# CHAPTER 4

# RESULTS AND DISCUSSION

## 4.1 Qualitative and quantitative analysis of synthetic pyrethroids

### 4.1.1 Retention time of synthetic pyrethroid insecticides

Retention times of individual synthetic pyrethroids in standard mixture are presented in Table 4.1. The pure and extracted standard show multiple peaks while a control sample did not show peaks that could be attributed to any studied pesticide (Figure 4.1). Similar result was previously reported by Jing et al. (2010) multiple peaks were observed for several pyrethroids because the existence of isomers. In control extracts, crop sample without addition of insecticides passed through all steps. The purpose of control extract was to check accuracy of the method.

Table 4.1 Retention times of synthetic pyrethroids in standard mixture

| Synthetic pyrethroid insecticides | Retention time (minutes) |
|---|---|
| Lambda cyhalothrin 1 | 10.204 |
| Lambda cyhalothrin 2 | 11.292 |
| Lambda cyhalothrin 3 | 11.508 |
| Permethrin 1 | 12.389 |
| Permethrin 2 | 12.541 |
| Cyfluthrin 1 | 11.816 |
| Cyfluthrin 2 | 13.122 |
| Cyfluthrin 3 | 13.237 |
| Cyfluthrin 4 | 13.347 |
| Cyfluthrin 5 | 13.394 |

Table 4.1 Retention times of synthetic pyrethroids in standard mixture (continued)

| Synthetic pyrethroid insecticides | Retention time (minutes) |
|---|---|
| Cypermethrin 1 | 12.277 |
| Cypermethrin 2 | 13.544 |
| Cypermethrin 3 | 13.660 |
| Cypermethrin 4 | 13.777 |
| Cypermethrin 5 | 13.826 |
| Fenvalerate 1 | 14.897 |
| Fenvalerate 2 | 15.230 |
| Deltamethrin 1 | 14.238 |
| Deltamethrin 2 | 15.813 |
| Deltamethrin 3 | 16.207 |

**4.1.2 Linearity of synthetic pyrethroids**

Linearity regression equations and regression coefficients ($R^2$) of individual synthetic pyrethroids standard mixture are present in Table 4.2. The calibration curves were linear with correlation coefficients ($R^2$) between area ratio of sample and internal standard for all insecticides were 0.9835 to 0.9972. The linearity ranged differs by their compounds from 5 – 500 ng/mL.

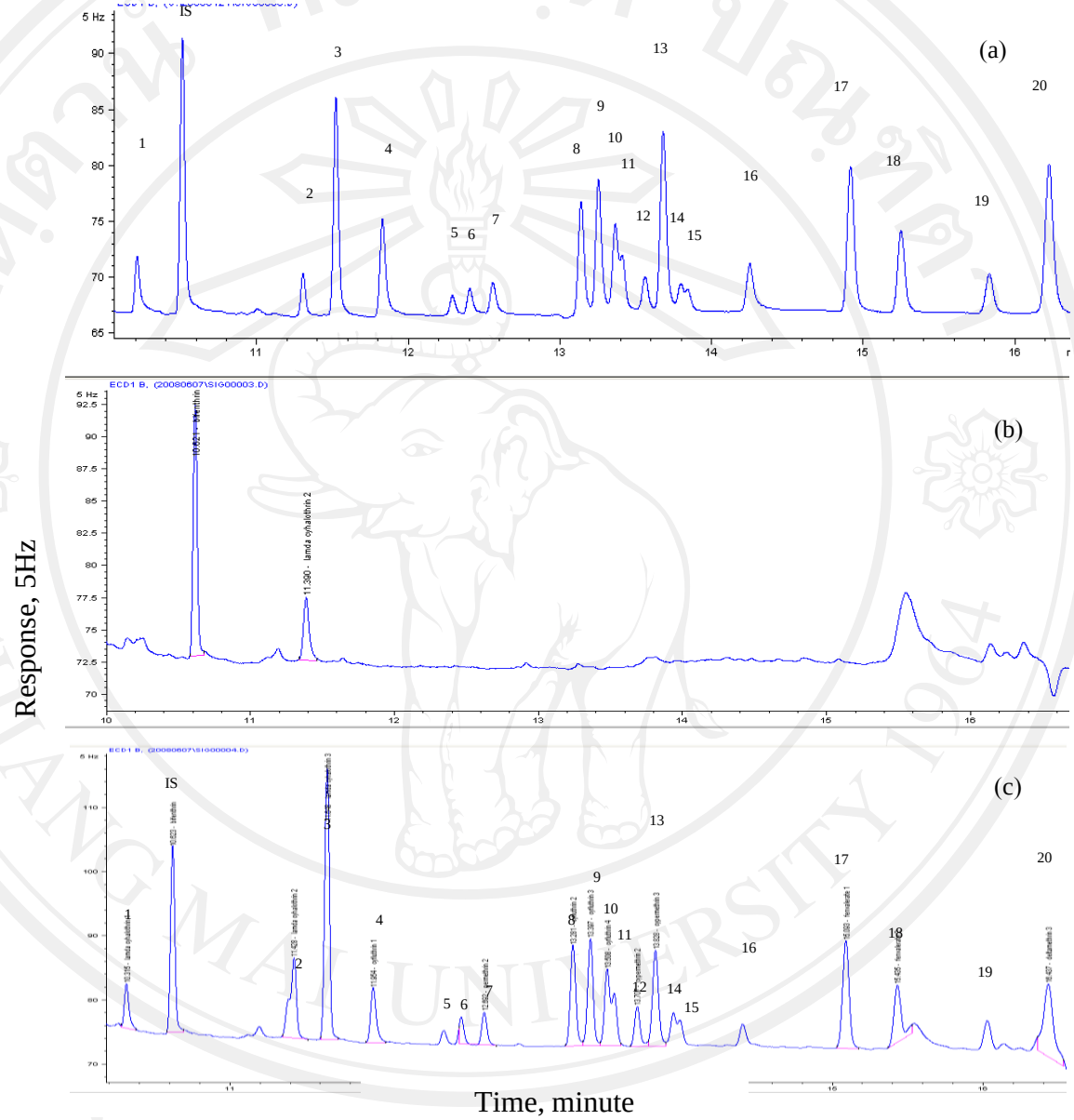


**Fig 4.1** GC-ECD chromatogram of (a) a pure standard a control extract, (b) an extraction internal standard solution and (c) an extracted standard solution. The compounds are numbered as follows: bifenthrin (IS)20ng/mL, 1,2,3=lambda cyhalothrin 10ng/mL, 4,8,9,10,11=cyfluthrin 20ng/mL 6,7=permethrin 5ng/mL, 5,12,13,14,15 = cypermethrin 5 ng/mL, 17,18=fenvalerate 20ng/mL and 16,19,20=deltamethrin 20ng/mL

Table 4.2 Linearity regression equations and correlation coefficients ($R^2$) of individual synthetic pyrethroids standard mixture

| Synthetic pyrethroid insecticides | Linearity regression equations | $R^2$ |
|---|---|---|
| 1. Lambda cyhalothrin | Y = 103.3 X + 0.49 | 0.9961 |
| 2. Bifenthrin (IS) | Y = 1X + 0 | 1.0000 |
| 3. Permethrin | Y = 54.0 X – 0.11 | 0.9835 |
| 4. Cyfluthrin | Y = 97.86 X + 0.59 | 0.9972 |
| 5. Cypermethrin | Y = 128.33 X + 1.68 | 0.9872 |
| 6. Fenvalerate | Y = 52.1 X + 0.55 | 0.9899 |
| 7. Deltamethrin | Y = 61.7 X + 0.22 | 0.9941 |

X: amount ratio of sum peaks of individual synthetic pyrethroids to internal standard (amt ratio)

Y: response ratio

$R^2$: regression coefficients

IS : internal standard

**4.2 Quality control of synthetic pyrethroid analysis**

**4.2.1 Level of detection of synthetic pyrethroid analysis**

The limits of detection (LOD) and the limits of quantification (LOQ) of individual synthetic pyrethroid are present in Table 4.3. LOD and LOQ ranged vary depending on its component. Values of LOD differ from 0.001 for permethrin and cypermethrin, 0.003 for lambda cyhalothrin and 0.005 μg/L for cyfluthrin, fenvalerate and deltamethrin. In addition, LOQ ranged from 0.005 for lambda cyhalothrin, 0.0025

for permethrin and cypermethrin to 0.01 mg/kg cyfluthrin, fenvalerate and deltamethrin. These results show the method has suitable range with lower value when compare to maximum residue limits (MRLs) recommended by Thai agriculture commodity and food standard (2006) and Codex alimentarius pesticide residues in food.

Table 4.3 Limit of detection (LOD) and limit of quantification (LOQ) of individual synthetic pyrethroids

| Synthetic pyrethroid insecticides | LOD (mg/kg) | LOQ (mg/kg) |
|---|---|---|
| 1. Lambda cyhalothrin | 0.003 | 0.005 |
| 2. Permethrin | 0.001 | 0.025 |
| 3. Cyfluthrin | 0.005 | 0.01 |
| 4. Cypermethrin | 0.001 | 0.025 |
| 5. Fenvalerate | 0.005 | 0.01 |
| 6. Deltamethrin | 0.005 | 0.01 |

**4.2.2 Recovery of synthetic pyrethroids' analysis**

Percent recoveries of the six pyrethroids from fortified crop at three concentrations are presented in Table 4.4. Recoveries ranged 96.8% to 109.3%, from 86.5% to 96.9 % and 83.8% to 98.4% for spiked at low, medium and high level, respectively. The standard deviation were 2.1 to 12.4, 5.5 to 10.0 and 1.8 to 16.5 for all synthetic pyrethroids at the spiking levels of low, medium and high level, respectively. The result of this study showed that the values of recoveries percentage in all synthetic pyrethroids were in range of acceptable criteria recommended by CODEX (70-110%).

Table 4.4 Recoveries percentage (%) and standard deviation (SD) of individual synthetic pyrethroids at low, medium and high spiked levels

| Synthetic pyrethroid insecticides | low spiked levels | | medium spiked levels | | high spiked levels | |
|---|---|---|---|---|---|---|
| | concentration (ng/mL) | Recoveries (%)±SD | concentration (ng/mL) | Recoveries (%)±SD | concentration (ng/mL) | Recoveries (%)±SD |
| 1. Lambda cyhalothrin | 10 | 98.4±12.4 | 20 | 92.2±5.7 | 50 | 89.0±15 |
| 2. Permethrin | 5 | 106.9±2.7 | 10 | 93.1±4.7 | 20 | 84.8±16.5 |
| 3. Cyfluthrin | 20 | 109.3±9.7 | 50 | 96.9±5.5 | 100 | 98.4±3.3 |
| 4.Cypermethrin | 5 | 96.8±6.9 | 10 | 86.5±10.0 | 20 | 89.5±9.4 |
| 5. Fenvalerate | 20 | 102.7±3.9 | 50 | 94.1±6.4 | 100 | 89.6±1.8 |
| 6. Deltamethrin | 20 | 105.2±2.1 | 50 | 96.5±9.4 | 100 | 83.8±3.0 |

### 4.2.3 Synthetic pyrthroids of intra- and inter- batches of pooled samples

Variations of intra-batch and inter-batch of pooled vegetable and fruit samples are presented in Table 4.5. Ten of pooled vegetable and fruit samples were analyzed for both intra- and inter-batch. The intra-batch recoveries percentage ranged from 87.9 for cypermethrin to 102.1 for bifenthrin and CV percentage ranged from 1.9 for bifenthrin to 7.4 for deltamethrin. The recoveries of inter-batch ranged from 81.0 for permethrin to 113.9 for fenvalerate and CV percentage ranged from 5.8 for bifenthrin to 15.7 for permethrin. Both of them were acceptable for criteria determine at less than 21%. (Pihlström, 2010)

Table 4.5 Intrabatch and interbatch analytical average recovery (%) ± SD of six synthetic pyrethroids in a pooled sample (spike concentration 100µg/kg)

| Synthetic pyrethroid insecticides | Intra- batch (n=10) | | Inter- batch (n=10) | |
|---|---|---|---|---|
| | %recovery±SD | %CV | %recovery ±SD | % CV |
| 1. Lambda cyhalothrin | 97.9±3.7 | 3.8 | 102.6±9.9 | 9.6 |
| 2. Bifenthrin (IS) | 102.1±1.9 | 1.9 | 107.2±6.2 | 5.8 |
| 3. Permethrin | 92.7±5.1 | 5.5 | 81.0±12.8 | 15.7 |
| 4. Cyfluthrin | 98.9±1.8 | 1.8 | 91.8±10.3 | 11.3 |
| 5.Cypermethrin | 87.9±1.7 | 2.1 | 94.5±14.4 | 15.3 |
| 6. Fenvalerate | 98.7±4.3 | 4.3 | 113.9±11.6 | 10.3 |
| 7. Deltamethrin | 92.8± 6.9 | 7.4 | 89.7 ± 8.0 | 8.9 |

**4.3 Qualitative and quantitative analysis of 3-PBA**

**4.3.1 Retention times of 3-PBA in urine analysis**

This study detected 3-phenoxybenzoic acid (3PBA), a common metabolite of pyrethroid insecticides. Retention times, linearity regression equations and regression coefficients ($R^2$) of 3-PBA are presented in Table 4.6. Moreover, peaks of 2-PBA which served as an internal standard at 20 ng/mL and 3-PBA at 10 ng/mL are show in Figure 2.

Table 4.6 Retention times, linearity regression equations and regression coefficients ($R^2$) of 3-PBA

| synthetic pyrethroid metabolite | Retention time (minutes) | Linearity regression equations | $R^2$ |
|---|---|---|---|
| 2 PBA (IS) | 9.931 | Y = 1X + 0 | 1.0000 |
| 3 PBA | 10.212 | y = 3.10x + 2.02 | 0.9939 |

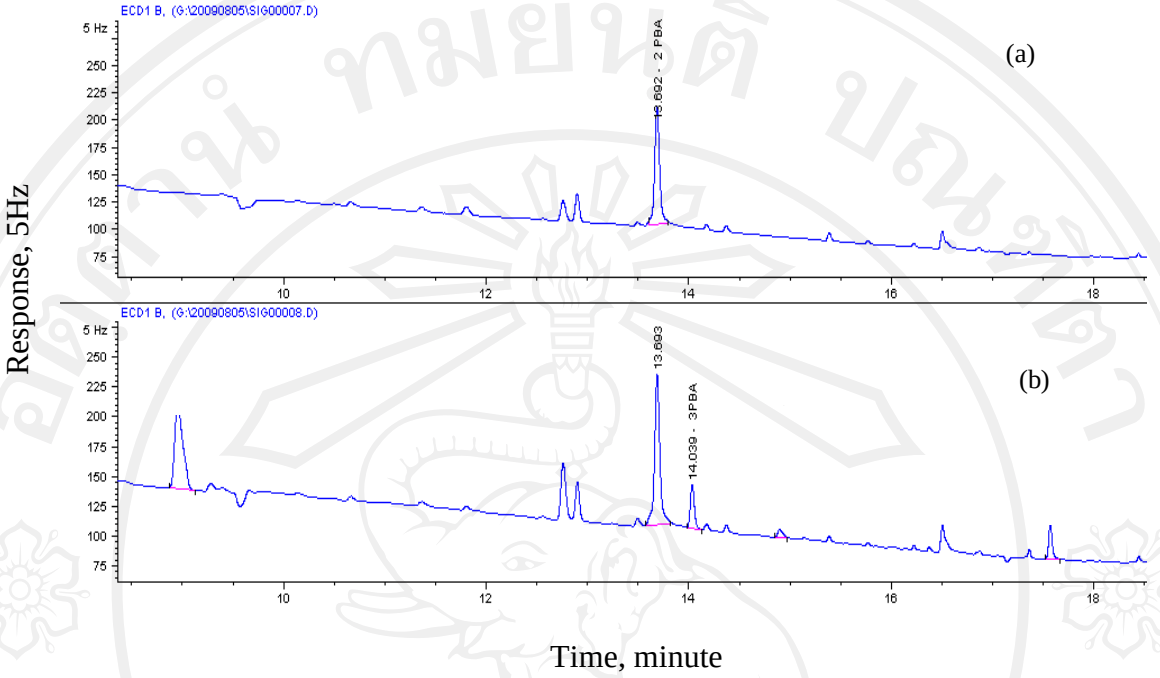


**Fig 4.2** GC-ECD chromatogram for a (a) pure standard 2-PBA (IS) 20ng/mL (b) pure standard 2-PBA (IS) 20ng/mL and 3-PBA 10ng/mL

### 4.3.2 Quality control of 3-PBA analysis

#### 4.3.2.1 Recovery, limited of detection (LOD), and limit of quantification (LOQ) of 3-PBA

Recovery studies were performed at 50 ng/mL fortification of 3-PBA and 2-PBA (internal standard), at least five times in a pooled sample urine. These samples were prepared by spike 3 PBA solutions in methanol. Percent recovery of 3-PBA at 91.4, and percent CV at 0.13. For limit of detection, and limit of quantification of 3-PBA are presented in Table 4.7. These results were acceptable when compared to the reference value of German population which determines urinary concentration of metabolites of pyrethroid insecticides at not exceed 2 ng/mL (Human Biomonitoring Commission of the German Federal Environment Agency 2005) .

Table 4.7 Average recoveries (%recoveries), limit of detection (LOD), and limit of quantification (LOQ) of 3-PBA

| Synthetic pyrethroid metabolite | %recovery ±SD | %CV | LOD (µg/l) | LOQ (µg/l) |
|---|---|---|---|---|
| 3 PBA | 91.4±2.29 | 0.13 | 0.8 | 1 |

**4.3.2.2. 3-PBA variations of intra- and inter-batches of pooled samples**

Variations of intra-batch of pooled urine samples are present in Table 4.8. Sixteen of pooled urine samples were analyzed for intra-batch and 8 of pooled urine samples were analyzed for inter-batch variations. For intra-batch, percent CV at 0.18 for 3-PBA. For inter-batch, percent CV at 0.12 for 3-PBA. Both of them were in the acceptable criteria, which were less than 21%

Table 4.8 Intra-batch and inter-batch variation of recovery (%)±SD of 3-PBA in spiked pooled sample

| Synthetic pyrethroid metabolite | Intra- batch variation (n=16) | | Inter- batch variation (n=8) | |
|---|---|---|---|---|
| | %recovery±SD | %CV | %recovery±SD | % CV |
| 3-PBA | 88.1±2.98 | 0.18 | 88.9±3.75 | 0.12 |

**4.4 Results and discussion of section 1**

**Development method for detecting synthetic pyrethroid residues in vegetable and fruits using GC-ECD**

**4.4.1 Extraction of synthetic pyrethroid residues from various solvents**

In this study, fruits and vegetables extraction efficiency for synthetic pyrethroid residues of four solvent extractions were compared in Table 4.9. Four solvents consisted of ethyl acetate, dichloromethane, acetonitrile, and acetone. Dichloromethane obtained highest recoveries percentage in most of synthetic pyrethroid residues except in deltamethrin. Recoveries percentage of dichloromethane ranged from 28% in deltamethrin to 90% in cypermethrin and fenvalerate.

Table 4.9 Percentage recovery of synthetic pyrethroid residues for ethyl acetate, dichloromethane, acetonitrile, and acetone

| Synthetic pyrethroid insecticides | %recovery | | | |
|---|---|---|---|---|
| | EA | DCM | ACN | acetone |
| lamda cyhalothrin | 54 | 68 | 2 | 3 |
| Permethrin | 27 | 69 | 1 | 1 |
| Cyfluthrin | 37 | 79 | 4 | 3 |
| Cypermethrin | 63 | 90 | 6 | 3 |
| fenvalerate | 34 | 90 | 8 | 4 |
| Deltamethrin | 82 | 28 | 13 | 6 |

**4.4.2 Effectiveness of clean up procedure for eliminating the interference in vegetable and fruits sample**

In this study, the effectiveness of various solid-phase extraction cartridges to clean up sample which comprise SAX/PSA, activated carbon, and octadecyl ($C_{18}$) were determined. From initial study, due to inability to remove pigment from sample of octadecyl ($C_{18}$), the researcher decided to stop the study on this solid-phase extraction. Further study, activated carbon has better ability to remove pigment from sample than SAX/PSA. Similar to recoveries percentage, activated carbon have higher value than SAX/PSA in all synthetic pyrethroid insecticides. Recoveries percentage of activated carbon range from 63.3% in deltamethrin to 101.0 in bifenthrin and standard deviation range from 1.14 in bifenthrin to 20.41 in deltamethrin.

Table 4.10 Percentage of recoveries and standard deviation of synthetic pyrethroid residues for activated carbon and SAX/PSA

| Synthetic pyrethroid insecticides | activated carbon (n=3) | SAX/PSA (n=3) |
|---|---|---|
| 1. Lambda cyhalothrin | 91.7±8.44 | 7.4±5.07 |
| 2. Bifenthrin (IS) | 101.0±1.14 | 28.3±6.56 |
| 3. Permethrin | 82.5±8.29 | 65.8±52.46 |
| 4. Cyfluthrin | 78.2±15.49 | 34.0±13.90 |
| 5.Cypermethrin | 67.5±15.85 | 26.5±12.27 |
| 6. Fenvalerate | 92.6±14.02 | 57.0±35.87 |
| 7. Deltamethrin | 63.3±20.41 | 52.4±6.02 |

**4.4.3 Effectiveness of extraction of synthetic pyrethroid residues in various matrices**

In this study, six synthetic pyrethroid insecticides various matrices vegetable and fruit samples are summarized in table 4.11. Four vegetable and fruit samples consist of cabbage, kale, longan, and tangerine were determined. From this study, the recoveries of synthetic pyrethroid in vegetable and fruit samples are higher than 80% except permethrin in cabbage and kale at 76 and 69 respectively. Recoveries percentage of various matrices vegetable and fruit samples range from 69% in permethrin of kale to 131 in cyfluthrin of cabbage and standard deviation range from 1.66 in deltamethrin of tangerine to 33.1 in lambda cyhalothrin of kale.

Table 4.11 Percentage of recoveries and standard deviation of synthetic pyrethroid residues in various matrices.

| Synthetic pyrethroids | Cabbage | Kale | Longan | Tangerine |
|---|---|---|---|---|
| 1. Lambda cyhalothrin | 108±15.9 | 82±33.1 | 99±3.50 | 94±9.32 |
| 2. Bifenthrin (IS) | 100±5.31 | 87±4.2 | 99±4.98 | 103±6.01 |
| 3. Permethrin | 76±19.1 | 69±25.2 | 93±6.79 | 91±10.20 |
| 4. Cyfluthrin | 131±24.9 | 107±20.5 | 118±15.3 | 97±8.68 |
| 5.Cypermethrin | 83±21.6 | 96±16.5 | 104±13.9 | 99±3.32 |
| 6. Fenvalerate | 106±21.1 | 91±23.2 | 103±10.20 | 100±3.95 |
| 7. Deltamethrin | 95±14.3 | 100±12.6 | 92±5.10 | 100±1.66 |

### 4.4.4 Effectiveness of extraction of synthetic pyrethroid residues in rugged test

In this study, fruits and vegetables extraction efficiency for synthetic pyrethroid residues in rugged test were compared in Table 4.12. From initial study, three scientists obtained the recoveries of synthetic pyrethroid in vegetable and fruit samples are higher than 80%. Mean recoveries percentage of various scientists range from 90.8 % in deltamethrin to 118.9 in lambda cyhalothrin and CV percentage ranged from 0.68 for fenvalerate to 17.13 for bifenthrin.

Table 4.12 Percentage of recoveries and CV of synthetic pyrethroids' residues in rugged test.

| Synthetic pyrethroid metabolite | Scientist 1 %recovery ±SD | Scientist 2 %recovery ±SD | Scientist 3 %recovery ±SD | Mean %recovery ±SD | % CV |
|---|---|---|---|---|---|
| 1. Lambda cyhalothrin | 126.9±12.24 | 134.1±28.60 | 97.3±10.84 | 118.9±20.37 | 2.70 |
| 2. Bifenthrin (IS) | 92.4±6.29 | 96.4±4.40 | 95.8±7.04 | 95.4±2.57 | 17.13 |
| 3. Permethrin | 94.3±31.09 | 104.1±17.76 | 82.3±11.64 | 93.6±10.92 | 11.67 |
| 4. Cyfluthrin | 110.1±36.63 | 119.1±21.11 | 113.3±14.61 | 114.2±4.56 | 4.00 |
| 5.Cypermethrin | 85.5±64.71 | 104.1±48.29 | 95.5±8.96 | 95.0±9.28 | 9.76 |
| 6. Fenvalerate | 100.9±13.00 | 99.6±17.79 | 100.0±6.48 | 100.2±0.68 | 0.68 |
| 7. Deltamethrin | 85.8±26.79 | 89.8±9.86 | 96.8±3.95 | 90.8±5.56 | 6.13 |

**4.5 Result and discussion of section 2**

**Development method for detecting synthetic pyrethroid metabolite in urine using GC-ECD.**

**4.5.1 Extraction of synthetic pyrethroid metabolite in urine from various solvent**

In this study, urine extraction efficiency for synthetic pyrethroid metabolite of four solvent extractions. Four solvent consist of ethyl acetate, dichloromethane, tert buthyl methyl ether, and hexane. Ethyl acetate obtained highest recoveries percentage in most of synthetic pyrethroid metabolite in urine. Recoveries percentage of ethyl acetate at 91.4.

**4.5.2 Effectiveness of clean up procedure for eliminating the interference in urine sample**

In this study, the effectiveness of various solid-phase extraction cartridges to clean up sample which comprise SAX/PSA, activated carbon, and octadecyl ($C_{18}$) were determined. From initial study, due to inability to remove pigment from sample of SAX/PSA and activated carbon, the researcher decided to stop the study on these cartridges. Further study, $C_{18}$ has better ability to remove pigment and noticeable chromatographic interferences from sample the recoveries percentage at 78.9.

**4.6 Assess the exposure of synthetic pyrethroid insecticides among school children**

Exposure assessment should be based on combined questionnaire and marker data characterizing external and internal exposure. In this study external marker can be measured in vegetable and fruit samples, and internal biomarker relevant to children includes measurements in urine samples.

**4.6.1 Types and levels of synthetic pyrethroid in vegetables and fruits**

The common name and scientific name of vegetable and fruit samples present in Table 4.13.

Table 4.13 Common name and scientific name of vegetables and fruits.

| Common name | Sciencetific name |
|---|---|
| Cabbage | *Brassica oleracea* L.var.*capitata* |
| Kale | *Brassica oleracea* L.var.*acephala* DC. |
| Water spinach | *Impomoea aquatica* Forsk. |
| Cauliflower | *Brassica oleracea* L.var.*botrytis* L. |
| Chinese cabbage | *Brassica rapa* L. subsp. *pekinensis* (Lour.) Olsson. |
| Chinese mustard | *Brassica.camprestris* L. ssp. *Chinensis* (Lour.)Ruprecht. |
| Yard long bean | *Vigna unguiculata* var.*sesquipedalis* (*Vigna sinensis* var.*sesquipedalis* L.Verdc.) |
| Cucumber | *Cucumis sativus* L. |
| Sugar pea | *Pisum sativum* var.*macrocarpon* Ser. |

Table 4.13 Common name and scientific name of vegetables and fruits. (continued)

| Common name | Sciencetific name |
|---|---|
| Tangerine | *Citrus reticulate* |
| Guava | *Psidium guajava* |
| Apple | *Malus domestica* |
| Dragon fruit | *Hylocereu undatuss* |
| Mango | *Mangifere india* Linn. |
| Sand pear | *Pyrus pyrifolia* |
| Grape | *Vitis venefera* |
| Lychee | *Litchi chinensis* Sonn. |
| Rose apple | *Syzygium samarangense (Blume)* Merr.&L.M.Perry |

In June 2009, analysis of synthetic pyrethroid residues in vegetable and fruit samples, each of the lychee is a residue in the permethrin, cyfluthrin, lambda cyhalothrin, and cypermethrin were 0.07, 0.16, 0.22, and 3.24 mg/kg respectively, but for fenvalerate have a maximum residue on the grapes is 0.27 mg/kg and deltamethrin have a maximum residue on a tangerine at 0.76 mg/kg. Cucumber is a vegetable that has residue at least one substance that is minimal lambda cyhalothrin. Show in Table 4.14

Table 4.14 Mean±SD concentration of six synthetic pyrethroid residues in individual commodity in June 2009 (mg/kg)

| Commodities | lambda cyhalothrin | permethrin | cyfluthrin | cypermethrin | fenvalerate | deltamethrin |
|---|---|---|---|---|---|---|
| Cabbage (n=7) | 0.044 | 0.028±0.04 | 0.087±0.04 | 0.080±0.06 | 0.024±0.01 | 0.329±0.19 |
| Kale (n=5) | ND | 0.027±0.02 | 0.076±0.04 | 0.574±.67 | 0.055±0.03 | 0.555±0.24 |
| Chinese lettuce (n=5) | ND | 0.014 | 0.100±0.02 | 1.962±2.65 | 0.015 | 0.045±0.04 |
| Yard long bean (n=7) | ND | 0.080±0.05 | 0.081±0.02 | 1.121±1.44 | 0.031±0.02 | 0.050 |
| Long cucumber (n=8) | ND | ND | <LOQ | ND | <LOQ | ND |
| Sugar pea (n=3) | ND | 0.092±0.02 | 0.050±0.01 | 0.751±1.07 | 0.02 | 0.054±0.07 |
| Tangerine (n=6) | 0.097±0.02 | 0.085±0.06 | 0.052±0.02 | 0.645±0.55 | 0.031 | 0.757±0.26 |
| Apple (n=35) | ND | 0.033±0.02 | 0.092±0.08 | 0.060±0.06 | 0.017 | ND |
| Grape (n=2) | ND | ND | ND | 0.409±0.52 | 0.269±0.37 | ND |
| Lychee (n=5) | 0.218 | 0.073±0.07 | 0.164±0.06 | 3.238±4.86 | 0.075±0.03 | 0.074±0.03 |
| dragon fruit (n=5) | 0.054 | 0.024±0.01 | 0.116±0.03 | 0.165±0.07 | 0.020 | 0.071±0.01 |

Table 4.15 Mean±SD concentration of six synthetic pyrethroid residues in individual commodity in March 2010 (mg/kg)

| Commodities | lambda cyhalothrin | permethrin | cyfluthrin | cypermethrin | fenvalerate | deltamethrin |
|---|---|---|---|---|---|---|
| Cabbage (n=5) | 0.38±0.32 | 0.06±0.01 | 0.11±0.04 | 0.10±0.03 | 0.68±0.93 | 1.55±1.87 |
| Kale (n=4) | 0.29±0.19 | 0.02 | 0.11±0.13 | 0.19±0.20 | 0.03 | 3.79±5.36 |
| Water spinash (n=2) | 0.70 | 0.04 | 0.19±0.20 | 0.17±0.14 | 0.54±0.52 | 0.42 |
| Cauliflower (n=3) | 0.27±0.07 | 0.04±0.02 | 0.15±0.07 | 0.09±0.01 | 0.11±0.01 | 0.48 |
| Chinese cabbage(n=4) | 0.63±0.30 | 0.03±0.04 | 0.07±0.01 | 0.34±0.60 | 0.20±0.02 | 1.53±2.15 |
| Chinese mustard (n=6) | 0.256±0.03 | 0.09±0.06 | 0.27±0.25 | 0.13±0.07 | 0.35±0.22 | 0.55±0.35 |
| Yard long bean (n=3) | 0.27±0.07 | 0.04±0.02 | 0.15±0.07 | 0.09±0.01 | 0.11±0.01 | 0.48 |
| Long cucumber (n=5) | 0.25±0.20 | 0.11±0.12 | 0.14±0.06 | 0.04±0.03 | 0.34±0.37 | 0.48±0.08 |
| Tangerine (n=4) | 0.29±0.05 | 0.07 | 0.27 | 2.69±1.81 | 0.14 | 0.45±0.30 |
| Apple (n=4) | 0.39 | 0.39±0.42 | 0.06±0.01 | 0.07±0.08 | 0.09±0.06 | 0.38 |
| Guava (n=3) | 0.12±0.07 | 0.22±0.18 | 0.31±0.13 | 0.10±0.09 | 0.06±0.03 | 0.42±0.18 |
| Sand pear (n=3) | 0.14±0.12 | 0.02 | 0.07±0.09 | 0.10±0.08 | 0.26±0.27 | 0.19 |
| Mango (n=5) | 0.08±0.09 | 0.06±0.07 | 0.20±0.33 | 0.06±0.06 | 0.41±0.41 | 0.27±0.16 |
| Rose apple (n=3) | 0.13±0.06 | 0.11±0.12 | 0.03 | 0.64±0.84 | 2.05±1.81 | 1.39±1.90 |

Analysis of synthetic pyrethroid residues in vegetable and fruit samples separate by commodities found, kale is the highest deltamethrin in 3.79 mg/kg, tangerine is the highest cypermethrin at 2.69 mg/kg, rose apple is the highest fenvalerate at 2.05 mg/kg, chinese cabbage is the highest lambda cyhalothrin at 0.63 mg/kg, apple is the highest permethrin at 0.39 mh/kg, and Guava is the highest cyfluthrin at 0.31 mg/kg, as shown in Table 4.15

The mean level of pyrethroid insecticides in vegetable and fruit samples in June 2009 present in table 4.16. Cyfluthrin has the highest positive sample at 26 samples of vegetable. Cypermethrin has the highest positive sample at 20 samples of fruit. The result also shows the highest mean value of cypermethrin in fruits at 1.062 mg/kg and 0.907 mg/kg in vegetable samples. In addition, the mean value of others pesticides in vegetables are 0.286 mg/kg of deltamethrin, 0.082 mg/kg of cyfluthrin, 0.050 mg/kg of permethrin, 0.044 mg/kg of lambda cyhalothrin and 0.030 mg/kg of fenvalerate. The following mean value of pesticide in fruit samples from cypermethrin are deltamehrin (0.317 mg/kg), lambda cyhalothrin (0.113 mg/kg), cyfluthrin (0.105 mg/kg), fenvalerate (0.072 mg/kg), and permethrin (0.052 mg/kg).

Table 4.16 Mean levels of six synthetic pyrethroid residues in vegetable and fruit samples in Fang district in June 2009 (mg/kg fresh weight).

| Synthetic pyrethroid insecticides | Vegetable (n=35) | | Fruit (n=21) | |
|---|---|---|---|---|
| | mean value (mg/kg) | No. of positive samples | mean value (mg/kg) | No. of positive samples |
| 1. Lambda cyhalothrin | 0.044 | 1 | 0.113 | 5 |
| 2. Permethrin | 0.050 | 12 | 0.052 | 16 |
| 3. Cyfluthrin | 0.082 | 26 | 0.105 | 19 |
| 4. Cypermethrin | 0.907 | 25 | 1.062 | 20 |
| 5. Fenvalerate | 0.030 | 19 | 0.072 | 15 |
| 6. Deltamethrin | 0.286 | 19 | 0.317 | 14 |

The mean level of pyrethroid insecticides in vegetable and fruit samples in March 2010 present in table 4.17. Cypermethrin has the highest positive sample at 25 samples of vegetable and 21 samples of fruit. The result also shows the highest mean value of cypermethrin in fruits at 0.655 mg/kg., while deltamethrin shows the highest mean value in vegetables at 1.382 mg/kg. In addition, the mean value of others insecticides in vegetables are 0.407 mg/kg of fenvalerate, 0.388 mg/kg of lambda cyhalothrin, 0.153 mg/kg of cypermethrin, 0.147 mg/kg of cyfluthrin and 0.074 mg/kg of permethrin. The following mean value of pesticide in fruit samples from cypermethrin are deltamehrin (0.551 mg/kg), fenvalerate (0.532 mg/kg), permethrin (0.181 mg/kg), lambda cyhalothrin (0.170 mg/kg), and cyfluthrin (0.160 mg/kg).

Table 4.17 Mean levels of six synthetic pyrethroid residues in vegetable and fruit samples in Fang district in March 2010 (mg/kg fresh weight).

| Synthetic pyrethroid insecticides | Vegetable (n=31) | | Fruit (n=22) | |
|---|---|---|---|---|
| | mean value (mg/kg) | No. of positive samples | mean value (mg/kg) | No. of positive samples |
| 1. Lambda cyhalothrin | 0.388 | 24 | 0.170 | 15 |
| 2. Permethrin | 0.074 | 20 | 0.181 | 15 |
| 3. Cyfluthrin | 0.147 | 21 | 0.160 | 15 |
| 4. Cypermethrin | 0.153 | 25 | 0.655 | 21 |
| 5. Fenvalerate | 0.407 | 19 | 0.532 | 18 |
| 6. Deltamethrin | 1.382 | 22 | 0.551 | 11 |

According to the Thai agricultural commodity and food standard (2006), the MRLs of pesticide was varies by commodities as shown in table 4.18. The mean values of pesticide in most samples were generally exceeded the MRLs, except cypermethrin in cabbage, fenvalerate in mango and deltamethrin in yard long bean. The highest percentage was found in cypermethrin of sugar pea while deltamethrin of yard long bean showed the lowest percentage.

Table 4.18 The MRLs of pesticide in commodities and number of samples above MRLs

| Insecticides | Commodities | Mean value (mg/kg) | MRLs [a] (mg/kg) | Percentage of samples exceeded MRLs |
|---|---|---|---|---|
| Lambda | Cabbage | 0.327 | 0.2 | 23.1 |
| cyhalothrin | Cauliflower | 0.273 | 0.2 | 66.7 |
| Cypermethrin | Cabbage | 0.087 | 0.1 | 30.8 |
| | Yard long bean | 0.810 | 0.05 | 80.0 |
| | Sugar pea | 0.751 | 0.05 | 100.0 |
| | Chinese cabbage | 1.962 | 1 | 40.0 |
| Fenvalerate | Mango | 0.408 | 1 | 20.0 |
| Deltamethrin | Cabbage | 0.838 | 0.2 | 76.9 |
| | Kale | 1.770 | 0.5 | 62.5 |
| | Yard long bean | 0.192 | 0.2 | 10.0 |
| | Cauliflower | 0.478 | 0.1 | 33.3 |
| | Chinese mustard | 1.962 | 0.5 | 33.3 |

[a] Thai agricultural commodity and food standard. 2006.

Furthermore, the percentage of sample above MRLs of different international standard has been presented in table 4.19. Samples with permethrin were not detected when compare to the Codex MRLs. Percentage of the samples above MRLs of Codex and Thailand have similar trend in all insecticides with range from 4.6 to 19.3 %. In addition, the European standards which have lower concentration limit shows higher percentage of sample exceed the MRLs in range of 22 to 55 %.

Table 4.19 Comparison of the percentage of sample exceeded Codex, Thailand, and the European standard.

| Insecticides | Codex MRLs [a] (mg/kg) | Percentage of sample exceeded MRLs | Thai MRLs [b] (mg/kg) | Percentage of sample exceeded MRLs | EU MRLs [c] (mg/kg) | Percentage of sample exceeded MRLs |
|---|---|---|---|---|---|---|
| Lambda cyhalothrin | 0.05-0.5 | 4.6 | 0.2-0.5 | 4.6 | 0.02-1 | 26.6 |
| Permethrin | 0.5-5 | ND | No | No | 0.02-0.05 | 22.0 |
| Cyfluthrin | 0.1-2 | 0.9 | No | No | 0.02-0.5 | 55.0 |
| Cypermethrin | 0.2-2 | 7.3 | 0.05-2 | 15.6 | 0.02-2 | 44.0 |
| Fenvalerate | 0.2-2 | 0.9 | 0.1-10 | 0.9 | 0.02-0.05 | 50.5 |
| Deltamethrin | 0.02-2 | 19.3 | 0.05-0.5 | 18.3 | 0.02-0.5 | 48.6 |

[a] Codex alimentarius; Pesticide Residues in Food. Maximum Residue Limits
[b] Thai agricultural commodity and food standard. 2006.
[c] The European commission, Council Directive "76/895/EEC" Insecticides MRLs Selected fruit & Vegetables

### 4.6.2 Levels of 3-PBA in urine

In this study, the concentration of 3-PBA in urine metabolite samples data for our study in 4 subdistricts of the studied areas, Fang district, Chiang Mai province.

In table 4.20 show, characteristic of school children in this studied include sex, age, family types, use of insecticide in agricultural area, and school children into the agricultural area. The study found that female student in the agricultural area, 52.8% in those aged 11-12 years, and 50.7 %. School children from agriculture families at 68.6%, and the use of insecticide in agriculture area 52.4%. Students to work in agriculture area 62.1%

Table 4.20 Characteristic of the study subject

| Variables | June 2009 (N=290) | March 2010 (N=285) |
|---|---|---|
| Gender | | |
| Male | 136 (46.9) | 134 (47.2) |
| Female | 154 (53.1) | 156 (52.8) |
| Age | | |
| ≤ 10 | 104 (35.7) | 107 (37.4) |
| 11-12 | 147 (50.7) | 140 (49.3) |
| ≥ 12 | 39 (13.4) | 73 (25.8) |
| Family types | | |
| Agricultural family | 199 (68.6) | 195 (68.7) |
| Non-agricultural family | 90 (31.0) | 88 (31.0) |
| Use of insecticide in agricultural area | | |
| Use insecticide | 152 (52.4) | 147 (51.8) |
| Non-use insecticide | 138 (47.6) | 137 (48.2) |
| School children into the agricultural area | | |
| Yes | 180 (62.1) | 177 (62.3) |
| No | 110 (37.9) | 107 (37.7) |

In analyzing by gender showed that 3PBA in urine by the second period, gender did not affect the concentration of 3 PBA. Shown in Table 4.21

Table 4.21 Concentration of 3 PBA metabolite detected in urine samples classified by gender

| Gender | Statistic | June 2009 (N=290) | | March 2011 (N=285) | |
|---|---|---|---|---|---|
| | | Unadj creatinine | Adj creatinine | Unadj creatinine | Adj creatinine |
| Male | Mean±SD | 18.45±37.9 | 0.14±0.3 | 4.91±7.3 | 0.04±0.1 |
| | Min | 1.06 | 0.013 | 1.00 | 0.004 |
| | Max | 198.99 | 1.59 | 47.07 | 0.46 |
| Female | Mean±SD | 19.82±27.0 | 0.13±0.3 | 4.35±7.2 | 0.04±0.1 |
| | Min | 1.31 | 0.004 | 1.02 | 0.007 |
| | Max | 134.63 | 1.45 | 49.32 | 0.46 |
| Total | Mean±SD | 18.93±33.3 | 0.14±0.3 | 4.68±7.2 | 0.04±0.1 |
| | Min | 1.06 | 0.004 | 1.00 | 0.004 |
| | Max | 198.99 | 1.59 | 49.32 | 0.46 |

Note: Unadjust creatinine (μg/L) and adjusted creatinine (μg/g creatinine)

Table 4.22 Mean concentration of 3 PBA metabolite detected in urine samples classified by school in June 2009

| school | N | Percent detection | Unadjust creatinine (μg/L) | | Adjust creatinine (μg/g creatinine) | |
|---|---|---|---|---|---|---|
| | | | Mean±SD | Maximum | Mean±SD | Maximum |
| Ban Lai Fang | 23 | 47.83 | 6.15±5.6 | 18.32 | 0.47±0.4 | 1.36 |
| Ban Mae Kha | 35 | 71.43 | 16.7±25.5 | 102.94 | 1.22±3.9 | 15.91 |
| Ban MaeLuang | 26 | 96.15 | 33.43±31.4 | 113.25 | 2.81±3.5 | 12.98 |
| Ban Tung Hluk | 16 | 81.25 | 18.19±31.3 | 131.29 | 1.90±2.7 | 8.38 |
| Ban Mae Ngon | 21 | 57.43 | 7.17±12.1 | 33.37 | 0.77±0.9 | 2.71 |
| Ban Lan | 28 | 82.14 | 21.10±22.6 | 79.74 | 1.53±2.1 | 7.9 |
| Ban Mung Chum | 26 | 69.23 | 21.09±18.3 | 53.65 | 1.74±1.4 | 3.48 |
| Ban San Sai Klong Noi | 43 | 79.07 | 15.51±31.3 | 134.63 | 1.53±3.2 | 14.54 |
| Rangsri | 72 | 61.10 | 25.58±46.2 | 198.99 | 1.99±3.2 | 11.72 |

The study found that the amount 3PBA separate school in June 2009, most school found that Ban Mea Luang 96.15% and the average concentration of most the concentration unadjusted creatinine was 33.43±31.4 μg/L and adjusted creatinine was 2.81±3.5 μg/g creatinine. Show in Table 4.22

Table 4.23 Mean concentration of 3 PBA metabolite detected in urine samples classified by school in March 2010

| school | N | Percent detection | Unadjust creatinine | | Adjust creatinine | |
|---|---|---|---|---|---|---|
| | | | Mean±SD | Maximum | Mean±SD | Maximum |
| Ban Lai Fang | 21 | 71.43 | 3.55±13.9 | 47.07 | 0.30±1.3 | 4.28 |
| Ban Mae Kha | 35 | 82.86 | 4.54±9.1 | 36.97 | 0.32±0.8 | 3.03 |
| Ban MaeLuang | 25 | 88.00 | 4.67±2.6 | 9.39 | 0.54±0.5 | 1.61 |
| Ban Tung Hluk | 17 | 82.35 | 4.92±13.5 | 38.74 | 0.57±1.6 | 4.62 |
| Ban Mae Ngon | 19 | 94.74 | 2.76±3.1 | 10.65 | 0.25±0.1 | 0.6 |
| Ban Lan | 27 | 81.48 | 3.06±2.6 | 10.05 | 0.29±0.4 | 1.23 |
| Ban Mung Chum | 26 | 88.46 | 5.87±4.1 | 17.39 | 0.65±0.3 | 1.57 |
| Ban San Sai Klong Noi | 39 | 84.62 | 6.98±9.5 | 49.32 | 0.67±1.1 | 4.64 |
| Rangsri | 76 | 75.00 | 4.80±4.7 | 22.47 | 0.37±0.7 | 4.09 |

The study found that the amount 3PBA separate school in March 2010, most school found that Ban Mea Nong 94.74%, and the average concentration of 3PBA higher concentration unadjusted creatinine was 6.98±9.5μg/L in Ban San Sai school and adjusted creatinine was 0.67±1.1μg/g creatinine. Show in Table 4.23.

Table 4.24 Concentration of 3-PBA in urine samples collected from school children in June 2009.

| Community | % pos | Dilution adjustment | GM (μg/l) | Max (μg/l) | Percentiles | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | | 25th | 50th | 75th | 95th |
| Mae Kha (n=58) | 62.1 | Unadjusted | 11.74 | 102.9 | 5.30 | 14.28 | 26.02 | 59.80 |
| | | Cre.adj | 0.09 | 1.6 | 0.04 | 0.08 | 0.22 | 0.75 |
| Mae Ngong (n=63) | 81.0 | unadjusted | 19.32 | 131.3 | 8.53 | 20.82 | 39.24 | 94.67 |
| | | Cre.adj | 0.10 | 0.8 | 0.06 | 0.11 | 0.20 | 0.53 |
| Mon Pin (n=54) | 75.9 | unadjusted | 16.03 | 79.7 | 6.17 | 21.10 | 33.13 | 60.57 |
| | | Cre.adj | 0.13 | 0.8 | 0.06 | 0.15 | 0.32 | 0.55 |
| Wiang (n=115) | 68.7 | unadjusted | 19.78 | 199.0 | 9.90 | 25.12 | 50.46 | 113.72 |
| | | Cre.adj | 0.16 | 1.5 | 0.08 | 0.18 | 0.39 | 0.93 |
| Total (n=290) | 71.4 | unadjusted | 17.41 | 199.0 | 7.35 | 18.93 | 39.41 | 87.14 |
| | | Cre.adj | 0.12 | 1.6 | 0.06 | 0.14 | 0.26 | 0.83 |

N= Number of samples, % pos = The positively percentages, Cre.adj = Creatinine adjusted, GM = Geometric mean, Max = Maximum, Concentration of individual 3-PBA presented in unit of μg/l for non-creatinine adjusted results and μg/g creatinine adjusted result

In June 2009, 3-PBA was detected most frequently in Mae Ngong (81.0%) followed by Mon pin (75.9%), Wiang (68.7%), and Mae Kha (62.1%), respectively. The result also shows the highest geometric mean value of 3 PBA unadjusted in waing at 19.78 μg/l. In addition, the geometric mean value of others area in Mae Ngong at 19.32 μg/l, Mon Pin at 16.03 μg/l, and Mae Kha at 11.74 μg/l. Shown in Table 4.24.

Table: 4.25 Distribution of 3-PBA in urine samples collected from school children in March 2010.

| Community | % pos | Dilution adjustment | GM (μg/l) | Max (μg/l) | Percentiles | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | | 25th | 50th | 75th | 95th |
| Mae Kha (N=56) | 78.6 | Unadjusted | 4.55 | 47.07 | 2.19 | 4.35 | 8.17 | 32.88 |
| | | Cre.adj | 0.04 | 0.43 | 0.02 | 0.03 | 0.09 | 0.28 |
| Mae Ngong (N=61) | 88.5 | unadjusted | 4.19 | 38.74 | 2.43 | 4.35 | 6.24 | 17.96 |
| | | Cre.adj | 0.61 | 6.70 | 0.33 | 0.60 | 0.88 | 2.41 |
| Mon Pin (N=53) | 84.9 | unadjusted | 4.18 | 17.39 | 2.23 | 4.58 | 6.79 | 10.75 |
| | | Cre.adj | 0.60 | 2.29 | 0.28 | 0.66 | 1.20 | 1.75 |
| Wiang (n=115) | 81.7 | unadjusted | 5.23 | 49.32 | 2.71 | 5.34 | 8.90 | 19.43 |
| | | Cre.adj | 0.71 | 6.73 | 0.39 | 0.67 | 1.24 | 2.67 |
| Total (N=285) | 83.2 | unadjusted | 49.32 | 4.67 | 2.53 | 4.68 | 8.39 | 19.26 |
| | | Cre.adj | 0.42 | 6.73 | 0.24 | 0.50 | 1.08 | 2.34 |

Note: N= Number of samples, % pos = The positively percentages, Cre.adj = Creatinine adjusted, GM = GeoMean, Max = Maximum, Concentration of individual 3-PBA presented in unit of μg/l for non-creatinine adjusted results and μg/g creatinine adjusted result

In March 2010, 3-PBA was detected most frequently in Mae Ngong (88.5%) followed by Mon pin (84.9%), Wiang (81.7%), and Mae Kha (78.6%), respectively. The result also shows the highest geometric mean value of 3 PBA unadjusted in waing at 5.23 μg/l. In addition, the geometric mean value of others area in Mae Kha at 4.55 μg/l, Mae Ngon at 4.19 μg/l, and Mon Pin at 4.18 μg/l. Shown in Table 4.25.

In figure 4.3, graph comparing the concentration of 3 PBA in urine samples with creatinine values were the result of the analysis in March 2010, there were 1.4 times higher than in June 2009. In subdistrict sepsrate analysis showed that 4.3 times higher than in Mae Kha district, 3.9 times higher than in Mae Ngon subdistrict, 2.9 times higher than in Mon Pin subdistrict, except in Wiang subdistrict in concentration higher than 4.1 times.

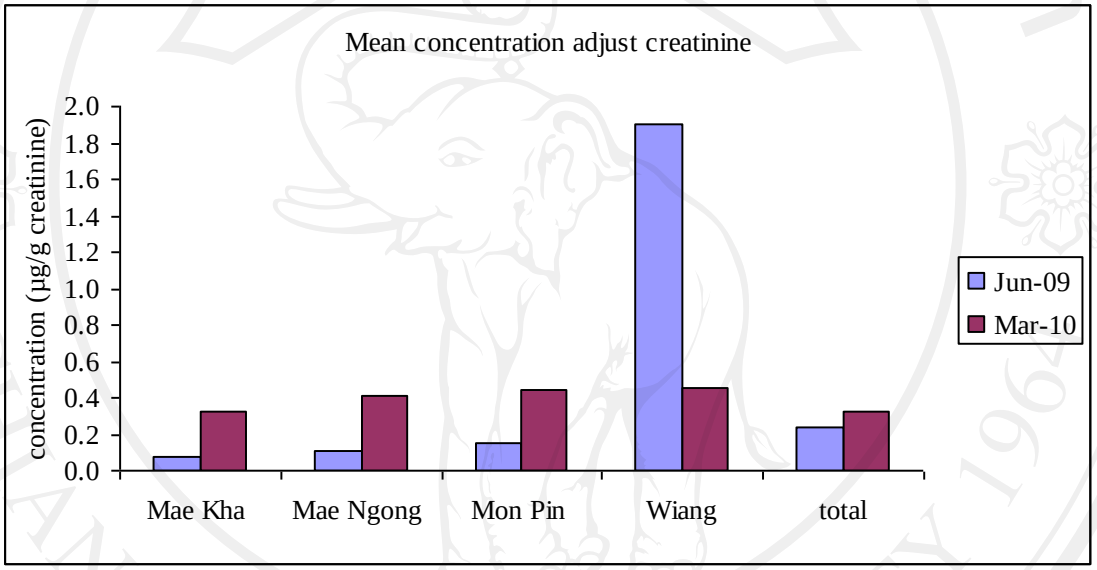


Figure 4.3 comparing the concentration of 3 PBA in urine samples