APPENDIX

MATERIALS

A1 Reagents of blood cholinesterase activity measurement

1) 5mM phosphate buffer, pH 7.7 (stock, 100X)

- Dissolved separately $NaH_2PO_4.2H_20$ (6.9 g) and $Na_2HPO_4.2H_20$ (8.9 g) in 100 mL distilled water
- Titrated $Na_2HPO_4.2H_20$ with $NaH_2PO_4.2H_20$ until pH 7.7
- Store at room temperature

2) 0.5 mM 5,5'-dithiobis-2-nitrobenzoic acid (DTNB), pH 7.7

- Dissolved DTNB (0.198 g) in 10 mL 5mM phosphate buffer pH 7.7 (stock)
- Added distilled water to about 900 mL and stirred on magnetic stirrer until well dissolved.
-Adjusted the pH 7.7 with 6N HCl and 6N NaOH.
-Filled up to 1,000 mL with water and stored at room temperature.

3) 156 mM acetylthiocholine iodide

- Dissolved acetylthiocholine iodide (0.4511g) in 10 mL distilled water
- Store at room temperature

4) 156 mM butyrylthiocholine iodide

- Dissolved butyrylthiocholine iodide (0.4948g) in 10 mL distilled water

- Store at room temperature

5) 12 mM Eserine

- Dissolved eserine (0.0390 g) in 10 mL distilled water

- Store at room temperature

A2 Reagents of salivary cholinesterase activity measurement

1) 1M phosphate buffer, pH 8 (stock, 10X)

- Dissolved each $NaH_2PO_4.2H_20$ (13.8 g) and $Na_2HPO_4.2H_20$ (17.8 g) in 100 mL distilled water

- Titrated $Na_2HPO_4.2H_20$ with $NaH_2PO_4.2H_20$ until pH 8

- Store at room temperature

2) 0.1 M 5,5'-dithiobis-2-nitrobenzoic acid (DTNB), pH 8

- Dissolved DTNB (0.396 g) in 10 mL 100% ethanol (C_2H_2OH)

3) 75 mM acetylthiocholine iodide

- Dissolved acetylthiocholine iodide (0.217 g) in 10 mL distilled water

- Store at room temperature

4) 75 mM butyrylthiocholine iodide

- Dissolved butyrylthiocholine iodide (0.238 g) in 10 mL distilled water

- Store at room temperature

A3 Reagents for Plasma sample preparation

1) 6N NaOH

- Dissolved NaOH (12 g) in 50 mL distilled water

- Store at room temperature

2) 6N HCl

-Added HCl 25 mL to 25 mL distilled water

-Mixed until well dissolved and stored at room temperature.

3) 0.2M Sodium acetate buffer, pH 4.5

- Weight Sodium acetate (16.41 g).

- Added distilled water to about 900 mL and stirred on magnetic stirrer until well dissolved.

-Adjusted the pH 4.5 with acetic acid.

-Filled up to 1,000 mL with water and stored at room temperature.

4) 1% acetic acid in a mixure of hexane and ethyl acetate (70 : 30 v/v)

-Added 140 mL of hexane to 60 mL of ethyl acetate.

-Pipetted out 2 mL of mixure solution and added 2 mL of acetic acid.

-Mixed until well dissolved and stored at room temperature.

A4 Reagents for ELISA

1) 0.1M Carbanate/bicarbonate coating buffer, pH 9.6

-Dissolved Na_2CO_3 (0.792 g) and $NaHCO_3$ (1.465 g) in 500 mL distilled water.

-Adjusted pH 9.6 with 6NNaOH

-Store at 4°C.

2) Phosphate buffer saline (PBS), pH 7.5 (stock, 10X)

- Weight NaCl (160 g), KH_2PO_4 (4 g), $Na_2HPO_4.2H_20$ (23 g) and KCl (4 g).

-Added distilled water to about 1900 mL and stirred on magnetic stirred until well dissolved.

-Adjusted the pH 7.5 with 6NHCl and 6NNaOH.

-Filled up to 2,000 mL with distilled water and stored at room temperature.

3) Citrate-acetate buffer, pH 5.5

- Weight Sodium acetate (13.61 g).

-Added distilled water to about 900 mL and stirred on magnetic stirred until well dissolved.

-Adjusted the pH 5.5 with acetic acid.

-Filled up to 1,000 mL with distilled water and stored at4°C.

4) 1% hydrogen peroxide

-Added 1mL of 30% H_2O_2 into 29 mL of distilled water.

-Store at 4°C.

5) 0.6% 3,3'5,5'-tetramethyl benzidine (TMB)

-Weight TMB (60 mg).

-Added 10 mL DMSO and stored in the dark at room temperature.

6) 4.6 Phosphate buffer saline with 0.05% Tween (PBST, Washing buffer)

-Added tween-20 (0.5 mL) into phosphate buffer saline pH, 7.5 (1,000 mL) and mix well.

7) 0.5% BSA (Blocking reagent)

-Weight BSA (0.125g) dissolved in PBS, pH 7.5 (25 mL)

8) TMB substrate working solution

-Added Citrate buffer, pH 5.5 (12.5 mL), 0.6% TMB (200 µl) and 1% hydrogen peroxide (50µl)

-Mixed all together until well dissolved

9) 2N H_2SO_4 (Stopping reagent)

-Added 222 mL of 2N H_2SO_4 into 778 mL of distilled water.

CURRICULUM VITAE

Name	Ms.Sarunya Thiphom
Date of birth	October 26, 1981
Education background	2012: Ph.D. (Enviromenatal Science), Chiang Mai University, Thailand. 2004-2005: Grad. Dip. in Teaching Profession, Faculty of Education, Chiang Mai University, Thailand. 2000-2004: B.Sc. Biology Hons. Faculty of Science, Chiang Mai University, Thailand.
Work experience	I had 8 months experiences in laboratory about enzyme purification and immunoassay in Department of Entomology and Nematology, University of California, Davis, Unites state, 2009
Scholarships	2000-2004: Promotion and Producing Capability Teacher in Science and Mathematics Project (PCT ST), The Institute for the Promotion of Teaching Science and Technology (IPST), Thailand. 2005-2010: Golden Jubilee PhD program/The Thailand Research Fund (RGJ/TRF) (Contract PHD/0184/2547), Thailand.

Presentation

1) Poster: Sarunya Thiphom, Tippawan Prapamontol,Ampica Mangklabruks, Somporn Chantara, Chaisuree Suphavilai. Levels of butyryl-cholinesterase activities in human plasma and saliva: a preliminary study of salivary test for pesticide exposure assessment. RGJ-Ph.D. Congress VIII, Jomtien Palm Beach Hotel & Resort, Pattaya, Chonburi, Thailand, April 20-22, 2007.

2) Oral presentation: Sarunya Thiphom, Tippawan Prapamontol, Ampica Mangklabruks, Somporn Chantara, Chaisuree Suphavilai, Bruce D. Hammock, Shirley J. Gee. Evaluation of human exposure to pyrethroid insecticides. RGJ-Ph.D. Congress XI, Jomtien Palm Beach Hotel & Resort, Pattaya, Chonburi, Thailand, April 1-3, 2010.

3) Oral presentation: Sarunya Thiphom, Tippawan Prapamontol, Ampica Mangklabruks, Somporn Chantara, Chaisuree Suphavilai, Bruce D. Hammock. A Novel ELISA Method for Assessing Pyrethroid Insecticide Exposure by Detecting 3-PBA-Adduct in Plasma and Its Application in Farmer andConsumer Groups. RGJ Seminar Series 84, UNISERVE, Chiang Mai University,Thailand, September2, 2011.

Publication

1) Thiphom, S., Prapamontol, T., Chantara, S., Mangklabruks, A., Suphavilai, C., Ahn, K.C., Gee, S.J., Hammock, B.D., 2012. An enzyme-linked

2) immunosorbent assay for detecting 3-phenoxybenzoic acid in plasma and its application to farmers and consumers. Anal Methods 4 (11), 3772-3778.

3) Thiphom, S., Prapamontol, T., Chantara, S., Mangklabruks, A., Suphavilai, C., 2013. A method for measuring cholinesterase activity in human saliva and its application to farmers and consumers. Anal Methods 5 (18), 4687 – 4693.

4) Thiphom, S., Prapamontol, T., Chantara, S., Mangklabruks, A., Suphavilai, C., Ahn, K.C., Gee, S.J., Hammock, B.D., 2013. Determination of the pyrethroid insecticide metabolite 3-PBA in plasma and urine samples from farmer and consumer groups in northern Thailand. J Environ Sci Health [B], Ms.#B-1787, Accepted 2 August 2013.