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).