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.