CHAPTER 3

MATERIALS AND METHODS

3.1 Apparatus

(1) Digestion block, Model DS-40, Tecator Technology, Denmark

(2) Eluting vacuum, VacElut SPS-24, Varian, USA

(3) Harmony mixture, UZUSIO VTX-3000L, Vertex,

(4) Homogenizer, IKA ULTRA-TURRAX T25 digital, staufen, Germany.

(5) Gas chromatograph (HewlettePackard 6890) equipped with electron capture detector (ECD), the separation was accomplished by capillary column (HP-5 (5% phenylmethylpolysiloxane with 30 m × 0.25 mm, 0.25 μm film thickness), and computerized data handling system (HP3365 series chemstation)

(6) Glass tube with Teflon lined screw caps 16 x 26 mm

(7) Micro pipette, Pipetman P20, P100, P200, P1000 and P5000, Gilson, France

(8) Oak Ridge Teflon centrifuge tubes FEP, 50 mL, NALGENE®

(9) Rotavaper, R-210, Buchi, Germany

(10) Ultrasonic cleaner, Cavitator, Mettler, USA

3.2 Chemicals

(1) Acetone, grade for organic residue analysis, JT.Baker, USA

(2) Acetonitrile, grade for organic residue analysis, JT.Baker, USA

(3) Anhydrous sodium sulfate (Na_2SO_4), Pro analysis, Merck, Germany

(4) Bond-elute cartridge, octadecyl (C_{18}), 500 mg, Varian, USA

(5) Ethyl acetate, grade for organic residue analysis, JT.Baker, USA

(6) Activated carbon , modified from carbon/PSA cartridge

(7) Hexane, grade for organic residue analysis, JT.Baker, USA

(8) Hydrochloric acid (HCl), Pro analysis, Merck, Germany

(9) Methylene chloride , grade for organic residue analysis, JT.Baker, USA

(10) Methanol, grade for organic residue analysis, JT.Baker, USA

(11) *N,N'*-Diisopropylcarbodiimide, purum,≥99.8%, Fluka, Germany

(12) Pentafluorobenzyl bromide (PFBBr), purum,≥99.8%,, Fluka, Germany

(13) Potassium carbonate (K_2CO_3), Pro analysis, Merck, Germany

(14) Reference synthetic pyrethroid standard from Laboratory of Dr. Ehrenstorfer GMbH, Asuberg, Germany :

1) Lambda cyhalothrin ($C_{23}H_{19}ClFeNO_3$) 98.0%

2) Bifenthrin ($C_{23}H_{22}ClF_3O_2$) 99.5%

3) Permethrin ($C_{21}H_{20}Cl_2O_3$) 99.9%

4) Cyfluthrin ($C_{22}H_{18}Cl_2FNO_2$) 94.5%

5) Cypermethrin ($C_{22}H_{19}ClNO_3$) 92.5%

6) Fenvalerate ($C_{25}H_{22}ClNO_3$) 99.5%

7) Deltamethrin ($C_{22}H_{19}Br_2NO_3$) 99.0%

(15) Sodium chloride (NaCl) , Pro analysis, Merck, Germany

(16) Sodium hydrogen carbonate ($NaHCO_3$), Pro analysis, Merck, Germany

(17) SAX/PSA (6 mL), 1 g, International Sorbent Technology (IST), UK

(18) Tert bytyl methyl ether, grade for organic residue analysis, Merck, Germany

(19) Toluene, grade for organic residue analysis, JT.Baker, USA

(20) 1,1,1,3,3,3-Hexafluoroisopropanol puriss.p.a., for GC,≥99.8%, Fluka, Germany

(21) 2 Phenoxybenzoic Acid, ≥99.8%, Fluka, Germany

(22) 3 Phenoxybenzoic Acid, ≥99.8%, Fluka, Germany

(23) Filter paper No.1, Whatman, USA

(24) High purity helium gas (99.999%), TIG, Bangkok, Thailand

(25) Ultra high purity of nitrogen gas (99.999%), TIG, Bangkok, Thailand

3.3 Scope of study

3.3.1 Study area

1) Field study

This study was conduced in an agricultural community in 4 subdistricts (Mon Pin subdistrict, Mae Kha subdistrict, Mae Ngon subdistrict, and Wiang subdistrict) of the study areas. Fang district, Chiang Mai province, Thailand.

2) Laboratory

This study used laboratory equipment and analysis at the Center for Pollution and Environmental Health Research, Research Institute for Health Sciences, Chiang Mai University

3.3.2 Studied populations

1) Fruits and vegetables sampling were purchased from local markets during the rainy season in June 2009 and the second winter, in March 2010 to detect synthetic pyrethroid insecticides. The samples were obtained from the vegetable and fruit. By choosing to eat difference kinds of student eat the most. For the fourth subdistricts includes, Wiang subdistrict collected from Fang Kanlaya market and Chock Thane market, Mae Kha subdistrict collected from the Mae Kha market, Mae Nong subdistrict collected from Mae Nong market and Nong Yaw market, and Mon Pin subdistrict sampling from Pa Bong market.

In June 2009, samples collected 35 samples of six kinds of vegetable and a total of 21 samples of five kinds of fruits. In March 2010, samples collected 31 samples of 8 kinds of vegetables and a total of 22 samples of six kinds of fruits.

2) Urine samples were collected from students during the rainy season in June 2009 (Semester 1) and the second winter, in March 2010 to detect 3 PBA. For the fourth subdistricts includes, Wiang subdistrict collected from Ban San Sai Klong Noi school, and Rangsri Witaya school, Mae Kha subdistrict collected from Ban Lai Fang school and Ban Mea Kha school, Mae Nong subdistrict collected from Ban Mae Luang Uppathum school, Ban Tung Lung school, and Ban Mae Ngong Ke Lek school, and Mon Pin subdistrict collected from Ban Lan school, Ban Muang Chum school. Number of urine samples collected from school children, in June 2009, all of 290 samples and total of 285 samples collected in March 2010.

3.4 Methodology

3.4.1 Methodology of section 1:

Development of method for detecting synthetic pyrethroid residues

Development of a method for detecting synthetic pyrethroid residues in vegetables and fruits by using GC-ECD were included lambda cyhalothrin, cyfluthrin, cypermethrin, fenvalerate and deltamethrin. This experiment has been developed for analysis of residues of individual synthetic pyrethroid compounds in variety of vegetables and fruits. To reach optimize conditions and evaluate further, various extraction solvents and partitioning conditions were examined. Primarily study for

limit of detection (LOD) levels of each synthetic pyrethroid was obtained by injection pyrethroid at different concentration to determine the minimum value that GC-ECD can detect. Four crops were selected as representative of high matrix enhanced are composed of kale, cabbage, longan and orange. The samples were fortified with synthetic pyrethroid and analyzed at three different levels (low, medium and high levels) show in Table 3.1. Therefore, the determinations of effectiveness to extract clean-up by using various solid phase extraction (SPE) cartridges were investigated to reduce the matrix effect from the vegetable and fruit samples.

Table 3.1 different levels of fortified with synthetic pyrethroid for analyzed

Synthetic pyrethroid	Difference concentration (ng/mL)		
	Low	Medium	High
lambda cyhalothrin	10	20	50
permethrin	5	10	20
cyfluthrin	20	50	100
cypermethrin	5	10	20
fenvalerate	20	50	100
Deltamethrin	20	50	100

3.4.1.1 Analysis of synthetic pyrethroid pesticides

(1) Preparation of synthetic pyrethroid stocks standard were stepped as follow:

1) All volumetric flasks were cleaned 3 times with isooctane and rinsed with 3 times methanol and 3 times acetone.

2) Individual synthetic pyrethroids were prepared approximate 1 mg/mL in ethyl acetate (0.01g/10mL).

3) Individual intermediate and working standards were prepared from stock standards and the series of working standard mixture has been presented in Table 3.2.

Table 3.2 Series of working standard mixture (M_1-M_8) of synthetic pyrethroid insecticides (ng/mL in ethyl acetate)

Synthetic pyrethroid	M_1	M_2	M_3	M_4	M_5	M_6	M_7	M_8
lambda cyhalothrin	10	20	50	100	150	200	250	300
permethrin	5	10	20	50	100	150	200	250
cyfluthrin	20	50	100	150	200	250	300	500
cypermethrin	5	10	20	50	100	150	200	250
fenvalerate	20	50	100	150	200	250	300	500
Deltamethrin	20	50	100	150	200	250	300	500
bifenthrin (IS)	20	20	20	20	20	20	20	20

IS = internal standard

(2) Preparation of a calibration curve

Standard stock solution intermediate and working standard solutions of a mixture of synthetic pyrethroids and internal standard solution were prepared in ethyl

acetate and stored at -20°C until analysis. Eight serial dilutions (5, 10, 20, 100, 150, 200, 250 and 300 ng/mL) were prepared from stock solution and 20 ng/mL of bifenthrin which is the appropriate amounts of internal standard stock solution were added. One µl of solutions were injected and analyzed by GC-ECD for three times at each concentration level and determined from their areas of peak.

3.4.1.2 Selection of the extraction solvent

In the process of the solvent to extract the fruit and vegetables using the method of (fillion, 2000). Weigh 20 g sample of the extraction solvent 50 mL and then clean up with a carbon/amine cartridge. Extraction-partition solvents are depending on the substance to analyze and commodity extraction. In this study, the extraction efficiency of ethyl acetate, acetonitrile, acetone, and dichloromethane were compared. The efficiency of the extraction solvent was checked by recovery value.

3.4.1.3 Effectiveness of extraction clean up procedure for eliminating the interference in sample

In the study of clean up process, the clean up procedure using the modified method. Weigh 5 g sample extracted with dichloromethane and clean up with a variety of cartridge. Sample clean up should be considered to improve compatibility of samples to GC analysis and reduce matrix effect. In this study, the effectiveness of various solid-phase extraction cartridges to clean up sample which comprise SAX/PSA, activated carbon, and C_{18} were determined.

3.4.1.4 To validate the method for determining synthetic pyrethroid

For limit of detection (LOD) levels, tests were repeated with dichloromethane as extraction solvent.

(1) Preparation of spiked samples

Pooled samples were prepared from fruit and vegetable collected from untreated commodities. Samples of vegetables and fruit are blender, then all kinds. Samples collected during the same period. Vegetable and fruit samples were weighed in the rate of 1:1 by weight, depending on the number of samples collected for analysis. Samples were homogenized and stored at -20°C until analysis. For recovery experiment, an aliquot of standard mixture solution was added to a 5 g defrosted pool sample. The spiked sample matrix was allowed to stand for one hour before extraction.

(2) Qualitative and quantitative analysis of synthetic pyrethroid insecticides

Individual of synthetic pyrethroid were qualified by the retention time and quantified by peak area.

(3) Quality control of synthetic pyrethroids' analysis

1) Levels of detection

Limit of detection (LOD) and limit of quantification (LOQ) were analyzed. Series of working standard mixtures include M_1, M_2, M_3, and M_4 were analyzed 5 times for calculation of the standard deviation (S.D.). Synthetic pyrethroid level (X axis) and S.D. (Y axis) of individual series were plotted as a linear curve for determining Y-intercept. Limit of detection was calculated as described below:

Limit of detection (LOD) = 3 x Y-intercept

Limit of quantification (LOQ) = 10 x Y-intercept

2) Percent of recovery

Pool samples were unspiked and spiked with known level of individual synthetic pyrethroid in some samples for analysis of recovery. It was calculated as described below:

Recovery (%) = ((Cs – Cu) / Ck) x 100

Where: Cs = the individual pyrethroid levels in spike vegetable (mg/kg)

Cu = the individual pyrethroid levels in unspike vegetable (mg/kg)

Ck = the known spiked levels in individual pyrethroid (mg/kg)

3) Intra- and inter- batch variation

For intra batch analysis, five replicates of pooled samples were analyzed at the same time for calculating average recovery, S.D. and coefficient of variation (% CV) of synthetic pyrethroid insecticides.

For inter batch analysis, at least one aliquot pooled sample was applied in each batch (n=10) and analyzed the synthetic pyrethroid levels for monitoring synthetic pyrethroid variation.

3.4.1.5 Effectiveness of extraction of synthetic pyrethroid insecticide residues in various matrices

This studied to test the ability to extract of synthetic pyrethroid insecticide residues in fruits and vegetable with the variety of color and other elements. The researcher then tested the fruits and vegetables with difference colors. In spite of the dark color is orange, and kale. And colorless are cabbage and longan.

3.4.1.6 Effectiveness of extraction of synthetic pyrethroid insecticide residues in rugged test

This studied, test the rugged of the extraction, three scientist determined that the three samples were extracted by the deleloped extraction process, all three of samples obtained were analyzed by GC-ECD injection by the same person.

3.4.2 Methodology of section 2:

Develop a method for detecting a synthetic pyrethroids' metabolite in urine

3.4.2.1 Analysis of synthetic pyrethroid metabolites

(1) Preparation of synthetic pyrethroids' metabolite stock standard were stepped as follows:

1) All volumetric flasks were cleaned with 3 times isooctane and rinsed with 3 times methanol and 3 times acetone.

.2) 3-PBA and 2-PBA were prepared 1 mg/mL in acetonitrile (0.01g/10mL).

3) Individual intermediate and working standards were prepared from stock standards and series of working standard mixture are presented in Table 3.3.

Table 3.3 Series of working standard mixture (M_1-M_8) of synthetic pyrethroid metabolite (ng/mL in isooctane)

Synthetic pyrethroid metabolite	M_1	M_2	M_3	M_4	M_5	M_6	M_7	M_8
3-PBA	0.5	1	10	20	50	100	200	300
2-PBA (IS)	20	20	20	20	20	20	20	20

IS = internal standard

(2) Preparation of calibration curve

Standard stock solution were prepared in acetonitrile and stored at -20°C. Intermediate and working standard solutions (3-PBA) and internal standard (2-PBA) solution was prepared in isooctane. Eight serial dilutions (0.5, 1, 10, 20, 50, 100, 200, and 300 ng/mL) were prepared from stock solution and the appropriate amounts of internal standard stock solution were added 20 ng/mL These solutions were analyzed by GC-ECD for three times at each concentration level and determined from their areas of peak.

3.4.2.2 Selection of the extraction solvent

Extraction-partition solvents are depending on the substance to analyze and commodity extraction. In this study, the extraction efficiency of hexane, *tert*-butyl methy ether (*t*-BME), acetone, and dichloromethane were compared. The efficiency of the extraction solvent was checked by recovery value.

3.4.2.3 To compare the effectiveness of extraction clean up

Effectiveness of various solid-phase extraction cartridges to clean up sample which comprise SAX/PSA, activated carbon, and C_{18} were determined.

3.4.2.4 To validate the method for determining synthetic pyrethroid

For limit of detection (LOD) levels, tests were repeated with *tert*-butyl methyl ether as extraction solvent.

(1) Qualitative and quantitative analysis of synthetic pyrethroid

3-PBA was qualified by the retention time and quantified by peak area. 2-PBA solution was used an internal standard

(2) Quality control of Pyrethroid analysis

1) Levels of detection

Limit of detection (LOD) and limit of quantification (LOQ) were analyzed. Series of working standard mixture include M_1, M_2, M_3, and M_4 were analyzed 5 times for calculation of the standard deviation (S.D.). Synthetic pyrethroid level (X axis) and S.D. (Y axis) in individual series were plotted as a linear curve for determining Y-intercept. Limit of detection was calculated as described below:

Limit of detection (LOD) = 3 x Y-intercept

Limit of quantification (LOQ) = 10 x Y-intercept

2) Percent of recovery

Pooled samples were unspiked and spiked with known level of 3-PBA in some samples for analysis of recovery. It was calculated as described below:

Recovery (%) = ((Cs – Cu) / Ck) x 100

Where: Cs = the individual 3 PBA levels in spike urine (ng/mL)

Cu = the individual pyrethroid levels in unspike urine (ng/mL)

Ck = the known spiked levels in pyrethroid metabolite (ng/mL)

3) Intra- and inter- batch variation

For intra batch analysis, sixteen replicates of pooled samples were analyzed at the same time for calculating average recovery, S.D. and coefficient of variation (%CV) of synthetic pyrethroid insecticides.

For inter batch analysis, at least one aliquot pooled sample was applied in each batch (n=8) and analyzed the synthetic pyrethroid levels for monitoring synthetic pyrethroid variation.

3.4.3 Methodology of research section 3:

Assess the exposure of synthetic pyrethroid insecticides among school children in agricultural community

3.4.3.1 Environmental monitoring

(1) Environmental sample

Fruits and vegetables were purchased from local markets in 4 subdistricts of the study areas. The vegetables in experiment were included cabbage, kale, water spinach, cauliflower, chinese cabbage, chinese mustard, yard long bean, cucumber, and sugar pea, while the fresh fruits included tangerine, guava, apple, dragon fruit, mango, sand pear, lychee, rose apple and grape.

The sample weight was at least one kilogram for small and medium size fresh produces and two kilograms for large size fresh produces. In addition, the sample of pears, oranges and apples were purchased for ten each (Codex Alimentarius., 2000).

One kilogram of the collected vegetables and fruits were quartered and half of them were randomly selected (approximately 500 grams) for homogenized by blender

(IKA ULTRA-TURRAX T25 digital, Germany). Then, 50 grams aliquot of blended sample was kept and reserved in plastic container at temperature of -20°C until used.

Table 3.4 Common name and sciencetific name of vegetables and fruits collected in June 2009.

Common name	Sciencetific name	No of samples
Cabbage	*Brassica oleracea* L.var.*capitata*	7
Kale	*Brassica oleracea* L.var.*acephala* DC.	5
Chinese cabbage	*Brassica rapa* L. subsp. *pekinensis* (Lour.) Olsson.	5
Yard long bean	*Vigna unguiculata* var.*sesquipedalis* (*Vigna sinensis* var.*sesquipedalis* L.Verdc.)	7
Cucumber	*Cucumis sativus* L.	8
Sugar pea	*Pisum sativum* var.*macrocarpon* Ser.	3
Tangerine	*Citrus reticulate*	6
Guava	*Psidium guajava*	3
Apple	*Malus domestica*	2
Grape	*Vitis venefera*	5
Lychee	*Litchi chinensis* Sonn.	5

Table 3.5 Common name and sciencetific name of vegetables and fruits collected in March 2010.

Common name	Sciencetific name	No of samples
Cabbage	*Brassica oleracea* L.var.*capitata*	6
Kale	*Brassica oleracea* L.var.*acephala* DC.	3
Water spinach	*Impomoea aquatica* Forsk.	2
Cauliflower	*Brassica oleracea L.var.botrytis L.*	3
Chinese cabbage	*Brassica rapa L. subsp. pekinensis (Lour.) Olsson.*	4
Chinese mustard	*Brassica.camprestris* L. ssp. *Chinensis* (Lour.)Ruprecht.	5
Yard long bean	*Vigna unguiculata* var.*sesquipedalis* (*Vigna sinensis* var.*sesquipedalis* L.Verdc.)	3
Cucumber	*Cucumis sativus* L.	5
Tangerine	*Citrus reticulate*	4
Guava	*Psidium guajava*	4
Apple	*Malus domestica*	3
Mango	*Mangifere india* Linn.	3
Sand pear	*Pyrus pyrifolia*	5
Rose apple	*Syzygium samarangense (Blume)* Merr.&L.M.Perry	3

(2) Sample preparation for synthetic pyrethroid residues analysis

Sample preparations were modified from the extraction and clean up steps which presented in figure 2.1 as follow: Five grams of vegetable and fruit sample was transferred into a 50 mL Teflon centrifuge tube. 50 µL of bifenthrin (0.02 µg/mLl) which used as internal standard was added into each sample. 5 g of Na_2SO_4 were added to the sample. Each sample was extracted with 10 mL dichloromethane (DCM) and then vortex-mixed for 30 seconds. The samples were placed in ultrasonicator bath at 35ºC for 5 minutes.Re-extraction with 10 mL dichloromethane (DCM) and then vortex-mixed for 30 seconds. Extracts were filtered through a Whatman No.1 paper with Na_2SO_4. The supernatants were pooled in 50 mL centrifuge tube.

In clean up procedure, precondition of activated carbon cartridge (self-modified), they were conditioned with 2 x 1 mL of distilled water, 2 x 1 mL of EA, and 2 x 1 mL of DCM. The supernatants were loaded in the cartridge at 8 – 10 mm Hg pressure. The cartridges were eluted with 8 x 1 mL of DCM.

Extracts were evaporated to dryness by rotary evaporation at 37°C under reducing pressure. The final volume of concentrated extract was 1 mL with ethyl acetate, then determined by gas chromatography with electron capture detection.

(3) Gas chromatography condition

One µl of eluate was injected and analysis by using GC – ECD. The GC analysis consisted of a Hewlett-packard model 6890 Series GC System equipped with a ^{63}Ni ECD, a fused silica capillary column: HP5 (5% phenylmethylpolysiloxane with 30 m × 0.25 mm, 0.25 µm film thickness), and computerized data handing data system (HP 3365 Series Chemstation). Programming temperature was 300ºC for

detection port and 250ºC for injection port (splitless mode). Temperature of programming oven was increased as follows: initial temperature of 100°C for 1 min, first ramp 15 °C /minute ramp rate to 250°C for 1 minute, second ramp 5°C /minute ramp rate to 280°C. and final temperature hold at 280°C for 3 minute. High purity helium (99.993%) was used as a carrier gas at flow rate of 1.5 mL/min and ultra high purity nitrogen (99.999%) was used as a makeup gas.

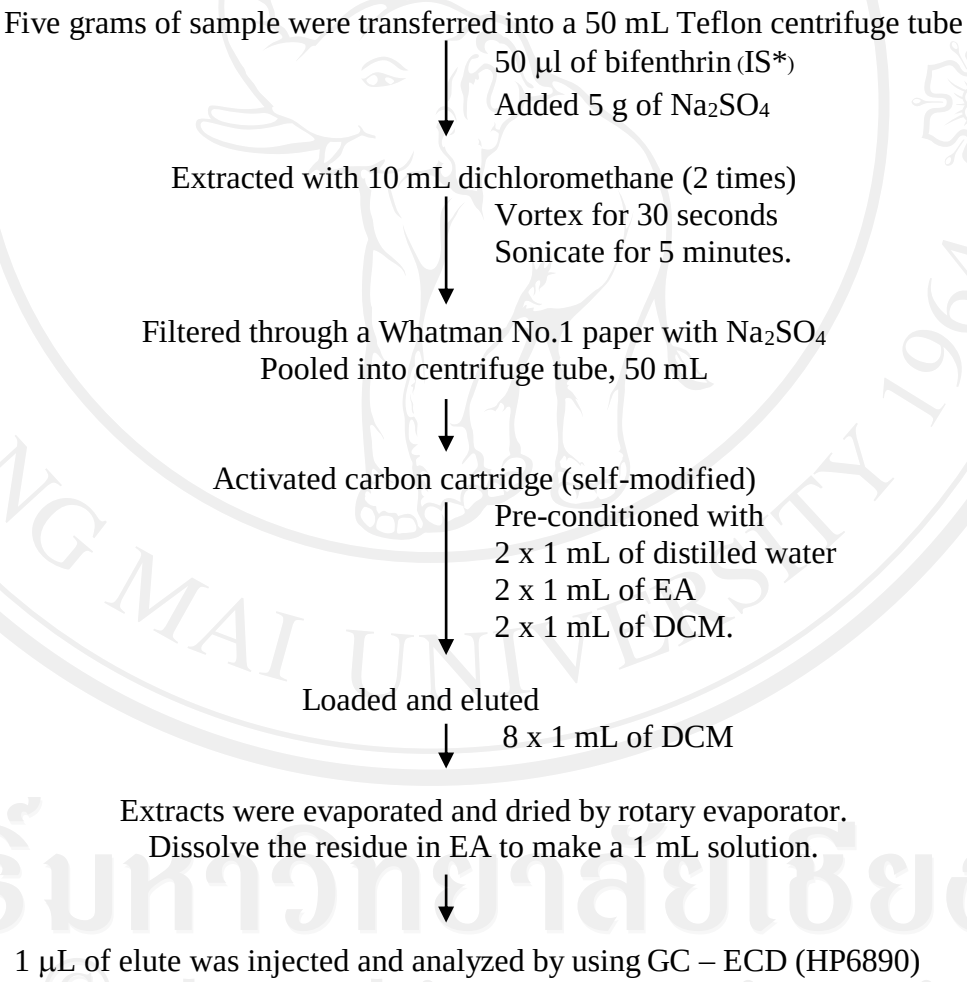

Figure 3.1 Flow diagrams of sample extraction and cleanup steps
Note: *IS = internal standard

3.4.3.2 Biological monitoring

(1) Studied group

In June 2009 and March 2010, the school children in grade 5 to 6 from nine school (Ban Lai Fang school, Ban Mea Kha school, Ban Mae Luang Uppathum school, Ban Tung Lung school, Ban Mae Ngong Ke Lek school, Ban Lan school, Ban Muang Chum school, Ban San Sai Klong Noi school, and Rangsri Witaya school) in four subdistricts of Fang district, Chiang Mai province were selected. The school children who eligible for the inclusion, willing to participate the study, sign written consents of children and parent were enrolled and interviewed for socio-demographic data. Data on questionnaire consisted of personal data, environmental data, health data, and diet behavior.

Approximately 50 mL morning urine was collected from selected children on Monday and Thursday (represent 1 week). All samples were collected by the participant in a small jar, and then given on field personal data to label. Samples were composited, labeled with an appropriate barcode system, and kept at -20 °C.

(2) Creatinine measurement

Urine creatinine concentration is also checked during standard urine drug tests. High creatinine levels indicate a pure test, whereas low amounts of creatinine in the urine indicate a manipulated test, either through the addition of water in the sample or by drinking excessive amounts of water.

In this study, the analysis of creatinine in the clinical chemistry laboratory, Faculty of Medicine, Maharaj Nakorn Chiang Mai Hospital, Chiang Mai University, Chiang Mai. In the analysis of urine creatinine measurements were performed

according to the Roche "Creatinine Plus Assay" using a Roche Hitachi Automatic Analyzer Model 917 (Hitachi, Inc., Japan).

(3) Analytical methods

Sample preparation for urinary pyrethroid analysis was modified from Leng *et al.* (2005) and Aprea *et al*., (1997). Extraction and clean up steps were done as follows: Two milliliters of urine sample was transferred in a screw cap test tube. Fifty microliters of 2 PBA (1µg/mL) which used as internal standard was added in each sample and then mixed for 30 seconds. 500 µL of concentration hydrochloric acid (37%) was added. The test tubes were covered with screw caps and hydrolyzed at 100° C in a block heater for 2 hours.

Each sample was extracted with 2 mL of ethyl acetate (EA) and shaken at 1500 rpm for 5 minutes. The test tubes with samples were placed in the centrifuge at 2500 round per minute (rpm) for 5 minutes. Organic layer was separated in new test tube. Re-extraction with two milliliter of ethyl acetate (EA) and shaken at 1500 rpm for 5 minutes. The test tubes with samples were placed in the centrifuge at 2500 round per minute (rpm) for 5 minutes

In clean up procedure, pre-condition of C_{18} – cartridge, they were conditioned with 4 mL of methanol and 5 mL of distilled water. The supernatants were loaded in the cartridge at 10 mm Hg pressure. The cartridges were eluted with 5 mL of acetone and dried at 10 mm Hg pressure for 5 minutes. The organic layer was dried under a gentle stream of nitrogen.

The residue was dissolved in 250 µl acetronitrile. For derivatization, 10 µl of HFIP and 20 µl of DIC were added. The solution was slightly mixed for 10 minutes in a vertex mixture. Then 1 mL of a 1M sodium hydrogencarbonate

solution was added for water free and condition pH value, mixed to 10 minutes. Finally, 500 µl of isooctane were added to redissolve and transferred to micro vial.

(4) Gas chromatography condition for urinary 3-PBA analysis

One µl of eluate was injected and analyzed by using GC – ECD. The GC analysis consist of a Hewlett-Packard model 6890 Series GC System equip with a ^{63}Ni ECD, a fused silica capillary column: HP5 (5% phenylmethylpolysiloxane with 30 m × 0.32 mm, 0.25 µm film thickness), and computerize data handing data system (HP 3365 Series Chemstation). Programming temperature was set at 300^{o}C for detection port and 250^{o}C for injection port (splitless mode). Temperature of programming of oven was as follows: initial temperature of 100°C for 0.5 min, first ramp 10°C /minute ramp rate to 150°C for 0.5 minute, second ramp 4°C /minute ramp rate to 180°C, third ramp 30°C /minute ramp rate to 270°C and final temperature hold at 280°C. High purity helium (99.993%) was used as a carrier gas at flow rate of 1.5 mL/min and ultra high purity nitrogen (99.999%) was used as a makeup gas.

3.5 Statistical analysis

Descriptive statistic, including arithmetic mean (AR), geometric mean (GM), standard deviation (SD), minimum (Min), and Maximum (Max) were analyzed for determination of synthetic pyrethroid residues in vegetable and fruit.

Computer programs were used to analyze data. Descriptive statistics, including AR, GM, and SD, Min, Max and percentile were analyzed. Compared mean concentration of 3-PBA in urine sample from school children for 2 collections period.

3.6 Ethical approval

The study was approved by Human Experimentation Committee, Research Institute for Health Sciences (RIHES), Chiang Mai University (Certificate of Ethical Clearance No. 6393(25)/0357, 4 February 2009).