

BOTTLE AND BIOCHEMICAL ASSAYS ON TEMEPHOS RESISTANCE IN *AEDES AEGYPTI* IN THAILAND

Visut Saelim¹, William G Brogdon², Jirasak Rojanapremsuk³, Saravudh Suvannadabba⁴, Wongdyan Pandit³, James W Jones⁵ and Ratana Sithiprasasna⁵

¹Pattani Provincial Public Health Office, Pattani, Thailand; ²Vector Biology and Toxicology Section, National Center for Infectious Diseases, Center for Disease, Control and Prevention, Atlanta, GA, USA; ³Faculty of Public Health, Mahidol University, Bangkok, Thailand;

⁴Department of Diseases Control, Ministry of Public Health, Nonthaburi, Thailand;

⁵Department of Entomology, US Army Medical Component, Armed Forces Research Institute of Medical Sciences, Bangkok, Thailand

Abstract. The bottle bioassay measuring the time-mortality rate is a simplified procedure for detecting insecticide resistance. It can be used with a biochemical microplate assay to identify the mechanism involved. This integrated approach was used to detect temephos resistance in *Aedes aegypti* from Nonthaburi (lowest use) and Roi Et (highest use). *Ae. aegypti* BKK1 laboratory strain was used as the susceptible reference strain. The appropriate concentration of insecticide for bottle bioassay was determined empirically for *Ae. aegypti* BKK1 strain and found to be in the range of 800-1,050 µg/bottle. The time-mortality rate at 800 µg/bottle was 170±8.66 minutes, significantly different from the time-mortality rates in the 850, 900, 950, and 1,050 µg/bottle ($p=0.008$) concentrations, which were 135±15.00, 140±8.66, 135±15.00, and 125±8.66 minutes, respectively. The cut-off concentration selected for resistance detection was 850µg/bottle. The time-mortality rate for the Roi Et strain was 382±26.41 minutes, significantly higher than the Nonthaburi (150±25.10 minutes) and BKK1 strains (145±20.49 minutes) ($p<0.001$). The temephos resistance ratio (RR_{100}) for the *Ae. aegypti* Roi Et strain was 2.64-fold higher at lethal time (LT_{100}) than for the reference *Ae. aegypti* BKK1 strain. The mean optical density (OD) value from the biochemical microplate assay for the non-specific esterase of the Roi Et strain was higher than the mean OD for the non-specific esterase of both the Nonthaburi and BKK1 strains. Insensitive acetylcholinesterase was not found to be responsible for the resistance in the field-collected mosquitos. This study suggests that esterase detoxification is the primary cause of resistance in the *Ae. aegypti* population from Roi Et. Both the bottle bioassay and the biochemical microplate assay were proven to be promising tools for initial detection and field surveillance for temephos resistance.

INTRODUCTION

Dengue fever (DF) and its most serious complications, dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS), is one of the most important arboviral diseases worldwide. In Thailand, DHF was first reported in 1949. In recent years, increasingly larger outbreaks have occurred, causing a major public health problem. There were 60,330, 37,929, 99,410,

126,348, 24,826, and 17,582 cases of DF/DHF reported by the Epidemiological Division, Ministry of Public Health (MOPH) during the years 1995-2000, respectively. Important vectors in Thailand are *Aedes aegypti* and *Ae. albopictus*. The former is thought to be the vector in more urban areas, whereas the latter is the vector in rural areas. *Ae. aegypti* is more predominant in dengue transmission. For this disease, where a vaccine is not available, the best way to control dengue and DHF is to eradicate the *Ae. aegypti* mosquitos or reduce their numbers to the level where viral transmission to man is interrupted. Vector control, by reducing the *Ae. aegypti* population is, at present, the only way to prevent the spread of disease. It consists of environmental

Correspondence: Ratana Sithiprasasna, Department of Entomology, US Army Medical Component, Armed Forces Research Institute of Medical Sciences, 315/6 Rajvithi Road, Bangkok 10400, Thailand.
Tel: 66 (0) 2644-5777; Fax: 66 (0) 2354-7885
E-mail: ratanas@afirms.org

management, biological, genetic and chemical control. Integration of these methods can lead to maximize *Ae. aegypti* reduction. Insecticides have been frequently used for vector control programs throughout the world. This is because insecticides can kill a great number of vectors within a short time and can reduce their densities sufficiently to suppress the vector population. Using the same insecticide in the same area for a long time can lead to resistance. The development of resistance, particularly to larvicides and adulticides, is an important issue. Mosquitos need to adapt themselves to the insecticide to be able to survive.

In Thailand, the first reported *Ae. aegypti* pilot control project took place in 1964 in Bangkok. Dichloro diphenyl trichloroethane (DDT) was applied to the inner surfaces of the treated houses at a dosage of 2 g/m² and all water containers in every house were treated with a DDT suspension of 1 ppm. Reinspections were carried out every 2 weeks and all water containers were treated with a 5% DDT suspension as a perifocal spray (Gratz, 1993). DDT resistance in *Ae. aegypti* was first reported in Bangkok in 1966. A high level of resistance to DDT and other organochlorine insecticides was discovered. This led to the use of organophosphates as insecticides. *Ae. aegypti* has demonstrated the ability to develop resistance to organophosphates in the British Virgin Islands, Santo Domingo, Dominican Republic and Antigua (Mekuria *et al*, 1991).

Since 1972, the Division of Medical Entomology of the Thai Department of Medical Sciences has studied integrated control measures and community participation in the control of *Ae. aegypti*, following WHO recommendations. In the first campaign, adult volunteers visited houses to eliminate unneeded water containers, and apply temephos (in the form of Abate 1% sand granules) or tablets containing methoprene to water-storage pots found to contain *Aedes* larvae (Curtis, 1991). In the year 2000, the Department of Disease Control, MOPH reported a heavy use of temephos during the period 1994-1998, about 9 times higher than malathion, DDT and permethrin. Their use has an increasing trend.

It is difficult to overcome resistance, as resistance mechanisms have become more complicated. The most effective way to fend off resistance is by close surveillance of susceptibility in the vector population.

The major mechanism for resistance is the target site. This occurs when the insecticide no longer binds to its target. Detoxifies enzyme-based resistance occurs when enhanced levels of modified activities of esterases, oxidases, glutathione S-transferases (GST) prevent the insecticide from reaching its site of action (Brogdon and McAllister, 1998). In mosquitos (Diptera: Culicidae), esterase is the primary mechanism for organophosphate insecticide resistance (Hemingway and Karunaratne, 1998).

The bottle bioassay describes a time-mortality rate that uses a simplified procedure for detecting resistance and give a good correlation with the biochemical microplate assay to verify the results, and identify the mechanisms involved in the insecticide resistance. This integrated approach was used to detect the resistance to temephos insecticide in adult *Ae. aegypti* from highest and lowest temephos use areas.

The information obtained in this study will be beneficial to mosquito control programs and in the development of strategies to overcome insecticide resistance.

MATERIALS AND METHODS

Temephos use

The Department of Disease Control, MOPH, Thailand has provided temephos for use at the provincial level for the years 1997-2001 throughout the country. The amount of temephos used by each province was based on distribution by the Department of Disease Control, MOPH. Use was compared to select only the highest and lowest temephos use provinces for this study.

Reference strain mosquitos

Aedes aegypti BKK1 strain, a temephos susceptible strain, was used as a reference strain for comparison with mosquitos collected from highest and lowest temephos use areas. This strain is maintained at the insectary, Department of Entomology, US Army Medical Component,

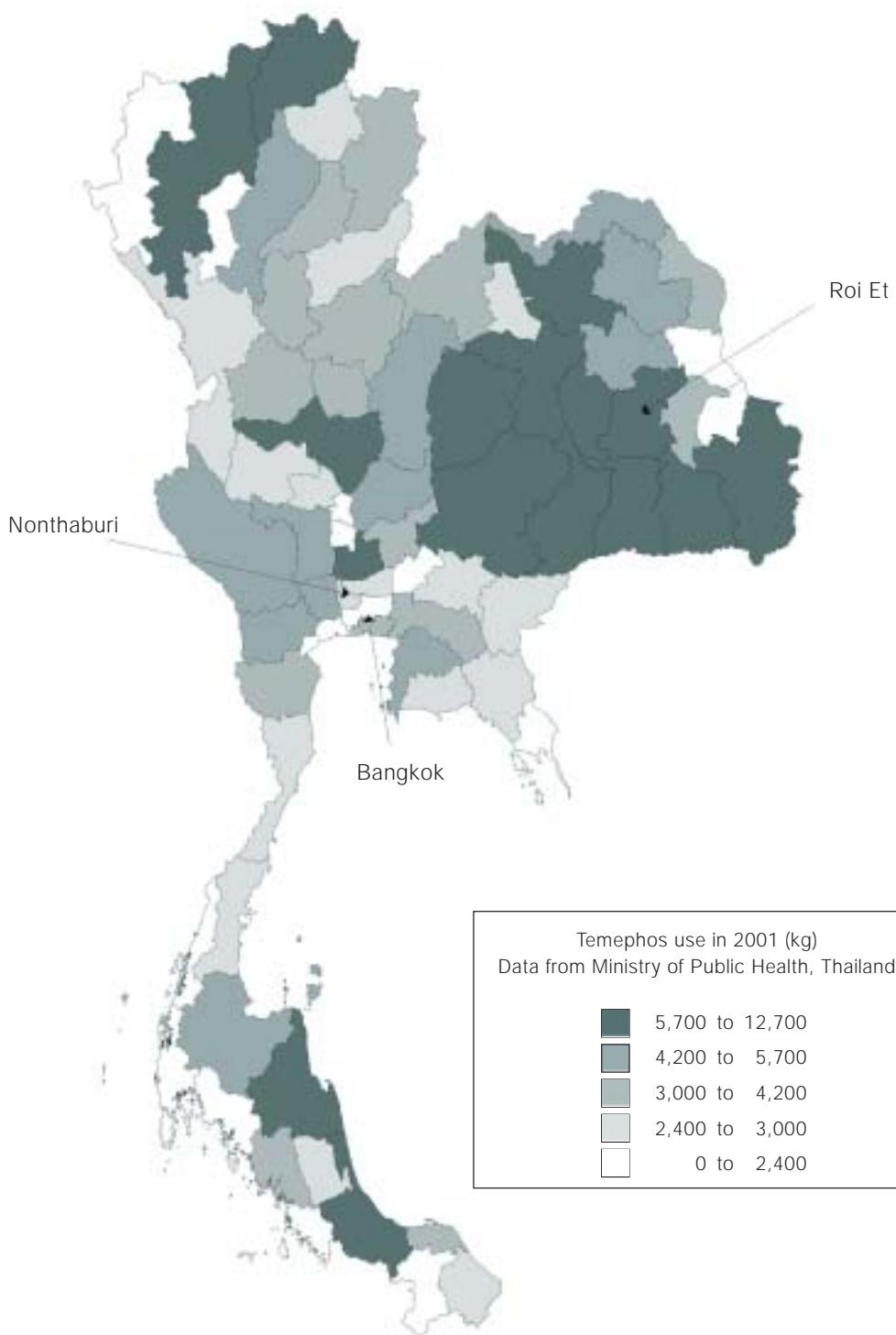


Fig 1-Thematic map showing distribution of temephos use for the year 2001 in different provinces of Thailand plotted on provincial boundary layer in a geographic information system.

Armed Forces Research Institute of Medical Sciences (USAMC-AFRIMS) since 1992.

Field-collected mosquito

During April and May 2002, field strains of *Ae. aegypti* eggs were collected by ovitrap from houses in Tha Sai subdistrict, Muang district, Nonthaburi Province (lowest temephos use) and from Pho Phan subdistrict, Muang district, Roi Et Province (highest temephos use). They were maintained and reared to adults, without exposure to temephos, in the insectary at the Department of Entomology, USAMC-AFRIMS. The 7-day-old F2 progeny were used for the experiments.

Insecticide

Technical grade (95.3% purity) temephos was provided by BASF (Thai) Limited.

Insecticide susceptibility test

The bottle bioassays and biochemical microplate assays followed the standard tests of Brogdon and Dickinson, 1983; Brogdon, 1984a,b,c; Brogdon and Barber, 1987; Brogdon, 1988a,b,c; Brogdon, 1989.

Data analysis

Bottle bioassay. Time-mortality rates for the *Ae. aegypti* BKK1 susceptible strain and the field-collected mosquito was calculated by a) descriptive statistics (mean and standard deviation), b) One-way ANOVA for significant differences in the time-mortality rates, and c) resistance ratios (RR_{100}), which were calculated by dividing the 100% time-mortality rate of the field-collected mosquitos by the 100% time-mortality rate obtained from the baseline susceptibility of the reference strain.

Biochemical microplate assays. The amount of total protein for the *Ae. aegypti* BKK1 susceptible strain and the field-collected mosquito was calculated by a) descriptive statistics (mean, standard deviation, minimum and maximum), and b) Kruskal Wallis test for the significant difference in the amount of protein.

Non-specific esterase. The optical density (OD), of the *Ae. aegypti* BKK1 susceptible strain and the field-collected mosquito was analyzed by a) descriptive statistics (mean, standard deviation, minimum and maximum), and b) any absorbance

reading higher than the upper range limit of the absorbance value for the susceptible strain.

Acetylcholinesterase and insensitive acetylcholinesterase. The percentage of residual AChE activity in the *Ae. aegypti* BKK1 susceptible strain and the field-collected mosquitos was calculated by descriptive statistics (mean, standard deviation, minimum, and maximum). Resistant individuals were those displaying residual AChE activity. The percentage of residual AChE activity was calculated by dividing the presence of AChE with propoxur (insensitive acetylcholinesterase assay) by the activity in the control (acetylcholinesterase assay). The resistance frequency was the number of mosquitos with residual AChE activity levels above the upper limit of the range measured in the susceptible strain.

RESULTS

The means for the mortality rates at 15 minutes intervals for *Ae. aegypti* BKK1 strains (Fig 2) were calculated from 3 replicates of 6 concentrations of temephos. Each concentration required various lengths of time to kill the mosquitos. The 100% mortality times for the *Ae. aegypti* BKK1 exposed to the 6 concentrations of temephos, 800, 850, 900, 950, 1,000, and 1,050 µg/bottle were 180, 150, 150, 150, 135, and 135 minutes, respectively. The means and standard deviations of the time-mortality rates at lethal time in which 100% mortality occurs (LT_{100}) to temephos are shown in Table 1. Using 800, 850, 900, 950, 1,000, and 1,050 µg/bottle concentrations, they were 170 ± 8.66 , 135 ± 15 , 140 ± 8.66 , 135 ± 15.00 , 35 ± 0.00 , and 125 ± 8.66 minutes, respectively.

Comparison of the time-mortality rates between the different concentrations was done by the One-way ANOVA. It revealed that time-mortality rate of 800 µg/bottle concentration was significantly different from other concentrations ($p=0.008$). The dosage selected for resistance detection was 850 µg/bottle of temephos. This concentration gave similar time-mortality rates to concentrations of 900, 950, and 1,050 µg/bottle. Increasing the concentration did not cause the insecticide to penetrate the mosquito and the target site any faster. The time-mortality

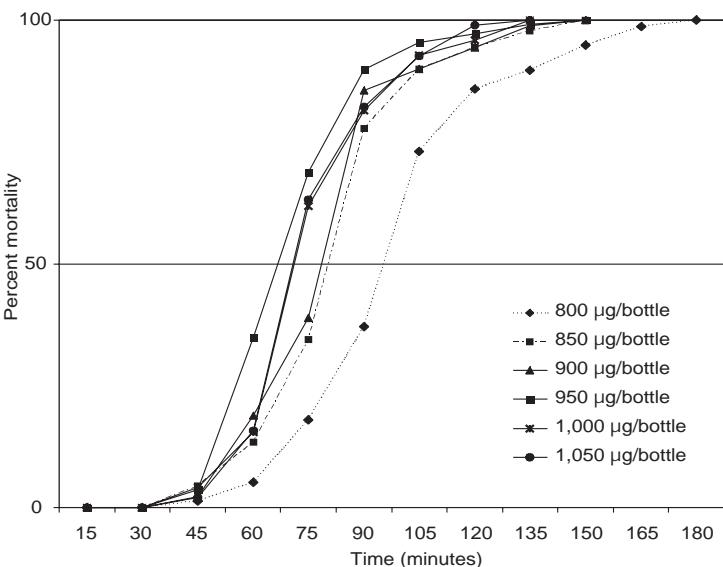


Fig 2-Percent mortality and mortality time of *Aedes aegypti* BKK1 exposed to different concentrations of temephos.

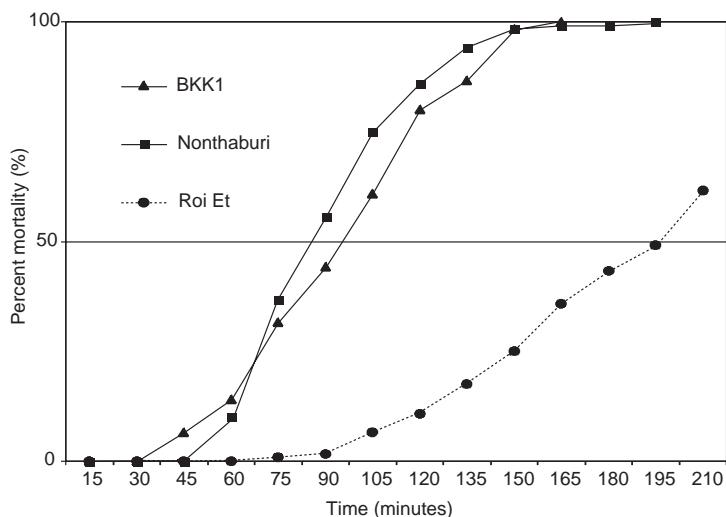


Fig 3-Percent mortality and mortality time of *Aedes aegypti* BKK1, Nonthaburi and Roi Et strains exposed to 850 µg/bottle temephos insecticide.

rates for *Ae. aegypti* in Nonthaburi, Roi Et, and in the BKK1 susceptible strains treated with 850 µg/bottle temephos are compared in Fig 3. Time-mortality rates were calculated from the average of 6 replicates of 20 mosquitos per replicate. Based on 100% mortality at 165 minutes in the BKK1 susceptible strain, the frequency of mortality rate was 99.17% for the Nonthaburi and

35.83% for the Roi Et strains. The means and standard deviations of the time-mortality rates at LT₁₀₀ values for temephos in *Ae. aegypti* from Nonthaburi, Roi Et, and BKK1 susceptible strains were 150±25.10, 382.50±26.41, and 145±20.49 minutes, respectively (Table 2). The resistance ratio at the lethal time 100% mortality for temephos in mosquitos was 1.03 fold for the Nonthaburi strain and 2.64 fold for Roi Et strain, when compared to the reference *Ae. aegypti* BKK1 strain.

Comparison of mean time-mortality rates between the field-collected mosquitos and BKK1 susceptible strain was done by the One-way ANOVA. It revealed that the time-mortality rate of the Roi Et strain was significantly different from both the Nonthaburi and BKK1 strains ($p<0.01$). The microplate assay measures levels of protein, non-specific esterase, acetylcholinesterase and insensitive acetylcholinesterase present in mosquitos. The means of the protein concentration in the *Ae. aegypti* BKK1, Nonthaburi, and Roi Et strains were 2.70±1.82, 2.40±1.47, and 2.21±1.49 mg, respectively (Table 3).

The statistical comparison of the means of the protein concentrations between the field-collected mosquitos and the BKK1 susceptible strains were done by the Kruskal Wallis test. This revealed that the amounts of protein in the field-collected mosquitos were not significantly different from the BKK1 strain ($p>0.05$). Table 4 presents the means, standard deviations, minimum, and maximum optical densities OD for non-specific esterase in the *Ae. aegypti* BKK1,

Table 1
Time-mortality rates at 100% mortality of
Aedes aegypti BKK1 strain exposed to
different concentrations of temephos
insecticide.

Concentration ($\mu\text{g}/\text{bottle}$)	Replicates (Number of bottles)	Mean \pm SD (minutes)	F	p-value
800	3	170 \pm 8.66 ^{a,b,c,d}	6.5	0.008
850	3	135 \pm 15.00 ^a		
900	3	140 \pm 8.66 ^b		
950	3	135 \pm 15.00 ^c		
1,000	3	135 \pm 0.00 [*]		
1,050	3	125 \pm 8.66 ^d		

^{a,b,c,d}The same letters were significantly different p<0.05

* Exclude the concentration of 1,000 $\mu\text{g}/\text{bottle}$

Table 2
Time-mortality rates at 100% mortality for
Aedes aegypti BKK1, Nonthaburi and Roi Et
strains exposed to a temephos insecticide
concentration of 850 $\mu\text{l}/\text{bottle}$.

Strain	Replicates (Number of bottles)	Mean \pm SD (minutes)	F	p-value
BKK1	6	145 \pm 20.49 ^a	189.678	<0.001
Nonthaburi	6	150 \pm 25.10 ^b		
Roi Et	6	382.50 \pm 26.41 ^{a,b}		

^{a,b}The same letters are significantly different, p<0.05

Nonthaburi and Roi Et strains. These were 1.27 \pm 0.18, 1.48 \pm 0.18, and 3.83 \pm 0.76, respectively. The maximum OD for the *Ae. aegypti* BKK1, Nonthaburi, and Roi Et strains were 1.80, 2.00, and 4.20, respectively. The minimum OD for the *Ae. aegypti* BKK1, Nonthaburi, and Roi Et strains were 0.70, 1.10, and 1.13, respectively. The non-specific esterase levels for the Nonthaburi, Roi Et, and BKK1 susceptible strains are shown in the Fig 4. The *Aedes aegypti* BKK1 susceptible strain OD for non-specific esterase ranged from 0.7 to 1.8. In the field-collected mosquitos from both the Nonthaburi and Roi Et strains, the OD for the esterase activity was

higher than in the BKK1 susceptible strain. The frequencies of distribution for 4 mosquitos (2.63%) and 141 mosquitos (92.76%) in the Nonthaburi and Roi Et strains, respectively, were above the resistance threshold (OD values higher than 1.8).

The absorbance values for the acetylcholinesterase assay and the insensitive acetylcholinesterase assay displayed residual AChE activity. The percentage of residual AChE activity in individual mosquitos was calculated by dividing the activity in the presence of propoxur (insensitive acetylcholinesterase assay) by the activity in the control (acetylcholinesterase assay). The means and standard deviations for the percentages of residual AChE activity in the *Ae. aegypti* BKK1, Nonthaburi and Roi Et strains were 2.14 \pm 1.40, 2.31 \pm 1.65, and 2.22 \pm 1.10%, respectively. The maximum percentages for the residual AChE activity in the *Ae. aegypti* BKK1, Nonthaburi, and Roi Et strains were 8.85, 8.20, and 6.53%, respectively (Table 5). The minimum percentages for the residual AChE activity in the *Ae. aegypti* BKK1, Nonthaburi, and Roi Et strains were 0.14, 0.13, and 0.15, respectively. The percentages of the residual AChE activity in the presence of propoxur in the Nonthaburi, Roi Et, and BKK1 susceptible strains are shown in Fig 5. The *Aedes aegypti* BKK1 susceptible strain gave a percentage of residual AChE activity range of 0.1-8.8%. Both the Nonthaburi and Roi Et strains gave percentages of residual AChE activity lower than the BKK1 susceptible strain. There was no evidence of insensitive acetylcholinesterase based on organophosphate (OP) resistance mechanisms in the Nonthaburi and Roi Et strains.

DISCUSSION

At a concentration of 850 $\mu\text{g}/\text{bottle}$ temephos insecticide resistance detection with the bottle bioassay, the time-mortality rate at 100% mortality in the mosquitos from Roi Et (382.50 \pm 26.41 minutes) was higher than the time-mortality rates in the Nonthaburi (150 \pm 25.10 minutes) and BKK1 (145 \pm 20.49 minutes) susceptible strains. The longest time required for 100% mortality in *Ae. aegypti* BKK1

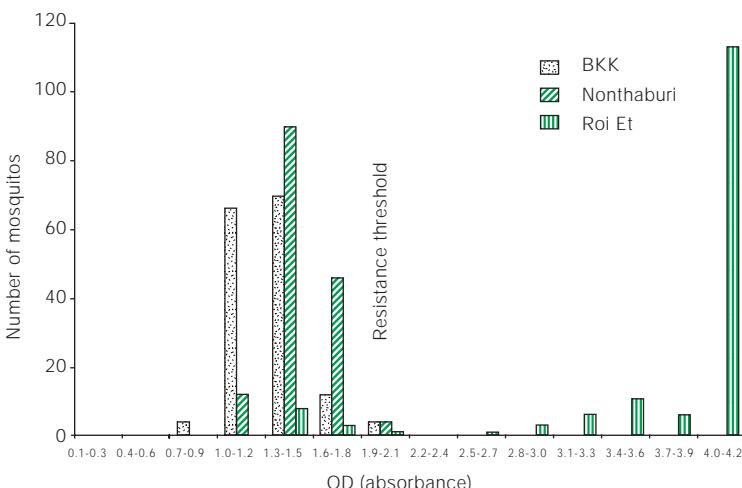


Fig 4—Values of non-specific esterase by microplate assay in three strains of adult *Aedes aegypti*.

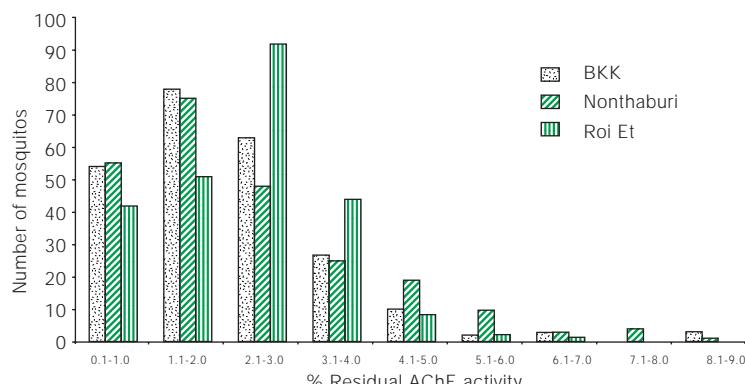


Fig 5—Frequency of distribution of percentages of residual acetylcholinesterase activity in the presence of propoxur in three strains of adult *Aedes aegypti*.

susceptible strain was 165 minutes, while the percentages of mortality were 99.17 in the Nonthaburi and 35.83 in the Roi Et strains. The resistance ratio to temephos in the Roi Et strain was 2.64 fold at the LT_{100} value when compared to the *Ae. aegypti* BKK1 strain. As the amount of protein in the field-collected mosquitos was not different from the *Ae. aegypti* BKK1 strain, only the OD for the non-specific esterase was concerned. The non-specific esterase in the Roi Et strain, with heavy use of temephos, was the highest when compared to the Nonthaburi and BKK1 susceptible strains. This result correlated well with the bottle bioassay.

There was no evidence of insensitive ace-

tylcholinesterase in the field-collected mosquitos. This may be the first finding of an elevated non-specific esterase mechanism in temephos resistance in *Ae. aegypti* in the Roi Et Province. The results show that the time-mortality rate and non-specific esterase-based insecticide-resistance was higher in mosquitos from the areas with the heaviest temephos use. Peiris and Hemingway (1993) also reported that high levels of esterase enzymes are often associated with the development of resistance. In mosquitos (Diptera: Culicidae) elevated esterase is the primary mechanism for organophosphate (OP) insecticide resistance. Resistance appears because the esterase and the insecticide interact more readily than the insecticide's target site. When esterases are present in an equal molar ratio with insecticides, they can effectively sequester the insecticides and slowly hydrolyze them (Scott, 1995). The mechanism of resistance to temephos in the *Ae. aegypti* Roi Et strain is based on elevated levels of esterase but not on the insensitive acetylcholinesterase, as confirmed by biochemical microplate assays.

Lee (1991) and Lee *et al* (1992) did not find the presence of insensitive acetylcholinesterase in *Ae. aegypti* larvae collected from Selayang (Selangor), Taman Sri Gombak (Selangor), Kangar (Perlis), and Kuala Trengganu in the State of Malaysia, and suggested that resistance or tolerance is most likely due to elevated levels of non-specific esterase. Melena and George (1995) found low levels of resistance to temephos ($RR_{95}=2.9$ -fold) in an *Ae. aegypti* population collected from the Falcon State of Venezuela. Biochemical tests showed significantly greater amounts of esterase activity,

Table 3
Amounts of protein (mg) in adult *Aedes aegypti* BKK1, Nonthaburi and Roi Et strains.

Strain	N	Min	Max	Mean ± SD	p-value
BKK1	152	0.03	8.48	2.70±1.82 ^a	0.059
Nonthaburi	152	0.20	7.44	2.40±1.47 ^b	
Roi Et	152	0.03	11.42	2.21±1.49 ^c	

^{a,b,c} The same letters are significantly different, p<0.05 (Kruskal Wallis test).

Table 4
Optical density (OD) for non-specific esterase in adult *Aedes aegypti* BKK1, Nonthaburi and Roi Et strains.

Strain	n	Min	Max	Mean±SD
BKK1	152	0.70	1.8	1.27±0.18
Nonthaburi	152	1.10	2.0	1.48±0.18
Roi Et	152	1.13	4.2	3.83±0.76

Table 5
Percentages of residual acetylcholinesterase (AChE) activity in adult *Aedes aegypti* BKK1, Nonthaburi, and Roi Et strains.

Strain	n	Min	Max	Mean±SD
BKK1	240	0.14	8.85	2.14±1.40
Nonthaburi	240	0.13	8.20	2.31±1.65
Roi Et	240	0.15	6.53	2.22±1.10

whereas insensitive acetylcholinesterase was not involved in OP resistance. Margaret and George (1999) found a high level of temephos resistance ($RR_{95}=180.6$ fold) in *Ae. aegypti* from Tortola, British Virgin Islands. Elevated esterase activity was present in this Tortola strain but no evidence of insensitive acetylcholinesterase was found.

Esterase is the most important enzyme involved in organophosphate, carbamate, and pyrethroid insecticide detoxification. These insecticide groups contain ester linkages susceptible to hydrolysis by esterase. Organophosphates and carbamates have cross-resistance because they have the same, or very similar, modes of action. Resistance to one usually de-

notes in resistance in the other (Peiris and Hemingway, 1993). Magaret and George (1999) found *Ae. Aegypti*, from Tortola, British Virgin Islands, resistant to temephos ($RR_{95}=180.6$ -fold) and cross-resistant to other organophosphates (fenthion $RR_{95}=13.8$ -fold, malathion $RR_{95}=6.0$ -fold, chlorpyrifos $RR_{95}=5.8$ -fold, parathion $RR_{95}=8.8$ -fold), carbamate (propoxur $RR_{95}=4.8$ -fold) and pyrethroid (premethrin $RR_{95}=43.0$). *Aedes aegypti* Roi Et strain may be resistant to other organophosphates, carbamates, and pyrethroids. Further monitoring of esterase-based mechanisms of other organophosphates, carbamates, and pyrethroids, in the *Ae. aegypti* Roi Et strain by bottle bioassay will ensure that suitable alternative compounds will be chosen for mosquito control.

The resistance to temephos of *Ae. aegypti* mosquitos from the Roi Et Province is a consequence of the prolonged and heavy use of this insecticide in the control program. The Roi Et strain showed a consistent pattern of resistance to temephos insecticide. The information obtained in this study is useful for mosquito control programs and for the development of strategies in vector control. The present control program for *Aedes* mosquitos is the use of temephos or Abate®. The results of this study suggest that the usage of temephos for emergency dengue control should continue in Nonthaburi Province, but that temephos is probably no longer effective for the control of *Ae. aegypti* in Roi Et Province.

The bottle bioassay is easy to use and gives some indication of what mechanism is involved, if resistance is to be discovered. The results can be confirmed by the high-sensitivity of the biochemical microplate assay, a simple and rapid

assay that indirectly measures the different levels of enzyme mechanisms of resistance in susceptible and resistant mosquitos. These two methods proved to be promising tools for the initial detection and field surveillance of resistance. The findings reinforce the need for routinely monitoring insecticide efficacy, as this is a very important step in vector control programs.

REFERENCES

- Brogdon WG, McAllister JC. Insecticide resistance and vector control. *Emerg Infect Dis* 1998; 4: 605-13.
- Brogdon WG, Dickinson CM. A microassay system for measuring esterase activity and protein concentration in small samples and in high-pressure liquid chromatography eluate fractions. *Anal Biochem* 1983; 131: 499-503.
- Brogdon WG. A proposed new method under development for field detection and evaluation of insecticide resistance. *WHO/VBC/84.895*. 1984a.
- Brogdon WG. Mosquito protein microassay - I. Protein determinations from small portions of single-mosquito homogenates. *Comp Biochem Physiol* 1984b; 79B: 461-64.
- Brogdon WG. Mosquito protein microassay - II. Modification for potential field use. *Comp Biochem Physiol* 1984c; 79B: 465-9.
- Brogdon WG, Barber AM. Microplate assay of acetylcholinesterase inhibition kinetics in single-mosquito homogenates. *Pest Biochem Physiol*. 1987; 29: 252-9.
- Brogdon WG, Georgiou GP, Hemingway J, Pasteur N. Detection of insecticide resistance. *WHO/ VBC/ 88.3.1988a*.
- Brogdon WG. Microassay of acetylcholinesterase activity in small portions of single-mosquito homogenates. *Comp Biochem Physiol* 1988; 90C: 145-50.
- Brogdon WG, Hobbs JH, St Jean Y, Jacques JR, Charles LB. Microplate assay analysis of reduced fenitrothion susceptibility in Haitian *Anopheles albimanus*. *J Am Mosq Control Assoc* 1988b; 4: 152-8.
- Brogdon WG, Beach RF, Stewart JM, Castanaza L. Microplate assay analysis of the distribution of organophosphate and carbamate resistance in Guatemalan *Anopheles albimanus*. *Bull WHO* 1988c; 66: 339-46.
- Brogdon WG. Biochemical resistance detection: an alternative to bioassay. *Parasitol Today* 1989; 5: 56-60.
- Curtis CF. Control of disease vector in the community. London: Wolfe, 1991.
- Division of Epidemiology, Thailand Ministry of Public Health. Preliminary annual summary. *Epidemiol Surv Rep* 1995-2000.
- Gratz NG. Lessons of *Aedes aegypti* control in Thailand. *Med Vet Entomol* 1993; 7: 1-10.
- Hemingway J, Karunaratne SHPP. Mosquito carboxylesterase: a review of the molecular biology and biochemistry of a major insecticide resistance mechanism. *Med Vet Entomol* 1998; 12: 1-12.
- Lee HL. Esterase activities and temephos susceptibility in *Aedes aegypti* (L.) larvae. *Mosq Borne Dis Bull* 1991; 8: 91-4.
- Lee HL, Abimbola O, Singh KI. Determination of insecticide susceptibility in *Culex quinquefasciatus* adults by rapid enzyme microassays. *Southeast Asian J Trop Med Public Health* 1992; 23: 458-63.
- Mekuria Y, Gwinn TA, William DC, Tidwell MA. Insecticide susceptibility of *Aedes aegypti* from Santo Domingo, Dominican Republic. *J Am Mosq Control Assoc* 1991; 7: 69-72.
- Melena BM, George PG. Characterization of resistance to organophosphate, cabamate and pyrethroid insecticides in field populations of *Aedes aegypti* from Venezuela. *J Am Mosq Control Assoc* 1995; 11: 315-22.
- Margaret CW, George PG. Selection and characterization of temephos resistance in a population of *Aedes aegypti* from Tortola, British Virgin Islands. *J Am Mosq Control Assoc* 1999; 15: 315-20.
- Peiris HTR, Hemingway J. Characterisation and inheritance of elevated esterase in organophosphorus and carbamate insecticide resistant *Culex quinquefasciatus* (Diptera: Culicidae) from Sri Lanka. *Bull Entomol Res* 1993; 83: 127-32.
- Scott JA, ed. The molecular genetic of resistance: resistance as a response to stress. Florida Symposium on pesticide resistance. September, 1995.