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TITLE: Established Diagnostic Doses of Six Synthetic Pyrethroids, Currently Used for the Control of *Aedes aegypti* L., a Vectors of Dengue

NAME: Mr. Suntorn Pimnon

THIS THESIS HAS BEEN ACCEPTED BY

THESIS ADVISOR

(Miss Waraporn Juntarajumnong, Ph.D.)

THESIS CO-ADVISOR

(Professor Theeraphap Chareonviriyaphap, Ph.D.)

DEPARTMENT HEAD

(Professor Theeraphap Chareonviriyaphap, Ph.D.)

APPROVED BY THE GRADUATE SCHOOL ON _____

DEAN

(Associate Professor Gunjana Theeragool, D.Agr.)

THESIS

ESTABLISHED DIAGNOSTIC DOSES OF SIX SYNTHETIC PYRETHROIDS, CURRENTLY USED FOR THE CONTROL OF *AEDES AEGYPTI* (L.), A VECTORS OF DENGUE

SUNTORN PIMNON

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Suntorn Pimnon 2012: Established Diagnostic Doses of Six Synthetic Pyrethroids, Currently Used for the Control of *Aedes aegypti* L., a Vectors of Dengue. Master of Science (Entomology) Major Field: Entomology, Department of Entomology. Thesis Advisor: Miss Waraporn Juntarajumnong, Ph.D. 81 pages.

Establishing baseline insecticide discriminating doses is crucial in accurately determining susceptibility status and changing temporal patterns of physiological response in mosquito populations. Synthetic pyrethroids are the predominant chemicals used for controlling adult *Aedes aegypti* and *Aedes albopictus*, both vectors of dengue viruses, in Thailand. Presently, only 2 synthetic pyrethroids, (permethrin and *lambda*-cyhalothrin), have published diagnostic dose rates for monitoring *Ae. aegypti* insecticide resistance. This study established the diagnostic lethal concentrations (LC) for six different synthetic pyrethroids active ingredients available in Thailand for dengue vector control. The United States Department of Agriculture (USDA) insecticide-susceptible strain of *Ae. aegypti* was used to establish the baseline concentrations for subsequent susceptibility testing of field populations . Our findings obtained lower discriminating concentrations for *lambda*-cyhalothrin and permethrin than recommended by WHO, at 2.5 and 1.7-fold lower dosing, respectively. The susceptibility status of three different geographical populations of field collected *Ae. aegypti* were tested using standard WHO procedures All three field strains demonstrated varying levels of physiological resistance to each compound. Strong physiological resistance to permethrin was seen in the Nong Khai populations (6% mortality) and to deltamethrin from Khon Kaen and Nong Khai (4% mortality). We conclude that establishing the true baseline diagnostic concentration of an insecticide is of paramount importance in accurately determining the susceptibility status in field collected mosquitoes. If possible, discriminating doses should be established for all insecticides and test assays run concurrently with a known susceptible strain for more accurate monitoring of resistance in mosquito populations in Thailand.

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TABLE OF CONTENTS

Page

TABLE OF CONTENTS	i
LIST OF TABLES	ii
LIST OF FIGURES	iii
LIST OF ABBREVIATIONS	iv
INTRODUCTION	1
OBJECTIVES	4
LITERATURE REVIEW	5
MATERIALS AND METHODS	28
RESULTS AND DISCUSSION	36
Results	36
Discussion	46
CONCLUSION	51
LITERATURE CITED	52
APPENDIX	64
CURRICULUM VITAE	81

LIST OF TABLES

Table		Page
1	Percent mortality of a laboratory susceptible strain of *Aedes aegypti* (USDA) exposed to 6 different pyrethroids	38
2	Percent mortality of a laboratory susceptible strain of *Aedes aegypti* (USDA) exposed to 6 different pyrethroids using 3 different concentrations	39
3	Probit dose/mortality analysis of a laboratory susceptible strain of *Aedes aegypti* (USDA) exposed to 6 different pyrethroids using 3 different concentrations establishing lethal concentration for each AI.	40
4	Percent knockdown and mortality of a laboratory susceptible pyrethroids using diagnostic concentration	42
5	Percent knockdown and mortality of Kanchanaburi strain of *Aedes aegypti* using an established diagnostic concentration of each insecticide	43
6	Percent knockdown and mortality of Khon Kaen strain of *Aedes aegypti* using an established diagnostic concentration of each insecticide	44
7	Percent knockdown and mortality of Nong Khai strain of *Aedes aegypti* using an established diagnostic concentration of each insecticide	45

Appendix Table

1	WHO recommended insecticides for indoor residual spraying and established diagnostic concentration	65
2	Steps in calculating probit analyses computations for the fitting of a probit regression equation Probit analysis	66

LIST OF FIGURES

Figure		Page
1	World distribution of dengue viruses and their mosquito vector, *Aedes aegypti*, in 2006	8
2	*Aedes aegypti* mosquitoes life cycle	10
3	Alphacypermethrin chemical structure	16
4	Bifenthrin chemical structure	18
5	Cypermethrin chemical structure	19
6	Deltamethrin chemical structure	21
7	Lamdacyhalothrin chemical structure	23
8	Permethrin chemical structure	25
9	Map of the three localities of *Aedes aegypti* mosquitoes collection sites in Thailand	30
10	Insecticides susceptibility test (A) Selection of 25 female mosquitoes (B) Selection for female mosquitoes (3- 4 day-old) (C) Holding tube (D) Holding tube and treatment tube (E) Susceptibility test with 1h exposure to treated papers (F) Holding tube of which sugar pads are placed on top	34

Appendix Figure

1	On the probability of mortality (% mortarity) the concentration (dose)	68

LIST OF ABBREVIATIONS

Ae	=	Aedes
α	=	Alpha
Chi sq	=	Chi-squared
°C	=	degree Celsius
cm^2	=	square centimeter
L	=	Linnaeus
LC	=	Lethal concentrations
LD	=	Lethal dose
USDA	=	United States Department of Agriculture
ml	=	milliliter
%	=	Percent
MOPH	=	Ministry of Public Health
WHO	=	World Health Organization

ESTABLISHED DIAGNOSTIC DOSES OF SIX SYNTHETIC PYRETHROIDS, CURRENTLY USED FOR THE CONTROL OF *AEDES AEGYPTI* L., A VECTORS OF DENGUE

INTRODUCTION

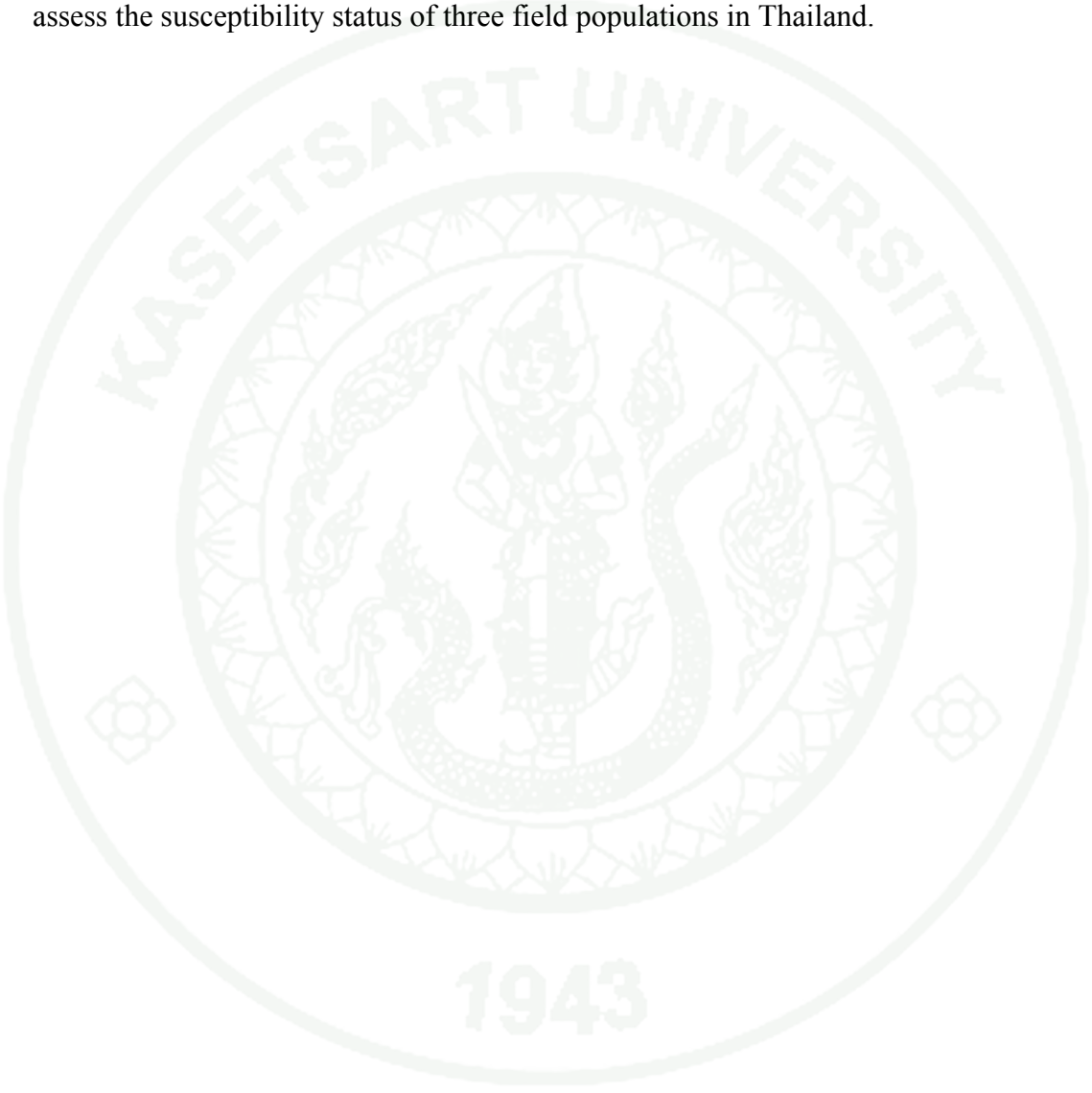
Many tropical and subtropical countries around the world present risk for dengue fever and dengue hemorrhagic fever (DF/DHF). Between 2.5 and 3 billion people (two-fifths the world's population) are at risk of contracting dengue, many of whom live in the Southeast Asian region (WHO, 2002). With an estimated 50-100 million people having symptomatic dengue infection each year, the majority of cases occur primarily in crowded, impoverished urban regions of the world (Gubler, 1998; Gibbons and Vaughn, 2002). In Southeast Asia, DHF, a severe manifestation of dengue, has shown a disturbing increase from an annual rate of < 10,000 in the 1960s to >200,000 in the 1990s (Gibbons and Vaughn, 2002). In Thailand, there were 48,514 reported dengue cases and 53 deaths in 2010 which represents a small fraction of the actual number of mild and asymptomatic infections that same period (MOPH 2010).The four different virus serotypes (DEN-1, 2, 3, 4) are transmitted by mosquitoes, primarily *Aedes aegypti* L., a highly efficient vector mosquito because of its close association with humans and exploitation of domestic and peridomestic environments, most notably in dense urban areas. As yet, no commercial multivalent dengue vaccine is available, therefore prevention of this disease remains almost entirely dependent on using methods of control that attack both adult and immature stages of the mosquito. Vector control remains the most effective means of reducing risk of virus transmission (Reiter and Gubler, 1997; WHO, 1999). Unfortunately, *Ae. aegypti* has confounded most organized control efforts to bring vector population densities below sustainable thresholds to eliminate transmission.

In Thailand, the standard vector control techniques are based on use of chemicals and source reduction of larval habitats. Many chemical compounds, including organophosphates, carbamates, synthetic pyrethroids, and so-called bio-rational pesticides (bacterial toxins and insect growth regulators) have been used in

national public health vector control programs (Reiter and Gubler, 1997; WHO, 1999). In Thailand, synthetic pyrethroids, e.g., deltamethrin, cyfluthrin, and permethrin, are common active ingredients in many commercial products designed for controlling household adult mosquitoes *Ae. aegypti*. However, control efforts have been hampered by the development of resistance to many of these insecticide compounds by *Ae. aegypti* throughout Thailand (Chareonviriyaphap *et al*. 1999, Somboon *et al*., 2003 Sathantriphop *et al*., 2006, Thanispong *et al*., 2008, 2010). The selection pressure for developing resistance to pyrethroids has largely been attributed to the frequent and pervasive use of the same chemical class of compounds and is believed to have a direct bearing on the effective management and prevention of vector-borne diseases in general (Hemingway and Ranson, 2000). Although there are a number of reports that described the status of pyrethroid resistance in *Ae. aegypti* populations in Thailand (Chadwick *et al*., 1977; Paeporn *et al*., 2004; Chareonviriyaphap *et al*., 1999; Yaicaharoen *et al*., 2005; Sathantriphop *et al*., 2006; Jirakanjanakit *et al*., 2007; Thanispong *et al*., 2008), all reports were based on use of 'diagnostic' doses established by the World Health Organization (WHO; 1998, 2006). To our knowledge, baseline insecticide susceptibility has not been established for detection of insecticide resistance in populations of *Ae. aegypti* in Thailand. This study aims were two-folds: 1) establishing the baseline susceptibility levels of six synthetic pyrethroids using the USDA susceptible standard strain of *Ae. aegypti* and 2) determining the susceptibility level of several field populations of *Ae. aegypti* based on these discriminating concentrations.

Synthetic pyrethroids are the predominant chemicals used for controlling adult *Aedes aegypti* and *Aedes albopictus*, both vectors of dengue viruses in Thailand. Establishing a baseline insecticide discriminating dose is crucial for determining susceptibility status and changing temporal patterns of physiological response over time in mosquito populations. Most insecticides used for the control of anopheline malaria vectors have well established and recommended discriminating ('diagnostic' doses) for routine monitoring of vector populations. However, currently very few insecticides have analogous discriminating doses by which to test the susceptibility of *Ae. aegypti*. Presently, only 2 synthetic pyrethroids, (permethrin and Lambda-

cyhalothrin), have published diagnostic dose rates for monitoring *Ae. aegypti*. The objective of this study was to establish the baseline diagnostic concentrations for six synthetic pyrethroids available in Thailand for dengue vector control. For purposes of accurate comparison, the baseline lethal concentrations derived from a fully insecticide-susceptible laboratory strain of *Ae. aegypti* were subsequently used to assess the susceptibility status of three field populations in Thailand.



OBJECTIVES

1. To establish the baseline susceptibility levels of six synthetic pyrethroids using the USDA susceptible standard strain of *Ae. aegypti* and to detect physiological resistance in *Ae. aegypti* strains using established diagnostic doses of synthetic pyrethroids

2. To determine the susceptibility level of field populations of *Ae. aegypti* from Kanchanaburi, Khon Kaen, Nong Khai provinces in Thailand

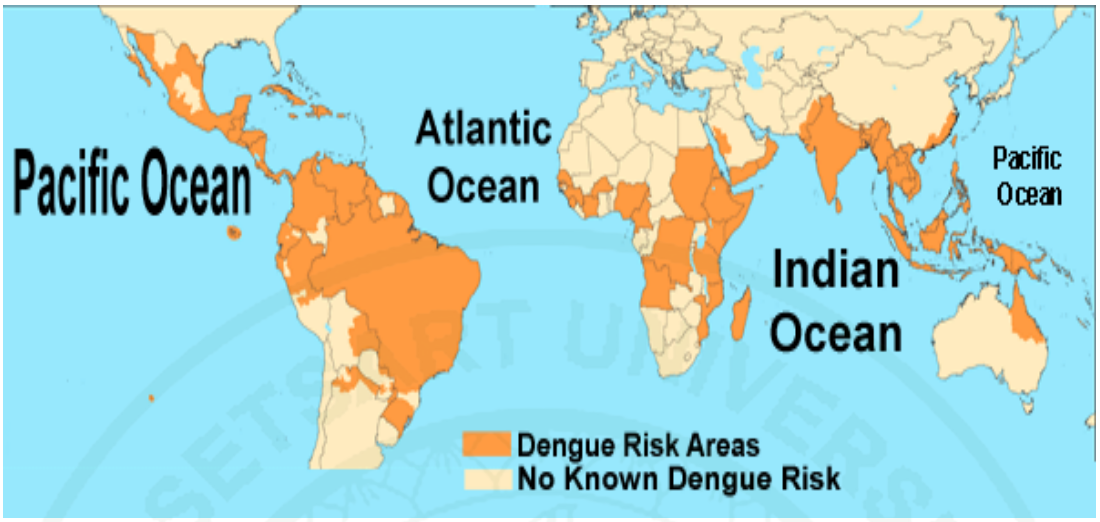
LITERATURE REVIEW

1. Dengue situation

Dengue fever (DF) and its most serious complication, dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS) are caused by one of four closely related viruses, separated into antigenically distinct virus serotypes DEN1, DEN2, DEN3 and DEN4 of the genus *Flavivirus*. This infection is transmitted from person to person by the bite of infected *Aedes* mosquitoes, principally *Aedes aegypti*. Illness can include a variety of other signs and symptoms ranging from fever, headache, joint pain, nausea and vomiting (typical dengue fever) to high fever, subdermal hemorrhage, hepatomegaly, lowered platelet count, high bleeding tendency (dengue hemorrhagic fever) to the same clinical signs and symptoms as DHF with circulatory failure, shock until death (dengue shock syndrome) (Anon, 1980). The incidence and geographic distribution of dengue have increased dramatically in recent decades. More than half the world's population now lives in areas at risk of infection. The first reported epidemics of dengue fever occurred in Asia, Africa, and North America between 1779-1780, the disease was identified and named in 1779. Initially, it was rather benign. A global pandemic began in Southeast Asia in the 1950s; by 1975, DHF had become a leading cause of hospitalization and death among children in many countries in those regions. In the 1980s, DHF began a second expansion into Asia when Sri Lanka, India and the Maldives Islands encountered their first DHF epidemic. Dengue has become more common since the 1980s, and by the late 1990s dengue was the most important mosquito-borne (viral) disease affecting humans after malaria (Gubler and Clark, 1995).

From 1955 to 1976, the WHO reported incidence worldwide was consistently fewer than 40,000 cases per year, but from 1977 to 2007, there have been epidemic cycles every 2-4 years and a gradual trend toward greater overall case numbers and more countries reporting cases. In this period, the lowest number of annual dengue cases reported was 110,000 in 1979, and the highest was 1.3 million cases in 2002.

In Thailand, the first published record of the incidence of dengue hemorrhagic fever (DHF) appeared before 1958 as 'influenza with hemorrhagic' manifestations, with only 50-100 cases reported per year (Ministry of Public Health, 2003).It was recognized as dengue hemorrhagic fever (DHF) following the first documented outbreak in 1958 (Nimmannitya, 1978). The first outbreak occurred only in Bangkok and Thonburi Province. After that this disease occurred in some provinces closed to Bangkok in 1963-1964 and spread to the province connected to Bangkok by transportation in 1965-1967. From the year 1978 then this disease has become recognized as one of the major public health problem (Wangrungsap, 1992). During the past four decades, there were reports that major epidemics of dengue occurred in every 2 to 4 years. Moreover, it seemed that the severity of disease is still unchanged. The number of annually reported cases has been increased gradually from 2,500 cases in 1958 to 174,285 cases, with 1,007 deaths, in 1987 (Ungchusak and Kunasol, 1988). Moreover, it was reported that severity of the disease has tended to increase. The worst outbreak of DHF in Thailand was in 1987, with the total of 171,630 cases and the death cases by DEN-3 was the dominant serotype (WHO, 1999). The second largest recorded epidemic of DHF (by cases) occurred in 1997-1998, the number of reported cases declined to 101,689, with 253 deaths in 1997. In 1998, a total of 129,954 cases with 424 deaths were reported during the period of epidemic However , there are sudden decreased in cases in year 1999-2000 because many control program were used during that time. However, in 2001-2002 there were a serious epidemic of dengue reported again in 2001-2002 with the number of 132,082 cases during the epidemic, in 2001 and 108,905 cases with 172 deaths in 2002. Data for the year 2003, the total number of DHF cases was 63,657 cases, with 75 deaths reported, in 2004 show decrease in reported cases from year 2003 to 39,135 cases with 48 deaths. In 2005 showed 45,893 cases with 71 deaths. The DHF cases showed sudden increase in year 2007 to 65,581 cases with 95 deaths from 46,829 cases with 59 deaths in 2006 (Annual report bureau of vector borne disease, 2007). Again the cases increase in 2008 to 87,494 with 101 deaths. Recently, during the eighth months of the year 2011, the total number of dengue cases was 34,744 with 25 deaths (Bureau of Epidemiology, 2011). Therefore, DHF is still the most important vector borne disease in Thailand nowadays.



: Areas with *Aedes aegypti* and dengue epidemic

: Areas infested with *Aedes aegypti*

Figure 1 World distribution of dengue viruses and their mosquito vector, *Aedes aegypti*, in 2008.

Source: CDC (2008).

2. *Aedes aegypti* mosquito

2.1 Biology

Aedes aegypti, is widely but sporadically distributed throughout the tropical and subtropical areas of the world. Its distribution appears to be related to the 20°C isotherm which roughly correlates with latitudes 40°N and 40°S (Kettle, 1984). It is a highly domesticated mosquito which can complete its entire life cycle within the confines of a single human dwelling. The female lays its eggs in small containers holding water in houses. It will also lay eggs in small amounts of peridomestic water which collects in tires, plastic containers and other debris associated with human settlement. When the embryo inside the egg of *Ae. aegypti* has developed to a certain stage the egg becomes resistant to desiccation. It may then enter diapause in which it

can remain for about a year. When the eggs are flooded they hatch and the larvae commence their development immediately. This ability of *Ae. aegypti* to produce diapausing eggs enables the species to survive in areas with prolonged dry seasons while the rapid hatching of the eggs on flooding and the speedy development of the immature stages are adaptations to breeding in temporary collections of water.

Adult *Ae. aegypti* emerging from indoors breeding sites can complete their cycle without going outside. Swarming is not an essential component of mating. Males orientate to females by responding to the female's wing beat and specific identification is achieved by a contact pheromone on the female. Although in domestic female *Ae. aegypti* autogeny is low, blood feeding presents no problem because the female is strongly anthropophilic and feeds readily on the human inhabitants of the dwelling (Gubler, 1997). The blood-fed female rests in the house while maturing her ovaries and then deposits her eggs in domestic water containers. The cycle is then complete. It is not surprising that a species with such modest requirements had readily adapted to laboratory colonization. The fact that *Ae. aegypti* produces diapausing eggs made it easy to disseminate material widely throughout the world, even before the days of air transport, and colonies of *Ae. aegypti* have been maintained for many years at centers in the northern hemisphere without the introduction of new genetic material. Such colonies form valuable material for studying biological processes but caution must be used in applying the results obtained on such material to "natural populations".

Aedes aegypti (Diptera: Culicidae), the principle vector of DF and DHF in the world, probably originated in the forest of Africa. It is widely distributed in the Southeast Asia, West Pacific, Africa and Americas (Christophers, 1960). This vector has also been known as the principal vector of urban yellow fever in Africa and Central and South America (Scanlon, 1965). It is a peridomestic species found not far from human dwelling. The life cycle of *Ae. aegypti* is a complete metamorphosis consisting of four developmental stage namely, egg, larva, pupa and adult.

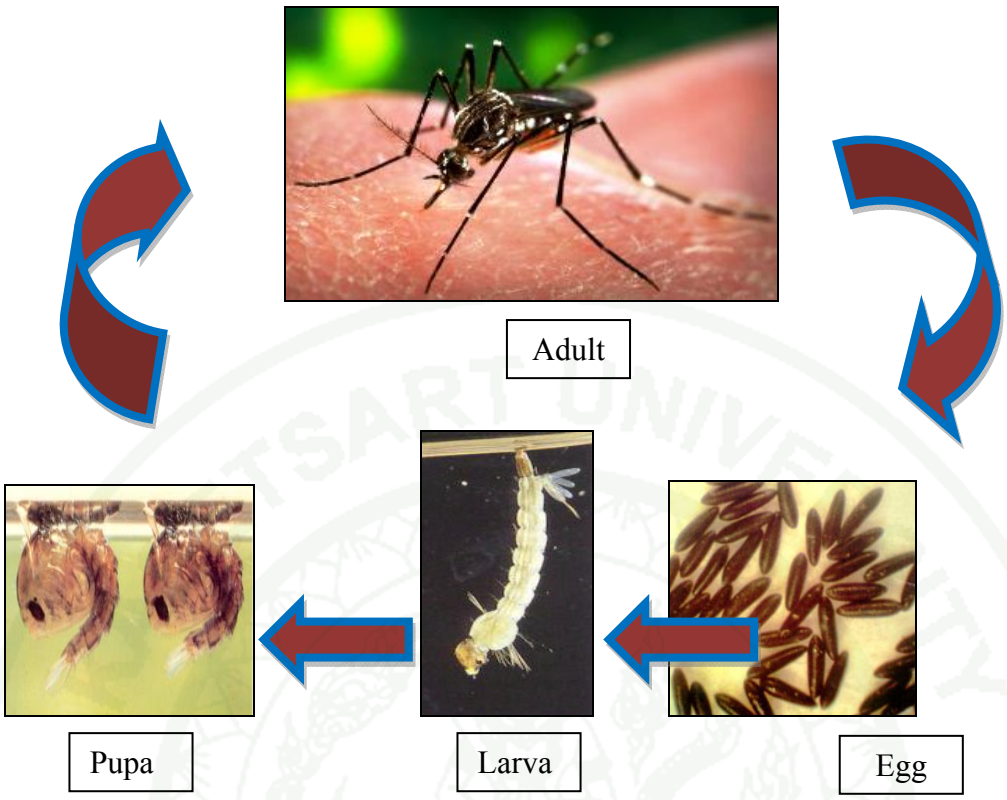


Figure 2 *Aedes aegypti* mosquitoes life cycle

Each of these stages can be easily recognized by its special appearance. The breeding sites of *Ae. aegypti* are typically located near houses in relatively clean water stored in containers, both indoors and outdoors, as well as miscellaneous vessels such as flower pot , ant traps also serve as breeding sites. The eggs are approximately 1 mm long. The freshly laid eggs are pale white and turn to black in color within a short time. Fertilized eggs are deposited singly just above the water line of containers. The eggs are capable of withstanding desiccation for a week or months. These eggs hatch only when they were flooded with water, but not all eggs hatch at the same time. Larvae are aquatic. The larvae have four instars. It consists of 3 portions of head, thorax and abdomen. When the larvae come to the water surface to breathe, air is take in siphon or air tube is located at the tip of the abdomen. Larvae dive to the bottom for short periods in order to feed or escape danger. The larvae feed on the aquatic micro biota that present in artificial containers. Factors involving in the duration of larvae development are temperature, food availability and larval density in the receptacles. The pupal period is quite short, usually 1-2 days. Pupa does not feed but swim and

float. Internal changes occur to transform the larvae to adult mosquito. Breathing is via trumpets (Pant and Self, 1993). After emerging, adult rest on the wall of the breeding site for a few hours to allow the exoskeleton and wings harden. Both sexes can mate within 24 hours after emergence. After mating, the female of *Ae. aegypti* requires a blood meal because of the development of the eggs. Human blood is preferred over other animals, often biting in doors or in sheltered areas near the house. Females bite and feed mainly during the day or early evening. Three to four days later the gravid females search for suitable places to deposit their eggs. They prefer to lay eggs one by one on a surface with a high degree of dark color, roughness and water absorption. This process is repeated until the mosquito dies. A female can lay an average of 100 and 150 eggs each gonotrophic cycle (Kettle, 1995).

2.2 Ecological habitats

Aedes aegypti is a mosquito found in peridomestic waters such as flower pots, tin cans, discarded tires, barrels, buckets, cisterns and all kinds of small collection of water around the houses. *Aedes* usually bites in the early morning (08.00-11.00 AM) and late afternoon (13.00- 16.00 AM). In shaded areas or when it is overcast, *Ae. aegypti* may bite at any time during the day. (Nelson *et al*., 1978; Lumsden, 1957; Sheppard *et al.,* 1969)

The urbanization and associated proliferation of man-made container habitats, such as temporaly water-storage containers, are responsible for the maintenance and spread of *Ae. aegypti*. In fact, dengue epidermics is frequency related to urbanization, which determines the numbers of available susceptible humans and the density of vector populations. However, in some situations the introduction of piped water does not appear to have reduced domestic breeding of *Ae. aegypti*, mainly because people still persist in storing drinking water (Service, 1989) or as they become affluent they become surrounded by ever increasing discarded trash, such as tin cans, which supports mosquito breeding. *Aedes aegypti* is an efficient vector, because it is a highly domestic species, breeding in all kinds water containers in or around houses. The

adults preferably rest inside houses. These characteristics make it has a much stronger vector-human contact than the other vectors.

3. Vector control

3.1 Biological control

Biological control or "biocontrol" is the use of natural enemies to manage mosquito populations. There are several types of biological control including the direct introduction of parasites, pathogens and predators to target mosquitoes. Effective biocontrol agents include predatory fish that feed on mosquito larvae such as mosquitofish (*Gambusia affinis*) and some cyprinids (carps and minnows) and killifish. Tilapia will also consume mosquito larvae. Invertebrate pathologists study these diseases in the hope that some of them can be utilized for mosquito management. Microbial pathogens of mosquitoes include viruses, bacteria, fungi, protozoa, nematodes, and microsproidia (Davidson 1981, Jahn 1986). Also used as biological control agent are the dead spores of varieties of the natural soil bacterium *Bacillus thuringiensis*, especially *Bt israelensis* (BTI). BTI is used to interfere in thedigestion systems of larvae. It can be dispersed by hand or dropped by helicopter in large areas. BTI is no longer effective after the larvae turn into pupae, because they stop eating. Integrated pest management (IPM) is the use of the most environmentally appropriate method or combination of methods to control pest populations. Typical mosquito-control programs using IPM first conduct larval and adult surveys, in order to determine the species composition, relative abundance, and seasonal distribution of adult and larval mosquitoes, and only then are the best and most effective methods of control utilized.

3.2 Environmental control

According to the larval habitat characters and the ease to manipulate in collecting the larvae, larval surveys are commonly used for *Aedes* species, and involve the collection of larvae or pupae. The immature stages are collected from all

water storages containers found both inside and outside houses. Information concerning the locality, date of survey, precise location and classification of the container or source is carefully recorded. (WHO, 1969)

The commonly used larval indices are as follows: House or premises index - This is the percentage of houses with one or more habitats positive for *Ae. aegypti* or related species. This index can be marked as HI. Container index - Percentage of Containers infested with larvae/pupae in examined containers. This index can be given as CI. Breteau index – Originally, this index was used to estimates percentage of infested *Ae. aegypti* in containers per number of inspected houses. This index can be referred as BI.

The Breteau Index is considered to be the best used indices (such as the House or premises Index and the Container Index) since it combines dwellings and containers and is more qualitative and of more epidemiological significance.

The House Index is most frequently used and understood. It also involves less labour. When the first positive container is located in a house, there is no need to proceed further. This index does not take into account the number of positive containers in an infested house.

The House Index gives an idea of the percentage of houses positive for vector breeding and hence the percent- age of the population at risk. If the index is high, transmission occurs easily to neighbouring houses, and if the index is low transmission occurs less rapidly.

The Container Index, although not so useful from the epidemiological point of view, is a useful comparative figure, especially when evaluation of control measures is being carried out.

3.3 Chemical control

Insecticides play an important role in controlling vector of some diseases such as mosquito, sand flies, tsetse flies, lice, fleas, and triatomid bugs (Hemingway and Ranson, 2000). Not only in Thailand but also in other countries of Asia, Americas, Europe and Africa, insecticide have been used in the control program of agricultural and public health (Chansang, 2003). The vectors control of *Aedes aegypti* in Thailand has relied on organophosphate and carbamate insecticides, including temephos, fenitrothion, malathion and propoxur since 1950 (Chareonviriyaphap *et al*., 1999; Yaicharoen *et al.,* 2005). DDT was used for insect control at the sometime. However, DDT was withdrawn for all agricultural uses in the beginning of 1983 and was completely stopped from public health use in 2001 as an indoor residual spray for malaria control (IRS) (Chareonviriyaphap *et al*., 1999). The reasons for the removal of DDT from malaria control in Thailand was because of reported vector resistance and perceived adverse impact on the environment. Synthetic pyrethroids have replaced widely used classes of insecticides such as organophosphates, carbamates, and chlorinated hydrocarbons since 1992, (Prasittisuk, 1985; Chareonviriyaphap *et al*., 2000). Lately, six pyrethroid insecticides such as alpha-cypermethrin, cyfluthrin, deltamethrin, etofenprox, lambda-cyhalothrin and permethrin have been recommended by World Health Organization (WHO) in the framework of the WHO Pesticide Evaluation Scheme (WHOPES) for the treatment of mosquito net (Zaim *et al.,* 2000; WHO, 2005), These insecticide are the most widely used in East African countries (Mosha *et al.,* 2008). In Thailand, during endemic seasons, deltamethrin and permethrin are main synthetic pyrethroids used to control adult *Aedes* mosquitoes through mass spraying (Annual report Bureau of Vector Borne Disease, 2007). In addition, temephos (abate) and organophosphate are frequently and widely used in domestic containers for the control of *Ae. aegypti* larvae in Thailand (Chareonviriyaphap *et al*., 1999).

Unfortunately, insecticide resistance is a serious problem facing the effective control of insect vector, especially in tropical countries because insecticides have been used intensively to control insect pests and vectors over the past 50 years.

The spread of resistance among arthropods has rendered many pesticides ineffective. Resistance is already present in several parts of the world (Chandre *et al.,* 1999; Hargreaves *et al.,* 2000). Resistance has been reported to every class of insecticides, including microbial agents and insect growth regulators (Armed Forces Pest Management Board, 2009). The development of pyrethroid resistance in major vector mosquitoes is a very serious concern. An issue of concern in vector control is whether DDT resistance confers cross-resistance to pyrethroids as a result of similar resistance mechanisms, same target site by modifying the gating kinetics of voltage-sensitive sodium channel (Lund and Narahashi, 1983). Resistance to pyrethroids is now widespread and has been reported in most regions where *Ae. aegypti* is established (Brengues *et al.,* 2003; Ponlawat *et al.,* 2005). Microbial larvicide resistance has been documented in the literature (Rodcharoen and Mulla, 1996; Zahiri *et al.,* 2002; Paul *et al.,* 2005). Cornel *et al.* (2002) reported methoprene (IRGs) resistance in California and Florida.

4. Pyrethroids used in mosquito control

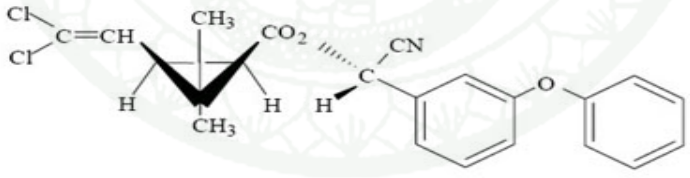
Synthetic pyrethroids are an important class of insecticides that have been proved to be effective in controlling arthropods of medical and veterinary importance. Synthetic pyrethroids such as deltamethrin, permethrin and alpha-cypermethrin are among the choice of current insecticides for vector control (Zerba, 1988). Pyrethroids from a group insecticidal esters, of which both the alcohol and carboxylic acid moieties may have isomeric forms so each pyrethroid chemical may be composed of several isomers. These insecticides show remarkably high toxicity and rapid action against a wide range of insects, but relatively low mammalian toxicity (Sathantriphop *et al*., 2006). Pyrethroids have been most commonly used for the impregnation of bed nets or indoor residual house sprays.

Pyrethroids act on the nervous system by modifying the gating kinetics of voltage-sensitive sodium channels (Lund and Narahashi, 1983). These so-called ‘axonic poisons’ interfere with the sodium channels of both the peripheral and central

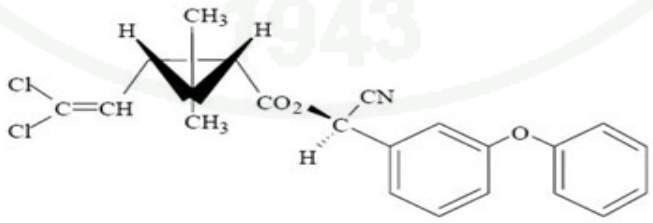
nervous system, thereby stimulating repetitive nerve discharges leading to rapid paralysis (knockdown) and death.

4.1 Alphacypermethrin

Alphacypermethrin [(*S*)-á-cyano-3-phenoxybenzyl-(1*R*,3*R*)-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane-carboxylate and (*R*)-á-cyano-3-phenoxybenzyl-(1*S*,3*S*)-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylate)] consist of 2 cis-isomers from the 8 isomers present in cypermethrin 13 Alphacypermethrin is a type II synthetic pyrethroid and contains an alpha-cyano group (Figure 3). Alphacypermethrin is a non-systemic, broad spectrum insecticidal pyrethroid with rapid knockdown activity. It is effective by contact and ingestion against target pests at relatively low application rates. It acts by preventing transmission of nerve impulses by blocking the passage of sodium ions through channels in nerve membranes preventing signals passing down axons. Typically this toxication results in a rapid "knockdown" and mortality. In public health it is used to control cockroaches, mosquitoes, flies and other insect pests. It is also used in animal health as an ectoparasiticide (WHO, 2007).



(*S*) (1*R*)-*cis*



(*R*) (1*S*)-*cis*-

Figure 3 Alphacypermethrin chemical structure.

Source: Department of Health and Ageing (2007).

4.2 Bifenthrin

Bifenthrin (2-methyl[1,1'-biphenyl]-3-yl)methyl 3-(2-chloro-3,3,3-trifluoro-1-propenyl)-2,2-dimethylcyclopropa (AgroChina,2008)(Figure 4). Bifenthrin is a member of the synthetic pyrethroid family of pesticides. Like most pyrethroid pesticides, bifenthrin affects the central and peripheral nervous system of insects causing paralysis (Miller and Salgado, 1985). Because of their high toxicity to aquatic organisms, bifenthrin products are registered as “restricted use pesticides”, to be sold only to and used by Certified Pesticide Applicators. In addition to Red Imported Fire Ant (RIFA) control, bifenthrin is used as a miticide and acaricide in orchards, nurseries and homes. Bifenthrin is a third-generation synthetic pyrethroid chemical. This group is characterized by greater photostability and greater insecticidal activity than previous pyrethroids (Mokry and Hoagland, 1989.) Little research has been done specifically on bifenthrin’s mode of action on invertebrates or vertebrates, however, most investigations have found that the pyrethroid family of pesticides demonstrate very similar effects on invertebrate nervous systems (Miller and Salgado, 1985). Pyrethroids utilize a number of different pathways to cause nervous system damage in invertebrates (Miller and Salgado, 1985). Significant among these is interference with sodium channel gating in the nerve cell endings (Lund and Narahashi, 1981) by acting on the sodium channels to depolarize the pre-synaptic terminals. Pyrethroid insecticides effectively paralyze organisms by severely limiting neuro-transmission (Salgado et al, 1983). This paralysis is often preceded by spastic activity of the organism due to the hyper-activity of nerve endings. The spastic activity is caused by sodium channels repeatedly polarizing and depolarizing, mimicking neuro-transmission where none is actually taking place.

Pyrethroids have also been shown to inhibit ATPase enzyme production (Clark and Matsumura, 1982). This is primary importance in understanding why aquatic organisms are much more susceptible to pyrethroid insecticides than terrestrial organisms. Freshwater aquatic organisms must maintain ionic balances and osmoregulation in an extremely dilute environment. Active transport at cellular walls is needed to maintain critical cellular ion levels against a concentration gradient.

ATPase enzymes provide the energy needed by cells to maintain this gradient. By inhibiting ATPase enzymes, pyrethroids breakdown the critical concentration gradient, eventually leading to death of the organism. Pyrethroids have the most serious effects on fish and gill breathing aquatic insects because of the large surface area available to de-ionize after ATPase enzymes inhibition (Siegfried, 1993). Bifenthrin is a non-alpha cyano pyrethroid insecticide and used as a miticide and acaricide in orchards, nurseries, and homes. Bifenthrin is potentially a good candidate insecticide for treatment of mosquito nets. Because of these is less toxic and no irritation after dermal application on abraded and intact skin.

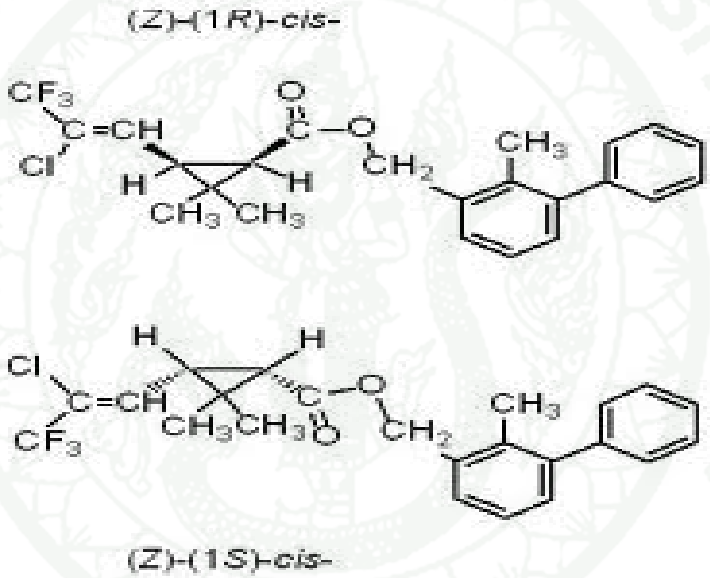


Figure 4 Bifenthrin chemical structure.

Source: AgroChina (2008).

4.3 Cypermethrin

Cypermethrin Cyano(3-phenoxyphenyl)methyl 3-(2,2-dichloroethenyl)-2,2-dimethylcyclopropanecarboxylate (AgroChina, 2008)(Figure 5). Cypermethrin is a synthetic pyrethroid insecticide containing three chiral centres, giving a racemic mixture of eight isomers comprising four diasterioisomeric pairs. The cypermethrins

are alpha-cyano- or type II pyrethroids. Cypermethrin was first evaluated by the 1979 JMPR, when a temporary ADI was established. New toxicological data were evaluated at the 1981 JMPR and an ADI of 0–0.05 mg/kg bw per day was established.

Cypermethrin is a synthetic pyrethroid used as an insecticide in large-scale commercial agricultural applications as well as in consumer products for domestic purposes. It behaves as a fast-acting neurotoxin in insects. It is easily degraded on soil and plants but can be effective for weeks when applied to indoor inert surfaces. Exposure to sunlight, water and oxygen will accelerate its decomposition. Cypermethrin is highly toxic to fish, bees and aquatic insects, according to the National Pesticides Telecommunications Network (NPTN). It is found in many household ant and cockroach killers, including Raid and ant chalk.

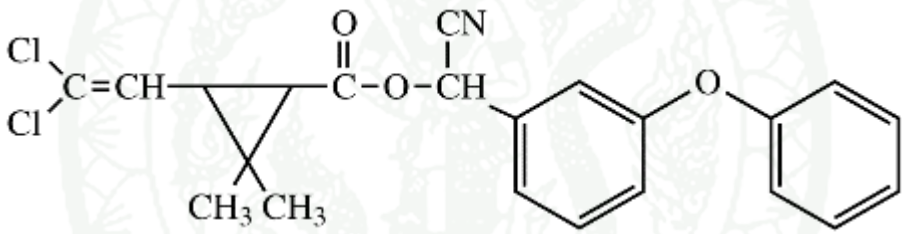


Figure 5 Cypermethrin chemical structure.

Source: AgroChina (2008).

4.4 Deltamethrin

Deltamethrin [(S)-á-cyyano-3-phenoxybenzyl(1R,3R)-3(2,2-dibromovinyl)-2,2-dimethylcyclopropane carboxylate], is a single stereoisomer pyrethroid (Hodgson *et al*., 1998) (Figure 6).Deltamethrin is a pyrethroid insecticide that kills insects by contact or digestion. It is used to control apple and pear suckers, plum fruit moths, caterpillars on brassicas, pea moth, aphids (apples, plums, hops), winter moths (apples and plums), codling and tortrix moths (apples), mealy bugs, scale insects, and whiteflies (glasshouse cucumbers, tomatoes, peppers, potted plants and ornamentals). It also controls numerous insect pests of field crops. Formulations

include emulsifiable concentrates, wettable powders, ULV, flowable and granules. Deltamethrin is a synthetic insecticide based structurally on natural pyrethrins, which rapidly paralyze the insect nervous system giving a quick knockdown effect. Deltamethrin has a rapidly disabling effect on feeding insects. For this reason, there is hope that it may be useful to control the vectors of "non-persistent" viruses that can be passed on the vector within a few minutes of starting to feed on the plant. Deltamethrin's mode of action is thought to be mainly central in action, or at least originate in higher nerve centers of the brain. Death of insects seems to be due to irreversible damaging of the nervous system. Deltamethrin poisoning occurs through cuticular penetration or oral uptake. The susceptibility of insects depends on a variety of factors according to the environmental conditions. Flies are the most susceptible to pyrethroid poisoning shortly before dawn. Many pyrethroids are not very active against cattle ticks, but some alpha cyano compounds (of which deltamethrin is one) have higher activity than organophosphates or amidines, the former standard compounds for this purpose. Deltamethrin has very good residual activity for outdoor uses (field crops, cattle dip and tsetse) and for indoor uses (mosquitoes, stable flies, horsefiles, fleas, cockroaches and stored product insects). Deltamethrin has very broad spectrum control. It is considered to be the most powerful of the synthetic pyrethroids. It is up to three orders more active than some pyrethroids.

Deltamethrin is used in the U.S. in the Environmental Health Market. It is being sold in many countries for agricultural, public health and livestock applications. The active ingredient of deltamethrin is found in a variety of commercial insecticide products. Trade names for products containing deltamethrin include Butoflin, Butoss, Butox, Cislin, Crackdown, Cresus, Decis, Decis-Prime, K-Othrin, and K-Otek.

Deltamethrin produces typical type II motor symptoms in mammals. Type II symptoms include a writhing syndrome in rodents, as well as copious salivation. The acute oral LD_{50} in male rats ranged from 128 mg/kg to greater than 5,000 mg/kg depending on the carrier and conditions of the study The LD_{50} for female rats was 52 mg/kg and other published values range from 31 to 139 mg/kg. Values ranging from 21 to 34 mg/kg were obtained for mice; while dogs had a report of LD_{50} of 300

mg/kg. The intravenous LD_{50} in rats and dogs was 2 to 2.6 mg/kg, and the dermal LD_{50} was greater than 2,940 mg/kg. The acute percutaneous LD_{50} for rats was reported to be greater than 2,000 mg/kg; greater than 10,000 mg/kg for quail; and greater than 4,640 mg/kg for ducks. The acute dermal LD_{50} for rabbits was greater than 2,000 mg/kg. No skin irritation and slight eye irritation were reported. Another study indicated skin irritation in rats and guinea pigs. The signs of poisoning produced in rats by deltamethrin are not the same as those produced by other pyrethroids. Especial characteristic are rolling convulsions. The site of action is considered to be central with little or none of the peripheral component demonstrated for other pyrethroids. The sequence of signs is clearly defined, progressing from chewing, salivation, pawing to rolling convulsions, tonic seizure, and death. Blood pressure begins to drop promptly, but slowly; it tends to normalize about the time choreoathetosis (abnormal movements of the body of a combined choreic and athetoid pattern) begins but falls precipitously prior to death. The early signs, including choreoathetosis, are reversible, but rats that exhibit a tonic seizure and shock almost always die promptly. Acute exposure effects in humans include the following: ataxia, convulsions leading to muscle fibrillation and paralysis, dermatitis, edema, diarrhea, dyspnea, headache, hepatic microsomal enzyme induction, irritability, peripheral vascular collapse, rhinorrhea, serum alkaline phosphatase elevation, tinnitus, tremors, vomiting and death due to respiratory failure. Allergic reactions have included the following effects: anaphylaxis, bronchospasm, eosinophilia, fever, hypersensitivity pneumonia, pallor, pollinosis, sweating, sudden swelling of the face, eyelids, lips and mucous membranes, and tachycardia. Studies have shown many cases of dermal deltamethrin poisoning after agricultural use with inadequate handling precautions, and many cases of accidental or suicidal poisoning by the oral route at doses estimated to be 2-250 mg/kg. Oral ingestion caused epigastric pain, nausea, vomiting and coarse muscular fasciculations. With doses of 100-250 mg/kg, coma was caused within 15-20 minutes.

Deltamethrin is a synthetic insecticide also belonging to the pyrethroid family. It is widely registered and used in agriculture and in public and animal health as a broad spectrum insecticide against noxious and disease-bearing insects

(dipterans, hemipterans) and acarines (ticks and mites). Deltamethrin is also widely used as an acaricide/insecticide for the control of ticks, mites and insect pests of livestock. Deltamethrin is non-systemic with contact and stomach action (WHO, 2007).

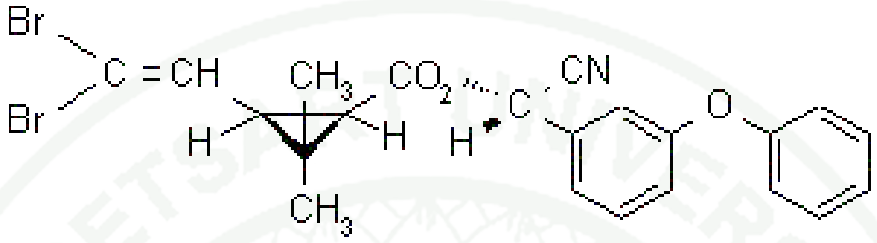


Figure 6 Deltamathrin chemical structure.

Source: Anonymous (2008).

4.5 Lamdacyhalothrin

Lambdacyhalothrin (R)-cyano(3-phenoxyphenyl)methyl (1S,3S)-rel-3-[(1Z)-2-chloro-3,3,3-trifluoro-1-propenyl]-2,2-dimeth (AgroChina,2008)(Figure 7). Lambdacyhalothrin is a synthetic pyrethroid insecticide and acaricide used to control a wide range of pests in a variety of applications. Pests controlled include aphids, Colorado beetles and butterfly larvae. Crops on which it may be applied include cotton, cereals, hops, ornamentals, potatoes, vegetables or others. It may also be used for structural pest management or in public health applications to control insects such as cockroaches, mosquitoes, ticks and flies which may act as disease vectors. Lambda cyhalothrin is available as an emulsifiable concentrate, wettable powder or ULV liquid, and is commonly mixed with buprofezin, pirimicarb, dimethoate or tetramethrin. It is compatible with most other insecticides and fungicides. Unless otherwise stated, data presented herein refer to the technical product. Lambda cyhalothrin is a Restricted Use Pesticide and so may be purchased and used only by

certified applicators. It is in EPA Toxicity Class II, and products containing it must bear the signal word WARNING.

Trade names for products containing lambda cyhalothrin include Charge, Excaliber, Grenade, Hallmark, Icon, Karate, Matador, Saber, Samurai and Sentinel. Lambda cyhalothrin is moderately toxic in the technical form, but may be highly toxic via some routes in formulation (e.g., as Karate). Available data indicate that lambda cyhalothrin is moderately toxic via the oral route in test animals. Reported oral LD_{50} values are 79 mg/kg and 56 mg/kg for male and female rats, respectively. The vehicle used was corn oil. The rat oral LD_{50} has also been reported as 144 mg/kg. The reported rat LD_{50} for the technical product is similar, 64 mg/kg. These indicate moderate acute toxicity via the oral route of exposure. No data were available regarding the acute toxicity of the technical compound via the inhalation route, but for Karate the reported 4-hour inhalation LD_{50}s were 0.175 mg/L and 0.315 mg/L for female and male rats, respectively. These data indicate a moderate to high toxicity via the inhalation route for the formulated product, Karate. The technical product has reported dermal LD_{50}s of 632 mg/kg and 696 mg/kg for male and female rats (vehicle used was propane-1,2-diol). It has also been found to be non-irritating to the skin of rabbits and non-sensitizing to the skin of guinea pigs but may cause mild eye irritation in rabbits. The formulated product, Karate, however, causes severe primary skin irritation in rabbits and mild skin sensitization in guinea pigs. Primary eye irritation also was observed with the technical product. In addition to the corrosive effects to skin and eyes, other acute effects due to exposure to lambda cyhalothrin, like those of other pyrethroids, will be mainly neuropathy (effects on the nervous system). Cyhalothrin may act on ion channels within the nerve cells (neurons) to disrupt proper function of the cells of both the peripheral and central nervous systems. At lower doses, this may take the form of stable, repetitive firing of the neuron, but high doses may result in depolarization of the nerve cell and blockage of conduction. These effects may result in observable effects such as: tingling, burning or numbness sensations (particularly at the point of skin contact); tremors, incoordination of movement, paralysis or other disrupted motor function; and confusion or loss of consciousness. Since most pyrethroids are generally absorbed only poorly through the

skin, the latter two systemic effects are unlikely unless the compound has been ingested. Effects are generally reversible due to rapid breakdown of the compound in the body. Similar to warrior compounds of the pyrethroid family, the observed toxicity of lambda cyhalothrin may vary according to not only the concentration of the active ingredient, but also according to the solvent vehicle.

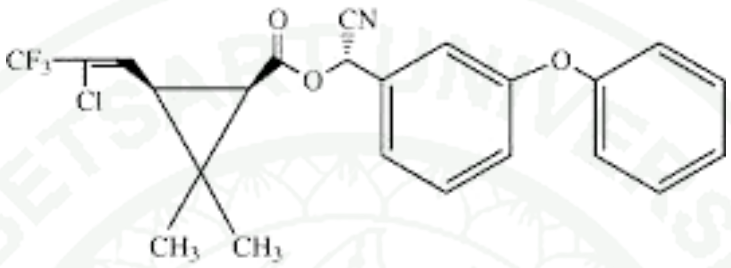


Figure 7 Lambdacyhalothrin compound structure.

Source: AgroChina (2008).

4.6 Permethrin

Permethrin [3-phenoxybenzyl-3-(2,2-dichlorovineyl)-2,2-dimethylcyclopropanecarboxylate] is a type I synthetic pyrethroid in that it is without an alphacyano groups. It has four stereoisomers (two enantiomeric pairs), molecules made up of the same atoms with different three-dimensional structures (Cox, 1998) (Figure 8). Permethrin is a broad spectrum synthetic pyrethroid insecticide, used against a variety of pests, on nut, fruit, vegetable, cotton, ornamental, mushroom, potato and cereal crops. It is used in greenhouses, home gardens, and for termite control. It also controls animal ectoparasites, biting flies, and cockroaches. It may cause a mite buildup by reducing mite predator populations. Permethrin is available in dusts, emulsifiable concentrate, smoke, ULV (ultra-low volume) and wettable powder formulations.

Permethrin is a moderately to practically non-toxic pesticide in EPA toxicity class II or III, depending on the formulation. Formulations are placed in class

II due to their potential to cause eye and skin irritation. Products containing permethrin must bear the signal word WARNING or CAUTION, depending on the toxicity of the particular formulation. All products for agricultural uses (except livestock and premises uses) are Restricted Use Pesticides (RUPs) because of their possible adverse effects on aquatic organisms. Restricted Use Pesticides may be purchased and used only by certified applicators.Trade names include Ambush, Cellutec, Dragnet, Ectiban, Eksmin, Exmin, Indothrin, Kafil, Kestrel, Pounce, Pramex, Qamlin, and Torpedo.

Permethrin is moderately to practically non-toxic via the oral route, with a reported LD_{50} for technical permethrin in rats of 430 to 4000 mg/kg. Via the dermal route, it is slightly toxic, with a reported dermal LD_{50} in rats of over 4000 mg/kg, and in rabbits of greater than 2000 mg/kg. Permethrin caused mild irritation of both the intact and abraded skin of rabbits. It also caused conjunctivitis when it was applied to the eyes. The 4-hour inhalation LC_{50} for rats was greater than 23.5 mg/L, indicating practically no inhalation toxicity. The toxicity of permethrin is dependent on the ratio of the isomers present; the cis-isomer being more toxic.

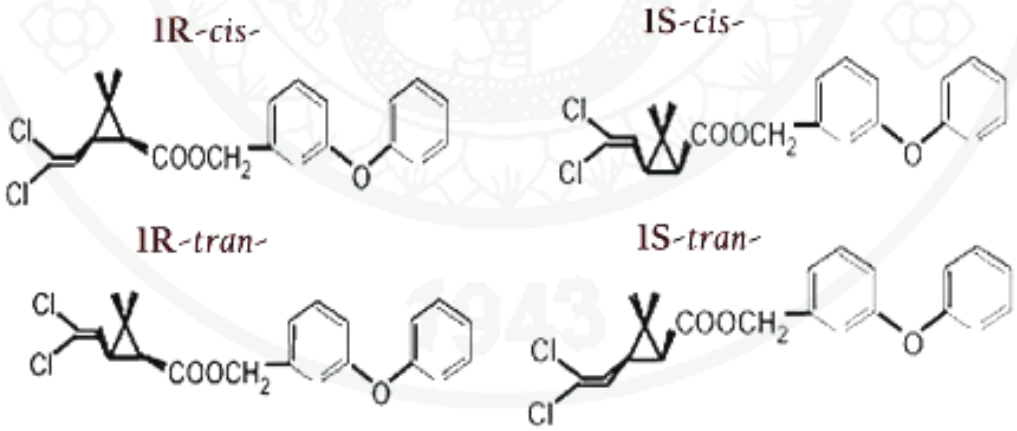


Figure 8 Permethrin chemical structure.

Source: Cox (1998).

5. Mode of action of pyrethroid insecticides on insects

These compounds act on the nervous system by modifying the gating kinetics of voltage sensitive sodium (Na+) channels (Bloomquist, 1994). Arthropod resistance to pyrethroids is characterized by a marked reduction in the intrinsic sensitivity of the insect nervous system to these compounds. Pyrethroids are synthetic chemicals whose structures mimic the natural insecticide pyrethrin. Pyrethrins are found in the flower heads of plants belonging to the family Compositae (e.g., chrysanthemums). These insecticides have a unique ability to knock down insects quickly. Synthetic pyrethrins (also known as pyrethroids) have been chemically altered to make them more stable Pyrethroids are axonic poisons (they poison the nerve fiber). They bind to a protein in nerves called the voltage-gated sodium channel. Normally, this protein opens causing stimulation of the nerve and closes to terminate the nerve signal. Pyrethroids bind to this gate and prevent it from closing normally which results in continuous nerve stimulation. This explains the tremors exhibited by poisoned insects. They lose control of their nervous system and are unable to produce coordinated movement.

6. Resistance of mosquitoes to insecticides

Insecticide resistance has been an increasing problem since the first report of resistance to DDT in the mosquitoes Aedes traetiorhynchus and Aedes solicitans in 1947, only a year after introduction of DDT for residual house spraying (Brown, 1986). The development of insecticide resistance by arthropod vectors is a primary concern for the management of human disease control. A few published papers on insecticide resistance in *Ae. aegypti* population have been reported in Thailand (Somboon et al., 2003; Jirakanjanakit *et al.*, 2007; Ponlawat *et al.*, 2005; Sathatriphop *et al.*, 2006). To date four major groups of insecticides, the organochlorines, organophosphates, carbamates and pyrethroids dominate the vector control market. All these insecticide target the insect nervous system. The cyclodiene organochlorines, such as dieldrin and gamma HCH target the GABA receptor on the nerve membranes. DDT and related organochlorines, along with pyrethroids, target the sodium channels on the nerve membrane, while the organophosphorus and

carbamate insecticides attack acetylcholinesterase at the nerve junction (Hemingway, 1997).

A single amino acid change in the receptor protein is responsible for the resistance. The a single amino acid change involved with resistance is an Ala^{302} to Ser change, within the second membrane spanning region of the $GABA_A$ receptor channel(ffrench-Constant *et al.,* 1993). The sodium channel insensitivity is a recessive mechanism and crossing experiments suggests that the gene responsible is on chromosome III (Ahn *et al.,* 1978). A single mutation (Leu to Phe) in the S6 thansmembrane segment of domain II of the sodium channel sequence is associated with '*kdr*' in houseflies (Williamson *et al.,* 1996). While a different mutation (Leu to His). The altered AChE is less susceptible to inhibition by these insecticides than its native counterpart and hence continues to turnover acetylcholine in the presence of insecticide. This mechanism confers cross-resistance to a broad range of organophosphorus and carbamate insecticides. The binding sites for these insecticide appear to be overlapping but non-identical (Hemingway, 1997).

The enzyme DDT dehydrochlorinase was in fact a glutathione S- transferase (GST), which rather than conjunating glutathione to the insecticide, uses it as a co-factor (Clark and Shamaan, 1984). A comparison of partially purified GSTs from resistant and susceptible *An. Gambiae* demonstrated resistance is assiociated with quantitative increases in multiple GSTs. The resistance gene is actually a regulator, which influences the expression of a range of GSTs in different life stages of the mosquito. This, is a relatively specific mechanism, where the enzyme involved targets the carboxylesterase bonds on the side chian of malathion (Hemingway, 1997). Biochemical analysis suggests that the underlying mechanism is likely to be a point mutation or structural gene rearrangement, which has resulted in an increase in substrate specificity for malathion (Hemingway), 1985; Herath *et al.*, 1987). The underlying molecular mechanism of esterase elevation in *Culex* is gene amplification (Vaughan and Hemingway, 1995; Vaughan *et al.*, 1995; Raymond *et al.*, 1989). The esterase appear to be affinity for the insecticides than the non-amplified esterases (Karunaratne *et al.,* 1995; Jayawardena *et al.*, 1994; Karunaratne *et al.,* 1993). This

enzyme system has previously been referred to as mfos, mixed function oxidases or multi-function oxidases in the literature. Monooxygenase is now generally used as this accurately reflects the general mechanism of the enzyme in transferring a single oxygen moiety to the substrate. The pyrethroids, organophosphorus and carbamate insecticides are all susceptible to monooxygenase degradation (WHO, 1997).

MATERIALS AND METHODS

1. Mosquito strains.

A susceptible standard strain of *Ae. aegypti* (USDA) was used to establish the baseline susceptibility levels of six synthetic pyrethroids that were used to determine the susceptibility status of three local strains of *Ae. aegypti*.

1. *USDA* laboratory strain was provided by the Center for Medical, Agricultural, and Veterinary Entomology, Gainesville, Florida, U.S.A. This colony has been maintained in colony for >40 yr.

2. *Kanchanaburi* strain (KB) was obtained in May 2010 as larvae from outdoor container habitats at Pu Teuy Village, Sai Yok District (13° 54’42.8’’N, 100 ° 26’58’’E), Kanchanaburi Province, an area west of Bangkok.

3. *Khon Kaen* strain (KK) was obtained in November 2010 as larvae from outdoor container habitats in Muang District (13° 57’23’’N, 100° 24’28’’E), Khonken Province, northeastern Thailand.

4. *Nong Khai* strain (NK) was obtained in November 2010 as larvae from outdoor container habitats in Maung District (13° 57’23’’N, 100° 24’28’’E), Nong Khai Province, northeastern Thailand.

2. Mosquito rearing

All mosquito larvae and pupae collected from each site were placed in an environmentally-controlled insectary located at KU Department of Entomology, Bangkok and reared to the adult stage. Adult mosquitoes were identified to species *Aedes aegypti* males and females were transferred to screened holding cages to allow free-mating. Females were provided 10% sugar solution soaked on cotton as sustenance and permitted to feed on live Guinea pig blood 3-4days after emergence.

Two days post-blood feeding, oviposition dishes were placed in the cages with gravid females. Eggs were properly conditioned and larval pans set for the next generation and reared using standard techniques and diet established at KU (Kongmee et al. 2004) All four cohorts were maintained separately and carefully segregated to avoid cross-genetic contamination and under identical laboratory-controlled conditions (25+3°C, 75±15 % RH, natural light:dark phase).

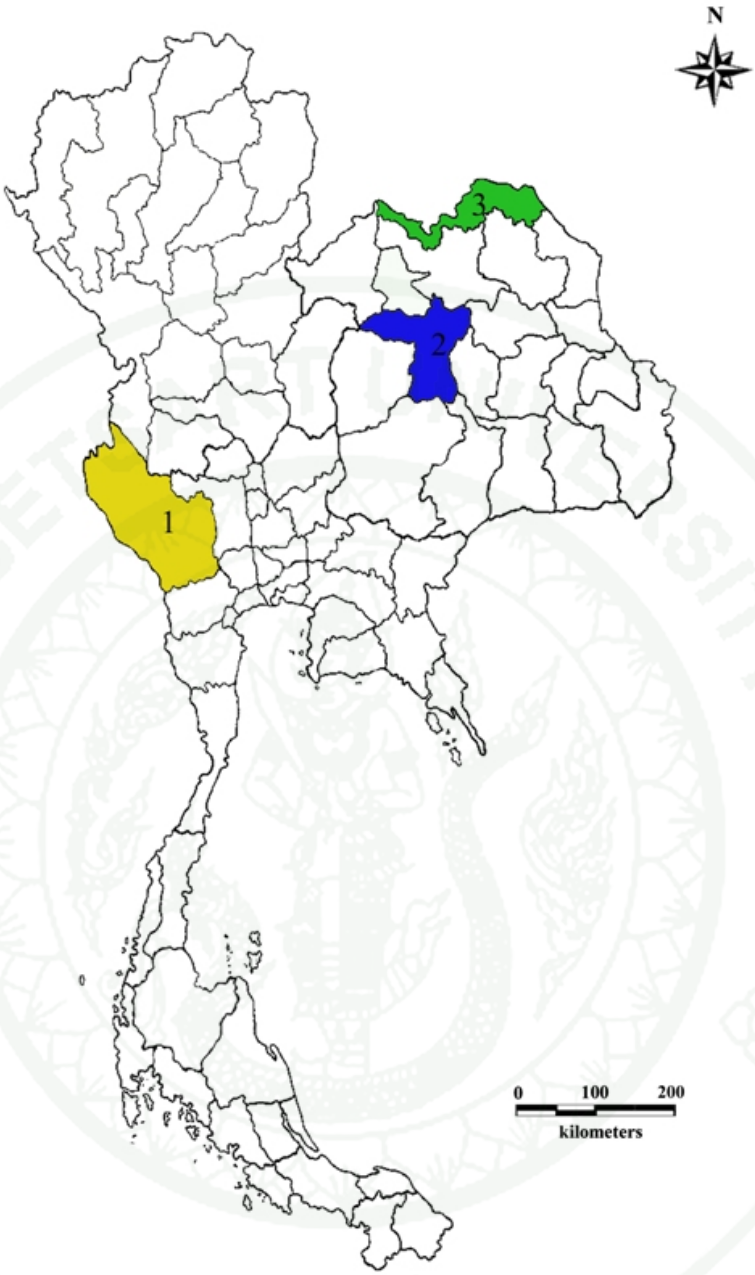


Figure 9 Map of the three localities of *Aedes aegypti* mosquitoes collection sites in Thailand.

Point of collection

1 = Sai Yok District, Kanchanaburi Province

2 = Muang Distric, Khon Kaen Province

3 = Tha Bo Distric, Nong Khai Province

3. Chemicals

Six insecticides were used in susceptibility testing and included

1. α -cypermethrin [(1a(S) 3a-(±)-cyano-(3-phenoxybenzyl) methyl 3-(2,2-dichlorovinyl)-2,2 dimethyl cyclopropanecarboxylate] (BASF Corp, Chicago, IL, purity 95%),

2. Bifenthrin [1 α 3 α (Z)-(±)-(2-Methyl[1,1-biphenyl])Methyl3-(2-chloro-3,3,3-trifluoro-1-propenyl)-2,2-dimethylcy clopropanecarboxylate] (Ladda Com, Bangkok, Thailand, purity 97%)

3. Cypermethrin[(±)α -Cyano-3-phenoxybenzyl(±)-3-(2,2-dichlorovinyl)-2-2-dimethl Cyclopropanecarboxylate](BASF Corp, Chicago, IL, purity 95%),

4. Deltamethrin [(S)- α -cyano-3-phenoxybenzyl (1R,3R)-3-(2,3-dibromovinyl)- 2,2-dimethylcyclopropanecarboxylate (BASF Corp, Chicago, IL, purity 99%),

5. λ-cyhalothrin(RS)- α -Cyano-3-phenoxybenzyl3-(2-chloro-3,3,3,-trifluoropropenyl)-2,2-dimethylcyclopropanecar boxylate (Syngenta Com, Bangkok, Thailand, purity 98%), and

6. Permethrin [3-phenoxybenzyl(1RS,3RS;1RS,3SR)- 3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylate] (Ladda Com, Bangkok, Thailand, purity 92%).

4. Insecticide impregnated paper

Separate rectangular test papers (Whatman® No. 1, 12×15 cm^2) were impregnated with each chemical active ingredient at a specified serial dilution use to establish baseline diagnostic concentration for each insecticide and a single diagnostic concentration to subsequently (LC_{99} x 2) as determined from to test USDA

susceptible strain. All papers were prepared in the laboratory at Department of Entomology, Faculty of Agriculture, Kasetsart University, Bangkok, Thailand. Technical grade active ingredient was diluted with silicon oil (non-volatile carrier) for uniform distribution of insecticide on papers. All papers were treated at the rate of 2 ml of the insecticide solution per 180 cm^2. Control papers will be impregnated with carrier diluents only (acetone and silicone oil).

5. Establishing baseline diagnostic lethal concentrations

The USDA susceptible strain of *Ae. aegypti* was used in a series of dose-response tests to establish the lethal concentrations required to kill 50% and 99% of the test population using each of six active ingredients (WHO, 1981). For all chemicals excluding α-cypermethrin, five different percent concentrations produced in a range of 2-fold serial dilutions were initially tested to determine the range of the 3 final concentrations used for establishing the baseline LC_{50} and LC_{99} values. For α-cypermethrin, only 3 initial concentrations in 10-fold serial dilutions were used to arrive at the final 3 used in establishing the baseline. The subsequent 3 final concentrations (produced as 2, 3, or 4-fold dilutions of active ingredient) used in baseline assays and dose-response analysis are presented in Table 3. Twenty-five, non-blooded female mosquitoes, approximately 3-5 days old, were tested per exposure tube. Treated papers were used only once and discarded. Four replicate assays were conducted for each dilution to derive a mean response and run concurrently with matching controls (without active ingredient) (Figure 10). To avoid spurious reporting of resistance in the field where none may exist, WHO routinely sets the diagnostic concentration at twice the minimum concentration that will kill 100% of susceptible mosquitoes (WHO, 2006). The double concentration of the LC_{99} for each active ingredient was designated the "diagnostic dose" or discriminating concentration and subsequently used for susceptibility tests using the *Ae. aegypti* field strains.

6. Susceptibility assay

The susceptibility level of each population to six synthetic pyrethroids was assessed by exposing 25 non-bloodfed, 3-5 day old female mosquitoes to a single established diagnostic dose established from the USDA standard strains. Mosquitoes were not deprived of nutritional sustenance (10% sugar solution) before testing. Standard testing procedures followed WHO recommendations (WHO, 1998). After 60 min exposure, test and control mosquitoes were transferred to separate holding containers and mortality was recorded after 24 hours post-exposure. Each trial design (population/chemical) was replicated 4 times using freshly treated papers no more than 3 times. Replicate trials were combined and a mean susceptibility level derived for each population tested. For further details see Chuaycharoensuk *et al.* (2011)

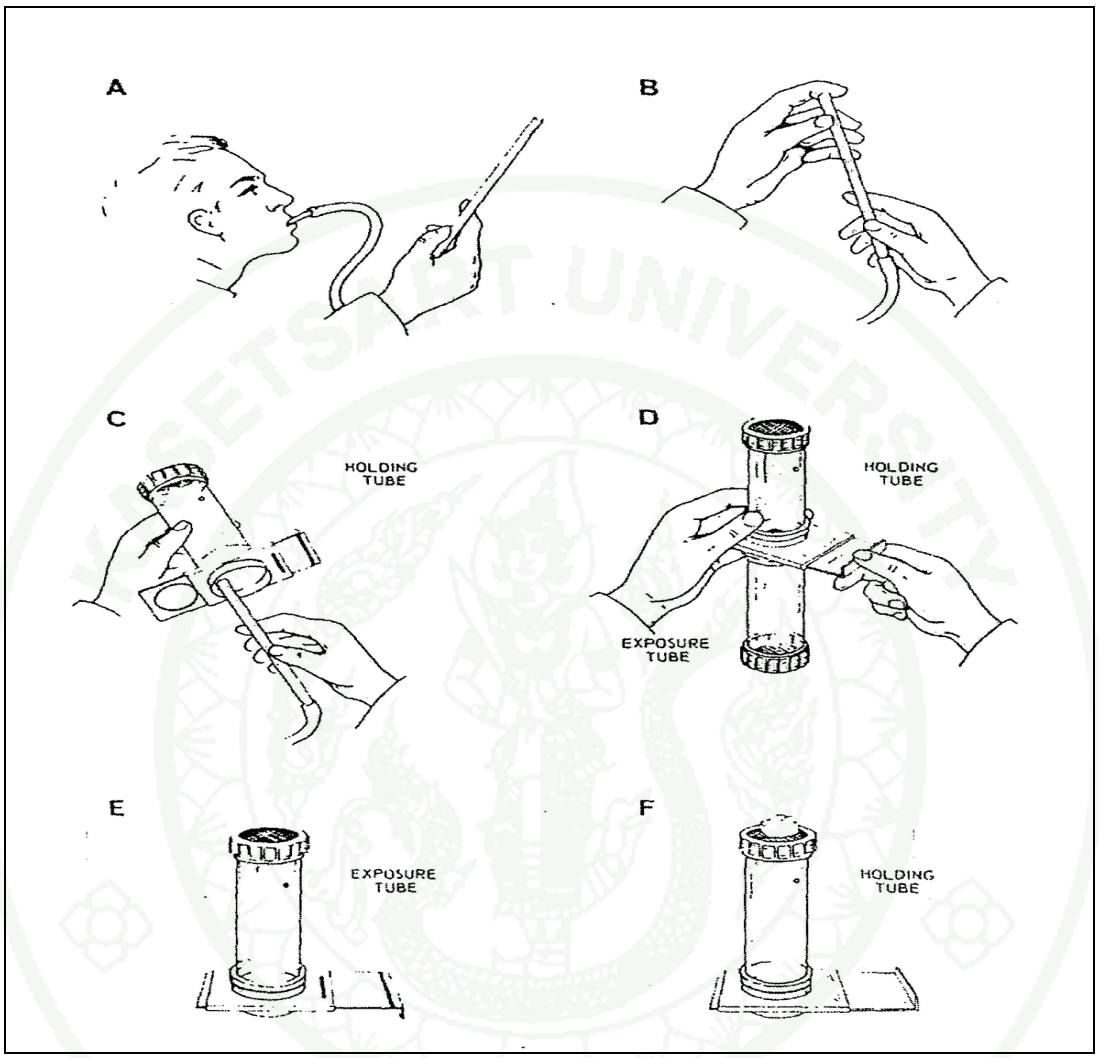


Figure 10 Insecticides susceptibility test (A) Selection of 25 female mosquitoes (B) Selection for female mosquitoes 3- 4 day-old (C) Holding tube (D) Holding tube and exposure tube (E) Susceptibility test with 1h exposure to treated papers (F) Holding tube for sugar pads are placed on top of tubes and 24 h post-exposure mortality observed.

Source: World Health Organization (1980).

7. Analysis

Control mortality was corrected using Abbott's formula (Finney, 1971). The LD_{99} value was calculated from a dosage-mortality regression line using the log-probit program from SAS Software version 9. (SAS, 2002). Pearson chi-square analysis was used for 'goodness of fit' tests. The estimate of LC_{99} was determined from four replicates conducted on the USDA susceptible strain of *Ae. aegypti*. Double the concentration for LC_{99} was determined as the "diagnostic dose" and used for susceptibility/resistance tests on *Ae. aegypti* field strains. Determination of resistance/susceptibility status was done according to WHO criteria (WHO, 1998).

Percent test mortality in susceptibility tests will be adjusted when the matched control mortalities are between 5% and 20%, using the following formula (Abbott, 1925) as % test mortality - % control mortality x 100 / 100 - % control mortality

$$\text{Abbott's formula} = \frac{\text{\% Test mortality - \% Control mortality}}{\text{100 - \% Control mortality}} \times 100$$

For susceptibility tests, the resistance status of adult mosquitoes to categorized based on World Health Organization criteria (WHO, 1981b).

98 - 100 %	mortality indicates complete susceptibility
80 - 97 %	mortality suggests the possibility of resistance that needs to be confirmed and monitored by repeat testing.
< 80 %	mortality strongly suggests resistance

RESULTS AND DISCUSSION

Results

The USDA susceptible strain of *Ae. Aegypti* was used in a series of dose-response tests to establish the lethal concentrations required to kill 50% and 99% of the test population using each six synthetic pyrethroids. All chemicals, except α-cypermethrin, five different percent concentration in a range of two fold serial dilution were initially tested to determine the range of the three final concentrations used for establishing the baseline 50% and 99% lethal concentration values (Table1). The subsequent three final concentrations in two fold dilutions of bifenthrin and permethrin, three fold dilutions of cypermethrin and four fold dilutions of deltamethrin and lambda cyhalothrin used in baseline assays and dose-response analysis are presented in Table 3. For α-cypermethrin, only three initial concentrations in ten folds serial dilutions were used to obtain the final three concentration used in establishing the baseline. The establishment was based on the insecticide doses exhibiting mortality ranging between 10% and 95% in the USDA susceptible strain. The results from Table 2 shows that the response of *Ae. aegypti* to each AI fit the linear model (P = 0.3191). Individual chemical goodness-of-fit tests ranged from *P* = 0.1333 to 0.9718 (Table 2). Therefore, the LC_{50} and LC_{99} values of 6 pyrethroids against *Ae. aegypti* (USDA) were determined using the log-probit analysis (Table 3). Permethrin produced the highest LC_{50} value (0.0007%). At LC_{99} values,cypermethrin had the greatest concentration (0.111%), whereas deltamethrin resulted in the lowest (0.002%). A single diagnostic concentration (double concentration of baseline LC_{99}) of α-cypermethrin (0.086%), bifenthrin (0.094%), cypermethrin (0.221%), deltamethrin (0.005%), λ-cyhalothrin (0.012%), and permethrin (0.147%) which were tested against *Ae. aegypti* (USDA) to confirm 100% mortality (Table 4), was subsequently used to determine the susceptibility of the 3 field populations of *Ae. aegypti* (Kanchanaburi, Khon Kaen, and Nong Khai. Results of susceptibility tests of 3 field populations and the USDA strain with the established diagnostic dose of 6 pyrethroids showed the ability of mosquitoes to survive the diagnostic dose after 1-h exposure to chemical and 24-h holding period. The

interpretation and criteria of insecticide susceptibility results were as follows: mosquitoes regarded as fully ‘‘susceptible’’ to an insecticide if the mean percent mortality was between 98% and 100%, as showing ‘‘incipient’’ resistance if between 80% and 97%, and ‘‘resistant’’ in operational terms of effectiveness if ,80% kill (WHO, 1998, 2006). In all trials, concurrent control (no insecticide, carrier compound only) mortality did not exceed 5%; therefore, final mean mortality did not require a correction factor. Complete mortality (100%) was observed in the USDA standard strain when exposed concurrently to the established discriminating doses of all 6 chemicals. The 3 field populations showed various levels of tolerance/resistance to the chemicals tested. Low to moderate incipient resistance (tolerance) to all 6 pyrethroids was seen in the Kanchanaburi population (Table 5), with mortality ranging between 88% (permethrin) and 97.98% (cypermethrin). The Khon Kaen population was found completely susceptible (100%) to cypermethrin and permethrin; however, incipient resistance was detected against α-cypermethrin (88.46% morality) and very strong resistance was seen with deltamethrin (0.0%), bifenthrin (9.7%), and λ-cyhalothrin (12.9%) (Table 6) . The Nong Khai strain demonstrated strong resistance to deltamethrin (3.92%), λ-cyhalothrin (11.1%), permethrin (6.12%), bifenthrin (14.14%), and cypermethrin (62.24%). The only chemical showing a high level of effectiveness with the Nong Khai population was α-cypermethrin (97.9% kill) (Table 7).

Table 1 Percent mortality of a laboratory susceptible strain of *Aedes aegypti* (USDA) exposed to 6 different pyrethroids.

Insecticide	Concentration (%)	USDA	
		No. tested	Dead (% ± SE)
α-cypermethrin	0.0005	96	14(14.58 ± 1.41)
	0.005	99	88(88.88 ± 0.51)
	0.05	100	100(100 ± 0)
Bifenthrin	0.003125	98	4(4.08 ± 0.33)
	0.00625	109	15(13.76 ± 0.35)
	0.0125	95	37(38.94 ± 2.23)
	0.025	89	59(66.29 ± 2.82)
	0.05	97	96(98.96 ± 0.22)
Cypermethrin	0.003125	98	1(1.02 ± 0.21)
	0.00625	98	12(12.2 ± 0.56)
	0.0125	93	41(44.08 ± 1.79)
	0.025	92	83(90.21 ± 0.23)
	0.05	94	87(92.55 ± 0.94)
Deltamethrin	0.00065	97	39(40.20 ± 3.67)
	0.0013	81	72(88.88 ± 1.13)
	0.0025	91	89(97.80 ± 0.27)
	0.005	91	91(100 ± 0)
	0.01	96	96(100 ± 0)
λ-cyhalothrin	0.0031	97	4(4.12 ± 0.55)
	0.0065	99	5(5.05 ± 0.38)
	0.0013	97	29(29.89 ± 0.66)
	0.0025	100	82(82 ± 0.72)
	0.005	94	88(93.61 ± 0.24)
Permethrin	0.00156	87	1(1.13 ± 0.21)
	0.00625	104	1(0.96 ± 0.19)
	0.025	99	35(35.35 ± 1.06)
	0.1	103	103(100 ± 0)
	0.25	98	98(100 ± 0)

Table 2 Percent mortality of a laboratory susceptible strain of *Aedes aegypti* (USDA) exposed to 6 different pyrethroids using 3 different concentrations.

Insecticide	Concentration (%)	% Mortality (No. of tested)		*P* > chi square
		Control	Treatment	
α-cypermethrin	0.00125	0 (85)	58.16 (98)	0.9419
	0.005	0 (85)	84.76 (105)	
	0.02	0 (85)	96.97 (99)	
Bifenthrin	0.0125	0 (95)	17.02 (94)	0.3082
	0.025	0 (95)	74.71(87)	
	0.05	0 (95)	100 (101)	
Cypermethrin	0.0077	1 (100)	59.43 (106)	0.1333
	0.023	1 (100)	90.82 (98)	
	0.07	1 (100)	96.26 (107)	
Deltamethrin	0.00031	0 (99)	9.09 (99)	0.8955
	0.00125	0 (99)	87.00 (100)	
	0.005	0 (99)	100 (98)	
λ-cyhalothrin	0.00037	0 (101)	4.04 (99)	0.8633
	0.0015	0 (101)	63.37 (101)	
	0.006	0 (101)	98.99 (99)	
Permethrin	0.03125	1(99)	24.75(101)	0.9718
	0.0625	1(99)	96.10(77)	
	0.125	1(99)	100(97)	

Table 3 Probit dose/mortality analysis of a laboratory susceptible strain of *Aedes aegypti* (USDA) exposed to 6 different pyrethroids using 3 different concentrations establishing lethal concentration for each AI.

Insecticide	Concentration (%)	LC_{50}	95% F.L.*	LC_{99}	95% F.L.	Diagnostic concentration ** (%)	*P* > chi square
α-cypermethrin	0.00125	0.0009	0.0004-0.0013	0.043	0.0220-0.1449	0.08630	0.9419
	0.005						
	0.02						
Bifenthrin	0.0125	0.0185	0.0171-0.0202	0.047	0.0396-0.0599	0.0938	0.3082
	0.025						
	0.05						
Cypermethrin	0.0077	0.0052	0.0031-0.0072	0.111	0.0662-0.2760	0.2212	0.1333
	0.023						
	0.07						

Table 3 (Continued)

Insecticide	Concentration (%)	LC_{50}	95% F.L.*	LC_{99}	95% F.L.	Diagnostic concentration ** (%)	*P* > chi square
Deltamethrin	0.00031 0.00125 0.005	0.0007	0.0006-0.0007	0.002	0.0020-0.0034	0.0049	0.8955
λ-cyhalothrin	0.00037 0.0015 0.006	0.0012	0.0010-0.0014	0.006	0.0043-0.0087	0.0116	0.8633
Permethrin	0.03125 0.0625 0.125	0.0379	0.0354-0.0407	0.073	0.0632-0.0922	0.1466	0.9718

*F.L. = Fiducial limits at 95% level of confidence.

**Diagnostic Concentration/Discriminating Dose calculation = 2 x LC_{99}

Table 4 Percent knockdown and mortality of a laboratory susceptible strain of *Aedes aegypti* (USDA) exposed to 6 different pyrethroids using diagnostic concentration.

Insecticides	Diagnostic concentration (%)	% Knockdown (No. of tested)	% Mortality (No. of tested)	
			Control	Treatment
α -cypermethrin	0.086	100(100)	1.03(97)	100(100)
Bifenthrin	0.094	100(97)	1.03(97)	100(97)
Cypermethrin	0.221	100(99)	1.03(97)	100(99)
Deltamethrin	0.005	100(101)	1.03(97)	100(101)
λ-cyhalothrin	0.012	100(103)	1.03(97)	100(103)
Permethrin	0.147	96.91(97)	0(100)	100(97)

Table 5 Percent knockdown and mortality of Kanchanaburi strain of *Aedes aegypti* using an established diagnostic concentration of each insecticide.

Insecticides	Diagnostic concentration (%)	% Knockdown (No. of tested)	% Mortality (No. of tested)	
			Control	Treatment
α -cypermethrin	0.086	100(92)	0(97)	97.83(92)
Bifenthrin	0.094	85.86(92)	0(97)	92.39(92)
Cypermethrin	0.221	100(99)	0(97)	97.98(99)
Deltamethrin	0.005	91.75(97)	0(97)	94.85(97)
λ-cyhalothrin	0.012	88.30(94)	0(97)	97.87(94)
Permethrin	0.147	82(100)	0(97)	88(100)

Table 6 Percent knockdown and mortality of Khon Kaen strain of *Aedes aegypti* using an established diagnostic concentration of each insecticide.

Insecticides	Diagnostic concentration (%)	% Knockdown (No. of tested)	% Mortality (No. of tested)	
			Control	Treatment
α –cypermethrin	0.086	100(104)	1.47 (100)	88.3(104)
Bifenthrin	0.094	96(93)	1.47 (100)	100(93)
Cypermethrin	0.221	0(96)	1.47(100)	8.9(96)
Deltamethrin	0.005	0(99)	0(99)	0.7(99)
λ-cyhalothrin	0.012	0(85)	1.33 (100)	11.8(85)
Permethrin	0.147	98(98)	1.33 (100)	100(98)

Table 7 Percent knockdown and mortality of Nong Khai strain of *Aedes aegypti* using an established diagnostic concentration of each insecticide.

Insecticides	Diagnostic concentration (%)	% Knockdown (No. of tested)	% Mortality (No. of tested)	
			Control	Treatment
α –cypermethrin	0.086	100(98)	2.02 (99)	97.96(98)
Bifenthrin	0.094	0(99)	2.02 (99)	14.14(99)
Cypermethrin	0.221	23.47(98)	2.02 (99)	62.24(98)
Deltamethrin	0.005	6.86(102)	0(95)	3.92(102)
λ-cyhalothrin	0.012	16.16(99)	2.02 (99)	11.11(99)
Permethrin	0.147	0(98)	2.02 (99)	6.12(98)

Discussion

By applying new, revised diagnostic concentrations of six synthetic pyrethroids, three field collected *Ae. aegypti* populations demonstrated varying physiological resistance based origin (geography) and chemical tested. The population from Kanchanaburi proved reasonably susceptible to all six active ingredients with the lowest mean mortality against permethrin. These results are compatible with previous work from this same area of Kanchanaburi with only slightly lower levels of resistance to permethrin and deltamethrin reported previously (Thanispong *et al.* 2008, Chuaycharoensuk *et al.* 2011). However, the other 2 Thai populations, Khon Kaen and Nong Khai, displayed high levels of physiological resistance to bifenthrin, deltamethrin and λ-cyhalothrin. Interestingly, the Nong Khai population was also found highly resistant to permethrin (6%) and significantly so (62%) with cypermethrin while Khon Kaen was completely susceptible to both compounds. In general, these results are consistent with recent resistance patterns seen with *Ae. aegypti* elsewhere in Thailand (Chareonviriyaphap *et al.* 1999, Prapanthadara *et al*. 2002, Ponlawat *et al.* 2005, Paeporn *et al.* 2005, Jirakanjanakit *et al.* 2007). Comparing all three local populations, α-cypermethrin proved to be the one chemical that provided the best overall mortality (88.46 to 97.96%).

Aedes aegypti is both a common nuisance mosquito and a constant public-health threat in Thailand serving as the primary vector of dengue/dengue hemorrhagic fever (DHF) (MOPH, 2010). One of the very few methods to effectively curb dengue transmission is to reduce a human-vector contact using insecticides (Reiter and Gubler ,1997, WHO, 1999, Jacobs, 2000). However, a major disadvantage with the routine long-term use of insecticides is the prospect a vector population may develop resistance to the active ingredient rendering it operationally useless (WHO, 1992, Roberts and Andre, 1994, Brogdon and McAllister 1998, Hemingway and Ranson 2000, Thanispong *et al.* 2008).

In Thailand, information on insecticide resistance in *Ae. aegypti*, the primary vector of dengue/dengue haemorrhagic fever, is relatively limited due to a shortage of

studies and comprehensive sustainable monitoring programs within the national public health vector control program. *Aedes aegypti* is one of the most efficient, well-adapted and widely distributed mosquitoes in the tropical and sub-tropical zones and has proven extremely recalcitrant to control (Gratz and Halstead, 2008). Among the commonly available control techniques, chemical control remains the most effective method to curb dengue transmission. Of the chemical categories (classes), synthetic pyrethroids are the most common and extensively used in both governmental and public sectors and still generally regarded as an effective adulticide (Chareonviriyaphap *et al*. 1999, Kongmee *et al.* 2004, Jirakanjanakit *et al*. 2007, MOPH, 2010). In Thailand, Ultra-Low Volume (ULV) application of deltamethrin has been used repeatedly to interrupt dengue transmission soon after the first dengue case has been reported. For general household use, a variety of low concentration, combination synthetic pyrethroids are widely available to for public to control household arthropod pests. Not unexpectedly, the continuous and repetitive contact with insecticides, especially pyrethroids, has resulted in various degrees of insecticide resistance in *Ae. aegypti* populations throughout Thailand. Admittedly, precisely how resistance has impacted dengue control efforts in Thailand has not been adequately evaluated.

Insecticide resistance in mosquito populations is considered one of the major factors undermining the success and impact of vector control programs (Brogdon and McAllister, 1998, Hemingway and Ranson, 2000). For several decades insecticide companies have continued to develop promising synthetic alternative compounds and formulations for public health use in private and governmental sectors to prevent dengue transmission (MOPH, 2010). Among the compounds for greatest interest have been the synthetic pyrethroids such as permethrin, cypermethrin, bifenthrin, deltamethrin, cyfluthrin, resmethrin, α-cypermethrin and tetramethrin (Chareonviriyaphap *et al*., 1999, Somboon *et al.* 2003, Paeporn *et al.* 2005, Ponlawat *et al.* 2005, Thanispong *et al.* 2008). Pyrethroids have earned a more favorable acceptance for the control of mosquitoes primarily because of their inherent properties of relatively low toxicity to humans and being highly effective at low concentrations by quickly immobilizing (knockdown) and killing insects. However, it has been this

over-reliance on a single class of compound that has contributed to widespread insecticide resistance in mosquito populations (Roberts and Andre 1994, Hemingway and Ranson, 2000). In Thailand, insecticide resistance in *Ae. aegypti* was first reported against DDT in Bangkok and Nakhon Ratchasrima (northeast Thailand) (Neely 1964). Subsequently, resistance to phosphorothioate (organophosphate) compounds was found present throughout the country before being reported in *Ae. aegypti* to synthetic pyrethroids (Chareonviriyaphap *et al.*, 1999, Jirakanjanakit *et al.* 2007, Thanispong *et al.*, 2008, Chuaycharoensuk *et al.*,2011).

Over the past 60 plus years, *Ae. aegypti* and other dengue vectors in different countries have developed resistance to commonly used insecticides (Brown and Pal 1971, WHO, 1999). Both baseline data (before the start of control operations), followed by routine or periodic insecticide susceptibility assays to operational chemicals used in a vector control program is of paramount importance for monitoring vector response over time. Although a number of studies on pyrethroid resistance in *Ae. aegypti* have been published, many have relied on using World Health Organization published diagnostic concentrations and conditions (e.g., exposure times) typically used for monitoring *Anopheles* mosquitoes (WHO 1981, 1998). Surprisingly, there is far less information or data supporting the standard diagnostic criteria for susceptibility testing of *Ae. aegypti* (WHO, 1992, 1999, 2006) For pyrethroids, only λ -cyhalothrin (0.03%) and permethrin (0.25%) have recommended diagnostic doses provided by WHO for determining the resistant status of *Ae. aegypti* (WHO, 1992, 1998).

Recently, the diagnostic doses of 2 commonly used synthetic pyrethroids, permethrin (0.9%) and deltamethrin (0.06%), were established using a reference susceptible strain (Bora Bora, Fr. Polynesia) of *Ae. aegypti* (Jirakanjanakit *et al.* 2007). However, both diagnostic doses were derived from a log-probit analysis which had a very high chi-square and low *P*value (< 0.005), indicating a relatively poor goodness-of-fit of the data . In general, susceptibility baselines and diagnostic doses of various compounds used for the control of *Ae. aegypti* are lacking and thus information derived on pyrethroid susceptibility may not be completely accurate or

operationally meaningful. Furthermore, the majority of data on insecticide susceptibility is limited to only a few areas in Thailand (Chareonviriyaphap *et al.* 1999, Jirakanjanakit et al. 2007, Prapanthadara *et al.* 2002, Ponlawat 2005, Thanispong et al. 2008, Chuaycharoensuk *et al.* 2011). Therefore, WHO (1998) has repeatedly recommended that baseline data on insecticide susceptibility should be gathered on a reference strain of *Ae. aegypti* before performing tests on field-collected populations.

This study did not investigate the possible metabolic and target site mechanisms involved in the resistance detected in the populations tested. Of those mechanisms most likely to be involved with conferring reduced susceptibility to pyrethroids, elevated or modified activities of esterases and/or monoöxygensaes in involved in metabolic detoxification of insecticides (Paeporn et al. 2004) and the possible presence of the *kdr* (knockdown resistance) mutation (Brogdon and McAllister 1998).

The susceptibility of adult *Ae. aegypti* to the six pyrethroid active ingredients were selected as these compounds currently represent the predominant chemical class utilized for space spray applications ('fogging') and treated materials (e.g., window curtains). Space spray ('fogging') application of synthetic pyrethroids remains method and insecticides of choice for adult *Aedes* control in Thailand (MOPH 2010). However, this was not always the case in Thailand as decades ago DDT (organochlorine), dieldrin (cyclodiene) and malathion (phosphorothioate) compounds had been extensively used to control vector mosquitoes (Bang *et al.* 1969, Gould *et al.* 1970, Lofgren 1970, Chareonviriyaphap *et al.* 1999). At that time, DDT was also widely used to control *Aedes* mosquitoes in Thailand (Neely 1964, Ponlawat *et al.* 2005). The first reports of DDT resistance in *Ae. aegypti* in Thailand were published in the 1960s (Neely 1964, Bang *et al.* 1969). Thereafter, resistance to temephos (larvicide), malathion and fenitrothion were reported as widespread in Thailand (Chareonviriyaphap *et al.* 1999) followed more recently by many reports of resistance to pyrethroids (Prapanthadara *et al.* 2002, Somboon *et al.* 2003. Ponlawat *et al.* 2005, Yaicharoen *et al.* 2005, Paeporn *et al.* 2004, 2005, Sathantriphop *et al.* 2006,

Jirakanjanakit *et al.* 2007, Thanispong *et al.* 2008, Chuaycharoensuk *et al.* 2011). Although DDT was last used in Thailand in 1994 the current susceptibility status of *Ae. aegypti* to various pyrethroids may have been impacted by persistent cross-resistance mechanisms between the two chemicals (Chadwick *et al.* 1977) that still persist in Thailand (Prapanthadara *et al.* 2002).

The use of chemicals as contact residual insecticides on indoor walls of homes has not been routinely used to directly control adult *Aedes* mosquitoes, although there is strong enough evidence to show it would likely provide longer-lasting control in some situations (Giglioli 1948, Lien *et al.* 1992, Sulaiman et al. 1993, Reiter and Gubler 1997, Doke *et al.* 2000) and even eradication (Halcrow 1954, Brown and Pal 1971) when compared to the far more transient effects of space spray applications. The fact that many pyrethroids also perform as contact excitants and spatial repellents on *Ae. aegypti* (Kongmee *et al.* 2004, Thanispong et al. 2010), exclusive of direct toxic action, lends further support for use of residual applied insecticides inside homes to reduce human-vector contact and disease transmission. Whether realistic or cost-effective in control programs has yet to be fully explored.

A dengue control program can be seriously compromised and valuable resource squandered without accurate information on insecticide susceptibility status of local *Aedes* vector populations. As dengue remains a major disease problem throughout much of Thailand, the monitoring of insecticide resistance in *Ae. aegypti* and *Ae. albopictus* should be increased in periodicity, geographical coverage and range of insecticides to assist vector control programs to anticipate and respond accordingly. Investigations of cross resistance to the similar or closely related synthetic compounds and in-depth discovery of the actual mechanisms responsible for resistance are needed. Knowledge of vector/pest susceptibility to pesticides, changing trends of resistance and their operational implications are basic requirements to guide optimum chemical use. Insecticide resistance monitoring must be an integral part of a viable vector-borne disease and pest control program.

CONCLUSION

The diagnostic dose of 6 commonly used synthetic pyrethroids, alphacypermethrin (0.086%), bifenthrin (0.094%) cypermethrin (0.0221%) deltamethrin (0.05%) lamdacyhalothrin (0.012%) and permethrin (0.0147%) were established using reference susceptible strain (USDA) of *Ae. aegypti*

Low to moderate incipient resistance to all 6 pyrethroids was seen in Kanchanaburi population. Completaly susceptible to cypermethrin and permethrin ;however, incipient resistance was detected against alphacypermethrin and very strong resistance was obtained with deltamethrin bifenthrin and lamdacyhalothrin. The Nong Khai strain demonstrated strong resistance to deltamethrin, lamdacyhalothrin, permethrin, bifenthrin and cypermethrin whereas completely susceptible to alphacypermethrin was observed.

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APPENDIX

Appendix Table 1 WHO recommended insecticides for indoor residual spraying and established diagnostic concentration

Insecticides	WHO Dosage	Established diagnostic
α -cypermethrin	0.02-0.03	0.086
Bifenthrin	0.025-0.05	0.094
Cypermethrin	-	0.221
Deltamethrin	0.02-0.025	0.005
λ-cyhalothrin	0.02-0.03	0.012
Permethrin	-	0.147

Note: WHO recommendations on the use of pesticides in public health are valid ONLY if linked to WHO specifications for their quality control.

Appendix Table 2 Steps in calculating probit analyses computations for the fitting of a probit regression equation Probit analysis

dose	x	n	r	p (y)	Empirical probit	Y	x	Yo	A
0.029(20)	1.30	99	96	96	6.75	6.8	1.30	∞	∞
0.005(5)	0.69	105	89	84	6.23	6.2	0.69	1.6429	5.1497
0.00125(1.25)	0.009	98	57	58	5.18	5.2	0.09	3.7187	2.5573

Appendix Table 2(Continued)

dose	w	w	W	Wx	wx^2	Wxy	Wy	Wy^2	Y	nw
0.029(20)	0.180	0.180	18.82	23.14	0.3042	156.3705	120.280	811.923	6.8	17.82
0.005(5)	0.370	0.370	38.85	26.80	0.176157	167.0044	242.035	1507.8881	6.2	38.85
0.00125(1.25)	0.627	0.627	61.446	5.530	0.5078	28.6461	318.290	1648.743	5.2	61.446
				55.47	680.605					118.116

Probit analysis

mean x = $\sum nwx/\sum nw$ =	0.6933
mean y = $\sum nwy/\sum nwy$ =	6.0666
$1/\sum nw$ =	0.008466
$\sum nwx)^2/= \sum nw$ =	26.04999
$\sum nwx (\sum nwy)/ \sum nw$ =	319.6278
$(\sum nwy)^2/\sum nw$ =	3921.7647
$\sum nwx^2$ =	49.11
$\sum nwxy$ =	170.3362
$\sum nwxy^2$ =	3978.891
$\sum x^2$ =	2.1742
$\sum xy$ =	13.586
$\sum y^2$ =	111.72
$(\sum xy^2) /\sum x^2$ =	84.895
b =	6.2487

Y = mean of y+b (x-mean of x) =

= 6.0667 + 6.2487 (X- 0.6933)

= 6.0667 + 6.2487X – 4.3322

Y = 1.7345 + 6.2487X=

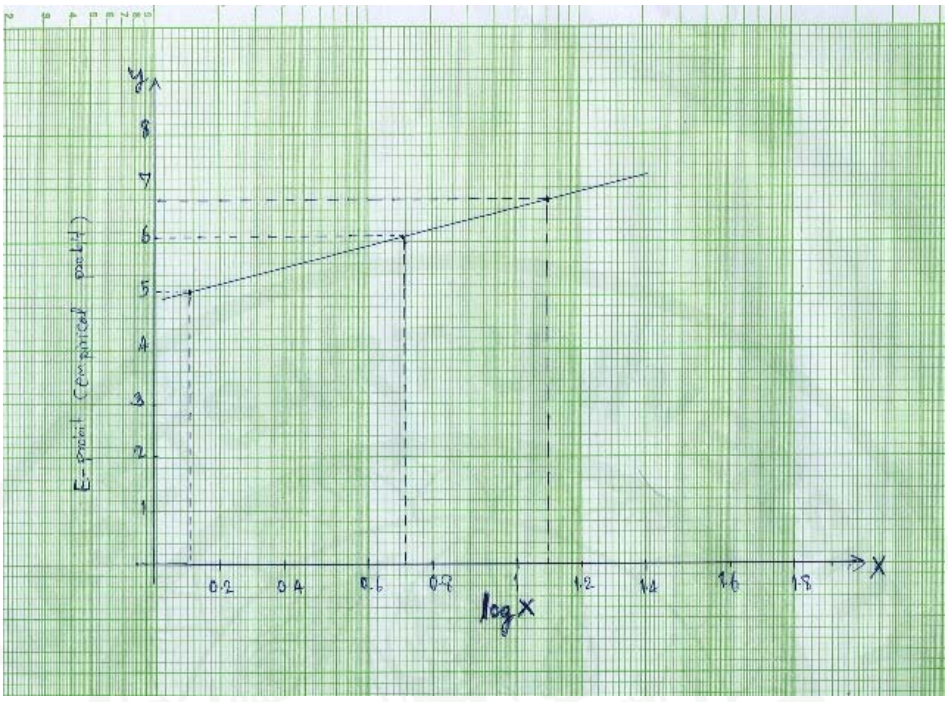
= 5.1967 + 4.8767(X- 2.2346)

$g = t^2/b^2 \sum x^2$ =

= 3.8416/ 39.046 X 2.1742

= 3.8416/ 84.8943

g = 0.045



Appendix Figure 1 On the probability of mortality (% mortarity) the concentration (dose)

Alphacypermethrin

Algorithm converged.

Goodness-of-Fit Tests

Statistic	Value	DF	Pr > ChiSq
Pearson Chi-Square	0.0053	1	0.9419
L.R. Chi-Square	0.0053	1	0.9419

Response-Covariate Profile

Response Levels	2
Number of Covariate Values	3

Since the chi-square is small (p > 0.1000), fiducial limits will be calculated using a t value of
1.96.

Type III Analysis of Effects

Effect	DF	Wald Chi-Square	Pr > ChiSq
Log10(dose)	1	41.9345	<.0001

Analysis of Parameter Estimates

Parameter	DF	Estimate	Standard Error	95% Confidence Limits		Chi-Square	Pr > ChiSq
Intercept	1	4.2103	0.5455	3.1410	5.2795	59.56	<.0001
Log10(dose)	1	1.3803	0.2131	0.9625	1.7981	41.93	<.0001
_C_	0	0.0000	0.0000	0.0000	0.0000		

Probit Procedure

Probit Model in Terms of Tolerance Distribution

MU	SIGMA
-3.0502857	0.72448725

Estimated Covariance Matrix
for Tolerance Parameters

	MU	SIGMA
MU	0.010889	-0.009091
SIGMA	-0.009091	0.012517

The SAS System 14:25 Wednesday, December 22, 2010

Probit Procedure

Probit Analysis on dose

Probability	dose	95% Fiducial Limits	
0.01	0.0000184	1.95971E-6	0.0000617
0.02	0.0000290	3.75173E-6	0.0000877
0.03	0.0000386	5.66309E-6	0.0001097
0.04	0.0000480	7.71786E-6	0.0001298
0.05	0.0000573	9.92657E-6	0.0001489
0.06	0.0000666	0.0000123	0.0001673
0.07	0.0000759	0.0000148	0.0001853
0.08	0.0000855	0.0000175	0.0002032
0.09	0.0000951	0.0000204	0.0002209
0.10	0.0001050	0.0000235	0.0002386
0.15	0.0001581	0.0000420	0.0003284
0.20	0.0002188	0.0000666	0.0004238
0.25	0.0002891	0.0000988	0.0005280
0.30	0.0003714	0.0001406	0.0006441
0.35	0.0004683	0.0001946	0.0007755
0.40	0.0005837	0.0002645	0.0009262
0.45	0.0007222	0.0003553	0.00110
0.50	0.0008907	0.0004738	0.00131
0.55	0.00110	0.0006295	0.00157
0.60	0.00136	0.0008360	0.00188
0.65	0.00169	0.00111	0.00230
0.70	0.00214	0.00148	0.00287
0.75	0.00274	0.00199	0.00371
0.80	0.00363	0.00269	0.00507
0.85	0.00502	0.00371	0.00754
0.90	0.00755	0.00536	0.01284
0.91	0.00834	0.00583	0.01466
0.92	0.00928	0.00639	0.01696
0.93	0.01044	0.00705	0.01991
0.94	0.01192	0.00786	0.02386
0.95	0.01385	0.00889	0.02937
0.96	0.01652	0.01027	0.03754
0.97	0.02053	0.01222	0.05083
0.98	0.02739	0.01538	0.07624
0.99	0.04316	0.02203	0.14489

NOTE: The above quantiles and fiducial limits refer to effects due to the independent variable
and do not include any effect due to the natural threshold.

Obs	tot	dose	RESPONSE	N	LDOSE	obs	pobs	prob	xbeta	std
1	1	0.00125	57	98	-2.90309	0.58163	0.20607	0.58050	0.20317	0.12121
2	2	0.00500	89	105	-2.30103	0.84762	1.02628	0.84948	1.03419	0.10211
3	3	0.02000	96	99	-1.69897	0.96970	1.87636	0.96892	1.86520	0.1977

Bifenthrin

Algorithm converged.

Goodness-of-Fit Tests

Statistic	Value	DF	Pr > ChiSq
Pearson Chi-Square	1.0386	1	0.3082
L.R. Chi-Square	1.6813	1	0.1948

Response-Covariate Profile

Response Levels	2
Number of Covariate Values	3

Since the chi-square is small (p > 0.1000), fiducial limits will be calculated using a t value of
1.96.

Type III Analysis of Effects

Effect	DF	Wald Chi-Square	Pr > ChiSq
Log10(dose)	1	95.3022	<.0001

Analysis of Parameter Estimates

Parameter	DF	Estimate	Standard Error	95% Confidence Limits		Chi-Square	Pr > ChiSq
Intercept	1	10.0143	1.0267	8.0020	12.0266	95.14	<.0001
Log10(dose)	1	5.7860	0.5927	4.6244	6.9477	95.30	<.0001
_C_	0	0.0000	0.0000	0.0000	0.0000		

Probit Model in Terms of Tolerance Distribution

MU	SIGMA
-1.7307843	0.17283086

Estimated Covariance Matrix
for Tolerance Parameters

	MU	SIGMA
MU	0.000324	-0.000014
SIGMA	-0.000014	0.000313

The SAS System 14:25 Wednesday, December 22, 2010

Probit Procedure

Probit Analysis on dose

Probability	dose	95% Fiducial	Limits
0.01	0.00736	0.00572	0.00877
0.02	0.00821	0.00653	0.00962
0.03	0.00879	0.00711	0.01020
0.04	0.00926	0.00758	0.01066
0.05	0.00966	0.00798	0.01105
0.06	0.01001	0.00833	0.01140
0.07	0.01033	0.00866	0.01172
0.08	0.01063	0.00896	0.01201
0.09	0.01090	0.00924	0.01228
0.10	0.01116	0.00951	0.01253
0.15	0.01231	0.01069	0.01366
0.20	0.01330	0.01173	0.01464
0.25	0.01421	0.01268	0.01555
0.30	0.01509	0.01358	0.01644
0.35	0.01594	0.01446	0.01733
0.40	0.01680	0.01534	0.01823
0.45	0.01768	0.01621	0.01918
0.50	0.01859	0.01709	0.02018
0.55	0.01954	0.01801	0.02126
0.60	0.02056	0.01896	0.02246
0.65	0.02167	0.01996	0.02379
0.70	0.02290	0.02105	0.02531
0.75	0.02431	0.02227	0.02710
0.80	0.02598	0.02368	0.02928
0.85	0.02808	0.02539	0.03209
0.90	0.03095	0.02768	0.03607
0.91	0.03169	0.02826	0.03711
0.92	0.03251	0.02890	0.03828
0.93	0.03344	0.02962	0.03961
0.94	0.03451	0.03044	0.04115
0.95	0.03577	0.03140	0.04299
0.96	0.03731	0.03256	0.04525
0.97	0.03929	0.03404	0.04822
0.98	0.04209	0.03611	0.05247
0.99	0.04691	0.03961	0.05997

NOTE: The above quantiles and fiducial limits refer to effects due to the independent variable
and do not include any effect due to the natural threshold.

Obs	tot	dose	RESPONSE	N	LDOSE	obs	pobs	prob	xbeta	std
1	1	0.0125	16	94	-1.90309	0.17021	-0.95332	0.15939	-0.99696	0.14898
2	2	0.0250	65	87	-1.60206	0.74713	0.66547	0.77180	0.74480	0.12653
3	3	0.0500	101	101	-1.30103	1.00000	.	0.99355	2.48656	0.2710

Cypermethrin

Algorithm converged.

Goodness-of-Fit Tests

Statistic	Value	DF	Pr > ChiSq
Pearson Chi-Square	2.2540	1	0.1333
L.R. Chi-Square	2.2587	1	0.1329

Response-Covariate Profile

Response Levels	2
Number of Covariate Values	3

Since the chi-square is small (p > 0.1000), fiducial limits will be calculated using a t value of
1.96.

Type III Analysis of Effects

Effect	DF	Wald Chi-Square	Pr > ChiSq
Log10(dose)	1	42.5656	<.0001

Analysis of Parameter Estimates

Parameter	DF	Estimate	Standard Error	95% Confidence Limits		Chi-Square	Pr > ChiSq
Intercept	1	4.0074	0.5037	3.0201	4.9948	63.29	<.0001
Log10(dose)	1	1.7580	0.2695	1.2298	2.2861	42.57	<.0001
_C_	0	0.0000	0.0000	0.0000	0.0000		

Probit Procedure

Probit Model in Terms of Tolerance Distribution

MU	SIGMA
-2.2795884	0.56883986

Estimated Covariance Matrix
for Tolerance Parameters

	MU	SIGMA
MU	0.007313	-0.005900
SIGMA	-0.005900	0.007602

The SAS System 14:25 Wednesday, December 22, 2010

Probit Procedure

Probit Analysis on dose

Probability	dose	95% Fiducial	Limits
0.01	0.0002495	0.0000426	0.0006525
0.02	0.0003566	0.0000708	0.0008606
0.03	0.0004472	0.0000977	0.00103
0.04	0.0005303	0.0001245	0.00117
0.05	0.0006092	0.0001517	0.00130
0.06	0.0006855	0.0001793	0.00143
0.07	0.0007602	0.0002077	0.00155
0.08	0.0008340	0.0002369	0.00167
0.09	0.0009073	0.0002669	0.00178
0.10	0.0009804	0.0002979	0.00189
0.15	0.00135	0.0004694	0.00243
0.20	0.00174	0.0006731	0.00297
0.25	0.00217	0.0009162	0.00353
0.30	0.00264	0.00121	0.00413
0.35	0.00317	0.00156	0.00477
0.40	0.00377	0.00198	0.00549
0.45	0.00446	0.00250	0.00629
0.50	0.00525	0.00313	0.00721
0.55	0.00619	0.00391	0.00828
0.60	0.00732	0.00489	0.00956
0.65	0.00870	0.00613	0.01116
0.70	0.01044	0.00771	0.01324
0.75	0.01271	0.00975	0.01612
0.80	0.01582	0.01244	0.02047
0.85	0.02042	0.01609	0.02772
0.90	0.02815	0.02160	0.04181
0.91	0.03041	0.02312	0.04634
0.92	0.03309	0.02486	0.05187
0.93	0.03630	0.02689	0.05877
0.94	0.04026	0.02933	0.06765
0.95	0.04530	0.03234	0.07952
0.96	0.05203	0.03622	0.09628
0.97	0.06170	0.04158	0.12198
0.98	0.07739	0.04987	0.16739
0.99	0.11059	0.06619	0.27645

NOTE: The above quantiles and fiducial limits refer to effects due to the independent variable
and do not include any effect due to the natural threshold.

Obs	tot	dose	RESPONSE	N	LDOSE	obs	pobs	prob	xbeta	std
1	1	0.0077	63	106	-2.11351	0.59434	0.23872	0.61484	0.29196	0.11814
2	2	0.0230	89	98	-1.63827	0.90816	1.32953	0.87022	1.12741	0.10655
3	3	0.0700	103	107	-1.15490	0.96262	1.78190	0.97599	1.97716	0.20577

Deltamethrin

Algorithm converged.

Goodness-of-Fit Tests

Statistic	Value	DF	Pr > ChiSq
Pearson Chi-Square	0.0172	1	0.8955
L.R. Chi-Square	0.0339	1	0.8539

Response-Covariate Profile

Response Levels	2
Number of Covariate Values	3

Since the chi-square is small (p > 0.1000), fiducial limits will be calculated using a t value of
1.96.

Type III Analysis of Effects

Effect	DF	Wald Chi-Square	Pr > ChiSq
Log10(dose)	1	110.4787	<.0001

Analysis of Parameter Estimates

Parameter	DF	Estimate	Standard Error	95% Confidence Limits		Chi-Square	Pr > ChiSq
Intercept	1	12.9557	1.2348	10.5356	15.3759	110.09	<.0001
Log10(dose)	1	4.0736	0.3876	3.3140	4.8332	110.48	<.0001
_C_	0	0.0000	0.0000	0.0000	0.0000		

Probit Procedure

Probit Model in Terms of Tolerance Distribution

MU	SIGMA
-3.1803899	0.24548141

Estimated Covariance Matrix
for Tolerance Parameters

	MU	SIGMA
MU	0.000841	-0.000020
SIGMA	-0.000020	0.000545

The SAS System 14:25 Wednesday, December 22, 2010

Probit Procedure

Probit Analysis on dose

Probability	dose	95% Fiducial Limits	
0.01	0.0001772	0.0001263	0.0002258
0.02	0.0002068	0.0001521	0.0002581
0.03	0.0002280	0.0001711	0.0002810
0.04	0.0002454	0.0001868	0.0002997
0.05	0.0002605	0.0002007	0.0003158
0.06	0.0002741	0.0002132	0.0003303
0.07	0.0002866	0.0002248	0.0003436
0.08	0.0002983	0.0002357	0.0003560
0.09	0.0003094	0.0002461	0.0003677
0.10	0.0003199	0.0002560	0.0003789
0.15	0.0003674	0.0003011	0.0004293
0.20	0.0004102	0.0003420	0.0004748
0.25	0.0004509	0.0003809	0.0005184
0.30	0.0004908	0.0004191	0.0005618
0.35	0.0005309	0.0004572	0.0006060
0.40	0.0005720	0.0004959	0.0006521
0.45	0.0006148	0.0005358	0.0007009
0.50	0.0006601	0.0005774	0.0007536
0.55	0.0007087	0.0006213	0.0008114
0.60	0.0007617	0.0006683	0.0008759
0.65	0.0008207	0.0007197	0.0009493
0.70	0.0008879	0.0007769	0.00103
0.75	0.0009665	0.0008425	0.00114
0.80	0.00106	0.0009205	0.00127
0.85	0.00119	0.00102	0.00144
0.90	0.00136	0.00115	0.00169
0.91	0.00141	0.00119	0.00176
0.92	0.00146	0.00123	0.00183
0.93	0.00152	0.00127	0.00192
0.94	0.00159	0.00133	0.00203
0.95	0.00167	0.00139	0.00215
0.96	0.00178	0.00146	0.00231
0.97	0.00191	0.00156	0.00253
0.98	0.00211	0.00170	0.00284
0.99	0.00246	0.00194	0.00342

NOTE: The above quantiles and fiducial limits refer to effects due to the independent variable
and do not include any effect due to the natural threshold.

Obs	tot	dose	RESPONSE	N	LDOSE	obs	pobs	prob	xbeta	std
1	1	.00031	9	99	-3.50864	0.09091	-1.33518	0.09058	-1.33716	0.17613
2	2	.00125	87	100	-2.90309	0.87000	1.12639	0.87068	1.12962	0.15734
3	3	.00500	98	98	-2.30103	1.00000	.	0.99983	3.58219	0.3574

Lamdacyhalothrin

Algorithm converged.

Goodness-of-Fit Tests

Statistic	Value	DF	Pr > ChiSq
Pearson Chi-Square	0.0297	1	0.8633
L.R. Chi-Square	0.0290	1	0.8648

Response-Covariate Profile

Response Levels 2
Number of Covariate Values 3

Since the chi-square is small (p > 0.1000), fiducial limits will be calculated using a t value of
1.96.

Type III Analysis of Effects

Effect	DF	Wald Chi-Square	Pr > ChiSq
Log10(dose)	1	102.4681	<.0001

Analysis of Parameter Estimates

Parameter	DF	Estimate	Standard Error	95% Confidence Limits		Chi-Square	Pr > ChiSq
Intercept	1	9.9000	0.9815	7.9764	11.8237	101.74	<.0001
Log10(dose)	1	3.3886	0.3348	2.7325	4.0447	102.47	<.0001
_C_	0	0.0000	0.0000	0.0000	0.0000		

Probit Procedure

Probit Model in Terms of Tolerance Distribution

MU	SIGMA
-2.9215395	0.29510397

Estimated Covariance Matrix
for Tolerance Parameters

	MU	SIGMA
MU	0.000991	-0.000020
SIGMA	-0.000020	0.000850

The SAS System 14:25 Wednesday, December 22, 2010

Probit Procedure

Probit Analysis on dose

Probability	dose	95% Fiducial Limits	
0.01	0.0002466	0.0001629	0.0003296
0.02	0.0002968	0.0002042	0.0003863
0.03	0.0003338	0.0002356	0.0004275
0.04	0.0003646	0.0002623	0.0004614
0.05	0.0003918	0.0002861	0.0004911
0.06	0.0004165	0.0003081	0.0005180
0.07	0.0004395	0.0003287	0.0005429
0.08	0.0004611	0.0003482	0.0005662
0.09	0.0004817	0.0003669	0.0005884
0.10	0.0005015	0.0003850	0.0006096
0.15	0.0005924	0.0004693	0.0007069
0.20	0.0006762	0.0005482	0.0007968
0.25	0.0007576	0.0006253	0.0008845
0.30	0.0008389	0.0007025	0.0009732
0.35	0.0009220	0.0007810	0.00107
0.40	0.00101	0.0008621	0.00116
0.45	0.00110	0.0009466	0.00127
0.50	0.00120	0.00104	0.00138
0.55	0.00130	0.00113	0.00151
0.60	0.00142	0.00123	0.00166
0.65	0.00156	0.00135	0.00183
0.70	0.00171	0.00148	0.00203
0.75	0.00189	0.00163	0.00228
0.80	0.00212	0.00181	0.00260
0.85	0.00242	0.00204	0.00304
0.90	0.00286	0.00236	0.00370
0.91	0.00298	0.00245	0.00389
0.92	0.00311	0.00254	0.00410
0.93	0.00327	0.00265	0.00434
0.94	0.00345	0.00278	0.00463
0.95	0.00366	0.00293	0.00498
0.96	0.00394	0.00312	0.00544
0.97	0.00430	0.00337	0.00605
0.98	0.00484	0.00373	0.00698
0.99	0.00582	0.00438	0.00875

NOTE: The above quantiles and fiducial limits refer to effects due to the independent variable
and do not include any effect due to the natural threshold.

Obs	tot	dose	RESPONSE	N	LDOSE	obs	pobs	prob	xbeta	std
1	1	00037	4	99	-3.43180	0.04040	-1.74602	0.04190	-1.72908	0.20335
2	2	.00150	64	101	-2.82391	0.63366	0.34157	0.62962	0.33084	0.11086
3	3	.00600	98	99	-2.22185	0.98990	2.32257	0.99113	2.37100	0.255

Permethrin

Algorithm converged.

Goodness-of-Fit Tests

Statistic	Value	DF	Pr > ChiSq
Pearson Chi-Square	0.0012	1	0.9718
L.R. Chi-Square	0.0025	1	0.9602

Response-Covariate Profile

Response Levels	2
Number of Covariate Values	3

Since the chi-square is small (p > 0.1000), fiducial limits will be calculated using a t value of 1.96.

Type III Analysis of Effects

Effect	DF	Wald Chi-Square	Pr > ChiSq
Log10(dose)	1	69.3205	<.0001

Analysis of Parameter Estimates

Parameter	DF	Estimate	Standard Error	95% Confidence Limits		Chi-Square	Pr > ChiSq
Intercept	1	11.5477	1.4115	8.7813	14.3142	66.93	<.0001
Log10(dose)	1	8.1255	0.9759	6.2127	10.0383	69.32	<.0001
_C_	0	0.0000	0.0000	0.0000	0.0000		

Probit Procedure

Probit Model in Terms of Tolerance Distribution

MU	SIGMA
-1.4211675	0.12306912

Estimated Covariance Matrix for Tolerance Parameters

	MU	SIGMA
MU	0.000226	0.000035
SIGMA	0.000035	0.000218

The SAS System 14:25 Wednesday, December 22, 2010

Probit Procedure

Probit Analysis on dose

Probability	dose	95% Fiducial Limits	
0.01	0.01961	0.01600	0.02237
0.02	0.02119	0.01766	0.02386
0.03	0.02225	0.01881	0.02486
0.04	0.02309	0.01971	0.02565
0.05	0.02379	0.02048	0.02631
0.06	0.02441	0.02115	0.02689
0.07	0.02496	0.02175	0.02741
0.08	0.02546	0.02231	0.02789
0.09	0.02593	0.02282	0.02833
0.10	0.02637	0.02331	0.02875
0.15	0.02827	0.02540	0.03056
0.20	0.02987	0.02716	0.03212
0.25	0.03132	0.02873	0.03357
0.30	0.03268	0.03018	0.03496
0.35	0.03399	0.03156	0.03635
0.40	0.03529	0.03288	0.03776
0.45	0.03659	0.03417	0.03922
0.50	0.03792	0.03545	0.04077
0.55	0.03929	0.03674	0.04242
0.60	0.04074	0.03806	0.04421
0.65	0.04229	0.03944	0.04618
0.70	0.04399	0.04090	0.04840
0.75	0.04590	0.04251	0.05096
0.80	0.04813	0.04433	0.05402
0.85	0.05086	0.04652	0.05786
0.90	0.05452	0.04938	0.06315
0.91	0.05544	0.05009	0.06451
0.92	0.05646	0.05087	0.06602
0.93	0.05760	0.05174	0.06772
0.94	0.05891	0.05273	0.06968
0.95	0.06043	0.05387	0.07199
0.96	0.06227	0.05524	0.07480
0.97	0.06461	0.05697	0.07842
0.98	0.06786	0.05934	0.08352
0.99	0.07331	0.06326	0.09227

NOTE: The above quantiles and fiducial limits refer to effects due to the independent variable
and do not include any effect due to the natural threshold.

Obs	tot	dose	RESPONSE	N	LDOSE	obs	pobs	prob	xbeta	std
1	1	0.03125	25	101	-1.50515	0.24752	-0.68230	0.24749	-0.68240	0.13583
2	2	0.06250	74	77	-1.20412	0.96104	1.76287	0.96110	1.76362	0.26072
3	3	0.12500	97	97	-0.90309	1.00000	.	0.99999	4.20965	0.53863

CURRICULUM VITAE

NAME : Mr. Suntorn Pimnon

BIRTH DATE : December 22, 1980

BIRTH PLACE : Srisaket, Thailand

EDUCATION :

INSTITUTE	**DEGREE**
Bansomdejchaopraya Rajabhat University	B.S. (Biology)

POSITION : Scientist

WORK PLACE : Department of Parasitology and Entomology Faculty of Public Health, Mahidol University