, Bangkok

5. How long has your family lived in this region?

   .... (days / months/ years)

6. Have children ever had breastmilk?

   ( ) No ( ) Yes: Many much ....

Part II Factors related to children:

1. Father/Mother/Parent education level

   ( ) Illiterate ( ) Primary school ( ) Secondary school

   ( ) High school ( ) University and gradate ( ) Others ....

2. Main source of Father/Mother/Parent incomes

   ( ) Trade ( ) Contractors ( ) Government official

   ( ) Employee ( ) Others...............

Part III Factors related to PYR exposure:

1. What type of pyrethroids insecticide products that you use?

   ( ) Mosquito coil ( ) Mosquito repellent spray ( ) Mosquito t raps

   ( ) Mosquito gels ( ) Termite ( ) Others …………

2. Please indicate the names of type pyrethroids insecticide products you've used in your household?

Name of pyrethroids insecticide products	Frequency
…………………………………………………………	………………
…………………………………………………………	………………

3. Frequency of insecticide use in household (times/month)

   ( ) 1 time ( ) 2 times ( ) 3 times ( ) ≥ 4 times

4. Store for keep pyrethroids insecticide products

   ………………………………………………………………………………………………

5. Frequency of floor-cleaning

   ( ) Every day ( ) 2-3 day/time ( ) 4-5 day/time

   ( ) Once a week ( ) Never ( ) Others …………

6. How is the floor cleaned? (You can select more than one choice)

   ( ) Wet mop ( ) Broom ( ) Electrolux

   ( ) Others ……………

7. Frequency of open windows or doors

   The windows

   1) Morning: ( ) 06.00-08.00 AM. ( ) 08.00-10.00 AM ( ) 10.00-12.00 AM.

      ( ) all morning ( ) Others ……….. ( ) Never open

   2) Afternoon ( ) 12.00-14.00 PM. ( ) 14.00-16.00 PM. ( ) 16.00-18.00 PM.

      ( ) all afternoon ( ) Others ……….. ( ) Never open

The doors at

3) Morning: ( ) 06.00-08.00 AM. ( ) 08.00-10.00 AM ( ) 10.00-12.00 AM.

( ) all morning ( ) Others .... ( ) Never open

4) Afternoon ( ) 12.00-14.00 PM. ( ) 14.00-16.00 PM. ( ) 16.00-18.00 PM.

( ) all afternoon ( ) Others .... ( ) Never open

8. Frequency of insecticide control operators by officer

   ( ) every one month ( ) every 2 month

   ( ) Never ( ) others ....

Part IV Personal hygiene

1. How long does your child spend traveling outside ?

   ( ) every week ( ) every month ( ) Never ( ) Others ....

2. How long does your child spend traveling outside the home in the Hr/day ?

   ( ) ≤ 1 hr. ( ) 2 -3 hrs. ( ) ≥ 4 hrs.

   ( ) Never ( ) Others ....

3. Where is your child go to travel outside

   Locations

   1) ....

   2) ....

4. How long does your child play at household floor in the day?

   ( ) ≤ 1 hr. ( ) 2 -3 hrs. ( ) ≥ 4 hrs.

   ( ) Never ( ) Others ....

5. Where does your child spend time for playing in the day?

   (You can select more than one choice)

( ) Floor indoor ( ) Bedroom ( ) Living room

( ) Outside at home ( ) Others ....

6. How many time does your child play dirt or soil in the day?

   ( ) 1 – 2 times ( ) 3 – 5 times ( ) ≥ 6 times ( ) Never

7. How many time does your child wash his/her hands in the day?

   ( ) 1 – 2 times ( ) 3 – 5 times ( ) ≥ 6 times ( ) Never

8. How many time does your child wash foot in the day?

   ( ) 1 – 2 times ( ) 3 – 5 times ( ) ≥ 6 times ( ) Never

9. How many time does your child take a shower or bath in the day?

   ( ) 1 – 2 times ( ) 3 – 5 times ( ) ≥ 6 times ( ) Never

10. How frequently does your child walk barefeet inside household

   ( ) ≥ 11 times ( ) 6 – 10 times ( ) 1 – 5 times ( ) Never

11. How frequently does your child walk barefeet outside household

   ( ) ≥ 11 times ( ) 6 – 10 times ( ) 1 – 5 times ( ) Never

12. How frequently does your child has an illness in the past sixth months?

   ( ) ≥ 6 times ( ) 3 -5 times ( ) 1 – 2 times ( ) Never

Part V Contact variable behavior:

Contact variable behavior		Frequency/day			
		Often (≥ 11 times)	Sometime (6 – 10 times)	Almost never (1 – 5 times)	Never (0 time)
1	How frequently does your child catch or touch furniture to mouth (table, chair or empty space etc.)?				
2	How frequently does your child catch or touch bottle/dish to mouth?				
3	How frequently does your child put object to mouth (spoon, wall papers or something nearly children etc.)?				
4	How frequently does your child put hand to mouth?				
5	How frequently does your child put hands or suck fingers into mouth in the day?				
6	How frequently does your child put hand on the floor to mouth?				
7	How frequently does your child put toy to mouth (football etc.)?				
8	How frequently does your child catch or touch hair to mouth?				
9	How frequently does your child catch or touch skin to mouth?				
10	How frequently does your child catch or touch clothes to mouth?				
11	How frequently does your child catch or touch Grass/vegetation to mouth (orange, cucumber, carrot and grape etc.)?				
12	How frequently does your child crawling across on the floor?				

Part VI Home Characteristics:

1. Type of household

   ( ) Contemporary structure ( ) Permanent structure

   ( ) Condominium ( ) Apartment

   ( ) Other ....

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

   ( ) Cement floor ( ) Laminate floor

   ( ) Tile floor ( ) Dirt floor

3. Direction of your front household

   ( ) West ( ) East ( ) South ( ) North ( ) Other ....

4. Reported pesticide application in the last 1 months

   ( ) 1. Yes ( ) 2. No

5. Reported insecticide sighting in the home

   ( ) Mosquito ( ) Cockroach ( ) Ant

   ( ) Termite ( ) Others ....

6. Are you raising animals?

   ( ) Yes: What kind of animals ....

   ( ) No

7. How often do you use shampoo to get rid of ticks?

   ( ) Every day ( ) Every 3 days ( ) Every 5 days

   ( ) Every week ( ) Every mouth ( ) Never

   ( ) Others ....

Part VII Dietary habit:

1. How many of your child spend time for eat outside the household

   ( ) Everyday ( ) 2-3 times/week ( ) 1 time/week ( ) Never

   ( ) Others ....

2. How many of your child spend time for eat on the floor inside the household

   ( ) Everyday ( ) 2-3 times/week ( ) 1 time/week ( ) Never

   ( ) Others ....

3. How many of your child wash hands before eating

   ( ) Everyday ( ) Never ( ) Others ....

4. Vegetable washing soaking time

   ( ) Everyday ( ) Never ( ) Others ....

5. Fruits washing soaking time

   ( ) Everyday ( ) Never ( ) Others ....

6. Fruits peeled

   ( ) Everyday ( ) 2-3 times/week ( ) 1 time/week ( ) Never

   ( ) Others ....

Part VI Home Characteristics:

1. Type of household

   ( ) Contemporary structure ( ) Permanent structure

   ( ) Condominium ( ) Apartment

   ( ) Other ....

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

   ( ) Cement floor ( ) Laminate floor

   ( ) Tile floor ( ) Dirt floor

3. Direction of your front household

   ( ) West ( ) East ( ) South ( ) North ( ) Other ....

4. Reported pesticide application in the last 1 months

   ( ) 1. Yes ( ) 2. No

5. Reported insecticide sighting in the home

   ( ) Mosquito ( ) Cockroach ( ) Ant

   ( ) Termite ( ) Others ....

6. Are you raising animals?

   ( ) Yes: What kind of animals ....

   ( ) No

7. How often do you use shampoo to get rid of ticks?

   ( ) Every day ( ) Every 3 days ( ) Every 5 days

   ( ) Every week ( ) Every mouth ( ) Never

   ( ) Others ....

Part VII Dietary habit:

1. How many of your child spend time for eat outside the household

   ( ) Everyday ( ) 2-3 times/week ( ) 1 time/week ( ) Never

   ( ) Others ....

2. How many of your child spend time for eat on the floor inside the household

   ( ) Everyday ( ) 2-3 times/week ( ) 1 time/week ( ) Never

   ( ) Others ....

3. How many of your child wash hands before eating

   ( ) Everyday ( ) Never ( ) Others ....

4. Vegetable washing soaking time

   ( ) Everyday ( ) Never ( ) Others ....

5. Fruits washing soaking time

   ( ) Everyday ( ) Never ( ) Others ....

6. Fruits peeled

   ( ) Everyday ( ) 2-3 times/week ( ) 1 time/week ( ) Never

   ( ) Others ....

APPENDIX C

Questionnaire (Thai version)

รหัส ....

วันที่: ....

แบบสอบถามเกี่ยวกับการรับสัมผัสสารกำจัดแมลงในบ้านเรือน

ข้อมูลผู้ตอบแบบสอบถาม

ความสัมพันธ์กับเด็ก .... อายุ..

ส่วนที่ 1 ข้อมูลทั่วไป

1. เด็กอายุ

   ( ) 2 ขวบ...........เดือน ( ) 3 ขวบ................ เดือน

2. เพศ

   ( ) ชาย ( ) หญิง

3. น้ำหนัก (กิโลกรัม) .... ส่วนสูง (เซนติเมตร) ....
4. ที่อยู่ปัจจุบัน: แขวง .... เขต .... กรุงเทพมหานคร
5. เด็กอาศัยที่บ้านนี้นานเท่าไหร่ .... (ปี/เดือน)
6. เด็กเคยได้ดื่มนมแม่หรือไม่

   ( ) ไม่ใช่ ( ) ใช่ จำนวน .... เดือน

ส่วนที่ 2 ข้อมูลที่เกี่ยวข้องความสัมพันธ์กับเด็ก:

1. ท่านได้รับการศึกษาสูงสุดระดับใด

   ( ) ไม่ได้เรียน ( ) ประถมศึกษา ( ) มัธยมศึกษาตอนต้น

   ( ) มัธยมศึกษาตอนปลาย ( ) ปริญญาตรี ( ) อื่นๆ ....

2. อาชีพหลักของท่านคืออะไร

   ( ) ค้าขาย ( ) รับจ้างทั่วไป ( ) รับราชการ

   ( ) พนักงานเอกชน ( ) อื่นๆ ....

ส่วนที่ 3 ข้อมูลที่เกี่ยวข้องกับการสัมผัสสารกำจัดแมลงในบ้านเรือน:

1. บ้านของท่านใช้สารกำจัดแมลงแบบไหน (ตอบได้มากกว่า 1 ข้อ)

   ( ) ยากันยุงแบบม้วน ( ) ยากันยุงแบบพ่น ( ) เครื่องดักยุง

   ( ) ยากันยุงแบบเจล ( ) ยาฆ่าปลวก ( ) อื่นๆ ....

2. โปรดระบุชื่อสารกำจัดแมลงที่ท่านใช้ภายในบ้านเรือน

ชื่อสารกำจัดแมลง (ยี่ห้อ)	ประเภท	ความถี่ในการใช้/เดือน
....	....	....
....	....	....

3. ท่านใช้สารกำจัดแมลงบ่อยแค่ไหน (ครั้ง/เดือน)

   ( ) 1 ครั้ง ( ) 2 ครั้ง ( ) 3 ครั้ง ( ) 4 ครั้ง ( ) อื่นๆ ....

4. ท่านเก็บสารกำจัดแมลงไว้บริเวณใดของบ้าน

   ....

5. ท่านทำความสะอาดพื้นบ้านบ่อยแค่ไหน

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

   ( ) สัปดาห์ละครั้ง ( ) 5. ไม่เคย ( ) อื่น ....

6. ท่านได้ใช้อุปกรณ์ใดทำความสะอาดพื้นบ้าน (สามารถเลือกได้มากกว่า1 ข้อ)

   ( ) ผ้าเปียก ( ) ไม้กวาด ( ) เครื่องดูดฝุ่น

   ( ) อื่น ....

7. ท่านเปิดประตูหรือหน้าต่างบ้านของท่านบ่อยและนานแค่ไหน (สามารถเลือกได้มากกว่า 1 ข้อ)

   เปิดหน้าต่าง

   ช่วงเช้า: ( ) 06.00-08.00 น. ( ) 08.00-10.00 น. ( ) 10.00-12.00 น.

   ( ) ทั้งวัน ( ) อื่นๆ .... ( ) ไม่เคยเปิด

   ช่วงบ่าย: ( ) 12.00-14.00 น. ( ) 14.00-16.00 น. ( ) 16.00-18.00 น.

( ) ทั้งบ่าย ( ) อื่นๆ........... ( ) ไม่เคยเปิด

เปิดประตู

ช่วงเช้า: ( ) 06.00-08.00 น. ( ) 08.00-10.00 น. ( ) 10.00-12.00 น.

( ) ทั้งวัน ( ) อื่นๆ .... ( ) ไม่เคยเปิด

ช่วงบ่าย: ( ) 12.00-14.00 น. ( ) 14.00-16.00 น. ( ) 16.00-18.00 น.

( ) ทั้งบ่าย ( ) อื่นๆ........... ( ) ไม่เคยเปิด

8. เจ้าหน้าที่สาธารณสุขมีการพ่นยากันยุงในหมู่บ้านของท่านบ่อยแค่ไหน

( ) เดือนละครั้ง ( ) เดือนละ 2 ครั้ง

( ) 2 เดือนครั้ง ( ) ไม่มี ( ) อื่นๆ ....

ส่วนที่ 4 ข้อมูลเกี่ยวกับสุขลักษณะของเด็ก:

1. ท่านพาบุตรของท่านออกไปเที่ยวหรือเล่นนอกบ้านบ่อยแค่ไหน

( ) ทุกๆสัปดาห์ ( ) ทุกๆเดือน ( ) ไม่เคย ( ) อื่นๆ ....

2. บุตรของท่านใช้เวลาเล่นบริเวณนอกบ้านบ่อยแค่ไหน (ชั่วโมง/วัน)

( ) ≤ 1 ชั่วโมง ( ) 2 -3 ชั่วโมง ( ) ≥ 4 ชั่วโมง

( ) ไม่เคย ( ) อื่นๆ ....

3. สถานที่ข้างนอกที่บุตรของท่านใช้เวลาเล่นบ่อยที่สุด

ชื่อสถานที่

1) ....

2) ....

4. บุตรของท่านใช้เวลาในการเล่นบริเวณพื้นบ้านบ่อยแค่ไหนต่อวัน

( ) ≤ 1 ชั่วโมง ( ) 2 -3 ชั่วโมง ( ) ≥ 4 ชั่วโมง

( ) ไม่เคย ( ) อื่นๆ ....

5. บุตรของท่านใช้เวลาในการเล่นแต่ละวันบริเวณพื้นที่ใดบ้าง (เลือกได้มากกว่า 1 ข้อ)

( ) บนพื้นในบ้าน ( ) บนพื้นห้องนอน ( ) บนพื้นห้องนั่งเล่น

( ) บริเวณระเบียงนอกบ้าน ( ) อื่นๆ ....

6. บุตรของท่านเล่นดินบริเวณบ้านบ่อยแค่ไหนต่อวัน

( ) 1 – 2 ครั้ง ( ) 3 – 5 ครั้ง ( ) ≥ 6 ครั้ง ( ) ไม่เคย

7. บุตรของท่านล้างมือบ่อยแค่ไหนต่อวัน

( ) 1 – 2 ครั้ง ( ) 3 – 5 ครั้ง ( ) ≥ 6 ครั้ง ( ) ไม่เคย

8. บุตรของท่านล้างเท้าบ่อยแค่ไหนต่อวัน

( ) 1 – 2 ครั้ง ( ) 3 – 5 ครั้ง ( ) ≥ 6 ครั้ง ( ) ไม่เคย

9. บุตรของท่านอาบน้ำบ่อยแค่ไหนต่อวัน

( ) 1 – 2 ครั้ง ( ) 3 – 5 ครั้ง ( ) ≥ 6 ครั้ง ( ) ไม่เคย

10. บุตรของท่านเดินเท้าเปล่าบ่อยแค่ไหนบริเวณภายในบ้าน

( ) ≥ 11 ครั้ง ( ) 6 – 10 ครั้ง ( ) 1 – 5 ครั้ง ( ) ไม่เคย

11. บุตรของท่านเดินเท้าเปล่าบ่อยแค่ไหนบริเวณนอกบ้าน

( ) ≥ 11 ครั้ง ( ) 6 – 10 ครั้ง ( ) 1 – 5 ครั้ง ( ) ไม่เคย

12. บุตรของท่านมีอาการเจ็บป่วยบ่อยแค่ไหนในช่วง 6 เดือนที่ผ่านมา

( ) ≥ 6 ครั้ง ( ) 3 – 5 ครั้ง ( ) 1 -2 ครั้ง ( ) ไม่เคย

ส่วนที่ 5 ข้อมูลเกี่ยวกับพฤติกรรมเด็ก:

ข้อมูลเกี่ยวกับพฤติกรรมเด็ก		ความถี่/วัน			
		บ่อย (≥ 11 ครั้ง)	บางครั้ง (6 – 10 ครั้ง))	ค่อนข้างน้อย (1 – 5 ครั้ง))	ไม่แคย (0 ครั้ง))
1	บุตรของท่านสัมผัสเฟอร์นิเจอร์เข้าปากบ่อยแค่ไหนต่อวัน (โต๊ะ เก้าอี้ ชั้นว่างของ เป็นต้น)				
2	บุตรของท่านสัมผัสหรือหยิบขวดน้ำหรือขวดนมเข้าปากบ่อยแค่ไหนต่อวัน				
3	บุตรของท่านสัมผัสหรือหยิบสิ่งของทั่วไป เข้าปากบ่อยแค่ไหนต่อวัน (ช้อน กระดาษ หรือสิ่งของที่อยู่ใกล้ๆเด็ก เป็นต้น)				
4	บุตรของท่านสัมผัสหรือเอาแขนเข้าปากบ่อยแค่ไหนต่อวัน				
5	บุตรของท่านสัมผัสหรือเอานิ้วมือข้าปากบ่อยแค่ไหนต่อวัน				
6	บุตรของท่านสัมผัสบริเวณพื้นบ้านแล้วเอานิ้วมือเข้าปากบ่อยแค่ไหนต่อวัน				
7	บุตรของท่านสัมผัสหรือหยิบของเล่นเข้าปากบ่อยแค่ไหนต่อวัน (ของเล่นที่เด็กชอบเล่น เช่น ลูกบอล เป็นต้น)				
8	บุตรของท่านสัมผัสหรือเอาเส้นผมตัวเองเข้าปากบ่อยแค่ไหนต่อวัน				
9	บุตรของท่านสัมผัสผิวหนังแล้วเอานิ้วเข้าปากบ่อยแค่ไหนต่อวัน				
10	บุตรของท่านสัมผัสเสื้อผ้าแล้วเอานิ้วเข้าปากบ่อยแค่ไหนต่อวัน				
11	บุตรของท่านสัมผัสหรือหยิบผักและผลไม้สดเข้าปากบ่อยแค่ไหนต่อวัน (ส้ม แตงกวา แครอท แอปเปิล องุ่น เป็นต้น)				
12	บุตรของท่านคลานบนพื้นบ่อยแค่ไหนต่อวัน				

ส่วนที่ 6 ข้อมูลเกี่ยวกับที่พักอาศัย:

1. ลักษณะของบ้านพักท่านเป็นอย่างไร

   ( ) สิ่งปลูกสร้างชั่วคราว .... ชั้น ( ) สิ่งปลูกสร้างถาวร .... ชั้น

   ( ) คอนโดมิเนี่ยม .... ชั้น ( ) อพาร์ทเม้นท์ .... ชั้น

   ( ) อื่นๆ ....

2. ลักษณะพื้นที่พักอาศัยของท่านเป็นแบบไหน

   ( ) พื้นซีเมน ( ) พื้นไม้ลามิเนต

   ( ) พื้นกระเบื้องเซรามิก ( ) อื่นๆ ....

3. ทิศทางหน้าบ้านหรือที่พักอาศัยของท่าน

   ( ) ทิศตะวันตก ( ) ทิศตะวันออก ( ) ทิศใต้ ( ) ทิศเหนือ ( ) อื่น ....

4. ประวัติการเคยใช้ยากำจัดแมลงบริเวณบ้านของท่าน ภายใน 1 เดือนที่ผ่านมา

   ( ) ใช้ ( ) ไม่ใช้

5. ประวัติการเคยใช้ยากำจัดแมลงบริเวณบ้านของท่าน (เลือกได้มากกว่า 1 ข้อ)

   ( ) ยากันยุง ( ) ยาฆ่าแมลงสาบ ( ) ยาฆ่ามด

   ( ) ยาฆ่าปลวก ( ) อื่นๆ ....

6. ที่บ้านพักอาศัยของท่านเลี้ยงสัตว์หรือไม่

   ( ) เลี้ยง: สัตว์อะไร ....

   ( ) ไม่เลี้ยง

7. ท่านได้ใช้แชมพูกำจัดเห็บ หมัดให้สัตว์เลี้ยง บ่อยแค่ไหน (กรณีที่เลี้ยงสัตว์ในบ้านพักอาศัย)

   ( ) ทุกวัน ( ) 3 วัน/ครั้ง ( ) 5 วัน/ครั้ง

   ( ) สัปดาห์ละครั้ง ( ) เดือนละครั้ง ( ) ไม่เคย

   ( ) อื่น ....

ส่วนที่ 7 ข้อมูลเกี่ยวกับพฤติกรรมการกินของเด็ก:

1. บุตรของท่านรับประทานอาหารนอกบ้านบ่อยแค่ไหน

   ( ) ทุกวัน ( ) สัปดาห์ละ 2 -3 ครั้ง ( ) สัปดาห์ละ 1 ครั้ง

   ( ) ไม่เคย ( ) อื่น ๆ ....

2. บุตรของท่านรับประทานอาหารบนพื้นบ้านบ่อยแค่ไหน

   ( ) ทุกวัน ( ) สัปดาห์ละ 2 -3 ครั้ง ( ) สัปดาห์ละ 1 ครั้ง

   ( ) ไม่เคย ( ) อื่น ๆ ....

3. บุตรของท่านล้างมือก่อนรับประทานอาหารบ่อยแค่ไหน

   ( ) ทุกครั้ง ( ) ไม่เคย ( ) อื่นๆ ....

4. ท่านล้างผักให้บุตรของท่านก่อนรับประทานบ่อยแค่ไหน

   ( ) ทุกครั้ง ( ) ไม่เคย ( ) อื่นๆ ....

5. ท่านล้างผลไม้ให้บุตรของท่านก่อนรับประทานบ่อยแค่ไหน

   ( ) ทุกครั้ง ( ) ไม่เคย ( ) อื่นๆ ....

6. บุตรของท่านรับประทานผักและผลไม้ออแกนิคบ่อยแค่ไหน

   ( ) ทุกวัน ( ) สัปดาห์ละ 2 -3 ครั้ง ( ) สัปดาห์ละ 1 ครั้ง

   ( ) ไม่เคย ( ) อื่น ๆ ....

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