

TABLE OF CONTENTS

| | Page |
|--|-------------|
| Acknowledgements | iii |
| Abstract in English | v |
| Abstract in Thai | vii |
| List of tables | xii |
| List of illustrations | xvi |
| List of abbreviations | xix |
| Chapter 1. Introduction | 1 |
| 1.1 The need and safe use of pesticides | 1 |
| 1.2 Study site of interest | 3 |
| 1.3 Objectives of the present study | 5 |
| Chapter 2. Literature Review | 6 |
| 2.1 Pesticide definition and history | 6 |
| 2.2 Organophosphate and carbamate pesticides | 7 |
| 2.3 Measurement of ChE activity | 15 |
| 2.4 Pesticides impact on population and community | 18 |
| 2.5 Environmental risk assessment | 20 |
| 2.6 ChE activity as biomarker of exposure to OPs and CAs in aquatic ecosystem | 24 |
| 2.7 Existing information of study site | 25 |

| | |
|--|----|
| Chapter 3. Methodology | 27 |
| 3.1 Location of research operation and data collection | 27 |
| 3.2 Materials and Methods | 29 |
| 3.2.1 Ecological works | 29 |
| 3.2.1.1 Physico-chemical parameters | 30 |
| 3.2.1.2 Biological components | 31 |
| 3.2.2 Toxicological tests | 33 |
| 3.2.2.1 Measurement of ChE activity | 34 |
| 3.2.2.2 <i>In vitro</i> Chironomid ChE activity inhibition test | 43 |
| Chapter 4. Results | 47 |
| 4.1 Ecological works | 47 |
| 4.1.1 Physico-chemical parameters of streams | 47 |
| 4.1.2 Cluster analysis of physico-chemical parameters | 51 |
| 4.1.3 Biological components | 51 |
| 4.1.4 Cluster analysis of biological components | 55 |
| 4.1.5 Correlation between physico-chemical parameters and biological components | 56 |
| 4.2 Toxicological test | |
| 4.2.1 ChE activity of chironomids | 57 |
| 4.2.2 <i>In vitro</i> chironomid ChE activity inhibition test | 59 |

| | |
|---|-----|
| 4.2.3 Cluster analysis analysis of toxicological tests and some biological components | 59 |
| Chapter 5. Discussion | 63 |
| 5.1 Physico-chemical parameters | 63 |
| 5.2 Biological components | 65 |
| 5.3 Chironomids ChE activity in Mae Sa Noi and the control stream | 67 |
| 5.4 <i>In vitro</i> inhibition test | 69 |
| Chapter 6. Conclusion | 72 |
| References | 75 |
| Appendices | 84 |
| Curriculum Vitae | 100 |

LIST OF TABLES

| Table | | Page |
|--------------|---|-------------|
| 3.1 | Concentrations of methyl-parathion for chironomid ChE inhibition test. In left column were concentrations of methyl-parathion, which they are prepared. After they were added onto test tubes for inhibition testing, they were diluted to concentrations that showed in the right column | 44 |
| 4.1 | Physico-chemical parameters in Mae Sa Noi and control streams at 1050, 900 and 700 mAMSL in dry and rainy season in 1999 to cold season in 2000 | 48 |
| 4.2 | Comparison of mean physico-chemical parameters between elevations (1050, 900 and 700 mAMSL), seasons (dry 1999, rainy 1999 and cold 2000) and streams (Mae Sa Noi and control streams) | 50 |
| 4.3 | Mean biological components except for indices in Mae Sa Noi and control streams at 1050, 900 and 700 mAMSL in dry and rainy seasons in 1999 to cold season in 2000 | 52 |

| Table | Page |
|--|-------------|
| 4.4 Comparison of means of biological components between elevations (1050, 900 and 700 mAMSL), seasons (dry 1999, rainy 1999 and cold 2000) and streams (Mae Sa Noi and control streams) | 54 |
| 4.5 Pearson correlation coefficient (r) between physico-chemical parameter; stream velocity and pH and mean total population density and mean chironomid population density | 56 |
| 4.6 Chironomid ChE activity in Mae Sa Noi and the control stream at elevation 1050, 900 and 700 mAMSL in rainy and cold seasons | 58 |
| 4.7 Comparison of mean chironomid ChE activity between elevations (1050, 900 and 700 mAMSL), seasons (dry 1999, rainy 1999 and cold 2000) and streams (Mae Sa Noi and control streams) | 58 |
| 4.8 Concentration of methyl-parathion that caused inhibition of chironomid ChE activity at 25%, 50% and 75% in Mae Sa Noi and the control stream at elevation 1050, 900 and 700 mAMSL in cold season | 60 |

| Table | Page |
|---|------|
| 4.9 Comparison of methyl-parathion concentration (μM) that cause inhibition of chironomid ChE activity for 25%, 50% and 75% <i>in vitro</i> condition, chironomid sampling from Mae Sa Noi and the control stream at elevation 1050, 900 and 700 mAMSL in cold season | 60 |
| A1. Mean of physico-chemical parameters, biological components, chironomid ChE activity ($\mu\text{mol}/\text{min}/\text{mg}$ protein) and concentrations of methyl-parathion causing 50% inhibition of chironomid ChE activity (50% inh M-P conc; μM) in the control stream at elevation 1050, 900 and 700 mAMSL (S1050, S900 and S700) respectively and in Mae Sa Noi stream at same elevation (M1050, M900 and M700) in dry 1999, rainy 1999 and cold season 2000 | 84 |
| A2. Taxa and number of macroinvertebrates sampled with surber sampler from the control and Mae Sa Noi Streams at 1050, 900 and 700 mAMSL in dry season | 86 |
| A3. Taxa and number of macroinvertebrates sampled with surber sampler from the control and Mae Sa Noi Stream at 1050, 900 and 700 mAMSL in rainy season | 88 |

| Table | Page |
|--|-------------|
| A4. Taxa and number of macroinvertebrates sampled with surber sampler from the control and Mae Sa Noi Stream at 1050, 900 and 700 mAMS L in cold season | 90 |
| A5. ChE activity ($\mu\text{mol}/\text{min}/\text{mg}$), absorbance at 412 nm and protein content of chironomids sampled from the control and Mae Sa Noi streams at 1050, 900 and 700 mAMS L in rainy season | 92 |
| A6. ChE activity ($\mu\text{mol}/\text{min}/\text{mg}$), absorbance at 412 nm and protein content of chironomids sampled from the control and Mae Sa Noi Stream at 1050, 900 and 700 mAMS L in cold season | 93 |
| A7. ChE activity ($\mu\text{mol}/\text{min}/\text{mg}$) at each concentration (μM) of methylparathion (MP), absorbance at 412 nm and protein content of chironomids sampled from the control and Mae Sa Noi Stream at 1050, 900 and 700 mAMS L in cold season | 94 |
| A8. Daily Rainfall (mm) in Chiang Mai province from 1 April 1999 to 29 February 2000. Source: Meteorology Center, Chiang Mai | 97 |
| A9. Organophosphates (OPs) and Carbamates (CAs) Pesticides used in Mae Sa Mai with their properties. Source: Stuetz (1999) | 99 |

LIST OF ILLUSTRATIONS

| Figure | Page |
|--|------|
| 2.1 Cholinergic nerve impulse transmission in synapsis, nerve endings or ganglia (Paasivirta, 1991) | 12 |
| 2.2 Parathion metabolism in mammals and insects (Matsumura, 1972) | 14 |
| 2.3 Reaction of parathion changing to paraoxon in parathion metabolism (Hassall, 1990) | 14 |
| 2.4 Flow chart shows application of bioindicators to indicate environmental impact (Modified from: Hellawell (1986), Calow (1993), Nagel (1995)) | 21 |
| 3.1 The map of Mae Sa Noi stream and its control stream on Doi Suthep-Pui mountain, Chiang Mai, Thailand (Source: Royal Thai Survey Department) | 28 |
| 3.2 Flow chart showed processes of chironomid ChE activity measurement (Modified from: Scaps <i>et al</i> (1997), Seto <i>et al</i> (1997)) | 35 |

| Figure | Page |
|---|------|
| 3.3 Flow chart showed processing of chironomid homogenization (Modified from: Scaps <i>et al</i> (1997)) | 38 |
| 3.4 Flow chart showed processes of chironomid ChE activity measurement (Modified from: Seto <i>et al</i> (1997)) | 39 |
| 3.5 Flow chart showed processes of chironomid protein determination | 42 |
| 3.6 Flow chart showed processing of <i>in vitro</i> Chironomid ChE activity inhibition test | 45 |
| 3.7 Flow chart showed all processes of chironomid ChE activity measurement and <i>in vitro</i> inhibition test | 46 |
| 4.1 Dendrogram show the grouping of physico-chemical parameters of each site; G = group | 51 |
| 4.2 Dendrogram show the grouping of macroinvertebrate community of each site, G = group | 55 |
| 4.3 Dendrogram show the grouping of ChE activities and susceptibility of all sites in cold season | 61 |

| Figure | Page |
|---|-------------|
| 4.4 Dendrogram show the grouping of ChE activities, susceptibility, mean total population density and mean chironomid population density of all sites in cold season | 61 |
| A1. Graph of mean population density (individual/m ²) and number of sample using for selection appropriate replications in macroinvertebrate sampling, A1a and A1b using raw data of population density from MS900 and MS700 respectively | 95 |
| A2 Graph show ChE activity ($\mu\text{mol}/\text{min}/\text{mg}$) at each concentration (μm) of methyl-parathion, of chironomids sampling from the control and Mae Sa Noi Stream at 1050, 900 and 700 mAMSL in cold season | 96 |
| A3 Monthly Rainfall (mm) in Chiang Mai province from April 1999 to February 2000. Source: Meteorology Center, Chiang Mai. Arrows show sampling time of ecological work and chironomids sampling | 98 |
| A4 Maximum, minimum and mean of air temperature ($^{\circ}\text{C}$) in Chiang Mai province from April 1999 to February 2000. Source: Meteorology Center, Chiang Mai. Arrows show sampling time of ecological work and chironomids sampling | 98 |

LIST OF ABBREVIATIONS

| | | |
|-------|---|--|
| Aa | = | Acetic acid |
| ACEA | = | Acetyl coenzyme A |
| ACh | = | Acetylcholine |
| AChE | = | Acetylcholinesterase |
| AChI | = | Acetylthiocholine Iodide |
| BSA | = | Bovine Serum Albumin |
| CAs | = | Carbamate pesticides |
| Ch | = | Choline |
| ChE | = | Cholinesterase |
| DTNB | = | 5,5' dithiobis-(2-nitrobenzoic acid) |
| FAO | = | Food and Agricultural Organization |
| GAP | = | Good Agricultural Practice |
| mAMSL | = | Meters above mean sea level |
| MRL | = | Maximum Residue Limit |
| OCs | = | Organocholine pesticides |
| OPs | = | Organophosphate pesticides |
| rpm | = | Revolution per minute |
| SED | = | Square Euclidean Distance |
| SPSS | = | Statistical Package for Social Science |
| WHO | = | World Health Organization |