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# THESIS

# IMPACTS OF AGRICULTURAL AND PUBLIC HEALTH INSECTICIDES ON *ANOPHELES MINIMUS* SPECIES COMPLEX, VECTORS OF MALARIA IN WESTERN THAILAND

**JINRAPA PHOTHIKASIKORN**

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The findings of this study indicated the strong association between malaria transmission and ethnic groups. There were at least six ethnic groups that are highly migratory due to the occupation activities in Thong Pha Phum and Sai Yok Districts of Kanchanaburi Province. Of 232 respondents interviewed, 202 knew the malaria disease. Under the same group of respondents, 102 are at risk of malaria during the epidemic period of dry season.

Insecticide usage in the six villages was surveyed. Bong Ti Noy Village (BTN), Sai Yok District was the highest pesticides used area whereas Mae Num Noy Village (MNN), Thong Pha Phum District was the lowest pesticides used area. There were significantly lower in mosquito density in the high-pesticide location (BTN) compared to the low-pesticide location (MNN) during the entire study period ($P<0.05$).

There is one prominent biting peak of *Anopheles minimus* (2100-2300 hrs) from both MNN and BTN. The average larval density fluctuated in two selected villages throughout the year with highest density in cool season. In addition, behavioural responded of two wild caught populations on *An. minimus* species A and C to operational field doses of three agricultural compounds, carbaryl, malathion and cypermethrin, and three public health chemicals, DDT, deltamethrin, and lambda-cyhalothrin were characterized using excito-repellency test system and conclude that contact irritancy and non-contact repellency was present in both test populations across alls six chemicals.

Jinrapa Phothikasikorn C. Ketavan 13 Oct 2006

Student's signature Thesis Advisor's signature

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Jinrapa Phothikasikorn

October, 2006

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# LIST OF ABBREVIATIONS

| | | |
|---|---|---|
| An | = | Anopheles |
| BTN | = | Bong Ti Noy village |
| Bst | = | Big stream |
| cm | = | Centimeter |
| Fig | = | Figure |
| g | = | Gram |
| HP | = | Hans' Pool |
| h | = | Hour |
| MNN | = | Mae Num Noy village |
| mg | = | Milligram |
| ml | = | Milliliter |
| *P* | = | Probability Value |
| Sst | = | Small stream |
| ST | = | Stream |
| WHO | = | World Health Organization |
| % | = | Percent |

# IMPACTS OF AGRICULTURAL AND PUBLIC HEALTH INSECTICIDES ON *ANOPHELES MINIMUS* SPECIES COMPLEX, VECTORS OF MALARIA IN WESTERN THAILAND

# INTRODUCTION

## General Introduction

*Anopheles rninimus* s.l. Theobald is one of the most efficient malaria vectors throughout the eastern Asia (Reid,1968; Rao,1984). In Thailand, the *An. rninimus* complex contains important vectors of malaria that are found exclusively in the forested hilly and clear forested foothill areas (Ayurakit-Kosol and Griffith, 1963; Sucharit *et a*l., 1988; Nutsathapana *et al*., 1986; Green *et al*., 1990). *Anopheles rninimus* s.l. was reported to be mostly endophilic and endophagic throughout its geographic range (Sambasivan, 1953). After DDT was introduced to interrupt malarial transmission, *An. rninimus* reportedly shifted to greater outdoor feeding and more zoophilic preferences for blood, particularly bovids (Ziegler, 1978; Ismail, 1978). Even though DDT resulted in significant reductions of indoor-feeding mosquitoes, this control method did not completely interrupt transmission of malaria. This has been attributed partly to exophagic behavior of portions of the population and the persistence of a small number of vectors that enter and successfully feed indoors (Ratanatham *et al*., 1988 ; Chareonviriyaphap *et al*., 2003). Similar observations have also reported from Vietnam (van Bortel *et al*., 1999) raising questions on behavioral variations within the *An. minimus* taxon.

Based on morphologic and genetic variations, at least two closely related species of the *An. minimus* complex have been documented in Thailand and both have been incriminated as efficient vectors of malaria (Sucharit *et al*., 1988 ; Ziegler, 1978). *Anopheles minimus* species A is the predominant species and distributed throughout the country (Green *et al*., 1990) whereas species C appears restricted along the western Thailand-Myanmar border, particularly in Kanchanaburi Province (Sucharit *et al*., 1988; Baimai, 1989). Additionally, *An. minimus* species D has been reported in Thailand, but sufficient information is lacking to support the proposed sibling species status (Baimai, 1989). Although *An. minimus* species A and C occur in sympatry in western Thailand, notable ecoethologic variation in feeding and resting behaviors, degree of anthropophily, and other bionomical aspects may influence vector capacities of these two sibling species (van Bortel *et al*., 1999 and Theophil *et al*., 2002).

*Anopheles rninirnus* species A has shown a much greater (five-fold difference) endophilic behavior compared with species C (van Bortel *et al*., 1999). The *An. rninimus* complex has also shown different response levels of response to intradomicilary use of insecticides (Harrison, 1980; Parajuli *et al*., 1981; Lien, 1991; Chareonviriyaphap *et al.*, 1999; 2001). In Thailand, indoor house spray has been routinely conducted to interrupt human-vector contact and transmission (Chareonviriyaphap *et al*., 2001). Understanding the behavioral responses of different species of mosquitoes, even closely related sibling species, to insecticides can facilitate vector control by selecting and implementing the most effective interventions possible and help to target the primary disease vectors.

Behavioral responses, namely insecticide avoidance, can be separated into two important and distinct categories: contact irritancy and non-contact repellency. Irritant responses result from physical contact with chemical-treated surfaces, whereas repellency is an avoidance response devoid of making actual contact with insecticides (Roberts *et al*., 1997). Although ehavioral responses have been recorded with various mosquito species and populations of Anopheles from Thailand using the excito – repellency test box (Chareonviriyaphap *et al*., 2001; 1997; Sungvornyothin *et al*., 2001; Kongmee *et al*., 2004) none have been recorded to compare the behavioral responses between species in the *An. minimus* complex (e.g., species A and C). Described herein are observations using the excito-repellency test system to quantitatively measure behavioral responses between wild caught populations of *An. minimus* species A and C exposed to recommended field concentrations of DDT, deltamethrin, and lambda-cyhalothrin (WHO, 1992). In addition, behavioral responses to three agrochemical compounds by *An. minimus* species were also characterized using the same excito-repellency test system. Risk factors, base line malaria knowledge of local people from different pesticide Land-use systems in malaria endemic area, biting peak and population dynamics of *Anopheles minimus* species A, from high and low agricultural insecticide area in the two villages at Kanchanaburi Province Thailand, are also included in this study project.

**Purpose of Research**

1. To investigate farmer's pesticide use, malaria knowledge, and risk factors in different land-use systems.
2. To study the biting peak and population dynamics of *Anopheles minimus* species A, from high and low agricultural insecticide in the villages.
3. To compare behavioral responses of *Anopheles minimus* species A and C when exposed to agricultural and public health insecticides.

**Scope of Research**;

This research is divided into 4 parts as follow;

**Part 1;** Risk factors and base line malaria knowledge from different pesticide land-use systems in malaria endemic area at Kanchanaburi Province, Thailand.

**Part 2;** Biting peak and population dynamics of *Anopheles minimus* species A, from high and low agricultural insecticide area in the two villages at Kanchanaburi Province, Thailand.

**Part 3**; Behavioral responses by *Anopheles minimus* species A and C to three agrochemicals.

**Part 4;** Behavioral responses by *Anopheles minimus* species A and species C to DDT and pyrethroids.

# LITERATURE REVIEW

**Part 1; Risk factors and base line malaria knowledge from different pesticide Land-use systems in malaria endemic area at Kanchanaburi Province,Thailand.**

Historically, little was known about the behavioral risk factors that would favor the complications and increased severity of malaria in Thailand. However, several studies have since reported on various aspects of behavior and malaria. A community-based study in Nong Rhee sub district in Kanchanaburi province showed that 78% of the people that visiting to the malaria clinics preferred self-medication as the first choice of treatment before go to malaria clinics (Fungladda *el al.*, 1982). The reason for this preference was that the cost of buying anti-malarial drugs from local drug dispensers were less than traveling to malaria clinics. A similar observation noted in the studies done by Hongvivatana *et al* (1985) and Fungladda and Sommani (1986) at malaria clinics in Kanchanaburi, Western Thailand. It was shown that the dominant pattern of treatment two-stage, the first was self-medication health center, non-professional injectionist clinic and other source of treatment (Fungladda and Butraporn, 1992). Results from studies on treatment-seeking patterns of populations in endemic communities have led to the speculation that delay in seeking health probably plays an important role for malaria control (Fungladda and Sornmani, 1986; Rauyajin, 1988; Fungladda *et al*., 1991). A reported by Rauyajin (1988) revealed that than half (53.1%) of malaria cases delayed seeking care from malaria clinics. Furthermore, a clinical based case-control study by Fungladda and Sornmani (1986) revealed that the average time-lag between the onset of clinical symptoms to the time of treatment-seeking of patients at malaria clinics were relatively longer among malaria patients (4.3 days) than among control patients (4.0 days). However, another study showed that the average interval between the time of first noticeable symptoms until seeking treatment was 3.1 days for malaria cases and only 2.5 days for non-malaria patients (Fungladda *et a*l., 1991). In Trat province, eastern Thailand malaria occurred less frequently among persons who had lived in the area for a long period of time, who had obtained education, and who had a sizable annual family income (Butraporn *et al*., 1986). Malaria was more frequent among forest workers and persons who occasionally went into the forest, and among persons whose houses were in close proximity of vector breeding sites. Current information on malaria epidemiology in north-west or west Thailand adjacent to the Myanmar border has suggested that malaria transmission is closely associated with the forest and with movement of the population leading to contact with intense foci of transmission (Singhanetra-Renard, 1986; Fungladda *el al*, 1987; Ketranesee *et al*. 1991).

Thus, malaria cases usually occur among people who are both living in or close to forests and working in them. However, it is difficult to compare between these two places, because of their delivery works and the incubation periods of the malarial symptoms (usually 1-3 weeks) (Molineaux, 1991). But Somboon *et al*. (1998) showed that movement for forest activities had a malaria risk about 4-6 times higher than other activities and about 13 times higher than staying in the villages.

Many previous studies which highlight these malaria-related social and behavioral risk factors in Thailand were to assessing the present body of knowledge to guiding principle in outlining innovative strategies to better implementation and effectiveness of the existing malaria control and in suggesting future research directions.

**Part 2**; **Biting peak and population dynamics of *Anopheles minimus* species A, from high and low agricultural insecticide area in the two villages at Kanchanaburi Province, Thailand.**

Several studies from malaria endemic areas in Thailand have investigated the population dynamics of *Anopheles minimus* s.l., especially in the north and western parts of the country. Ratanatham *et al*. (1988) studied the bionomics of *Anopheles minimus* s.l. and its role in malaria transmission in Pakchong district, Nakhon Ratchasima province. They found that mosquito densities varied from month to month, with a major peak between September and November. In February to May the density was low. Rattanarithikul *et al.* (1995) studied larval habitats of malaria vectors in north-western Thailand and reported that *Anopheles minimus* species A was found in flooded rice fields and stream margins and seemed to oviposit in the stable habitats (semi permanent). Suwonkerd *et al.* (1995) reported a bi-modal pattern of *An. minimus* female density with peaks in May-June and October-November. The seasonal patterns of adults corresponded to the larval populations in three mountainous villages in north Thailand. Overgaard *et al*. (2002) described that *An. minimus* larval density generally increased during the dry season because of the Variations in the physical and vegetation characteristics in the breeding habitat affect the density of larvae collected there. Rwegoshora *et al*. (2002) studied biting behavior and seasonal variation in the abundance of *Anopheles minimus* species A and C at Ban Phu Teuy, Kanchanaburi province. Both species had high densities in October-November and lowest in the end of the hot season, in June. Chareonviriyaphap *et a*l. (2003) reported low *Anopheles minimus* larval densities at the same location, Ban Phu Teuy, from collections in November and December. The blood feeding peaks by females occurred immediately after sunset (18.00-21.00 hrs) (Chareonviriyaphap *et al*. 2003). Attempts to study the correlation between the bionomics of *An. minimus* species A, and the use of chemical pesticides in agriculture in the same area in this research is interesting because eighty percent of the pesticides used in Thailand are for agricultural purposes. In the long term there is a high risk of mosquito insecticide resistance to develop due to the influence of these insecticides. Mulla *et al*. (1987) described that pest control operations, especially those involving the use of chemical pesticides in agricultural biomes, could have both useful for plant protection and harmful impacts on mosquito populations. Use of agrochemicals have often been blamed for increased insecticides resistance in disease vectors (Lines, 1988; Georghiou, 1990b; eg Georghiou *et al*., 1971; Chapin and Wasserstrom 1981; Brogdon *et al*., 1988). More over, use of agrochemicals in the areas could developed cross-resistance in mosquito vectors (Mulla *et al*., 1987). In Thailand, a diversification of the agricultural sector has had led to more pesticide-intensive cropping systems, such as fruit orchards (Jungbluth, 1996). Overgaard *et al*. (2003) found a decrease in anopheline density with an increase in fruit orchard area in northern part of Thailand. Furthermore, Chareonviriyaphap *et al*. (2004) described that behavioral avoidance and resistance development can occur in mosquitoes when they are exposed for along time to chemicals used in agriculture. Recent research in rural areas of Thailand found increased resistance in anopheline mosquito populations in agroecosystems with high insecticide use compared to systems with low insecticide use (Overgaard *et al*., 2005). Intensive agrochemical pest control in fruit orchards using organophosphates caused the resistance patterns in *An. maculatus s.s* (Overgaard *et al*., 2005).

**Part 3; Behavioral responses by *Anopheles minimus* species A and C to three agrochemicals.**

**Chemical pesticides** are still commonly used in Thailand for control of agricultural pests and disease vectors. Organophosphates, carbamates and synthetic pyrethroids are commonly used for agricultural purposes, whereas synthetic pyrethroids have become more popular and predominate for public health use (Chareonviriyaphap *et al,* 1999).

Malathion, cypermethrin (synthetic pyrethroids) and carbaryl are chosen as the test chemicals in this research, as they represent an the most important pesticides that used in the agricultural area in western Thailand (unpublished data).

**Malathion**

Malathion is an organophosphate (OP) insecticide that has been registered for use in the United States since 1956. It is used in agriculture, residential gardens, public recreation areas, and in public health pest control programs. When applied in accordance with the rate of application and safety precautions specified on the label, malathion can be used to kill mosquitoes without posing unreasonable risks to human health or the environment (U.S. EPA. 2002). The mosquito goes through four distinct stages during its life cycle: egg, larva, pupa, and adult. Malathion is an adulticide, used to kill adult mosquitoes. In mosquito control programs conducted by state or local authorities, it is applied by truck-mounted or aircraft-mounted sprayers. Malathion is applied as an ultra-low volume (ULV) spray. ULV sprayers dispense very fine aerosol droplets that stay aloft and kill mosquitoes on contact. ULV applications involve small quantities of pesticide active ingredient in relation to the size of the area treated. For mosquito control, malathion is applied at a maximum rate of 0.23 pounds (or about 2.5 fluid ounces) of active ingredient per acre, which minimizes exposure and risks to people and the environment (U.S. EPA. 2002).

In 1985, Kirnowardoyo S. study on the "Status of Anopheles malaria vectors in Indonesia" topic, and investigated that *An. aconitus* is susceptible to dieldrin and organophosphates i.e. malathion and fenitrotion. In 1986, Scott JG. And Georghiou GP. study on the "Malathion-specific resistance in *Anopheles stephensi* from Pakistan", and found that a strain of *Anopheles stephensi* from Pakistan (MalR) was 8.7-fold resistant to malathion and 6.7-fold cross-resistant to phenithoate. Mekuria Y. *et al*. (1994), reported that *Aedes taeniorhynchus*, *Ae. Sollicitans* and *Culex nigripalpus* were resistance to malathion (mortality: 1.0-54.4%, 72.1-81.0% and 46.2% respectively) from their topic of "Malathion resistance in mosquitoes from Chrleston and Georgetown counties of coastal South Carolina". Das NG., et al. (2000), investigated that malaria vectors in Rajmahal range, Bihar; *Anopheles maculatus, An. minimus, An. philippinensis, An. varuna and An. annularis* accounted 32.8 per cent of the total anophelines collected, were found susceptible to DDT (4 per cent) and malathion (5 per cent) in 30 min exposure. 2001, Dev *et al*., study on "An outbreak of *Plasmodium falciparum* malaria due to *Anopheles minimus* in central Assam, India", and reported that *An. minimus* was incriminated as a malaria vector during the study period. Results of susceptibility test revealed that the vector was still susceptible to both DDT and malathion at discriminating dosages. Somboon P., *et al*. (2003) study on the topic of "Insecticide susceptibility tests of *Anopheles minimus s.l., Aedes aegypti*, *Aedes albopictus,* and *Culex quinquefasciatus* in northern Thailand" and reported that the susceptibility of *Anopheles minimus* s.l., *Aedes aegypti*, *Ae. albopictus*, and *Culex quinquefasciatus* to insecticide in northern Thailand was monitored by using the WHO standard susceptibility test. The results revealed that, in general, *An. minimus* s.l. was still susceptible to DDT and permethrin, except in some areas where a slight increase in tolerance to DDT was observed. *Ae. aegypti* and *Ae. albopictus* were both highly resistant to DDT, but in some areas the former was also resistant to permethrin and deltamethrin. *Cx. quinquefasciatus* was resistant to DDT and etofenprox, with a slight increase in tolerance to permethrin, deltamethrin, malathion and fenitrothion. No resistance to lambda-cyhalothrin was detected in any of the species studied.

**Cypermethrin**

Cypermethrin is a pesticide that belong to the synthetic pyrethroids group, used for controlling various insects in agricultural.

Pyrethroids are synthetic chemical insecticides that act in a similar manner to pyrethrins, which are derived from chrysanthemum flowers. Pyrethroids are widely used for controlling various insects.

In 1990, Chadd EM. Use of an electrostatic sprayer for control of anopheline mosquitoes. The Electrodyn sprayer was compared with a compression sprayer (Hudson X-pert) for residual application of cypermethrin, a pyrethroid insecticide, to control the malaria vectors *Anopheles arabiensis* Patton and *An. funestus* Giles in experimental huts at Magugu in Tanzania. Overall mortality-rates of mosquitoes were 66% of both species in huts treated with 40 mg/m$^2$ Electrodyn, 43% *An.funestus* and 71% *An.arabien*sis due to 80 mg/m$^2$ Electrodyn formulation and 49% *An.funestus* and 64% *An.arabiensis* due to 80 mg/m$^2$ WP formulation (no significant differences). In 2001, Mostafa AA. and Allam KA. Studies on the present status of insecticides resistance on mosquitoes using the diagnostic dosages in El-Fayium Governorate, a spot area of malaria in Egypt and found that, larvae of *Culex pipiens* were resistant to temephos, fenitrothion, bromophos and fenthion and susceptible to malathion, permethrin and diazinon, while the adults were resistant to fenitrothion, permethrin and propoxur and susceptible to deltamethrin, cypermethrin and malathion.

**Carbaryl**

Carbaryl is a wide-spectrum carbamate insecticide which controls over 100 species of insects on citrus, fruit, cotton, forests, lawns, nuts, ornamentals, shade trees, and other crops, as well as on poultry, livestock and pets. It is also used as a molluscicide and an acaricide. Carbaryl works whether it is ingested into the stomach of the pest or absorbed through direct contact. The chemical name for carbaryl is 1-naphthol N-methylcarbamate (Extension Toxicology Network, 2001). Carbaryl is formulated as a solid which varies from colorless to white to gray, depending on the purity of the compound. The crystals are odorless. This chemical is stable to heat, light and acids under storage conditions. It is non-corrosive to metals, packaging materials, or application equipment. It is found in all types of formulations including baits, dusts, wettable powder, granules, oil, molassas, aqueous dispersions and suspensions (U.S. EPA, 1987). The oral LD50 of carbaryl ranges from 250 mg/kg to 850 mg/kg for rats, and from 100 mg/kg to 650 mg/kg for mice (National Library of Medicine, 1992 and U.S. EPA, 1987). The inhalation LC50 for rats is 0,005 to 0.023 mg/kg (EPA, 1987). Low doses can cause minor skin and eye irritation in rabbits, whose dermal LD50 has been measured at greater than 2,000 mg/kg (National Library of Medicine, 1992). Technical carbaryl has little potential for skin or eye irritation (Extension Toxicology Network, 2001).

1975, Ariaratnam V. and Georghiou GP., reported about carbamate resistance in *Anopheles albimanus.* Penetration and metabolism of carbaryl in propoxur-selected larvae. Chandre, F *et al*. (1997) descript the distribution of organophosphate and carbamate resistance in *Culex pipiens quinquefasciatus* from West Africa.

In 2001, Mostafa AA. and Allam KA. studies on the present status of insecticides resistance on mosquitoes using the diagnostic dosages in El-Fayium Governorate, a spot area of malaria in Egypt., and found that adult of *C. pipiens* were resistant to fenitrothion permethrin and propoxur (from carbamate group) but susceptible to deltamethrin, cypermethrin and malathion.

**Part 4; Behavioral responses by *Anopheles minimus* species A and species C to DDT and pyrethroids.**

One of the reliable methods that used in vector borne disease control is chemical measure. To believe that insecticides like DDT or synthetic pyrethroids are needed for the control of insect-borne human disease in Thailand as several evidences reported by Roberts et al. (1994), Chareonviriyaphap *et al*. (1997), Kongmee *et al*. (2004), Grieco *et al*. (1999).

**DDT**

DDT (Dichloro-Diphenyl-Trichloroethan) is the most well known organic pesticide and is sometimes referred to as dicophane or chlorophenothane. DDT was developed as the first modern insecticides early in World War II (htt://en.wikipedia.org/wiki/DDT).

DDT was first synthesized in 1874 by Othmar Ziedler, but its insecticidal properties were not discovered until 1939 by Paul Hermann Müller. It was initially used as the most potential compound to combat malaria, typhus and the other insect-borne human disease among both military and civilian populations. DDT was available for agricultural and commercial usages in the States in the late 1940s (U.S.EPA, 2002).

In 1943, DDT was used as both larvicide and adulticide to control anopheline mosquitoes, vector of malaria (Gahan *et al*, 1945). Years later, the use of DDT was spread to several malaria endemic countries including countries in Southeast Asia.

Thailand accepted the World Health Organization (WHO) plan for malaria eradication in 1950 by using DDT as an intradomicillary (Prasittisuk, 1985). Smyth and Roys (1955) reported that DDT had a specific effect on chemoreceptors and Soliman and Cutkomp (1963) found that it had effect on sensory hairs, perhaps causing irritability. However, Carson (1962) published the book “Silent Spring”, which reported that DDT caused cancer and harmed bird reproduction by thinning egg shells (Carson, 1962), which eventually led to the insecticide being banned for agricultural use in the USA, and was one of the signature events in the birth of the environmental movement (htt://en.wikipedia.org/wiki/DDT). In 1970s, many countries stopped the use of DDT for agriculture as it was believed to have a negative environmental impact (htt://en.wikipedia.org/wiki/DDT). Consequently, DDT was banned by administrator of the Environmental Protection Agency (U.S.EPA, 2002).

Although banned, DDT had been reported as the chemical of choice in malaria control worldwide (Brown, 1976). Several published reports claimed the potential function of DDT in disease control (Roberts *et al*., 2000; Bangs, 2000). Such reports presented the wonderful action of DDT in repelling mosquitoes and later referred to as excito-repellency (Kennedy, 1946; Muirhead-Thomson, 1960; Roberts, 1993). Roberts *et al*. (2000) recently examined DDT use in malaria control and provided compelling evidence to show that the combined effect of repellency and irritancy exerted the dominant action on mosquitoes in reducing human-vector contact inside sprayed house.

**Synthetic pyrethroids**

Synthetic pyrethroids are the current insecticides of choice for malaria control in Thailand. Pyrethroids have known great promise for pest control due to their low mammalian toxicity and remarkable potency at low level that quickly immobilizes, kills and repels insects (Prasittisuk, 1994 and Chareonviriyaphap *et a.l*., 1997).
In 1995, WHO described pyrethroids and accepted it as a terrific compound in vector control worldwide. Patipong (2000) reported that pyrethroids gained general acceptance for use in impregnating bednets and for indoor residual spraying, including deltamethrin, permethrin, and lambdacyhalothrin.

**Deltamethrin**

Deltamethrin is odorless synthetic pyrethroids. In 1990, Haug and Hoffman found that deltamethrin is a synthetic insecticide based structurally on pyrethrins, which rapidly paralyze the insect nervous system giving a quick knockdown effect (EXTOXNET, 1995). Deltamethrin has been used worldwide, ranging from agricultural uses to home pest control. Trade names for products containing deltametrin include Butolin, Butoss, Cislin, Crackdown, Cresus, Decis, Decis-Prime, K-Oytek (EXTOXNET, 1995). Deltamethrin was used in the US in environmental health market (Thomson, 1992). It was being sold in many countries for agricultural, public health and livestock application. Jana-Kara *et al*., (1995) reported that deltamethrin impregnated bed nets were used in the control of *Anopheles minimus*, in Assam, India. Their works showed the nets provided a high degree of personal protection against all the local species of human biting mosquitoes. Mittal *et al*., (2002) found that the susceptibility of *Anopheles culicifacies* decreased after years of deltamethrin use. In the same year, Chareonviriyaphap *et al*., (2002) studied on the susceptibilities of *Anopheles minimus*, the malaria vector in Thailand to deltamethrin and found that there was approximately a 22-fold increase in LD50 and a 27-fold increase in LD90 when the F10 generation was compared to the parent colony (F1).

Several studies on the behavioral responses of deltamethrin to malaria vectors (Chareonviriyaphap *et al*. 1997; 2004).

**Lambda-cyhalothrin**

Lambda-cyhalothrin is a synthetic pyrethroid discovered by ICI in the early 1980s and developed as an insecticide for agricultural and public health applications (Orica 1998). It is a broad spectrum insecticide effective at low rates of application against major insect pests in wide range of crops. The product also has the ability to prevent a build up of mite populations and acts both by direct contact with insects and after ingestion. The trade names of Lambda-cyhalothrin are Karate 50EC and Karate ULV (Orica, 1998)

In 1996 Nylon nets impregnated with lambdacyhalothrin provided 100% mortality of female mosquitoes that landed on treated fabrics were recorded (Ansari and Razdan, 2000). Sampath *et al*., (1998) described the implementation and acceptability of the trial for evaluated of lambdacyhalothrin impregnated bed nets in a malaria endemic area of India. Kamolratanakul *et al*., (2001) described the cost-effectiveness and sustainability of lambda-cyhalothrin treated mosquito net.

In Thailand, Sungvornyothin *et al*., (2001) studied the effects of behavioral avoidance of *An. minimus* (Diptera: culicidae) to lambda-cyhalothrin and showed a good responses of female mosquitoes to this compound. However, this compound was found to produce an allergenic affect to human host during the period of field trial. As a consequence, lamda cyhalothrin remains uncertain for the IRS/bed net in the country (Chareonviriyaphap pers. com).

# MATERIAL AND METHODS

**Part 1; Risk factors and base line malaria knowledge from different pesticide Land-use systems in malaria endemic area at Kanchanaburi Province,Thailand.**

**Farming systems survey (August – September, 2003),** the selected districts were Sai Yok and Thong Pha Phum district;

**Sai Yok District** was selected 2 places as follow;

1. Bong Ti Noy village, Wang Krajae sub district, Sai Yok district (N 14° 19', E 98° 59')
2. Pu Tuey village, Sai Yok district (N 14° 20’, E 98° 59’)

**Thong Pha Phum district** was selected 4 places as follow**;**

1. Mae Num Noy, only in the part of Rubber forest village, Huay Ka Yeng sub district (N 14° 35', E 98° 36')
2. Huy Bak Kok village, Huay Ka Yeng sub district (N 14° 40', E 98° 31')
3. U-long village, Ta Ka Nun sub district (N 14° 48', E 98° 40')
4. Thung Nang Khruan village, Cha Lae sub district (N 14° 53', E 98°46')

**Map of Thailand**

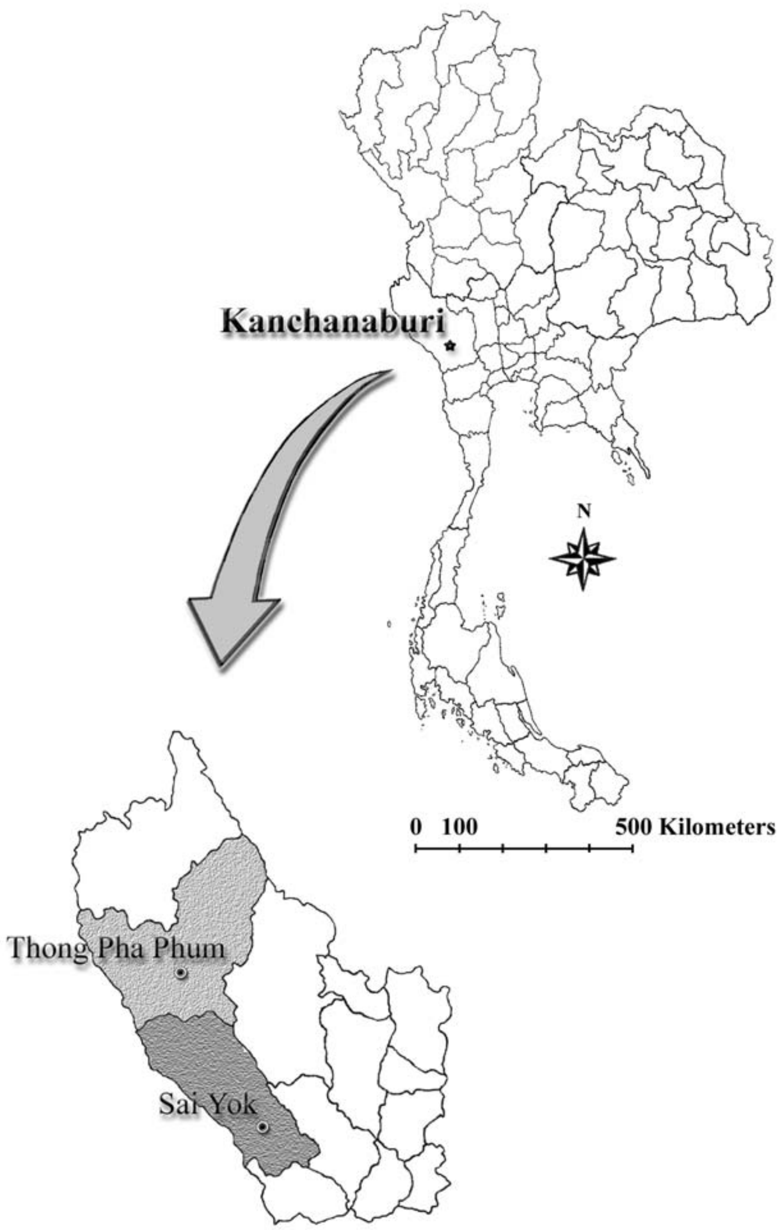



Figure1: Map showing the location for adult and larval of *Anopheles minimus* species complex collection.

## Field Survey


Bong Ti Noy

Mae Num Noy


Huy Bak Kok


Thung Nang Khruan


U-long


Pu Toey

Figure 2: Farmer system survey to six villages in Thong Pha Phum and SaiYok District, Kanchanaburi Province, Thailand.

**Interviews by questionnaire 1&2**

The six villages in Kanchanaburi province were selected, based on information on malaria prevalence, presence of anopheline larvae, land use and agricultural plant protection practices. Once villages were selected a probability sampling design was used to select households. During August – September, 2003, approximately 30% of the households in each village were selected by random sampling, visited, and the head of that household interviewed. The random selection was based on a list of village households from national census data or from data/maps from the Ministry of Public Health's Vector-Borne Disease Units. If household heads were not present, another respondent from that household was interviewed by using questionnaire 1.

**Questionnaire 1** (Q1) (see appendix).

This questionnaire provided information on three steps

Step1. General information;

This section contained questions on age, sex, occupation, ethnic group, number of adults and children in the household, time of residence in village, perceptions of the three largest problems and the three most important diseases in village, and patterns of travel outside village.

Step 2. Malaria knowledge;

This section contained questions on knowledge on malaria symptoms, how and in what season malaria is transmitted, mosquito biting times, mosquito larval sites, personal experience of malaria, mosquito preventive measures, and personal assessment of mosquito presence in houses after periods of agricultural insecticide applications.

Step 3. Malaria risk factors.

This section covered occupational and domestic risk factors. Questions were asked on how often and in what season they sleep in a field hut or in the forest; measures of mosquito protection; distances from their house to water, forest, agricultural field, and fruit orchards; house construction; window screening; bed nets; and types of animals kept near houses.

**Questionnaire 2** (Q2) (see appendix).

This questionnaire provided information on specific information on agricultural pesticide use, the 20 most important farmers in the village used questioned using Questionnaire 2 (Q2). The ‘most important farmers’ mean those farmers who had the largest area of perennial (e.g. fruits) and annual crops (e.g. rice, maize, etc.). At least 10 farmers were selected from this group. Q2 also provided information on insecticide use, concentration, amount, application date, how often in the past applied, etc.

**The statistic analysis**

The results from a six section undertaken simultaneously, containing information on farmer’s pesticide use, will be reported elsewhere. Data from questionnaires were entered, manipulated, and analyzed using SPSS software.

**Field Observation and Interviewing with Questionnaires**


Figure 3: Contacted to local peoples by interviewing with questionnaire 1 and questionnaire 2.

**Part 2**; **Biting peak and population dynamics of *Anopheles minimus* species A, from high and low agricultural insecticide area in the two villages at Kanchanaburi Province, Thailand.**

**Study Area**

The study sites are located in an endemic malarious area with both *Plasmodium falciparum* and *Plasmodium vivax* infections occurring (A1 area; see appendix). The principal malaria vectors in this region are *Anopheles minimus* A and species of the *Anopheles dirus* complex. The study sites were selected on the basis of agrochemical use. A questionnaire (Q2, see Part 1) was used to collect information on farmers' pesticide use. Based on the results obtained from this questionnaire the following villages were selected for further insect collections:

**Low pesticide village**

The village selected as having low pesticide agricultural use was Ban Mae Num Noy village (MNN), located in Huay Ka Yeng sub-district, Thong Pha Phum district, Kanchanaburi province, near the western border of Thailand. The study site is situated at latitude 14° 35' and longitude 98° 36', approximately 100 km from the High pesticide village. The village is surrounded by rubber plantations and hills. There are two large clean pools in the village formed by spring water damming up near the village. There is a small permanent stream nearby (Figure 4**).** The two selected locations where anopheline larvae were collected in this site were:

1. Pool (HP)

This is a pool which is located beside a road in the village. The central parts of the pool were absent of emergent aquatic vegetation. The depth of the pool was in general more than 1.0 m. Almost all of the pool area was covered with green algae and floating weeds in the sun-exposed sites. Grasses were growing around the pool and the vegetation sometimes entered the water close to the edge. A shallow waterway connected this pool with the second habitat (Figure 5).

2. Stream (ST)

This is a small stream running along a citrus garden in the village. The stream was narrow (usually less than 0.5m wide) and 0.1-0.2 m or less in depth. The stream connected to the end of HP pool and water flowed slowly in the dry season. Grasses and some emergent vegetation were growing along the margins of the stream. The water velocity was between 1.5-3.0 m/minute (Figure 6).


Figure 4: Map of Mae Num Noy village (MNN) (only part of Rubber forest village) showing 3 stations for adult collection and 10 points on 2 breeding sites for larvae collection.


Figure 5: Right picture is a pool **(HP)** which is located beside a road in the village. Left picture is a shallow waterway connected this pool with the ST habitat.


Figure 6: A small stream **(ST)** running along a garden in the village.

**High pesticide village**

The village selected as having high pesticide agricultural use was Bong Ti Noy village (BTN), located in Wang Krajae sub-district, Sai Yok district, Kanchanaburi province, near the western border of Thailand. The study site is situated at latitude 14$^{o}$ 19' and longitude 98$^{o}$ 59'. The village is located near the forest fringe, surrounded by hills, and with the presence of clean water bodies. There is one large stream running through the area and many small temporary streams are present that are water-filled only during the rainy season (Figure 7) The two selected locations where anopheline larvae were collected in this site were:

1. Big stream (Bst)

This stream is a large perennial stream which runs along the village. The larval collection site was a 20 m long stretch close to the village temple. The width of the stream varied between 5-10 m across and the depth was 0.2-0.5 m in the dry season. In the rainy season the width was more than 10 m and the depth more than 3.0 m. Emergent vegetation were growing near the edge with some green algae along sun-exposed areas. Grasses often grew along the margin of the stream. Stream water velocity was between 1.5-30.0 m/minute (Figure 8).

2. Small stream (Sst)

This stream is a relatively small perennial stream running along a road. The width varied between 0.3-1.0 m across and 0.05-0.5 m in depth. There was some emergent vegetation and grasses growing near the edge. The water volume and velocity was quite low in the dry season during the dry season (Figure 9).

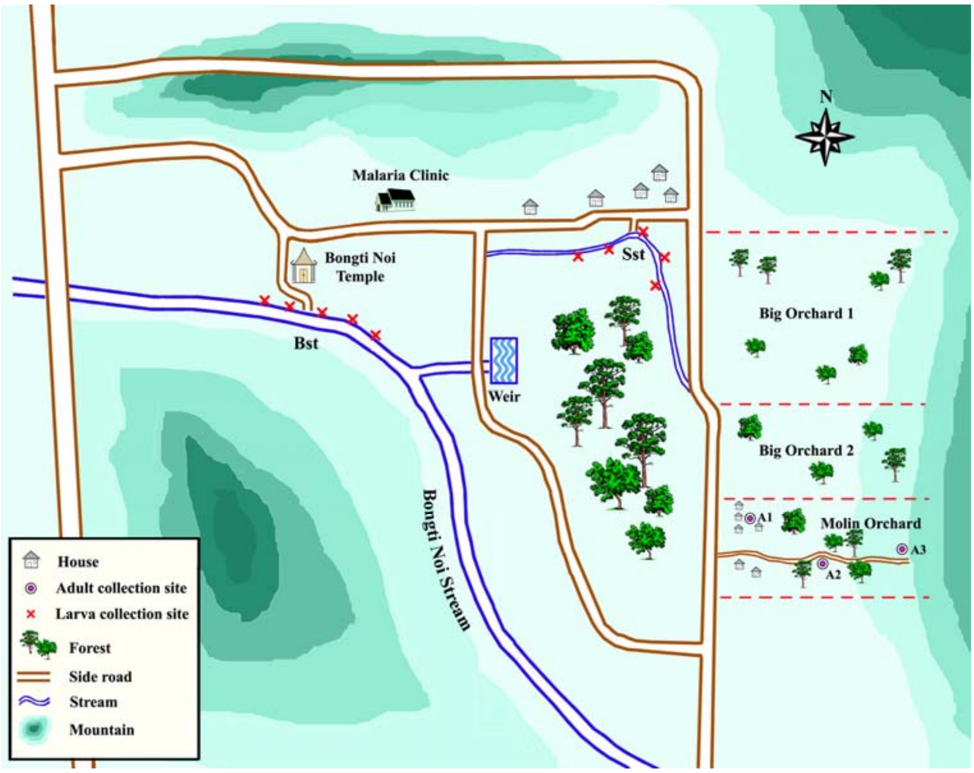



Figure 7: Map of Bong Ti Noy village (BTN) showing three stations for adult collection and 10 points on two breeding sites for larvae collection


Figure 8: A large perennial stream **(Bst)** which runs along the BTN village.

Figure 9: A small perennial stream **(Sst)** running along a road in BTN village.

## 2. Mosquito collections

### Adult sampling

Wild adult females of *An. minimus* A were collected monthly between October 2003 and September 2004 during three seasons by outdoor human bait catches (Figure 10) in each village. Adult collections were undertaken at three sites, approximately 200 m apart from each other, in each village. The three sites were a) inside village, b) in an orchard (for BTN) or in a rubber plantation (for MNN), and c) in the forest (Figure 11). Mosquito collections were undertaken during the night by two teams of two persons each at each site. The first team collected mosquitoes from 18.00 to 24.00 hours and the second team collected from 00.00-06.00 hours. Torches were used to observe mosquitoes and aspirators for collecting them. Collections were made two consecutive nights each month at the same sites. Collected mosquitoes were placed in separate marked plastic cups, one for each hour, and covered with netting material. All live mosquito specimens were provided with 10% sugar solution and transported to the field laboratory for morphological identification the following morning (Figure 12). During transport and storage mosquitoes were kept in larger containers covered with damp cotton towels to avoid desiccation. The humidity and temperature were recorded each hour.


Figure 10: Mosquitoes collecting by outdoor human bait catches

In the village site


In the rubber forest site (MNN) In the orchard site (BTN)


In the forest site


Figure 11: The three sites were undertaken during the night by two teams of two persons each at each site.


Figure 12:  Collected mosquitoes were placed in separate marked plastic cups, all alive mosquito specimens were provided with 10% sugar solution and transported to the field laboratory for morphological identification


Figure 13:  Species identification was carried out by stereo-microscope.

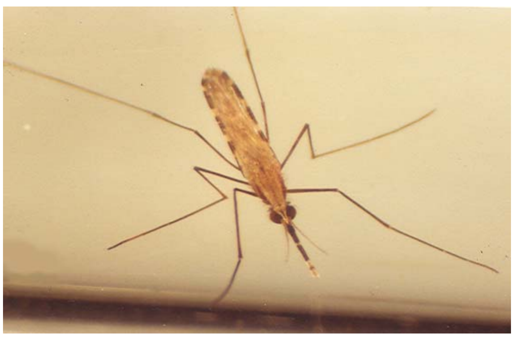


Figure 14:   *Anopheles minimus* species A

**Larval sampling**

At each breeding site, stream or pool, a section of about 20 m long and 0.05-0.20 m wide from the edge was selected and visited monthly (October 2003-September 2004). All breeding sites were closer than 3 kilometers away from the each village. Larval and pupal stages of anopheline mosquitoes were collected using the dipping method. Larvae were collected at four points at each selected habitat. The distance between each point was 5 m. Larval sampling was undertaken during daytime between 10.00-13.00 hrs with 30 dips per point (approximatly 120 dips per habitat) (Figure 15). Larvae were kept in a plastic bag half-filled with water from the respective habitat. The frequency of larval instars and pupae were recorded for one year.

**3. Identification of adult and larval mosquitoes**

Adults and larvae were brought to the field laboratory and the laboratory at the Department of Entomology, Kasetsart University for identification. Species identification was carried out by stereo-microscope, using morphological characters and keys by Peyton and Scanlon (1966), Rattanarithikul and Panthusiri (1994) and Harrison (1980). Larvae were identified alive and preserved (if dead) in the laboratory by the method described by Rattanarithikul and Panthusiri (1994).

**4. Data analysis**

Differences in mean numbers of *An. minimus* A mosquitoes between two villages, three sites, and three season were compared using analysis of variance (ANOVA) (SPSS Base 11, 2001, SPSS Inc.)

Larva sampling at MNN village

HP site

ST site

Larva sampling at BTN village


Bst site

Sst site

Figure 15: Larval and pupal stages of anopheline mosquitoes were collected using the dipping method

**Part 3; Behavioral responses by *Anopheles minimus* species A and C to three agrochemicals.**

**Test population**

*Anopheles minimus* species A was collected by human bait in Mae Nam Noi Village, Thong Pha-Phoom District, Kanchanaburi province Province (N 14° 35', E 98° 36') and *An. minimus* species C was collected by cow bait in Pu Teuy Village, Sai Yok District, Kanchanaburi (N 14$^{o}$ 20', E 98$^{o}$ 59')The province is located in western Thailand and borders Myanmar. The collected mosquitoes were kept in mosquito plastic cups, provided with 10% sugar solution and transported to the field laboratory for morphological identification the following morning. During transport and storage mosquitoes were kept in larger containers covered with damp cotton towels to avoid desiccation.

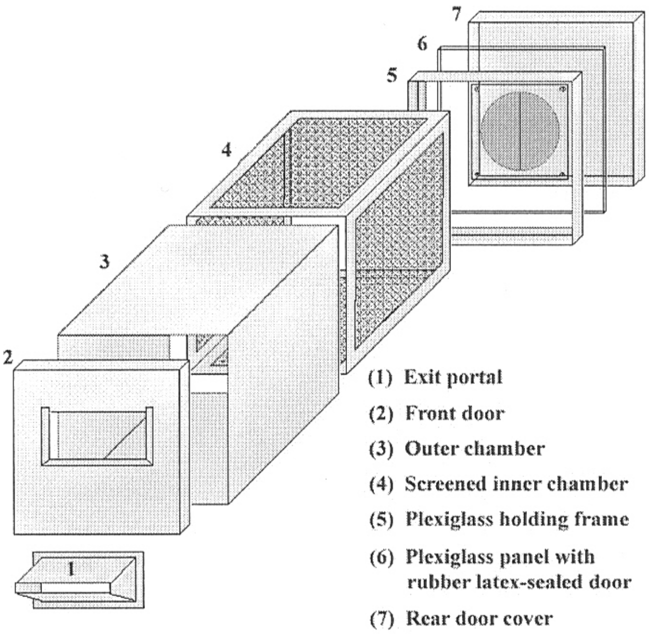
**Insecticide-treated papers**

Papers were impregnated using formulation grade insecticides at the operational field concentrations as recommended on the label. The concentrations used were 0.40 g/m$^2$ of carbaryl, 0.19 g/m$^2$ of malathion, and 0.04 g/m$^2$ of cypermethrin. All papers were treated at the rate of 12.5 ml of the insecticide solution per 0.0928 m$^2$ (26.5 x 35 cm).

**Excito-Repellency tests**

In this study, we used the improved test chamber for all tests as described in a recent publication (Chareonviriyaphap and Aum-Aong, 2000). Figure shows the stainless steel, collapsible excito-repellency escape chamber (34 X 32 X 32 cm), facing the front panel and escape runnel. The box comprises 4 side walls, a rear Plexiglas inner door, a rear outer door cover and a front door, and a removable exit portal (an escape funnel). Each wall is constructed of stainless steel sheet (0.7-mm thickness), which has an aluminum sliding rib on each end and a socket, providing a surface for the test paper holder in the middle. The test paper holder has 2 sides; a sheet of fine mesh iron screen net is permanently attached on side, and a panel to hold test papers to secure the panel on top is on the opposite side. A 0.8-cm gap between the test papers and screen prevents mosquitoes from making physical contact with the surface of test paper in the exposure windows during the non contact repellency trials. The test paper holder is convenient and functions similarly under contact and non contact conditions, depending on the purpose of the test. The holder simply has to be inverted to provide the proper conditions. A spring mechanism on side of the test paper holder secures it tightly when putting the holder into the socket. The front door is constructed of a stainless steel frame with stainless steel sheet affixed on the front side. The steel sheet has a trough for sliding the exit funnel into place. Two screws at end secure the funnel to the front panel. The inner rear door is constructed of a stainless steel frame and a transparent Plexiglas door that is attached to the frame. The Plexiglas door serves to seal the chamber and at the same time allow the investigator to look inside the exposure chamber before and after a test is conducted. A self-sealing 6-in. (15.5 cm)-diameter portal made of dental dam is used for placing test specimens inside the chamber and for removing the specimens from the chamber after each test. The outer rear door is constructed of stainless steel and is used to shut off all light inside the chamber when the test is being conducted. The last part is a removable exit runnel attached to the outside of the chamber. The escape runnel gap is a 20.5-cm-long and 1.5-cm-wide opening (Chareonviriyaphap *et al*., 2001) (Figure 16).

Tests were designed to compare two field populations of the two collected species in contact and non-contact exposures using insecticide treated papers and excito-repellency test chambers as described above. The tests were undertaken within 48 hours of capture of mosquitoes. Only female specimens were used in the tests. Mosquitoes were deprived of all nutrition supplies, except water for a minimum of 12 hours before exposure. All tests were performed in the field laboratory during daylight hours and each test was replicated three times. Temperatures and relative humidity were recorded during tests. Observations of escaping mosquitoes were made at 1 min intervals for 30 min. The number of dead or knockdown specimens was recorded separately for each exposure chamber, external holding cage, and control chambers (without insecticide). Escaped specimens and those remaining inside chambers, for both treatments and controls, were held separately in small holding containers, provided with 10% sugar solution, and mortalities were recorded after 24 hours.




Chareonviriyaphap, *et al*., 2001

Figure 16: Exito-repellency test chamber model.

Figure 17: Exito-repellency test chamber were designed to compare two field populations of the wild caught species in contact and non-contact exposures using insecticide treated papers**.**

**Data analysis**

A Kaplan-Meier survival analysis method was used to analyze and interpret the behavioral response data (Roberts *et al.*, 1997). Survival analysis was used to estimate the probability of escape time (ET) and compare differences in mosquito response between two field populations and two insecticides (Kleinbaum, 1995). In the analysis, mosquitoes that escaped were treated as “deaths” and those remaining in the test chamber were considered as “survivals” as previously described (Roberts *et al.*, 1997). The escape times (ET) for 30, 50, and 70 percent ($ET_{30}$, $ET_{50}$, and $ET_{70}$) of the test populations to escape were estimated from data collected at 1-min intervals. Patterns of escape response between treatment groups were determined using the log-rank method (Mantel and Haenzel, 1959). Statistical software (STATA®, city, state) was used in the analysis (Roberts *et al.*, 1997).

**Part 4; Behavioral responses by *Anopheles minimus* species A and species C to DDT and pyrethroids.**

**Mosquito collection**.

*Anopheles minimus* complex mosquitoes were identified based on morphologic keys (Harison,1980 and Peyton and Scanlon, 1966). Species were differentiated by the presence or absence of the humeral pale spot on the costal wing vein. *Anopheles minimus* A has a wing costa without the humeral pale spot whereas An. minirnus C has the humeral pale spot. A diagnostic enzyme, octanal dehydrogenase, indicated 95% concurrence with species A, which does not have the humeral pale spot. This spot is lacking in 73% of species C (Green *et al*., 1990). *Anopheles minimus* A and C adult females were collected off human volunteer baits during the evening hours (6:00 PM to 6:00 AM). These volunteers (collectors) worked for the Ministry of Public Health. Behavioral tests were performed within 24 hours of capture. All mosquitoes were starved of blood and sugar 24 hours before the tests (Sungvornyothin *et al*., 2001). Temperatures and relative humidity were recorded during the tests. Both populations were physiologically susceptible to DDT, deltamethrin, and lambdacyhalothrin (Chareonviriyaphap, T and others, unpublished data).

**Insecticide-treated papers**.

Analytical grade insecticide was impregnated on papers at operational field concentrations of 2 $g/m^2$ of DDT, 0.02 $g/m^2$ of deltamethrin, and 0.03 $g/m^2$ of lambda-cyhalothrin and prepared using diluent according to World Health Organization protocol (Busvine, 1958).

**Behavioral tests**

Tests were designed to compare two wild caught populations in contact versus non-contact exposures using three different insecticides. Identical, specially designed test chambers (four per test trial) were used for all bioassays as previously described (Chareonviriyaphap *et al*., 2001). The stainless steel outer chamber of excito-repeIlency testing device measures 34 cm 32 cm 32 cm (Figure 18), and faces the front panel with the single escape portal. The box is composed of a rear door cover, an inner Plexiglas glass panel with a rubber latex-sealed door, a Plexiglas holding frame, a screened inner chamber, an outer chamber, a front door, and an exit portal slot. Only female *An. minimus* specimens were used in excito-repellency tests. Mosquitoes were deprived of all nutrition and water for a minimum of 24 hours before exposure. Laboratory tests were performed during daylight hours only and each test was replicated four times. Observations were taken at one-minute intervals for 30 minutes. After each test was completed, the number of dead or knockdown specimens was recorded separately for each exposure chamber, external holding cage, and paired control chamber (without insecticide). Escaped specimens and those remaining inside the chamber, for both controls and treatments, were held separately in small holding containers with food and water and 24-hour mortalities were recorded.

**Data analysis**

A Kaplan-Meier survival analysis method was used to analyze and interpret the behavioral response data (Robert *et al*., 1997; Chareonviriyaphap *et al*., 1997; Sungvornyothin *et al*., 2001 and Chareonviriyaphap *et al*., 2002). Survival analysis was used to estimate the probability of escape time (ET) and compare differences in mosquito response among the two populations and three insecticides. Mosquitoes that escape were treated as deaths and those remaining in the test chamber were considered survivals (Chareonviriyaphap *et al*., 1997). The $ET_{50}$, $ET_{75}$ and $ET_{90}$ time in minutes for 50%, 75% and 90% of the test population to escape, respectidy, were estimated from data collected at one-minute intervals. Patterns of escape response were determined using the log – rank Method (Mantel and Haenzel, 1959). Stata statistical software (Stata Corp., College Station, TX) was used in the analysis (Robert *et al*., 1997).

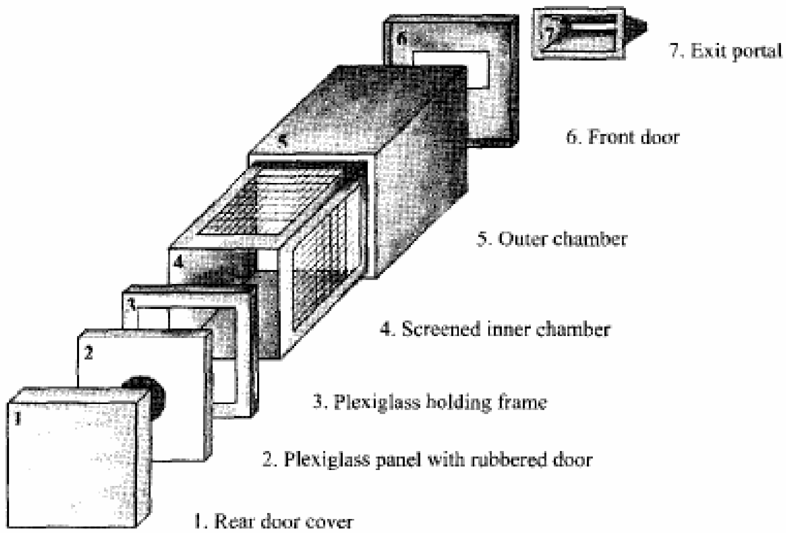



Figure 18: Excito-repellency test chamber used to study insecticide behavioral responses.

# RESULTS

**Part 1; Risk factors and base line malaria knowledge from different pesticide Land-use systems in malaria endemic area at Kanchanaburi Province,Thailand.**

**Questionnaire 1**

Table 1 show names of villages, sub-districts, districts, and provinces where the survey was undertaken. Table 2 shows number of inhabitants, huts, houses (households), and questionnaire respondents in each village. Table 3 shows demographic characteristics of interviewed household heads.

A total of 232 responded to the questionnaires in Kanchanaburi Provinces.

Sixty-five percent, 155 of all respondents, were household heads and 35% were closely related to the household head, most of them being either the wife or child of the household head.

About 77% and 23% of all respondents were male and female, respectively. The average age of household heads were 47 years, ranging from 20 to 83 years.

Approximately 70% of both respondents and household heads were born in Thailand, 22% were born in Myanmar.

## GENERAL INFORMATION

Table 1: Names and locations of surveyed villages.

| Village | Sub-district | District | Province |
|---|---|---|---|
| Mae Nam Noi (RFV)* | Huay Kayeng | Thong Pha Phoom | Kanchanaburi |
| Huay Bak Kok (07) | Huay Kayeng | Thong Pha Phoom | Kanchanaburi |
| U-Long (04) | Ta Kanun | Thong Pha Phoom | Kanchanaburi |
| Thung Nang Khruan (06) | Cha Lae | Thong Pha Phoom | Kanchanaburi |
| Bong Ti Noi (08) | Wang Krajae | Sai Yok | Kanchanaburi |

* Count only in the part of Rubber Forest Village (RFV)

Table 2: Village names, population, number of houses and huts, and total number of respondents.

| Village | Population[1] | No. of huts[1] | No. of houses | Respondents | % |
|---|---|---|---|---|---|
| Mae Nam Noi (RFV)* | 65 | 5 | 11 | 14 | 87.5% |
| Huay Bak Kok (07) | 1,397 | 31 | 338 | 52 | 14.09% |
| U-Long (04) | 2,797 | 92 | 685 | 77 | 9.9% |
| Thung Nang Kruan (06) | 948 | 9 | 235 | 57 | 23.36% |
| Bong Ti Noi (08) | 487 | 49 | 104 | 32 | 20.92% |

[1] According to NKM, Ministry of Public Health in 2546-2003

(RFV)* = Count only in the part of Rubber forest village

Table 3: Demographic and general characteristics of interviewed household heads in Kanchanaburi.

| Population characteristics | | % of respondents |
|---|---|---|
| Age groups (years) | | |
| | 20-29 | 8.3 |
| | 30-39 | 21.8 |
| | 40-49 | 27.4 |
| | 50-59 | 20.0 |
| | 60-69 | 16.0 |
| | 70+ | 6.5 |
| Gender | | |
| | Male | 76.8 |
| | Female | 23.2 |
| Country of birth | | |
| | Thailand | 70.4 |
| | Myanmar | 21.7 |
| No. of adults per household | | |
| | 1 | 3.8 |
| | 2 | 42.5 |
| | 3 | 24.1 |
| | 4 | 14.8 |
| | 5+ | 15.0 |
| No. of children per household | | |
| | 1 | 41.6 |
| | 2 | 34.8 |
| | 3 | 15.6 |
| | 4 | 6.5 |
| | 5+ | 1.6 |

Table 4: Household head ethnicity

| Ethnicity | Respondents | % of respondents |
|---|---|---|
| Thai | 128 | 55.17 |
| Karen | 19 | 8.20 |
| Mong | 4 | 1.72 |
| China | 3 | 1.29 |
| Burmese | 38 | 16.38 |
| Mon | 24 | 10.34 |
| Laos | 8 | 3.45 |
| Indian | 1 | 0.43 |
| No | 7 | 3.02 |
| Total | 232 | 100 |

Table 5: Year living in village

| Live in village | Respondents | % of respondents |
|---|---|---|
| Less than 1 year | 5 | 2.2 |
| 1-5 years | 32 | 13.8 |
| 5-10 years | 48 | 20.68 |
| More than 10 years | 147 | 63.36 |
| Total | 232 | 100 |

Table 6: Number of adult and children in the village

| | | Respondents | % of respondents |
|---|---|---|---|
| No. adult | 1.00 | 7 | 3.04 |
| | 2.00 | 106 | 46.09 |
| | 3.00 | 48 | 20.87 |
| | 4.00 | 37 | 16.09 |
| | 5.00 | 14 | 6.09 |
| | 6.00 | 14 | 6.09 |
| | 7.00 | 4 | 1.74 |
| | 8.00 | 0 | 0 |
| | 10.00 | 0 | 0 |
| | average | 32.86 | |
| | Total | 230 | 100 |
| No. children | 1.00 | 59 | 0.33 |
| | 2.00 | 66 | 37.29 |
| | 3.00 | 35 | 19.77 |
| | 4.00 | 15 | 8.47 |
| | 5.00 | 2 | 1.13 |
| | 12.00 | 0 | 0 |
| | average | 35.4 | |
| | Total | 177 | 100 |

Table 7: Respondents occupation

| | Respondents | % of respondents |
|---|---|---|
| Carpenter | 2 | 0.86 |
| Farmer | 131 | 56.47 |
| Trader | 5 | 2.16 |
| Employed | 49 | 21.12 |
| Government officer | 3 | 1.29 |
| Orchard | 25 | 10.78 |
| Repairman | 1 | 0.43 |
| Rubber plantation worker | 14 | 6.03 |
| Teacher/ Volunteer teacher | 2 | 0.86 |
| Total | 232 | 100.0 |

Table 8: Problems and diseases

| PROBLEM | Respondents | Disease | Respondents |
|---|---|---|---|
| No answer | 159 | No answer | 27 |
| Agricultural problems[1] | 25 | Malaria | 129 (55.6%) |
| Economical problems[2] | 7 | Fever | 42 |
| Bad communications[3] | 31 | HIV | 0 |
| No health center, no doctor | 3 | Influenza | 5 |
| Border problems[4] | 3 | Pink eye | 1 |
| ID Card/ Check card | 1 | Bone | 0 |
| Health/Sickness | 0 | Dengue | 15 |
| Narcotics | 0 | Diabetes | 0 |
| Education/Study | 1 | Diarrhea | 7 |
| Repellents/no net | 1 | Sickness | 1 |
| Destroy natural resources | 0 | TB | 0 |
| Difficult | 0 | Stomach ache | 2 |
| Electricity | 1 | Elephantiasis | 3 |
| Total | 232 | Total | 232 |

[1] Low price for my selling products, water shortage, destroyed products.
[2] Debt, economy, no salary, no work, poor.
[3] Bad road, no transport, no bus.
[4] Minorities, war, foreigners, border areas, migration of poor people.

Table 9: Travel out of the villages

| | | Respondents | % of respondents |
|---|---|---|---|
| Travel frequency | Less than 1 week | 121 | 77.42 |
| | 1-2 weeks /yr | 8 | 5.16 |
| | 1-4 weeks /yr | 16 | 10.32 |
| | 3-6 weeks /yr | 9 | 5.81 |
| | more than 1 month /yr | 3 | 1.94 |
| | Total | 155 | 100 |
| Traveled | within sub district | 21 | 13.54 |
| | within district | 54 | 34.84 |
| | within province | 36 | 23.23 |
| | other province | 43 | 27.74 |
| | outside country (to India) | 1 | 0.65 |
| | Total | 155 | 100 |

**MALARIA KNOWLEDGE**

Table 10: Malaria knowledge of the household heads

| | | **Respondents** | **% of respondents** |
|---|---|---|---|
| Know malaria | yes | 206 | 88.8 |
| | no | 26 | 11.2 |
| | Total | 232 | 100 |
| Malaria frequency | No answer | 3 | 1.29 |
| | once | 33 | 29.2 |
| | 2-3 times | 52 | 46.0 |
| | more than 3 times | 113 | 22.1 |
| | Never | 31 | 13.36 |
| | Total | 232 | 100 |
| Malaria symptoms | No answer | 4 | 1.72 |
| | Fever<br>Headaches<br>Shivering<br>Muscle pain<br>Nausea/vomiting | 220 | 94.83 |
| Other symptom name | Can not eat... | 1 | 0.43 |
| | Cough, yellow body | 1 | 0.43 |
| | Diarrhea | 3 | 1.29 |
| | Dizzy | 1 | 0.43 |
| | No feeling | 0 | 0 |
| | Stink | 1 | 0.43 |
| | Tired | 0 | 0 |
| | Yellow body | 1 | 0.43 |
| | Total | 232 | 100 |
| How malaria transmitted | No answer | 29 | 12.5 |
| | Mosquitoes bite | 175 | 75.43 |
| | Don't know | 25 | 10.78 |
| | other | 3 | 1.29 |
| | Total | 232 | 100 |
| Transmitted season | rainy season | 115 | 49.6 |
| | Hot season | 54 | 23.28 |
| | Cool season | 42 | 18.10 |
| | Don't know | 21 | 9.05 |
| | Total | 232 | 100 |
| Mosquito after insecticides | More | 25 | 10.78 |
| | Less | 86 | 37.07 |
| | No difference | 76 | 32.75 |
| | Don't know | 45 | 19.40 |
| | Total | 232 | 100 |

Table 11: Treatment after got malaria of the household heads

| | | Respondents | % of respondents |
|---|---|---|---|
| Treatment place | Malaria clinic | 154 | 66.38 |
| | Hospital | 67 | 28.88 |
| | Health clinic | 5 | 2.16 |
| | Health center | 2 | 0.86 |
| | Traditional practitioner | 3 | 1.29 |
| | Self treatment | 1 | 0.43 |
| | Total | 232 | 100 |
| Full course treatment | no answer | 8 | 3.45 |
| | yes | 218 | 93.97 |
| | no | 6 | 2.59 |
| | Total | 232 | 100 |

**Prevention**

Table 12: The preventative of malaria (mosquitoes)

| | | Respondents | % of respondents |
|---|---|---|---|
| Prevention | Impregnated bed nets | 193 | 83.20 |
| | Non- impregnated bed nets | 31 | 13.36 |
| | No bed nets | 8 | 3.45 |
| | Total | 232 | 100 |
| Other preventions | long sleeve cloth | 35 | 15.09 |
| | Insecticides spraying | 32 | 13.79 |
| | Repellent | 41 | 17.67 |
| | Mosquito coils | 81 | 34.91 |
| | Cleaning around the house | 25 | 10.78 |
| | Cleaning water stream | 11 | 4.74 |
| | Other | 7 | 3.02 |
| | Total | 232 | 100 |

Table 13: Number of bed nets in household (from 83% of 232 household head)

| | | Respondents | % of respondents |
|---|---|---|---|
| No. bed nets | 1,00 | 39 | 20.21 |
| | 2,00 | 74 | 38.34 |
| | 3,00 | 40 | 20.72 |
| | 4,00 | 33 | 17.10 |
| | 5,00 | 5 | 2.59 |
| | 6,00 | 1 | 0.52 |
| | 7,00 | 1 | 0.52 |
| | Total | 193 | 100 |

**RISK FACTORS**

**Work related**

Table 14: Frequency of household heads sleep in field hut

| | | Respondents | % of respondents |
|---|---|---|---|
| Sleep in field hut | never | 131 | 56.47 |
| | once a year | 62 | 26.72 |
| | once a month | 21 | 9.05 |
| | 2-3 nights/month | 9 | 3.89 |
| | 4 or more/month | 4 | 1.72 |
| | 1 or more/wk | 2 | 0.86 |
| | other | 3 | 1.29 |
| | Total | 232 | 100 |
| Kinds of field hut | Hut in rainy season | 6 | 2.59 |
| | Hut in cool season | 43 | 18.53 |
| | Hut in hot season | 50 | 21.55 |
| | Sleep in forest (no hut) | 11 | 4.74 |
| | Other | 122 | 52.59 |
| | Total | 232 | 100 |

Table 15: Animal around house

| | | Respondents | % of respondents |
|---|---|---|---|
| Animal around house | yes | 182 | 78.44 |
| | no | 50 | 21.55 |
| | Total | 232 | 100 |
| Domestic animal | Cat | 99 | 39.91% |
| | Dog | 149 | 60.08% |
| | Total | 248 | 100 |
| Other Animal | Cows | 128 | 36.36 |
| | Buffalo | 16 | 4.55 |
| | Pigs | 19 | 5.4 |
| | Chicken | 142 | 40.34 |
| | Ducks | 19 | 5.40 |
| | Birds | 3 | 0.85 |
| | Fish | 25 | 7.10 |
| | Total | 352 | 100 |

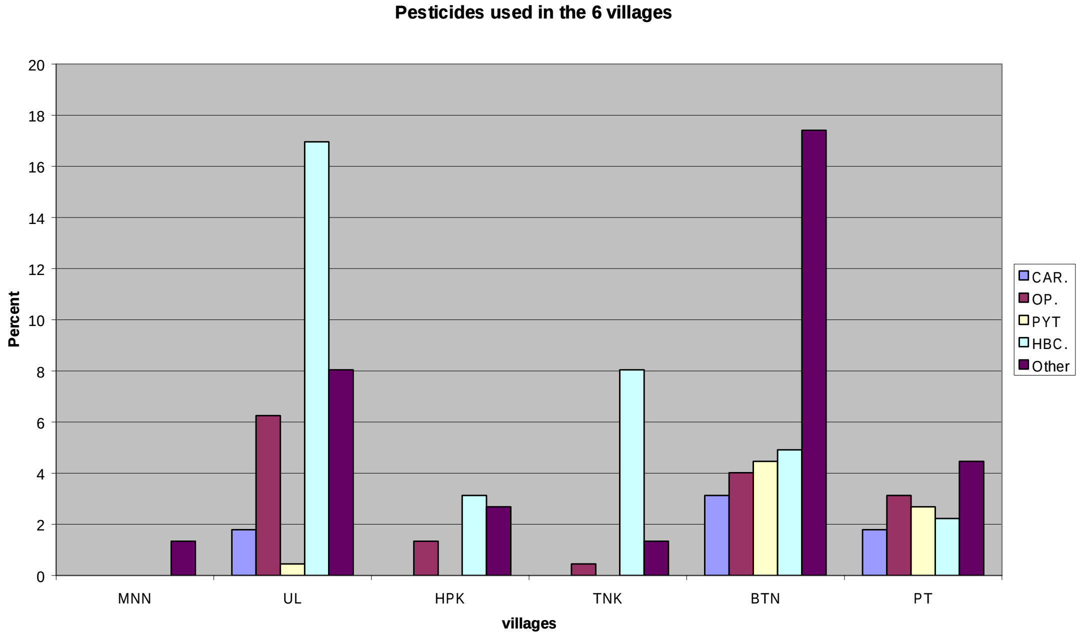
**Questionnaire 2**

The 1-20 most important farmers in the six selected villages were interviewed household heads specific information on agricultural pesticide used in their farms.
A total of 74 respondents to the questionnaires 2, from the six villages of two districts, were shown on table 4.1. The differentiation of percentage between chemical used in each village were shown on table 4.2, 4.3 and figure 1 – 3 respectively.

**Pesticides for agricultural plant protection**

Table 16: Pesticides used in each village

| Villages | No. of big farmer | Pesticide Groups | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Carbamate | | Organophosphate | | Pyrethroids | | Herbicides | | Other | |
| | | No. | % | No. | % | No. | % | No. | % | No. | % |
| Mae Num Noi (MNN) | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 3 | 1.34 |
| U - Long (UL) | 26 | 4 | 1.79 | 14 | 6.25 | 1 | 0.45 | 38 | 16.96 | 18 | 8.04 |
| Huy Pak Kok (HPK) | 12 | 0 | 0 | 3 | 1.34 | 0 | 0 | 7 | 3.13 | 6 | 2.68 |
| Thung Nang Klourn(TNK) | 15 | 0 | 0 | 1 | 0.45 | 0 | 0 | 18 | 8.04 | 3 | 1.34 |
| Bong Ti Noi (BTN) | 10 | 7 | 3.13 | 9 | 4.02 | 10 | 4.46 | 11 | 4.91 | 39 | 17.41 |
| PU Tuey ( PT) | 10 | 4 | 1.79 | 7 | 3.13 | 6 | 2.68 | 5 | 2.23 | 10 | 4.46 |
| Total | 74 | 15 | 6.71 | 34 | 15.19 | 17 | 7.59 | 79 | 35.27 | 79 | 35.27 |
| Total of chemical used | 224 | | | | | | | | | | |



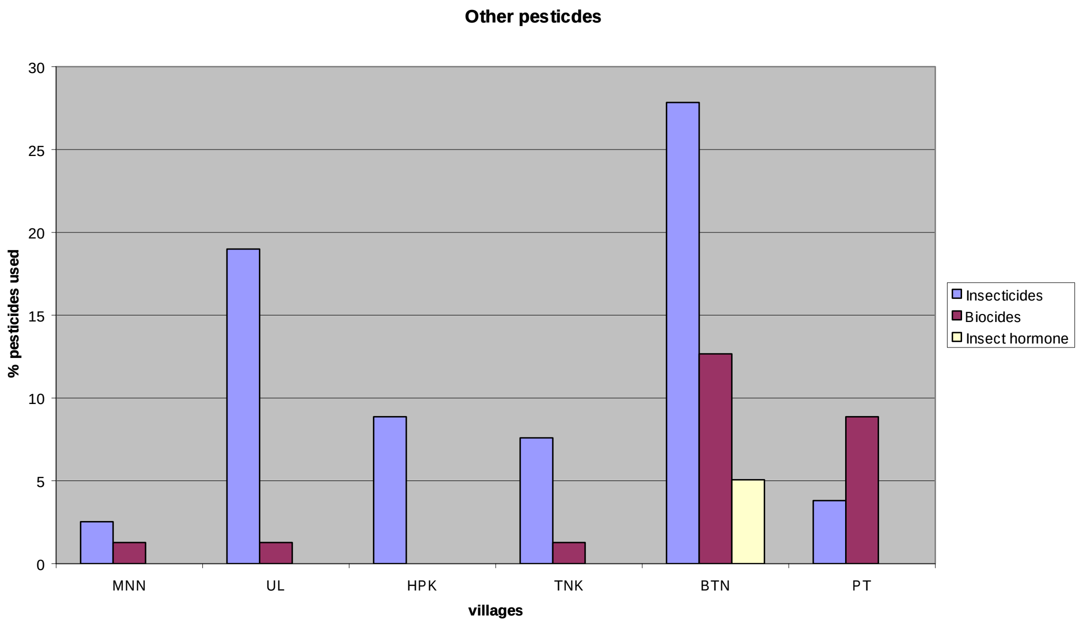

Villages: MNN = Mae Num Noi village ; UL = U-Long; HPK = Huy Pak Kok; TNK = Tung Nang Kruan; BTN = Bong Ti Noi; PT = Pu Tuey

Chemical groups : CAR = Carbamate; OP = Organophosphate; PYT = Pyrethroid; HBC = Herbicides

Figure 19: Pesticides used in each village

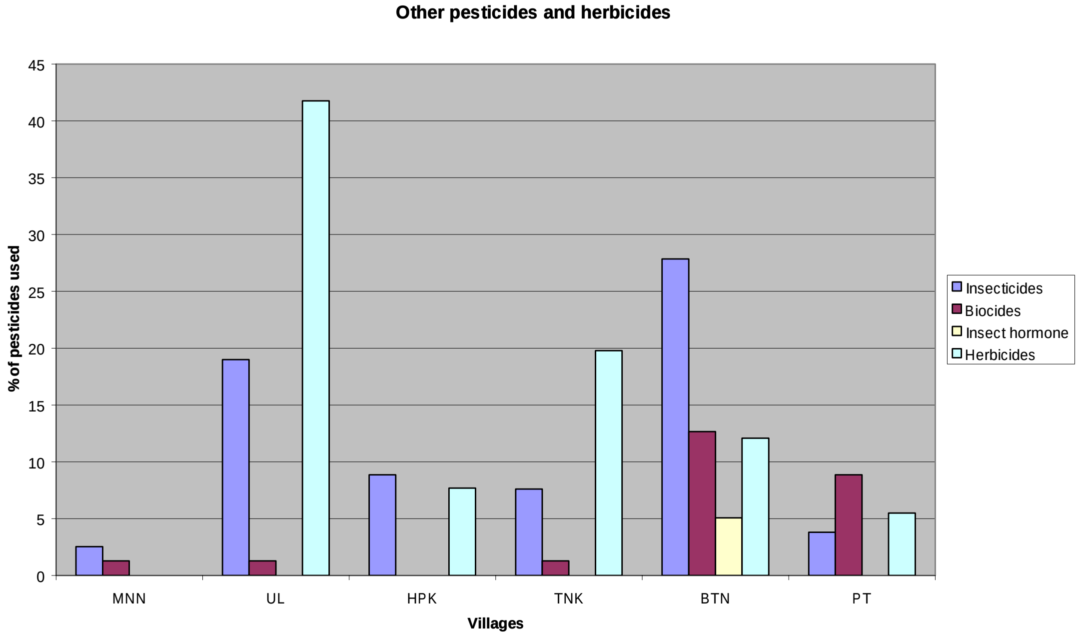
Table 17: Other pesticides used in each village

| Villages | of big farmer | Other pesticides | | | | | |
|---|---|---|---|---|---|---|---|
| | | Insecticides | | Biocides | | Insect hormone | |
| | | No. | % | No. | % | No. | % |
| Mae Num Noi (MNN) | 1 | 2 | 2.53 | 1 | 1.27 | 0 | 0 |
| U - Long (UL) | 20 | 15 | 18.99 | 1 | 1.27 | 0 | 0 |
| Huy Pak Kok (HPK) | 20 | 7 | 8.86 | 0 | 0 | 0 | 0 |
| Thung Nang Klourn(TNK) | 20 | 6 | 7.59 | 1 | 1.27 | 0 | 0 |
| Bong Ti Noi (BTN) | 20 | 22 | 27.85 | 10 | 12.66 | 4 | 5.06 |
| PU Tuey ( PT) | 10 | 3 | 3.8 | 7 | 8.86 | 0 | 0 |
| Total | 91 | 55 | 69.62 | 20 | 25.33 | 4 | 5.06 |




Villages: MNN = Mae Num Noi village
UL = U-Long
HPK = Huy Pak Kok
TNK = Tung Nang Kruan
BTN = Bong Ti Noi
= Pu Tuey

Figure 20: Other pesticides used in each village




Villages: MNN = Mae Num Noi village
UL = U-Long
HPK = Huy Pak Kok
TNK = Tung Nang Kruan
BTN = Bong Ti Noi
= Pu Tuey

Figure 21: Other pesticides and herbicides used in each village

**Part 2**; **Biting peak and population dynamics of *Anopheles minimus* species A, from high and low agricultural insecticide area in the two villages at Kanchanaburi Province, Thailand.**

**Adult *Anopheles minimus* species A collections**

A total of 1,899 adults of *Anopheles minimus* species A mosquitoes were collected from the six collection sites at the two locations (Figure 4 and 7). Collection size varied from month to month. The highest numbers collected were in October at both locations (Figure22 and Table 18).

There were highly significant differences between the two locations. The total number of female mosquitoes collected per month in MNN was 1,654 and in BTN with 245 and the corresponding averages were137.83 and 20.42 females per month in MNN and BTN, respectively ($P$ < 0.001). There were significant differences between the six collection sites in MNN and BTN ($P$ = 0.034) (Table 19). The seasonal differences between adult females were significantly different ($P$=0.014). The rainy season total for MNN was 917 females (mean 229.25 females per month) and for BTN 116 females (mean 29.0 females per month). The corresponding numbers for the cool season were 403 females (mean 100.75) for MNN and 10 females (mean 2.5) for BTN ($P$< 0.001) and for the hot season 334 females (mean 83.5) for MNN and 119 females (mean 29.75) for BTN. Female mosquito populations from MNN and BTN decreased in abundance during the cool to hot season, but the magnitude of decrease was greater in BTN than in MNN (Figure 22).

**Biting patterns of *An. minimus* A**

Results of the biting cycle of *An. minimus* A between the 2 locations selected in each season did not differ considerably. In general biting peak was 18.05-23.00 h both of MNN and BTN and the second peak was 01.05-.02.00 h (Figure 23). The highest collected number of mosquitoes was recorded on 21.05-23.00 h with 220 and 37 female mosquitoes from MNN and BTN respectively. The lowest collected number of mosquitoes were 85 and 5 from MNN and BTN respectively at 18.05-19.00 h (Table 20).

### Larval populations of *An. minimus* A

Mosquito larval survey results from December 2003 to September 2004 at the four breeding sites are summarized in Table 3 and figure 8. The total number of *An. minimus* A larvae collected were 1,184. The average larval density fluctuated similarly between the two villages. Anopheles larvae were prevalent throughout the year and appeared in high densities in October to December (late rain to cool) with stream velocities between 0.025-0.092 m/s. From January to May densities decreased. within an optimum 0.017 m/s of velocities of stream in the late dry season and 0.25 m/s in early rainy season both of MNN and BTN. However, there were no larvae at BTN in August, the middle of rainy season when stream velocities were at a maximum of over 0.42 m/ s.

Table 18: Number of *Anopheles minimus* species A collected through out the year compare between the two collecting sites, MNN and BTN.

| Month | Season | MNN/ low chemical | BTN/ high chemical |
|---|---|---|---|
| November | Cool | 172 | 1 |
| December | Cool | 74 | 2 |
| January | Cool | 69 | 1 |
| February | Cool | 88 | 6 |
| Mean | | 100.75 | 2.5 |
| Total | | 403 | 10 |
| March | Hot | 46 | 0 |
| April | Hot | 20 | 0 |
| May | Hot | 150 | 32 |
| June | Hot | 118 | 87 |
| Mean | | 83.5 | 29.75 |
| Total | | 334 | 119 |
| July | Rainy | 202 | 23 |
| August | Rainy | 122 | 2 |
| September | Rainy | 329 | 15 |
| October | Rainy | 264 | 76 |
| mean | | 229.25 | 29 |
| Total in rainy | | 917 | 116 |

Table 19: Number of *Anopheles minimus* species A collected through out of the year compare between the six collecting sites in Kanchanaburi province, Thailand.

| | | Collecting sites | | | | | |
|---|---|---|---|---|---|---|---|
| Month | Season | MNN/village | MNN/rubber | MNN/forest | BTN/village | BTN/orchard | BTN/ forest |
| November | Cool | 73 | 89 | 10 | 0 | 1 | 0 |
| December | Cool | 58 | 9 | 7 | 1 | 0 | 1 |
| January | Cool | 44 | 15 | 10 | 1 | 0 | 0 |
| February | Cool | 20 | 11 | 57 | 4 | 2 | 0 |
| March | Hot | 29 | 4 | 13 | 0 | 0 | 0 |
| April | Hot | 14 | 1 | 5 | 0 | 0 | 0 |
| May | Hot | 140 | 3 | 7 | 27 | 2 | 3 |
| June | Hot | 102 | 11 | 5 | 54 | 24 | 9 |
| July | Rainy | 118 | 73 | 11 | 20 | 2 | 1 |
| August | Rainy | 63 | 57 | 2 | 2 | 0 | 0 |
| September | Rainy | 82 | 241 | 6 | 6 | 5 | 4 |
| October | Rainy | 156 | 106 | 2 | 39 | 21 | 16 |
| mean | | 74.92 | 51.67 | 11.25 | 12.83 | 4.75 | 2.83 |
| Total | | 899 | 620 | 135 | 154 | 57 | 34 |

Table 20: Number of *Anopheles minimus* species A collected each hour through out the year compare between low chemical location (MNN) and high chemical location (BTN) in Kanchanaburi province, Thailand.

| Hours | MNN / low chemical | BTN / high chemical |
|---|---|---|
| 18.05-19.00 | 85 | 5 |
| 19.05-20.00 | 133 | 15 |
| 20.05-21.00 | 185 | 26 |
| 21.05-22.00 | 220 | 37 |
| 22.05-23.00 | 180 | 34 |
| 23.05-24.00 | 128 | 33 |
| 24.05-01.00 | 103 | 24 |
| 01.05-02.00 | 156 | 25 |
| 02.05-03.00 | 120 | 14 |
| 03.05-04.00 | 135 | 10 |
| 04.05-05.00 | 102 | 8 |
| 05.05-06.00 | 107 | 14 |
| Mean | 137.83 | 20.42 |
| Total | 1,654 | 245 |

Table 21: Larvae of *Anopheles minimus* species A collected each season through out the year compare between two collecting sites, MNN and BTN.

| | | **No. of *An. minimus* A (larvae)** | | | |
|---|---|---|---|---|---|
| | | **BTN** | | **MNN** | |
| Month | Season | Bst | Sst | HP | ST |
| November | Cool | 75 | 5 | 21 | 42 |
| December | Cool | 91 | 25 | 40 | 52 |
| January | Cool | 54 | 16 | 28 | 27 |
| February | Cool | 65 | 8 | 18 | 41 |
| mean | | 71.25 | 13.5 | 26.75 | 40.5 |
| Total in cool | | 285 | 54 | 107 | 162 |
| March | Hot | 47 | 6 | 28 | 34 |
| April | Hot | 34 | 0 | 12 | 17 |
| May | Hot | 21 | 2 | 10 | 5 |
| June | Hot | 39 | 6 | 10 | 31 |
| mean | | 35.25 | 3.5 | 15 | 21.75 |
| Total in hot | | 141 | 14 | 60 | 87 |
| July | Rainy | 0 | 7 | 28 | 12 |
| August | Rainy | 0 | 0 | 13 | 12 |
| September | Rainy | 5 | 3 | 6 | 20 |
| October | Rainy | 56 | 30 | 22 | 60 |
| mean | | 15.25 | 10 | 17.25 | 26 |
| Total in rainy | | 61 | 40 | 69 | 104 |

Table 22: Number of female and larvae of *Anopheles minimus* species A collected through out of the year compare between two collecting sites, MNN and BTN

| Month | MNN/ female | BTN/ female | MNN/ larvae | BTN/ larvae |
|---|---|---|---|---|
| Nov./cool | 85 | 5 | 63 | 80 |
| Dec./cool | 133 | 15 | 92 | 116 |
| Jan./cool | 185 | 26 | 55 | 70 |
| Feb./cool | 220 | 37 | 59 | 73 |
| Mar./hot | 180 | 34 | 62 | 53 |
| Apr./hot | 128 | 33 | 29 | 34 |
| May./hot | 103 | 24 | 15 | 23 |
| Jun./hot | 156 | 25 | 41 | 45 |
| Jul./ wet | 120 | 14 | 40 | 7 |
| Aug./wet | 135 | 10 | 25 | 0 |
| Sep./wet | 102 | 8 | 26 | 8 |
| Oct./wet | 107 | 14 | 82 | 86 |
| Mean | 137.83 | 20.42 | 49.08 | 49.58 |
| Total | 1,654 | 245 | 589 | 595 |

Table 23: The statistic of the caught female of *An minimus* A comparison between MNN and BTN in each season

| Source | Type III Sum of Squares | df | Mean Square | F | Significant |
|---|---|---|---|---|---|
| Corrected Model | 135384.375a | 5 | 27076.875 | 9.815 | 0 |
| Intercept | 150258.375 | 1 | 150258.375 | 54.467 | 0 |
| Location | 82720.042 | 1 | 82720.042 | 29.985 | 0 |
| Season | 30100 | 2 | 15050 | 5.456 | 0.014 |
| Location * Season | 22564.333 | 2 | 11282.167 | 4.09 | 0.034 |
| Error | 49656.25 | 18 | 2758.681 | | |
| Total | 335299 | 24 | | | |
| Corrected Total | 185040.625 | 23 | | | |

a. Rsquared = .732 (Adjusted R Squared = .657)

Table 24: The statistic of the collected larvae of *An minimus* A comparison between MNN and BTN in each season

| Source | Type III Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| Corrected Model | 9850.833a | 5 | 1970.167 | 3.222 | 0.03 |
| Intercept | 58410.667 | 1 | 58410.667 | 95.525 | 0 |
| Season | 8582.333 | 2 | 4291.167 | 7.018 | 0.006 |
| Location | 1.5 | 1 | 1.5 | 0.002 | 0.961 |
| Season * Location | 1267 | 2 | 633.5 | 1.036 | 0.375 |
| Error | 11006.5 | 18 | 611.472 | | |
| Total | 79268 | 24 | | | |
| Corrected Total | 20857.333 | 23 | | | |

a. Rsquared = .472 (Adjusted R Squared = .326)

Table 25: Climatological data as recorded from the meteorological station in Thong Pha Phum (TPP) and Sai Yok (SY) district Kanchanaburi Province, average in one year.

| Month | Relative humidity (average, %) | | Rain fall (average, mm) | |
|---|---|---|---|---|
| | TPP | SY | TPP | SY |
| Oct-03 | 95 | 96 | 2.06 | 6.1 |
| Nov-03 | 92 | 95 | 0 | 0 |
| Dec-03 | 93 | 93 | 0 | 0 |
| Jan-04 | 91 | 93 | 0.1 | 0.8 |
| Feb-04 | 88 | 91 | 0.4 | 0.8 |
| Mar-04 | 83 | 89 | 0.2 | 1.6 |
| Apr-04 | 83 | 88 | 0.4 | 3 |
| May-04 | 93 | 96 | 7.5 | 14 |
| Jun-04 | 94 | 96 | 12 .8 | 6.7 |
| Jul-04 | 95 | 96 | 5.2 | 4.1 |
| Aug-04 | 90 | 96 | 9.19 | 3.5 |
| Sep-04 | 90 | 96 | 5.03 | 5.6 |
| Total | | | 30.08 | 46.2 |

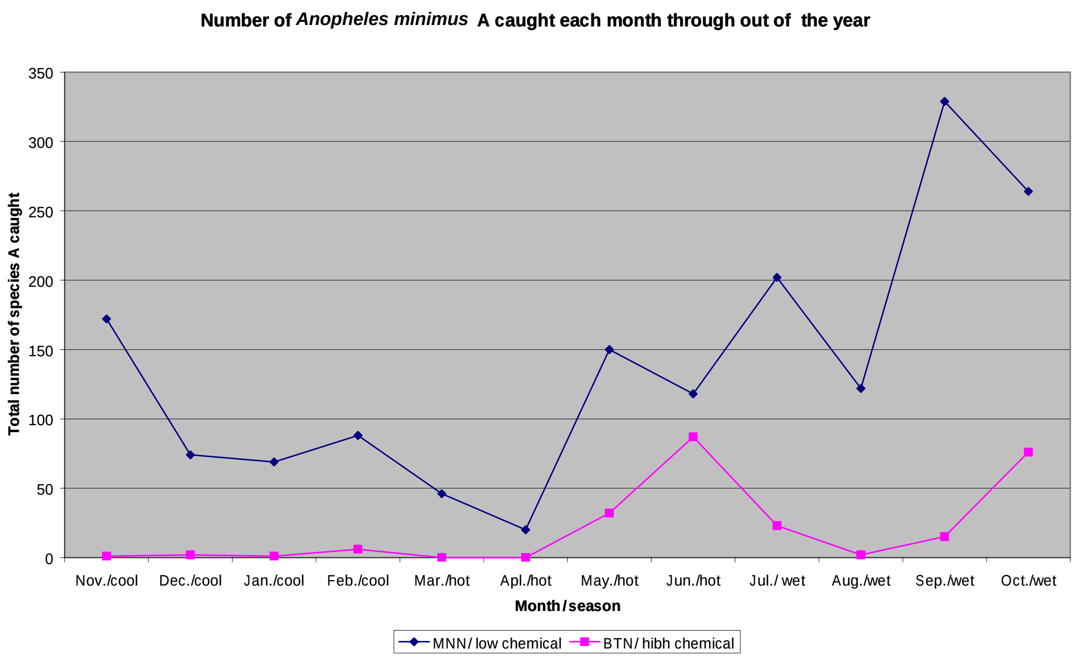



Figure 22: Number of *Anopheles minimus* species A collected through out the year compare between the two collecting sites, MNN and BTN.

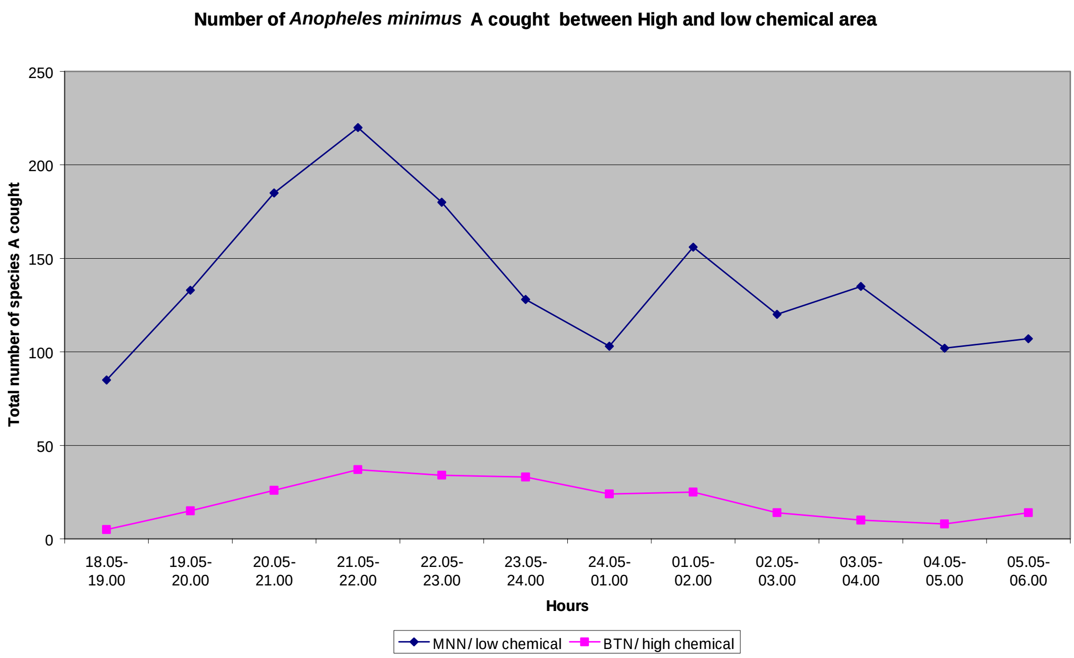



Figure 23: Number of *Anopheles minimus* species A collected each hour through out the year compare between the two collecting sites, MNN and BTN.

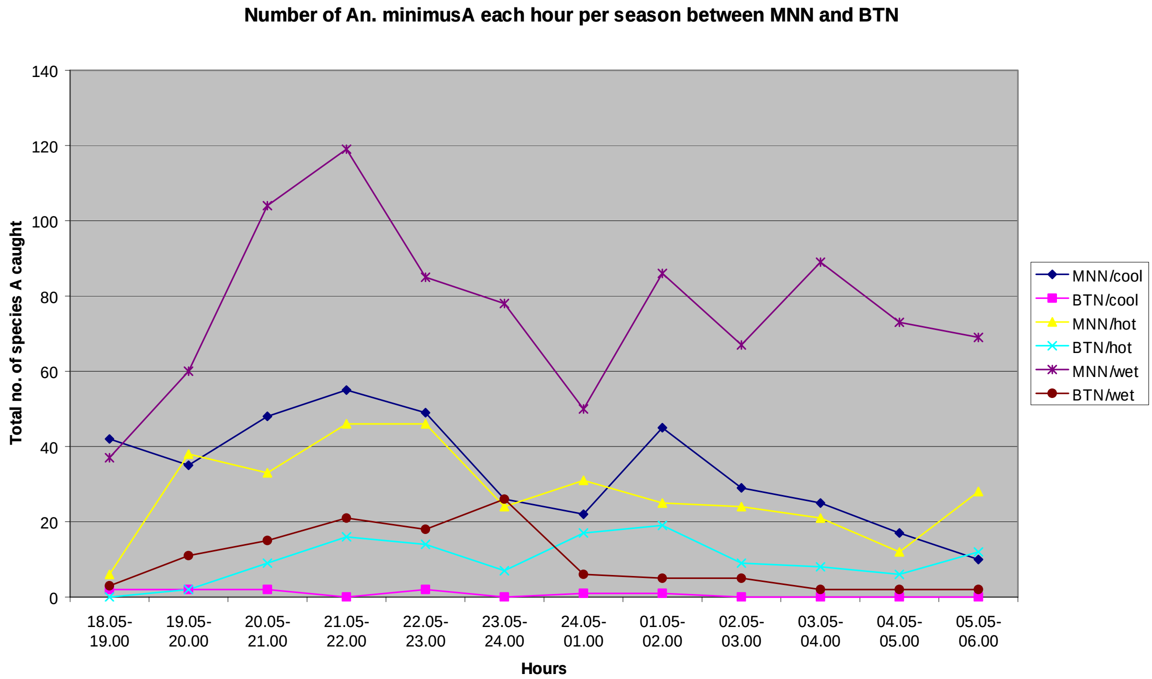



Figure 24: Number of *Anopheles minimus* species A collected each hour on three season compare between the two collecting sites, MNN and BTN.

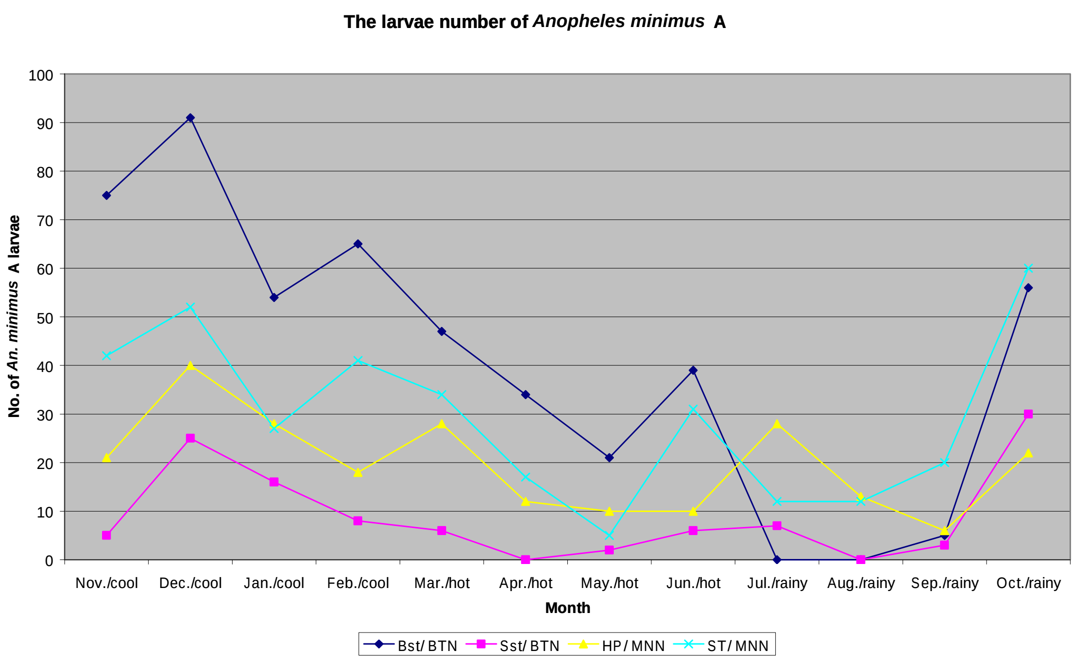



Figure 25: Larvae of *Anopheles minimus* species A collected through out the year compare between the four collecting sites in Kanchanaburi Province.

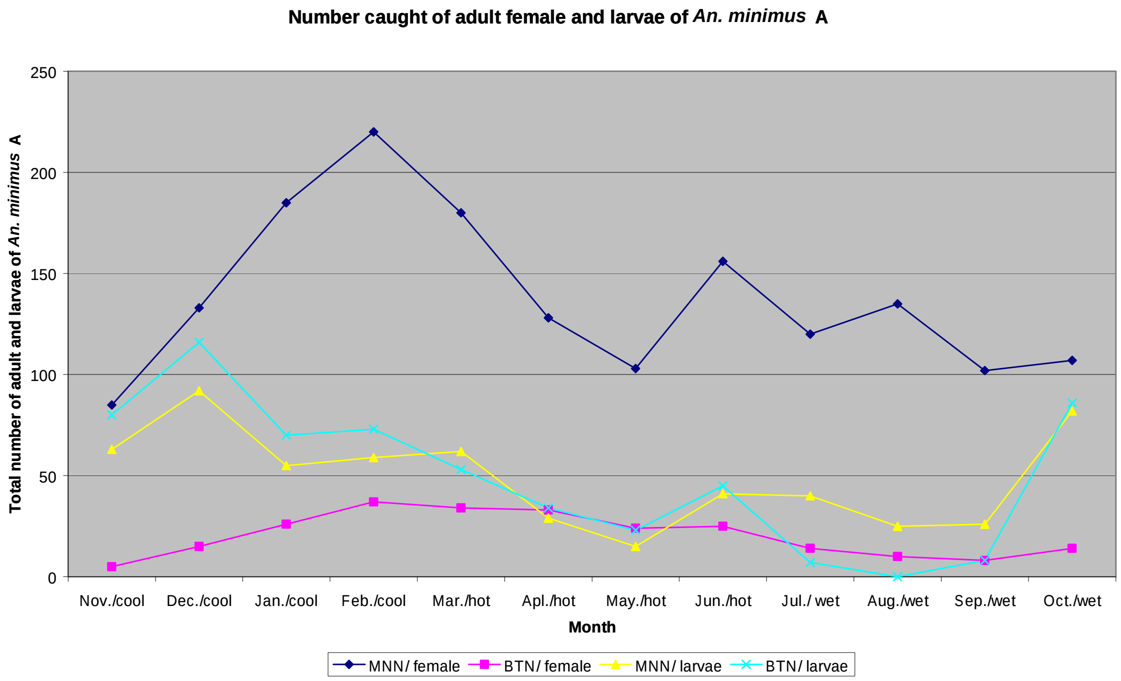



Figure 26: Number of female and larvae of *Anopheles minimus* species A collected through out the year compare between two collecting sites, MNN and BTN

**Part 3; Behavioral responses by *Anopheles minimus* species A and C to three agrochemicals.**

Presence of avoidance behavior to the tested chemicals was observed in the two strains of *Anopheles minimus* in the form of contact irritancy and non-contact repellency (Table 26). There were slightly different behaviors between the strains. In the contact trial, the escape response was stronger in species C than in species A when exposed to carbaryl (68% of species C and 46% of species A escaped; $P$=0.001) and cypermethrin (67% of species C and 58% of species A escaped; $P$=0.054) (Table 26, Figures 27 and 28). The opposite pattern was observed when mosquitoes were exposed to malathion. Here the escape response was significantly stronger in species A (85% escaped) than in species C (23% escaped) ($P$=0.0001). Similar escape patterns were observed for the repellency function in the non-contact trials. Escape response was significantly stronger in species C than in species A when exposed to carbaryl (80% of species C and 49% of species A escaped; $P$=0.001) and cypermethrin (64% of species C and 27% of species A escaped; $P$=0.001). As in the contact trial, the opposite pattern was observed for malathion. The escape response was significantly stronger in species A (52% escaped) than in species C (38% escaped) ($P$=0.001). Comparison between the contact and non-contact trials showed significant differences in escape response of *An. minimus* A and C across the three compounds $P$<0.05 ( $P$=0.001- 0.0001), except for carbaryl in species A, $P$>0.05 (P= 0.105) and cypermethrin in species C, $P$>0.05 ($P$=0.205). The trend seemed to be that species A tended to escape in higher numbers in the contact trials than in the non-contact trials (malathion and cypermethrin), whereas in species C more mosquitoes escaped in the non-contact compared to the contact trials (malathion and carbaryl). Mortalities after a 24-h holding period were in general higher for species A (0-21%) than species C (0-0.2%), those for mosquitoes that remained within the chambers compared to those that escaped (Table 26). The highest mortalities were found in species A that escaped in the contact and non-contact trials with cypermethrin (21% and 10%, respectively). Of those mosquitoes that remained within the chambers, 24-h mortalities were highest to carbaryl (contact: 16.7%; non-contact: 5.6%). Low mortalities were found in the other tests and there were no mortalities in any of the controls.

The escape times for 30%, 50%, and 70% of the two *An. minimus* strains ($ET_{30}$, $ET_{50}$ and $ET_{70}$) can not be obtained because many mosquitoes remained inside the exposure chambers after the test had ended (Table 27). In the contact trial with malathion, 70% of species A escaped after 14 minutes, whereas it can not be estimated from the species C. Carbaryl and cypermethrin used in the contact trials were not able to force more than 70% of the two species to escape within the 30 minute test period. In the non-contact trials, only carbaryl was able to force more than 70% of mosquitoes, in this case species C, to escape; this happened after 21 minutes. In the same test with species A, only 30% of the population escaped, and after 4 minutes.

Figures 27, 28 and 29 showed the proportions of mosquitoes remaining in the excito-repellency test chambers treated with carbaryl, malathion and cypermethrin, respectively. These proportions are served as an analytical data to develop patterns of escape rates and demonstrate probabilities for escaping from test chambers in contact vs. non contact (Figures 27, 28 and 29) In contact trials, escape rate of species A with carbaryl and cypermethrin were significantly lower than species C ($P$<0.05; Figures 27 and 29). In non contact trials with carbaryl and cypermethrin, the escape rate was dramatically higher for species C than species A ($P$<0.05; Figures 27 and 29). This phenomenon was not observed in non contact trials with malathion. With malathion, the escape rate was statistically higher for species A than species C ($P$<0.05; Figure 28).

Table 26: Percentage escape response and mortality of *Anopheles minimus* A and C exposed to carbaryl, malathion and cypermethrin in contact and noncontact trials

| | | | | | | | %Mortality | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | Treatment | | Control | | Treatment | | Control | |
| Test condition | Insecticide | Strain | No. tested | % Escape | No. tested | % Escape | Escape | Not Escape | Escape | Not Escape |
| Contact | Cabaryl | A | 77 | 46 | 76 | 19 | 8.6 | 16.7 | 0 | 0 |
| | | C | 78 | 68 | 77 | 27 | 0.2 | 0.1 | 0 | 0 |
| | Malathion | A | 65 | 85 | 60 | 22 | 0 | 0 | 0 | 0 |
| | | C | 78 | 23 | 80 | 12 | 0 | 0.1 | 0 | 0 |
| | Cypermethrin | A | 72 | 58 | 76 | 25 | 21 | 0 | 0 | 0 |
| | | C | 87 | 67 | 84 | 23 | 0.1 | 0.1 | 0 | 0 |
| Non-Contact | Cabaryl | A | 71 | 49 | 75 | 10 | 0 | 5.6 | 0 | 0 |
| | | C | 76 | 80 | 76 | 20 | 0 | 0 | 0 | 0 |
| | Malathion | A | 65 | 52 | 60 | 23 | 2.9 | 0 | 0 | 0 |
| | | C | 80 | 38 | 78 | 4 | 0.1 | 0.1 | 0 | 0 |
| | Cypermethrin | A | 73 | 28 | 77 | 12 | 10 | 1.9 | 0 | 0 |
| | | C | 85 | 63 | 83 | 34 | 0.1 | 0.1 | 0 | 0 |

Table 27: Estimated escape time (ET) at 30, 50, and 70 minutes for *Anopheles minimus A and C* in contact with 0.4 g/m$^2$ carbaryl, 0.19 g/m$^2$ malathin and 0.04 g/m$^2$ cypermethrin

| | | ET 30 | | ET 50 | | ET 70 | |
|---|---|---|---|---|---|---|---|
| Species | Insecticide | Contact | Non contact | Contact | Non contact | Contact | Non contact |
| A | Cabaryl | 10 | 4 | - | - | - | - |
| | Malathion | 2 | 4 | 7 | 16 | 14 | - |
| | Cypermethrin | 4 | - | 21 | - | - | - |
| C | Cabaryl | 4 | 2 | 18 | 8 | - | 21 |
| | Malathion | - | 18 | - | - | - | - |
| | Cypermethrin | 12 | 9 | 20 | 18 | - | - |

Table 28: Log-rank comparisons of escape responses between two species in contact and non contact trials.

| Insecticide | Contact trial<br>( *P* ) | Non-contact trial<br>( *P* ) |
|---|---|---|
| Carbaryl | 0.001 | 0.001 |
| Malathion | 0.0001 | 0.001 |
| Cypermethrin | 0.054 | 0.001 |

Table 29: Log-rank comparisons of escape responses between control and contact, contact and non-contact, and control and non contact trials for two strains of *An. minimus*

| Insecticide | Test Strain | Control vs. Contact ( *P* ) | Contact vs. Non-contact ( *P* ) | Control vs. Non-contact ( *P* ) |
|---|---|---|---|---|
| Carbaryl | Species A | 0.0001 | 0.105 | 0.0001 |
| | Species C | 0.0001 | 0.0001 | 0.0001 |
| Malathion | Species A | 0.001 | 0.001 | 0.001 |
| | Species C | 0.001 | 0.001 | 0.001 |
| Cypermethrin | Species A | 0.0001 | 0.0001 | 0.0001 |
| | Species C | 0.0001 | 0.205 | 0.0001 |

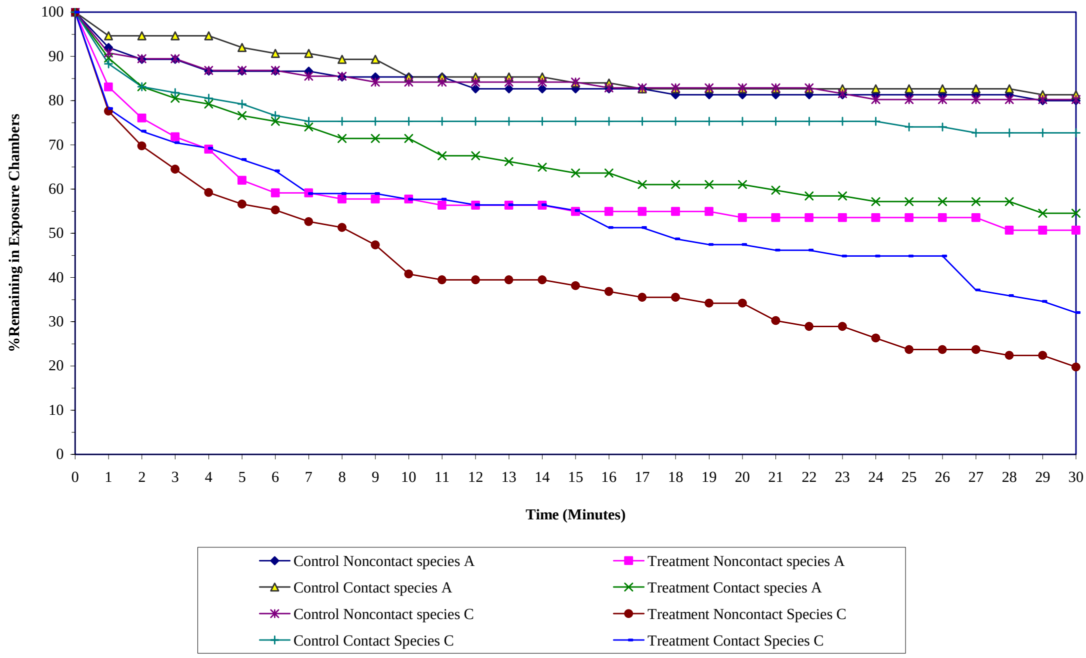



Figure 27: Escape probability of *Anopheles minimus* species A and C exposed to carbaryl and paired control chamber for contact and non-contact trials.

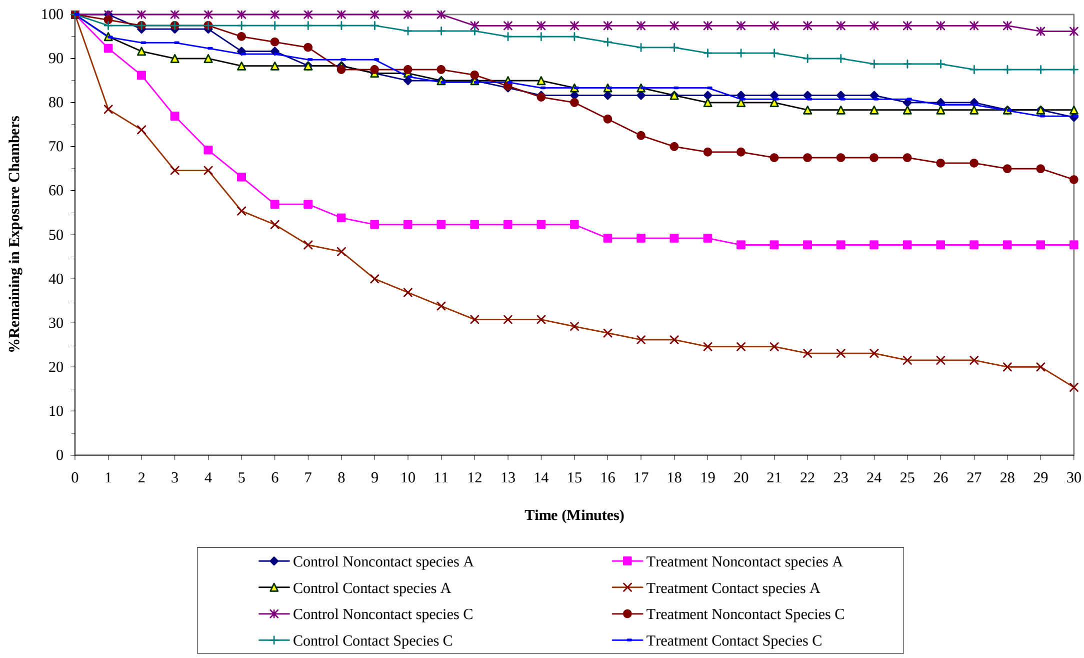



Figure 28: Escape probability of *Anopheles minimus* species A and C exposed to malathion and paired control chamber for contact and non-contact trials.

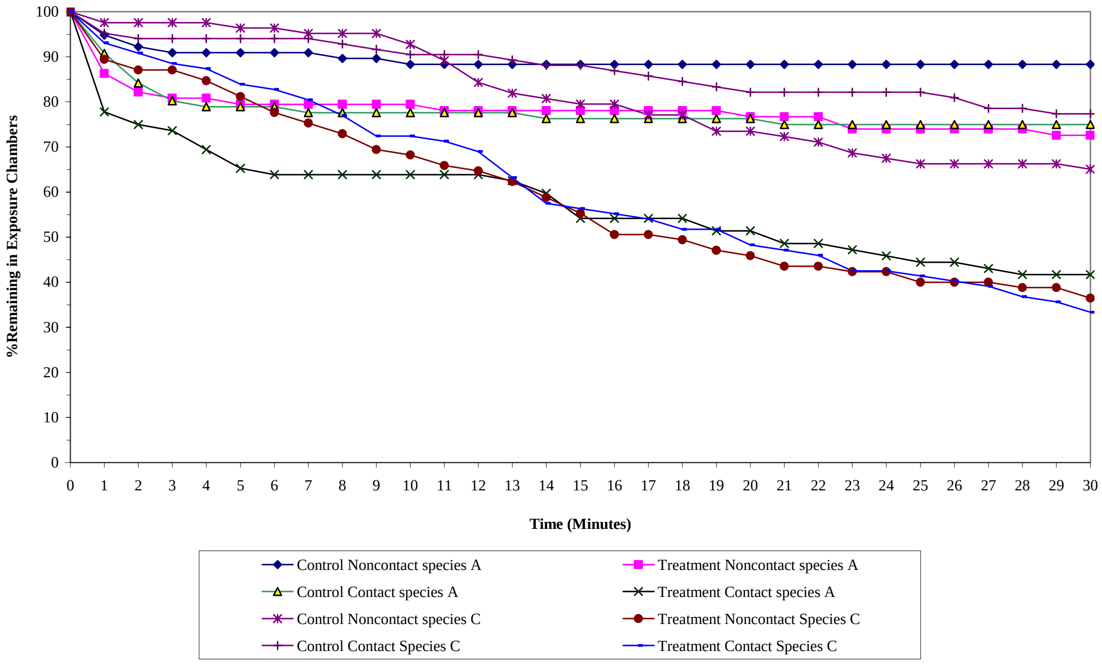



Figure 29: Escape probability of *Anopheles minimus* species A and C exposed to cypermethrin and paired control chamber for contact and non-contact trials.

**Part 4; Behavioral responses by *Anopheles minimus* species A and species C to DDT and pyrethroids.**

Two types of behavioral responses, contact irritancy and non-contact repellency, were observed with exposure to three insecticides and percent mortalities of escape and non-escape mosquitoes from control and treated chambers were recorded (Table 30). Patterns and rate of escape were stronger in *An.minimus* species A than *An. minimus* species C for all three compounds. In contact trials, percent escape of *An. rninimus* A (92-96%) was significantly ($P < 0.05$) higher than for *An. minimus* C (50-90%), regardless of compound used. Similarly, percent escape by species A was also greater than that by species C for the two synthetic pyrethroids. In general, a relatively low number of mosquitoes escaped from the control chambers (12-23% for contact and 10-15% for non-contact). Mortality rates of escaped mosquitoes from both test populations were low (0-13.3%), whereas those that remained in the test chamber (non-escape mosquitoes) showed much higher mortality rates (43-100%). All non-escape specimens of species A exposed to deltamethrin and lambda-cyhalothrin perished within 24 hours post-exposure (Table 30). High mortality rates (13.3%) of escaped mosquitoes from control chambers were observed with DDT. In non-contact trials, *An. minimus* species A demonstrated significantly strong escape responses to all three compounds compared with species C. After 30 minutes exposure, percent escape was approximately 96% for DDT, 92% for deltamethrin, and 87% for lambdacyhalothrin with *An. minimus* species A, while only 24% for DDT and deltamethrin and 18% for lambda-cyhalothrin with species C. Percent mortalities of escaped specimens of both populations were very low, ranging from 1.1% to 4.5%. Mortality was not seen in non-escaped *An. minimus* species A after the 24-hour holding period.

The escape patterns generated from insecticide-treated chambers are expressed in one-minute intervals for 50%, 75%, and 90% ($ET_{50}$, $ET_{75}$, and $ET_{90}$) of the test population to escape from exposure chambers (Table 31). In contact trials, the $ET_{50}$, $ET_{75}$, and $ET_{90}$ for *An. minirnus* species A were 5,12.5, and 24 minutes with DDT, 2.5, 6, and 16 minutes with deltamethrin, and 7, 23.5, and 30 minutes with lambdacyhalothrin, respectively. The $ET_{50}$ for *An. minimus* C was 5, 8.5, and 6 minutes for DDT (0.02 $g/m^2$). The $ET_{75}$, and $ET_{90}$ values for all three compounds for *An. minimus* species C could not be calculated (with one exception: lambdacyhalothrin $ET_{75}$ =12.5 minutes) because of insufficient numbers of mosquitoes escaping. Similarly, ET values for *An. minimus* species C in all non-contact trials could not be estimated. For non-contact trials, the $ET_{50}$, $ET_{75}$, and $ET_{90}$ values were 5, 4.5, and 14 minutes for DDT and 5.6, 8, and 25 minutes for deltamethrin. The $ET_{50}$ and $ET_{75}$ estimates were 6.5 and 23.5 minutes, respectively, for lambda-cyhalothrin.

Comparison of escape responses between *An.. rninimus* species A and C in contact and non-contact trials using log - rank analysis showed statistically significant differences in escape patterns between species in non-contact trials for all three compounds ($P < 0.05$). In contact trials, significant differences in escape responses between species were observed with DDT and deltamethrin ($P < 0.05$). Comparisons of escape responses between paired contact and control, contact and non-contact, and non-contact and control bioassays for each species for each compound were made. No significant differences between contact and non-contact escape for *An. rninimus* species A were observed ($P > 0.05$). Escape probabilities in contact and non-contact trials were significantly higher than those in paired controls for all bioassays.

Figures 2-5 show the proportions of mosquitoes remaining in the exposure and control chambers under different test conditions and chemical exposure. Strong repellency action was seen with *An.minirnus* species A against all three compounds, whereas significantly less escape reaction was observed with *An. minimus* species C (Figure 33). In non-contact tests, *An. minimus* species A demonstrated a stronger escape rate with DDT than with either deltamethrin or lambda-cyhalothrin (Figure 33). There were significant differences in escape responses seen in all contact trials compared with paired control and non-contact trials with *An. minimus* species C ($P <$ 0.05). Escape patterns in all non-contact repellency trials for *An. minimus* species A were significantly greater than paired controls.

Table 30: Percentage escape response and mortality of *Anopheles minimus* A and C exposed to DDT, deltamethrin and lambda-cyhalothrin in contact and non contact trials

| | | | | | | | %Mortality | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | Treatment | | Control | | Treatment | | Control | |
| **Test condition** | **Insecticide** | **Strain** | **No. tested** | **% Escape** | **No. tested** | **% Escape** | **Escape** | **Not Escape** | **Escape** | **Not Escape** |
| Contact | DDT | A | 85 | 92 | 85 | 12 | 0 | 42.8 | 0 | 0 |
| | | C | 100 | 77 | 100 | 15 | 1.3 | 0 | 13.3 | 1.2 |
| | Deltamethrin | A | 76 | 96 | 75 | 23 | 0 | 100 | 0 | 0 |
| | | C | 98 | 51 | 94 | 14 | 2 | 0 | 0 | 1.2 |
| | Lambdacyha-lothrin | A | 77 | 94 | 78 | 23 | 1.4 | 100 | 0 | 0 |
| | | C | 100 | 90 | 100 | 18 | 1.1 | 0 | 0 | 0 |
| Non-Contact | DDT | A | 85 | 96 | 83 | 27 | 0 | 0 | 0 | 0 |
| | | C | 100 | 24 | 100 | 10 | 0 | 0 | 0 | 0 |
| | Deltamethrin | A | 76 | 92 | 75 | 29 | 0 | 0 | 4.5 | 0 |
| | | C | 100 | 24 | 100 | 10 | 0 | 0 | 0 | 1.3 |
| | Lambdacyha-lothrin | A | 77 | 87 | 77 | 27 | 1.5 | 0 | 0 | 0 |
| | | C | 95 | 18 | 95 | 15 | 0 | 0 | 0 | 0 |

Table 31: Estimated escape time (ET) at 50, 75, and 90 minutes for *Anopheles minimus A and C* in contact with 2 g/m$^2$ DDT, 0.02 g/m$^2$ deltamethrin and 0.03 g/m$^2$ lambda-cyhalothrin

| | | ET 50 | | ET 75 | | ET 90 | |
|---|---|---|---|---|---|---|---|
| Species | Insecticide | Contact | Non contact | Contact | Non contact | Contact | Non contact |
| A | DDT | 5 | 2 | 12.5 | 4.5 | 24 | 14 |
| | Deltamethrin | 2.5 | 5.6 | 6 | 8 | 16 | 25 |
| | Lambdacyhalothrin | 7 | 6.5 | 23.5 | 23.5 | 30 | 0 |
| C | DDT | 5 | 0 | 0 | 0 | 0 | 0 |
| | Deltamethrin | 8.5 | 0 | 0 | 0 | 0 | 0 |
| | Lambdacyhalothrin | 7 | 6.5 | 12.5 | 0 | 0 | 0 |

Table 32: Log-rank comparisons of escape responses between two species in contact and non contact trials

| Insecticide | Contact trial ( *P* ) | Non-contact trial ( *P* ) |
|---|---|---|
| DDT | 0.001 | 0.0001 |
| Deltamethrin | 0.001 | 0.0001 |
| Lambda-cyhalothrin | 0.205 | 0.0001 |

Table 33: Log-rank comparisons of escape responses between control and contact, contact and non-contact, and control and non contact trials for two strains of *An. minimus*

| Insecticide | Test Strain | Control vs. Contact ( *P* ) | Contact vs. Non-contact ( *P* ) | Control vs. Non-contact ( *P* ) |
|---|---|---|---|---|
| DDT | Species A | 0.0001 | 0.205 | 0.0001 |
| | Species C | 0.0001 | 0.001 | 0.001 |
| Deltamethrin | Species A | 0.001 | 0.205 | 0.001 |
| | Species C | 0.001 | 0.0001 | 0.001 |
| Lambda-cyhalothrin | Species A | 0.001 | 0.117 | 0.0001 |
| | Species C | 0.0001 | 0.0001 | 0.105 |

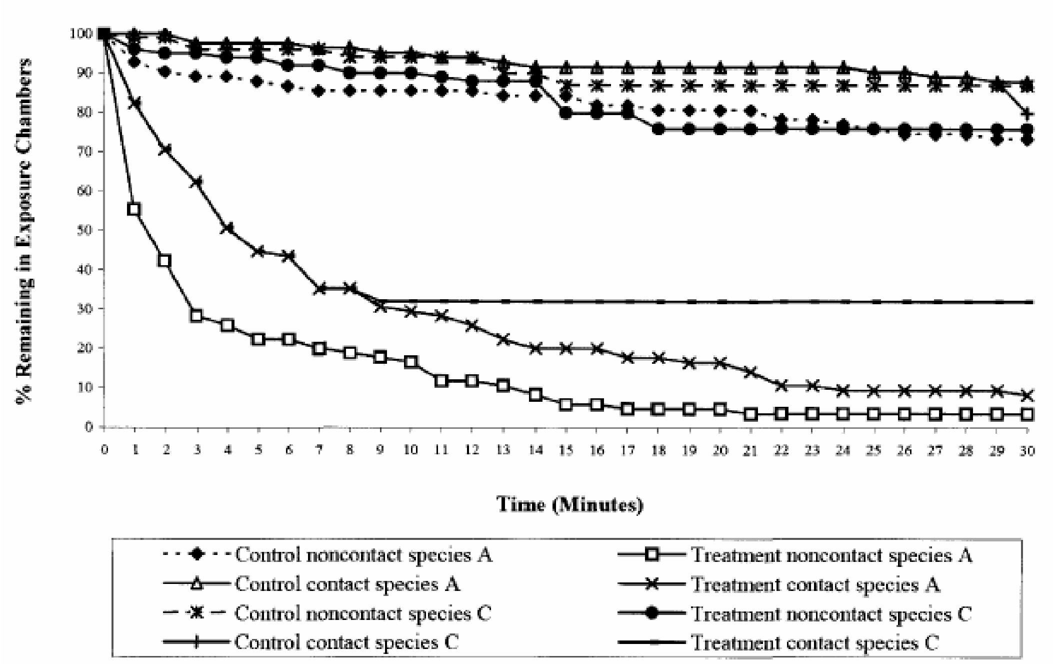



Figure 30: Escape probability of Anopheles minimus species A and C exposed to DDT and paired control chambers for contact and non-contact trials.

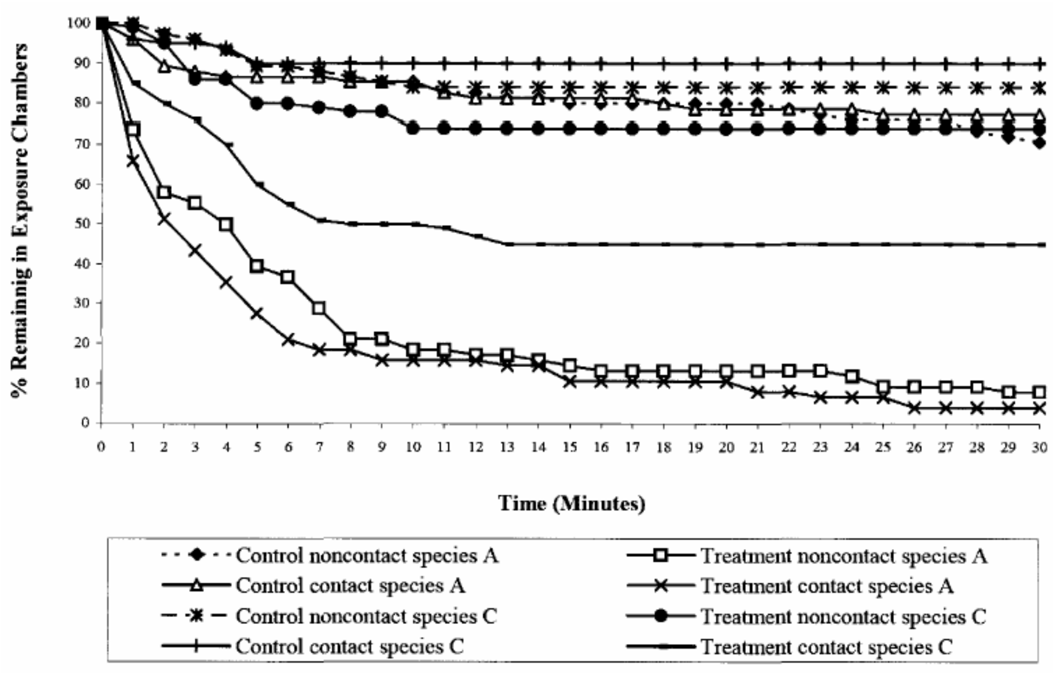



Figure 31: Escape probability of Anopheles minimus species A and C exposed to deltamethrin and paired control chambers for contact and non-contact trials.

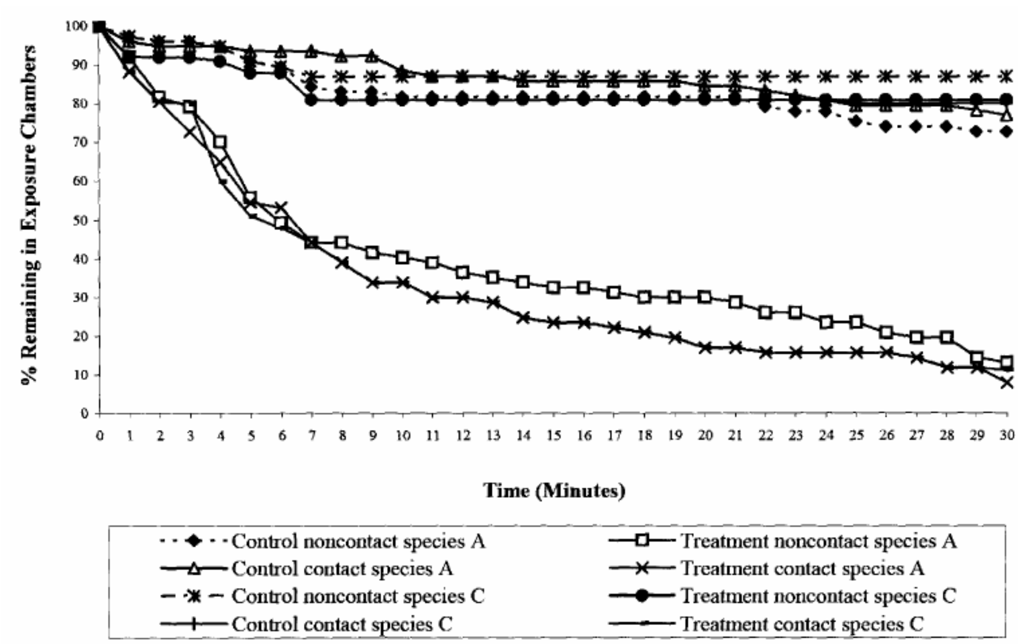



Figure 32: Escape probability of Anopheles minimus species A and C exposed to lambda-cyhalothrin and paired control chambers for contact and non-contact trials.

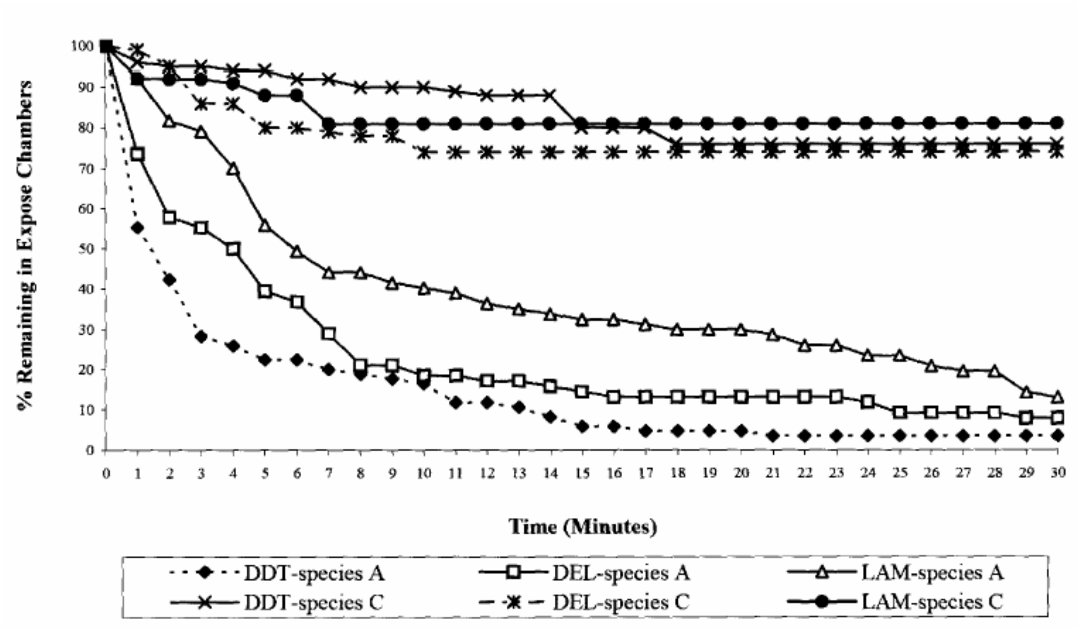



Figure 33: Escape probability of Anopheles minimus species A and C exposed to DDT, deltamenthrin (DEL), and lambda-cyhalothrin (LAM) in non-contact trials.

# DISCUSSION

**Part 1; Risk factors and base line malaria knowledge from different pesticide Land-use systems in malaria endemic area at Kanchanaburi Province,Thailand.**

**General Information**

A total of 232 persons responded to the questionnaires from Kanchanaburi province. Sixty-six percent, 155 of all respondent, were household heads and 34% were closely related to household heads, most of them being either a wife or a child.

About 77% of the household heads were male and 23% were female. The average age of household heads was 47 years, ranging from 20 to 83 years. Approximately 70% of household heads were born in Thailand, 22% were born in Myanmar (Table 3). There was 38/232=16.38 % of Burmese respondents in Kanchanaburi (Table 4). Ethnicity is often confused with nationality, therefore respondents sometimes considered themselves Thai in addition to belonging to another ethnic group. The largest ethnic group among the questioned household heads was Thai (55%). Other reported ethnic groups were Mon, Karen, Lao, and Mong in Kanchanaburi province.

More than 21% of the households had lived in their village 10 years or more (63%). Almost 14% had lived in the village between 1-5 years, and 2% between 0-1 year (Table 5).

The number of adults per household ranged from one to ten. In 46% of the households there were two adults, in 21% of households there were three adults, and in 16% of households there were four or more adults. The number of children per household ranged from one to twelve. In 0.33% of the households there was one child, in 37% of households there were two children, and in 20% of households there were three or more children (Table 6).

The questions on household head's occupation allowed multiple choices, since many are engaged in several income-bringing activities. Of the 232 interviewed households heads 131 (56%) responded that they were farmers and 49 (21%) that they were employed worker. Other answers were orchard owner (25), rubber plantation worker (14), and a range of other occupations (Table 7).

There was a large non-response on questions about respondents' perceptions on the three largest problems in the village (Table 8). However, the answers given can be grouped, in order of importance, into agricultural problems, economical problems, bad communications, no health services, and border problems. Agricultural problems included aspects on low price for selling products, water shortage, destroyed products, low crop yields, and expensive agricultural inputs (i.e. fertilizer and pesticides). Economical problems were debt, no job, no money, low income, and no savings. Bad communications meant bad roads and no public transport. Answers included in the border problems category were war at border, migration, minorities, foreigners, and unsafe area.

Respondents considered malaria the most important disease in the villages (129 answers out of 232). 56% in Kanchanaburi considered malaria is the most important disease. The second most important health problem was fever, and the third was dengue.  Other health problems of importance were diarrhoea, influenza, elephantiasis, and stomach aches.

More household heads in Kanchanaburi (67%) travelled outside their village, and they also travelled more frequently. The most common destinations were within the district (35%), another province (29%), within province (24%), and within sub-district (14%). The majority (77%) of the travelling household heads stayed less than a week outside the village. About 10% stayed away between one and four weeks. The main reasons for travelling were to visit relatives or friends (49%), for purchases (41%), and for work (18%).

### Malaria Knowledge

Of all respondents (232), in Kanchanaburi provinces, a total of 206, or 87%, knew about the disease malaria, and 11% did not know of the disease (Table 10).

An average of about 29% of respondents (33/232) reported having had malaria at least once, 42% two to three times, and 22% reported having had malaria more than three times. Malaria episodes among respondents occurred most commonly between 1-5 years ago (51%). Almost 30% of respondents reported having had malaria episodes more than five years ago. Thirty-seven or almost 16% of respondents reported more recent malaria episodes occurring within the last year.

When asked about malaria symptoms the 232 respondents that knew about malaria associated shivering, headaches, fever, nausea/vomiting, and muscle pain (95%) with the disease.

Three quarters of all 232 respondents knew that malaria is caused by mosquito bites and 50% knew that the main transmission season is during the rainy season. As many as 19% of all respondents did not associate the main malaria season with the rainy season, this pattern was pronounced for Kanchanaburi respondents. Half of the respondents reported that mosquitoes mainly bite during the early evening (16.00-20.00) and 35% associated biting with the late evening (20.00-24.00). Ten percent mentioned that mosquitoes bite all the night and 8% that they bite during the daytime (06.00-16.00).

Most respondents (46%) associated mosquito larval sites as water reservoirs, and 33% said that mosquitoes develop in slow-moving streams. Other reported larval sites were stagnant clean water (21%), rice fields (11%), and ground pools (8%).

Forty six percent of respondents in Kanchanaburi said they could differentiate between Anopheles and other mosquitoes.

There were fewer mosquitoes inside their houses after periods of agricultural insecticide spraying. Of the 232 household heads that answered this question, almost 19% said they didn't know and 33% said that there was no difference. However, interestingly, almost 37% said there were fewer mosquitoes after agricultural sprayings. Almost 11% said that there were more mosquitoes (Table 10).

Malaria clinics (66%) and hospitals (29%) were the most common places where respondents went for malaria treatment. Three of the respondents (1.3%) went to a traditional practitioner for treatment. More than 90% stated they took the full course of treatment (Table 11).

As preventive measures against malaria (or mosquitoes) 83% of all respondents said they used impregnated bed nets, 13% used non-impregnated bed nets. Other measures of prevention were, in order of importance, cleaning around the house (11%), cleaning of water streams (5%), spraying inside and outside of house (14%), commercial repellents (18%), mosquito coils (35%). and 15% wore long-sleeved shirts (Table 12). 83% of the Kanchanaburi respondents used bed nets in their households. One to three bed nets were the most common numbers of bed nets used in household heads' homes (76%) (1 net = 20%, 2 nets = 38%, 3 nets = 21%, 4 nets = 17%). Two bed nets were the most common number of bed nets used in the household heads' homes (38%) (Table 13).

**Malaria Risk Factors**

Only 101 out of 232 household heads, almost 44%, reported that they sometimes sleep in a field hut. It was most common to stay once a year in the field hut (Table 14).

Eighteen percent household heads stayed in the field hut during the cold season (Nov.-Feb.) and fifty (22%) during the hot season (Mar.-Jun.). The most common way to protect oneself during stays in the field hut was to use long sleeves, but other protection measures were also used, such as non-impregnated and impregnated bed nets were commonly used. Insecticide commercial repellents (28%), and mosquito coils (15%) were also used. Moreover it was also common to make a fire for protection against mosquitoes.

Of the 117 household heads that answered the question on how far their house is from water, e.g. stream, lake, pond, etc., almost 39% estimated the distance to less than 100 m (45/117=39%). Of 142 household head answers, 53% estimated the distance from their house to a forest to less than 100 m ( 75/142=53%). Of 143 household head answers, 63% estimated the distance from their house to an agricultural field to less than 100 m ( 90/143=63%). Of 138 household head answers, 60% estimated the distance from their house to a fruit orchard to less than 100 m (83/138=60%).

The majority of houses of household head, about 76% were made out of wood. Other house construction materials were often used in combinations with wood, such as bamboo, concrete, and corrugated sheet. 36% of houses were 5-10 years old, 31% were 1-5 years old, 28% were more than 10 years old. Only 5 percent of houses were constructed during the last year. 55% of 232 household heads said that their houses had some form of window screening.

More than three quarters of all 232 households had animals around their houses. The most common animals kept were chicken (40%) and pigs (5.4%). About 36% of households had cows around their houses. Other reported animals were ducks and buffaloes. Cattle were kept close to houses during the night, was slightly more common in Kanchanaburi.

Dogs 60% and cats 40% were the most common domestic animals kept by all households. Keeping domestic animals was common in Kanchanaburi.

In 57% of all households animals were kept inside houses. This was slightly more common in Kanchanaburi (62% of households), cats (36%) and dogs (32%) were the most common animals kept inside houses, and other animal were chicken and other birds (35%).

**Pesticides for agricultural plant protection**

The specific information on agricultural pesticide used was assign for interviewing the 20 most important farmers in each village by questioned using Questionnaire 2. However this work could not successful for the specific number of 20 for most important farmers in each village because it was up on the problems that found on that time. The first village, MNN, had only 1 big farmer because almost of the households were rubber plantation workers. BTN and PT were found only 10 big farmers in each of them because they quite small villages. Whereas UL was a very big village with more than one thousand populations and more than 25 big farmers in the village (interviewed 26 big farmers), 12 and 15 big farmers were interviewed in HPK and TNK respectively.

The results of the used pesticides survey from each village were shown that BTN was the highest chemical used area with almost 11% pyrethroid, 10% organophosphate and 8% carbamate respectively and with the highest other pesticides used (43%). The 43% of other pesticides used compost with 3 groups of insecticides, biocides and insect hormone as shown on table 4.2 and figure 2-3. MNN was the lowest chemical used area with 2 other insecticides (copper and fangkuran) and 1 biocide (white oil). The highest herbicides use found in UL, 38/224 chemicals used = 42% (Table 16 and Table 17, Figure 19, 20 and 21).

**Part 2**; **Biting peak and population dynamics of *Anopheles minimus* species A, from high and low agricultural insecticide area in the two villages at Kanchanaburi Province, Thailand.**

**Adult**

This study showed that there were lower densities of mosquitoes in the high-pesticide location (BTN) compared to the low-pesticide location (MNN) throughout three seasons. The reason for this phenomenon was possibly because of intensive use of agricultural insecticides used in the BTN location.. The effect of mosquitoes to insecticides in the BTN area may be cause from avoidance behavior of them that developed to insecticides used in both agricultural and vector control. Avoidance behavior in mosquitoes to insecticides was first described by Kennedy (1946). Irritancy, a result of physical contact with insecticide treated surfaces, was recognized even before the use of insecticides to control vector mosquitoes. Subsequent observations indicated that some insecticides also could induce a repellent effect, without actual physical contact with a treated surface. Repellency effects to insecticides used in malaria control have been reported in several anopheline species (Roberts and Alecrim, 1991, Roberts *et al*., 1997, Chareonviriyaphap *et al*., 1997). Overgaard *et al*. (2003) showed that the density and diversity of anopheles mosquitoes decrease as the area of fruit orchards increase. These reasons could have been important factors for the lower density of females observed in BTN compared to MNN. *An. minimus* A in Kanchanaburi province is normally abundant throughout the year (Department of Communicable Disease Control 1985-2001) as seen in the populations studied in MNN. The temporal variations of number of females caught from the MNN sites were similar to previous studies (Ismail *et al*., 1974; 1978, Ratanatham *et al*., 1988; Suwonkerd *et al*., 1995; Rwegoshora *et al*., 2002 ; Chareonviriyaphap *et al*., 2003). Mosquito densities increased from May and July and reached the highest at the end of rainy season (September) and early cool (November) season. The lowest densities were in the hot season (April). On the other hand, the combined mosquito female densities in the BTN sites had two peaks. The highest peak was in the late hot season (June) and the second, slightly smaller, peak was in the rainy season (October). Low numbers or no females were caught in the cool and early hot seasons (November to April) (Figure 22). The reasons for this phenomenon could possibly be the strategy of agricultural pest control in the village. To control insect pests in agricultural crops, the farmers in Bong Ti Noy village usually spray pesticides at the onset of the dry season or after the rainy season (November to April) (Pothikasikorn *et al*., unpublished data), to protection the spring of their crops from the pests attached. After the cool season until the onset of the hot season, the crops were harvested and the weather was too dry and hot, cause of decreasing of the pests and chemical used in the areas, hence the density of mosquitoes was appeared. However, other drastic environmental changes have also occurred in this location, such as deforestation, human settlement, and agricultural activities. These changes very likely affect the bionomics of anopheline mosquitoes, especially *An. minimus*, as suggested by Ismail *et al*. (1978) and Rao (1984).

Results of the biting cycle of this study showed no significant differences between MNN and BTN for all three seasons and three sites. The biting cycle showed two peaks throughout the night, between 20.05-23.00 hour and 01.05-02.00 hour. The blood feeding activity of *An. minimus* has been reported by several authors in Thailand. In Mae Tha Waw village, Tak province, this species exhibited a feeding activity throughout the night with peaks between 21.00-22.00 hours (Harbach *et al*., 1987). Ratanatham *et al*., (1988) reported two feeding peaks for *An. minimus* collected in Pakchong district, Nakhon Ratchasima province; the first and largest peak occurred during early evening (19.00-22.00 hours), and a second much weaker peak occurring at about 05.00 hours in the morning. Rattanarithikul *et al*. (1996) also reported two outdoor feeding periods for *An. minimus* from southern Thailand, one beginning from 18.00 to 23.00 hours, and a second, more moderate, peak beginning at 01.00 hours and declining throughout the second half of the night. Chareonviriyaphap *et al*. (2003) showed feeding activity in Ban Pu Tuey with two peaks of activity were seen in indoor and outdoor collections, regardless of season. The first peak was seen immediately after the sunset (18.00-19.00 hours) and the second peak was at dawn (05.00 hours). However, the feeding pattern in the present study (20.05-23.00 hours) was very similarly when compared with the broad time period (18.00-22.00 hours) of increased activity seen by Ratanatham *et al*. (1988). It seems that agrochemicals did not influence the biting cycle of *An. minimus* A female in these areas.

**Larvae**

From previous research, Hall *et al*. (1998) reported that, generally, there is an increase in pollution and a decline in species richness in catchments with increasing levels of human development. In this study, no significant difference in abundance of *An. minimus* A larvae was observed among the two locations, although the locations were different in the intensity of agrochemical use. Possibly, the selected breeding sites in BTN are situated more than 1 km from agricultural crop and fruit cultivation and there are large continuous areas of forest and grove. Moreover, both locations, MNN and BTN, were quite similar in plant growth-form in the breeding sites with aquatic, riparian and emergent plants present, although there were differences in the species. From several previous studies it has been shown that breeding habitat characteristics were crucial for mosquito population dynamics. Teng *et al*. (1998) suggested that many biotic and abiotic factors such as the water pollution were influence to the activity and efficacy of the mosquito larva density. How ever the unexplained 50% of the variation might be attributed to aquatic plants that provide shelter from flooding and predators (Savage *et al*., 1990). Muirhead Thomson (1940a) reported shade as an important factor for *An. minimus* larval presence. The result from this study indicated that differences in breeding habitat plant species did not affect average larval density between the two locations (figure 8). Another study reported that plant growth-form might be more important than plant species (Overgaard *et al*., 2002).

This study observed that the larval density of *An. minimus* species A was affected by amounts of rainfall (Table 8) and stream velocities in each season. There was high density in the late rain and cool seasons with stream velocities between 0.025 - 0.092 m/s and low densities during late cool to hot season within an optimum of velocities was 0.017 m/s. The average larval density fluctuated similarly throughout the year in the two villages. There were no larvae collected in BTN during August, the middle of rainy season, when the maximum stream velocities were more than 0.42 m/s. Muirhead Thomson (1940b) reported that *An. minimus* occurs in streams with velocities between 0.015 and 0.27 m/s, with in an optimum range of 0.015 and 0.165 m/s and showed that the mean flushing point for full-grown larvae was only 0.087 m/s. Overgaard et al. (2002) found that the most important single variable associated with larval density was current velocity. Thus, a plausible explanation for the relationship between mosquito larvae and water coverage of the streams in this study could be the amount of water and stream velocities decisively influencing the seasonal occurrence of *An. minimus* A larvae. Too much rain in the middle of the rainy possibly lowered the larval density by flooding or fast currents of water, while too little rain late in the dry season also lowered it by reducing the available larval habitat (Suwonkerd *et al*., 1995).

From this study, decreasing of mosquitoes to insecticides in the BTN area caused by avoidance behavior to insecticides used in both agricultural and vector control was very importance for the scientific knowledge to guiding principle in outlining innovative strategies to better implementation and effectiveness of the existing malaria control and in suggesting future research directions.

**Part 3; Behavioral responses by *Anopheles minimus* species A and C to three agrochemicals.**

Although behavioral responses to test compounds by malaria vectors have long been recognized, true chemical responses and important role of behavioral avoidance remain unclear. Until recently, a mathematical framework for understanding the repellent, irritant and toxic functions of chemicals to control diseases have been quantified (Roberts *et al*., 2000). Since then, several studies on clear behavioral responses by malaria vectors to public health compounds are progressively reported (Chareonviriyaphap *et al*., 2001; 2002;2003; 2004; Sunvornyouthin *et al*., 2002; Kongme *et al*., 2004; Pothikasikorn *et al*., 2005; Chareonviriyaphap *et al*., 2006; Stantripop *et al*., 2006). Apart from public compounds, several agrochemicals may exert behavioral responses to several insects (Roberts *et al*., 1994). There is lack of information as to how malaria vectors responds to agrochemicals currently used to protect agricultural crops. Irritability and repellency responses were quantitatively assessed using excito-repellency test system developed by Chareonviriyaphap *et al*. (2002).

This study represents the first report on the behavioral responses of two test populations of Minimus group, species A and C, to agrochemicals currently used in Thailand and two main avoidance responses: irritancy and repellency (excito-repellency) were documented. Differences in escape rate between the two species when exposed to the operational field doses of three agrochemicals were observed. In general, greater irritancy and repellency responses to carbaryl and cypermethrin were observed in species C than in those in species A. In contrast, malathion produced a higher irritancy and repellency responses in species A than C. Although differences in escape patterns, both contact irritancy and non contact repellency are involved in *An. minimus* escape responses. We know that both species were collected from different land used areas. Specifically, species A was collected from the village and forest fringe areas. This area is considered as a low level of an agrochemical insecticide use (A1 area or high risk area for malaria). Species C was obtained from low hill zone along the village margins and deemed a high level of agrochemical insecticide use area or lower risk area for malaria (A2) (Pothikasikorn *et al*. unpublished paper). In addition to agrochemicals, deltamethrin and malathion are being used in both areas to control malaria and dengue with the greater amount in the A1 area than those in the A2 area (Ministry of Public Health, 2005).

There is no clear explanation on the differences in rate of escape responses to agrochemicals between species A and C. However, it seems that great response of species A in both contact and non contact to malathion could be a certain extent from the routine residual chemicals of malathion being sprayed more frequently in the more disease–prone area. Previous report documented great repellency responses of species A to DDT and some pyrethroids in the area where routine residual chemical was applied (Pothikasikorn *et al*., 2005). In addition, differences in mosquito species, surrounding environment, ambient temperature during test, mosquito genetic background and time of the test could be significant factors affecting behavioral avoidance between two test species. Similarly, greater irritancy and repellency responses to carbaryl and cypermethrin in species C than species A could be from the more numerous exposure to routine use of species C to those test compounds that were used to protect crops. Species C was collected from the high agricultural area with high frequent uses of agrochemicals compared to species A. Great escape responses of specie C in the presence of cypermethrin and carbaryl may have evolved gradually as adaptations for avoiding toxic substance from the area (Chareonviriyaphap *et al*., 1997).

Most specimens from both species departed the treated surfaces and chambers before acquiring a lethal dose of test compounds (>79% of escape specimens survived). This indicated all three test compounds demonstrated strong excito-repellency function, not killing function. Prominent repellency function of carbaryl (80% escaped) and cypermethrin (63% escaped) in species C indicate the successful adaptation of this species to avoid the toxic substances. Strong and ambiguous behavioral responses in anopheline mosquitoes have been reported elsewhere (Pothikasikorn *et al*., 2005), indicating the limitation of physiological resistance in this mosquitoes as several previous published reports (Chareonviriyaphap *et al*., 2001; Sungvornypthin *et al*., 2001; Pothikasokorn *et a*l., 2005).

**Part 4; Behavioral responses by *Anopheles minimus* species A and species C to DDT and pyrethroids.**

In Thailand, *An. minimus* complex is comprised of at least two known species, species A and C, and both are important vectors of malaria in Thailand (Ayurakit-Kosol and Griffith, 1963; Sucharit *et al*., 1988; Green and Munstermann, 1990; Harrison, 1980). After DDT was introduced for malaria control in 1949, *An. minimus* reportedly became predominately an outdoor-feeding species (Nutsathapana *et al*., 1986), although it appears that feeding behavior varies depending upon geographic distribution. Thus, insecticides may have little to do with any purported genetic selection or shift from an indoor to outdoor-feeding behavior (Ratanatham *et al*., 1988). The failure to completely interrupt malarial transmission by *An. minimus* s.l. might be related to the behavioral diversity and innate response to insecticidal intervention ((Nutsathapana *et al*., 1986; Ismail *et al*., 1974).

Studies have attempted to quantitatively describe and resolve the ecoethologic differences (Ismail *et al*., 1978; Ratanatham *et al*., 1988; van Bortel *et al*., 1999; Rwegoshora *et al*., 2002; Ismail *et al*., 1975) genetic composition and diversity (Sucharit *et al*., 1988; Green *et a*l., 1990; van Bortel *et al*., 1999; Ismail *et al*., 1975; Sucharit and Choochote, 1982; Thanaphom *et a*l., 1990; van Bortel *et al*., 2000) and responses to intradomicilary use of DDT (Ismail *et al*., 1975) in this species complex. Experiments using recently colonized *An. minimus* species A exposed to deltamethrin clearly demonstrated the two primary avoidance responses: irritancy and repellency (excito-repellency) (Chareonviriyaphap *et al*., 2001). In this study, to compared both behavioral responses in the two sibling species of *An. rninimus* in Thailand to three different residual insecticides used in public health with hopes that such information will facilitate targeting of specific malaria vectors and increase the effectiveness of vector control activities.

After observed unambiguous behavioral avoidance responses in *An. minirnus* species A and C using an excito-repellency test system (Chareonviriyaphap *et al*., 2002). All three insecticides produced rapid and striking irritancy in both sibling species. Moreover, very strong repellency responses to each compound were observed in *An. minimus* species A. Repellency reactions were similar to those of a recent laboratory colony of *An. minirnus* species A from northern Thailand, which showed > 75% repellency to deltamethrin (Chareonviriyaphap *et al*., 2001). Repellency responses were relatively weak in *An. rninimus* species C, yet still significantly greater than the paired controls for all cases. Similarly, weak repellency of *An. minimus* species C from Pu Teuy Village (approximately 95% were confirmed as species C) to the three compounds was previously observed (Chareonviriyaphap *et al*., 2001). *Anopheles minimus* complex from Pu Tuey village in Kanchanaburi Province was exposed to operationally standard concentrations of DDT (2 $g/m^2$) and established medium lethal doses ($LD_{50}$) of deltamethrin and lambda-cyhalothrin that produced poor repellency activity (Chareonviriyaphap *et al*., 2001). The relative inability to detect chemical signals or odors without physical contact with insecticide in *An. minimus* species C may be driven by evolutionary processes different from those in species A. Since 1990, Pu Teuy village has been considered a low-risk area for malaria, which has resulted in routine residual chemicals being applied more sparingly compared with more malaria-prone areas of the country such as Mae Sot District (Department of Communicable Disease Control, 2004, unpublished data). The differences in proportion of total houses sprayed with insecticides (i.e., insecticide exposure pressure) could be a factor affecting the avoidance behavior of these two closely related species.

One of the key components in preventing malaria transmission has relied mainly on methods that interrupt human vector contact. (Evans, 1993; Robert and Andre, 1994; Roberts *et al*., 1984). Insecticides that have strong irritant and repellency attributes on vectors can perform this function without necessarily having to kill the mosquito to interrupt transmission. Repellency to insecticides in vectors has been recognized in several *Anopheles* mosquitoes (Lien, 1991; Chareonviriyaphap *et al*., 1999; 1997; Sungvornyothin *et al*., 2001; Kongmee *et al*., 2004; Roberts *et al*., 1984; 2000; Chareonviriyaphap *et al*., 2004). Compared with contact irritancy, this type of avoidance behavior could mitigate even more against selection of insecticide resistance in mosquito populations.

*Anopheles minimus* species A in Thailand, has been subjected to routine intradomicilary DDT spraying to interrupt malaria transmission for decades. DDT was applied either once or twice a year, especially in malaria-endemic areas of western Thailand. Although DDT was used for many years, no evidence of physiologic resistance has been detected in the *An. minirnus* complex. Showed that innate behavioral avoidance of insecticide-sprayed surfaces by mosquitoes has, and continues to play, a significant role in delaying or preventing resistance from developing. These findings confirm that strong behavioral avoidance of chemical residues is due to excito-repellent properties of these compounds and most likely contributes to interruption of feeding by mosquitoes and transmission of malaria.

These findings indicate differences in behavioral responses between two species of the *An. rninimus* complex in Thailand. All of these important observations can help explain some of the varying effectiveness of indoor residual spraying in various regions in Thailand. It is the understanding of behavioral avoidance and an appreciation for excito - repellency that indicate an important set of properties of residual insecticides and how they function to control disease transmission apart from contact toxicity alone.

# CONCLUSION

**Part 1; Risk factors and base line malaria knowledge from different pesticide Land-use systems in malaria endemic area at Kanchanaburi Province,Thailand.**

The findings of this study showed that persons in Kanchanaburi province were mixed with 6 ethnic groups. The largest ethnic group among the questioned household heads were Thai (55%). Other reported ethnic groups were Burmese, Mon, Karen, Lao, and Mong in Kanchanaburi province. A total of 232 persons responded to the questionnaires, sixty-six percent, 155 of all respondents, were household heads and 34% were closely related to household heads, most of them being either a wife or a child. A total of 206/232, or 87%, knew about the disease malaria, and 11% did not know of the disease. Almost 44% reported that they sometimes sleep in a field hut. It was most common to stay once a year in the dry season. More than three quarters of all 232 households had animals around and in side their houses. The most common animals kept were dogs, cats, cows, buffaloes, chicken and pigs (5.4%).

After pesticides used in agricultural, found that the mosquitoes were decrease. The results of the used pesticides survey from each village showed that BTN was the highest chemical used area with almost 11% pyrethroid, 10% organophosphate and 8% carbamate respectively and with the highest other pesticides used (43%).

**Part 2**; **Biting peak and population dynamics of *Anopheles minimus* species A, from high and low agricultural insecticide area in the two villages at Kanchanaburi Province, Thailand.**

From this research results, there were lower densities of mosquitoes in the high-pesticide location (BTN) compared to the low-pesticide location (MNN) throughout 3 seasons with highly significant. On other hand, the biting cycle of this study showed no significant different between MNN and BTN all of the three seasons and the three sites. The biting cycle was showed with two peaks between 18.05 - 23.00 and 24.05-02.00 hours throughout the night.

This study observed that the larval density of this species provided by amounts of rainfall and velocities in each season, high density in late rain and cool with velocities of stream between 0.025 - 0.092 m/s and decreasing during late cool to hot season within an optimum 0.017 m/s, but no larvae from BTN on August, the middle of rainy season with the maximum of stream velocities was over than 0.42 m/s.

The average larval density fluctuated similarly in two selected village locations through out the year, because the selected breeding sites in BTN are situated more than 1 km from agricultural crop and fruit cultivation and there are large continuous areas of forest and grove between, hence of less or no insecticides residue in the breeding sites.

**Part 3; Behavioral responses by *Anopheles minimus* species A and C to three agrochemicals.**

Behavioral responses of two wild caught populations on *Anopheles minimus* species A and C to operational field doses of three agricultural compounds, carbaryl, malathion and cypermethrin, were characterized using excito-repellency test system. Species A was collected from human bait at Mae Nam Noi Village Thong Pha-Phoom District whereas species C was obtained from cow bait at Pu Teuy Village, Sai Yok District, Kanchanaburi Province, western Thailand. Specimens from two strains were quickly escaped from direct contact with treated surfaces from three insecticides compared to the pair controls. Noncontact repellency response to cypermethrin and carbaryl was significantly pronounced in species C ($P<0.05$) whereas it was comparatively weak when treated against malathion. We conclude that contact irritancy is a major behavioral response of both strain when exposed directly to all three compounds whereas non contact repellency to cypermethrin plays a significant role in escape response in species A.

**Part 4; Behavioral responses by *Anopheles minimus* species A and species C to DDT and pyrethroids.**

Behavioral responses of two field populations of *Anopheles minirnus* complex species A and C for contact and non-contact actions of chemicals were compared during and after exposure to operational field concentrations of DDT (2 $g/m^2$), deltamethrin (0.02 $g/m^2$), and lambda-cyhalothrin (0.03 $g/m^2$) using an excito-repellency escape chamber. The two populations were collected from the Mae Sot District in Tak Province (species A) and the Sai Yok District in Kanchanaburi Province (species C) in western Thailand. Female mosquitoes of both populations rapidly escaped from chambers after direct contact with DDT, deltamethrin, and lambda-cyhalothrin. The non-contact repellency response to DDT and the two synthetic pyrethroids was pronounced with *An. minimus* species A; however, non-contact repellency was relatively weak with *An. rninimus* species C, but remained significantly greater than the paired controls ($P < 0.05$). We conclude that strong contact irritancy was present in both test populations, whereas non-contact repellency also played a significant role in the escape response of *An. minimus* species A.

# RECOMMENDATION

The following recommendations listed below will help improve this research project.

1. The questionnaires from part 1 should include the design on pair-matched case-control design by Mantel and Haenszel methods (1959) and all variables have to be analyzed by the method of multiple logistic regression. Because this research was designed to describe only basic survey information by description analysis.

2. For the comparison purpose, the study of behavioral avoidance by mosquitoes to several test chemicals (parts 3 and 4) should be conducted on the same species.

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# APPENDIX

## Appendix part1.1 Stratification of Malarious Areas

The Thai Malaria Control Programme stratifies the country into 4 categories :

(1) Control area with transmission (referred to as A)

This category is divided into **A1** and **A2**

- Perennial transmission area, where transmission is reported throughout the year or at least 6 months per year, is designated as $\mathbf{A_1}$
- Periodic transmission area, where transmission is reported 5 months or less per year, is disignated as $\mathbf{A_2}$

(2) Control area without transmission (referred to as**B**)

This category is divided into two subcategorized, namely $\mathbf{B_1}$ and $\mathbf{B_2}$.

- High risk are $\mathbf{B_1}$, transmission was not reported within the last 3 years but primary of secondary vectors are found. Consequently, the area is potentially suitable for malaria transmission (high and moderate receptivity).
- Low risk areas $\mathbf{B_2}$, transmission was not reported within the last 3 years and both primary and secondary vectors are not found. Suspected vector, however, may be found (low and non receptivity).

# Appendix part1.2. Questionnaire 1

# QUESTIONNAIRE 1 (Q1)

## 1. General information

Q1 No._______

1. Date:_______________________________

2. Address / Location: House no.: _______ Village: __________________

   Tambon:____________ Amphoe: _____________ Province: ____________

3. Respondant's name:______________ □ Male □ Female. Age:____________

4. Is respondant the head of the household? □ Yes □ No

***If "No", then ask the questions in the box below. Otherwise continue to Question 9.***

Please try to interview household heads only!

5. What is your relation with household head? I am the

   □ Husband □ Wife □ Father □ Mother □ Other: ……….………………

   of the household head.

6. Name of household head:___________________ □ Male □ Female

7. Age of household head: ____________________

8. What is the main occupation of household head? *(multiple choice possible)*

   □ Farmer □ Business □ Trader □ Merchant □ Housewife

   □ Government service □ Forest activity □ Factory □ Office

   □ Unemployed □ Other: ……………………….………………..

9. What is your ethnic group?

   □ Thai □ Karen □ Akha □ Mong □ Chinese □ Other:……………

10. No. of adults and children in household: Adults: ______Children: ______

11. How long have you lived in this village:

□ Less than 1 year □ 1-5 years □ 5-10 years □ More than 10 years

12. What is your main occupation? *(multiple choice possible)*

□ Farmer □ Business □ Trader □ Merchant □ Housewife

□ Government □ Forest activity □ Factory □ Office □ Unemployed

□ Other:…………………..

*If "Farmer" go to Questionnaire 2 after finishing Questionnaire 1.*

13. Name the 3 biggest problems in your village:

1………………………………………………………………………………

2………………………………………………………………………………

3………………………………………………………………………………

14. Name the 3 most important diseases in your village:

1………………………………………………………………………………

2………………………………………………………………………………

3………………………………………………………………………………

15. Do you ever travel outside of your village? □ Yes □ No

***If "Yes", then ask the questions in the box below. Otherwise continue to Question 21.***

16. How often do you travel outside village?

□ Less than once per year □ Once per year □ Twice per year

□ 3-6 times per year □ More than 6 times per year

17. Where do you travel?

□ Within tambon □ Within amphoe □ Within province □ Other provinces

□ Outside country (name country) ………………………………..

18. For how long time do you usually stay outside village?

□ Less than one week □ 1-4 weeks □ From 1-6 months

□ More than 6 months □ Other ……………………………

19. For what reason do you travel outside village?

□ Work (Labor) □ Purchases □ Visit relatives/friends □ Leisure

□ Business (what kind?) …………………………………………….

□ Other ………………..…………………… □ Unwilling to answer

20. If for work, what kind of work do you do outside village?

……………………………………………………………

□ Unwilling to answer

**2. Malaria Knowledge**

21. Do you know the disease malaria? □ Yes □ No

***If "Yes", then ask the questions in the box below. Otherwise continue to Question 33.***

22. Which malaria symptoms can you mention? *(multiple choice possible)*

    □ Fever □ Headaches □ Shivering □ Muscle pain

    □ Nausea/vomiting □ Others: ………………………………

23. Have you ever had malaria? □ Yes □ No ***(If "No" continue to Question 30)***
24. If "Yes", how many times? □ Once □ 2-3 times □ More than 3 times
25. When was the last time?

    □ Within last 3 months □ Between 3-12 months ago

    □ Between 1-5 years ago □ More than 5 years ago

26. When you got malaria <u>the last time</u> where were you treated?

    □ Malaria clinic (MC) □ Health clinic□ Hospital

    □ Traditional medical practitioneer □ Treat yourself □ No treatment

    □ Other …………………………………………………………

27. How far from your home is the place where you got treated (in km)? -----------
28. Did you take the full course of treatment? □ Yes □ No
29. How is malaria transmitted? □ Mosquito bite □ Don't know □ Other…………..
30. In what season is malaria transmitted? *(multiple choice possible)*

    □ Rainy season (June-October) □ Cold season (November-February)

    □ Hot season (March-May)

31. When do malaria mosquitoes bite? *(multiple choice possible)*

    □ Early morning (4.00-6.00) □ Day time (6.00-16.00)

    □ Early evening (16.00-20.00) □ Late evening (20.00-24.00)

    □ Night time (00.00-04.00) □ Don't know

32. Where do malaria mosquitoes breed? *(multiple choice possible)*

    □ Stagnant clean water □ Water reservoirs □ Slow-moving streams

    □ Rice fields □ Ground pools □ Others:………….

    □ Don't know

33. Do you know the difference between *Anophelines* (Yung Konplong) and *Culicines* (Yung Lai) by sight? □ Yes □ No

34. Which preventive measures against malaria (or mosquitoes) do you use? *(multiple choice possible)*

| | | |
|---|---|---|
| Cleaning of water streams | □ Yes | □ No |
| Cleaning around the house | □ Yes | □ No |
| Using impregnated mosquito bednets | □ Yes | □ No |
| Using non-impregnated mosquito bednets | □ Yes | □ No |
| Use of cloth(long sleeve) for prevention | □ Yes | □ No |
| Spraying inside and outside the house | □ Yes | □ No |
| Use commercial repellent | □ Yes | □ No |
| Use of mosquito coils | □ Yes | □ No |

Use traditional repellents (give details) ………………………………………..

Others: ……………………………………………………………………………

35. Are there more or less mosquitoes inside your house after application of insecticides in your fields?

□ More □ Less □ No difference □ Don't know

36. Additional information on malaria and mosquitoes given by respondant (optional)

______________________________________________________________

______________________________________________________________

## 3. Risk factors

### 3.1. Work related

37. How often do you sleep in a field hut?

□ Never □ Once a month □ 2-3 nights/month □ 4 or more times/month □ 1 or more times per week □ Other ……………

***If "Never" continue to Question 41.***

38. What season do you sleep in a field hut? *(multiple choice possible)*

□ Rainy season (June-October) □ Cold season (November-February)
□ Hot season (March-May)

39. How do you protect yourself from mosquito bites when you sleep in the field hut?
*(multiple choice possible)*

| | | |
|---|---|---|
| Using impregnated mosquito bednets | □ Yes | □ No |
| Using non-impregnated mosquito bednets | □ Yes | □ No |
| Use of cloth(long sleeve) for prevention | □ Yes | □ No |
| Spraying inside and outside the house | □ Yes | □ No |
| Use commercial repellent | □ Yes | □ No |
| Use of mosquito coils | □ Yes | □ No |

Use traditional repellents (give details) …………………………………………..
………………………………………………………………………………………
Others: ………………………………………………………………………………

40. How often do you sleep in the forest?

□ Never □ Once a month □ 2-3 nights/month
□ 4 or more times/month □ 1 or more times per week □ Other ………

***If "Never" continue to Question 44*.**

41. What season do you sleep in the forest? *(multiple choice possible)*

□ Rainy season (June-October) □ Cold season (November-February)

□ Hot season (March-May)

42. Do you use a bed net when you sleep in the forest?

*(multiple choice possible)*

| | | |
|---|---|---|
| Using impregnated mosquito bednets | □ Yes | □ No |
| Using non-impregnated mosquito bednets | □ Yes | □ No |
| Use of cloth(long sleeve) for prevention | □ Yes | □ No |
| Spraying inside and outside the house | □ Yes | □ No |
| Use commercial repellent | □ Yes | □ No |
| Use of mosquito coils | □ Yes | □ No |

Use traditional repellents (give details) ………………………………………..

…………………………………………………………………………………

Others: …………………………………………………………………………

3.2. Living area

43. Distance to water (irrigation stream/scheme/river/lake/wetland)

□ Less than 100 meters □ More than 100 meters

44. Distance to forest □ Less than 100 meters □ More than 100 meters

45. Distance to agricultural field □ Less than 100 meters □ More than 100 meters

46. Distance to fruit orchard □ Less than 100 meters □ More than 100 meters

47. Housing construction

□ Wood □ Bamboo □ Concrete □ Other: ………………….……

48. When was your house constructed?

□ During the last year □ 1-5 years ago □ 5-10 years ago

□ More than 10 years ago

49. Screening of windows? (multiple choice possible) □ Yes □ No

50. What kind of screening of windows?

□ Window curtain □ Mosquito netting □ Pane

□ Other ……………………………

51. Availability of mosquito bednets in household? □ Yes □ No

52. Number of mosquito bednets used in household? ______________

53. How many persons sleep in the house during the night? ______________

No. of children__________________ No. of adults ______________

3.3. Animals near or in the house

54. Are there animals around the house? □ Yes □ No

***If "No" continue to Question 58.***

55. What kind? *(multiple choice possible)*

□ Cows □ Buffalo □ Pigs □ Chicken □ Ducks

□ Others: ……□ Domestic animals: □ Dog □ Cat □ Birds □ Others……..

56. Do cattle (cows/buffaloes) stay around the house at night? □ Yes □ No

57. Are there animals in your house? □ Yes □ No

***If “No” finish interview.***

58. What kind? *(multiple choice possible)*

□ Domestic animals: □ Dog □ Cat □ Birds □ Others……………………..

# Appendix part1.3. Questionnaire 2

# QUESTIONNAIRE 2 (Q2)

Q2 No.______

**General information**

1. Date:________________________________

2. Address / Location**:** House no.: __________Village: _________________

Tambon: _______________Amphoe: _____________Province: _____________.

3. Respondant's name:____________________ □ Male □ Female

**Crop information**

4. What are your three most important crops (most important in terms of income)?
   1. ……………………………………………□ annual or □ perennial
   2. ……………………………………………□ annual or □ perennial
   3. ……………………………………………□ annual or □ perennial

5. What are your three major PERENNIAL (fruit) crops (in terms of area)?

***Crop P1 has the largest area; crop P2 the second largest and crop P3 the third largest.***

| **PERENNIAL CROP** | **P1** | **P2** | **P3** |
|---|---|---|---|
| Crop | | | |
| Area (rai) | | | |
| Age (months or yrs) | | | |
| Yield (kg/rai) | | | |
| Harvest time (which month) | | | |
| Pesticide information | Details in question 7 | Details in question 8 | Details in question 9 |

P=Perennial

6. What are your three major ANNUAL crops (in terms of area)?

***Crop A1 has the largest area; crop A2 the second largest and crop A3 the third largest.***

| ***ANNUAL CROP*** | **A1** | **A2** | **A3** |
|---|---|---|---|
| Crop | | | |
| Area (rai) | | | |
| Age (months or yrs) | | | |
| Yield (kg/rai) | | | |
| Harvest time (which month) | | | |
| Pesticide information | Details in question 10 | Details in question 11 | Details in question 12 |

A=Annual

**Pesticide information** (Continue on pages 129)

Page no..........

Date..................................

Respondant name............................................................

Pesticide used in............................................................

| No. | Brand name | AI (%) | Target pest org. | When applied (month) | How often applied | Quantity applied | Mixed with | How applied | Additional inf. |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | |