

Insecticide Susceptible/Resistance Status in *Aedes (Stegomyia) aegypti* and *Aedes (Stegomyia) albopictus* (Diptera: Culicidae) in Thailand During 2003–2005

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ABSTRACT Susceptibility baselines and diagnostic doses of the technical grade insecticides deltamethrin, permethrin, fenitrothion, and propoxur were established based on *Aedes aegypti* (L.), Bora (French Polynesia), a reference susceptible strain. Field-collected *Aedes* mosquitoes from each part of Thailand were subjected to bioassay for their susceptibility to the diagnostic doses of each insecticide. Almost all *Ae. aegypti* collected were incipient resistant or resistant to deltamethrin and permethrin, except those from some areas of Songkhla (southern) and Phan district of Chiang Rai (northern) province. Susceptibility to fenitrothion was found in mosquitoes from Bangkok (central), Chonburi (eastern), Chiang Rai, Kanchanaburi (western), and Songkhla, whereas they were resistant in almost all areas of Nakhon Sawan (north central) and Nakhon Ratchasima (northeastern) provinces. Most of *Ae. aegypti* were susceptible to propoxur except those from Mae Wong, Nakhon Sawan province. Various levels of insecticide resistance and susceptibility in adjacent areas revealed a focal susceptible/resistance profile in the country. It could be noted that almost all of *Ae. albopictus* were susceptible to the insecticides tested at the same diagnostic doses. In conclusion, resistance to pyrethroids (permethrin and deltamethrin) has developed in *Ae. aegypti* in most of the collected areas, suggesting that an alternative choice of insecticide or other control measures should be applied.

KEY WORDS *Aedes (Stegomyia)*, diagnostic dose, insecticide, Thailand

The incidence of dengue and dengue hemorrhagic fever (DF/DHF) in Thailand has increased cyclically since the first recognized outbreak in 1958. Various strategies have been implemented in controlling DF/DHF in Thailand. One important means to combat the disease is by the control of mosquito vectors *Aedes (Stegomyia) aegypti* (L.) and *Aedes (Stegomyia) albopictus* (Skuse). Several insecticides have been used to repress the spread of dengue transmission, particularly during epidemics (Bang and Pant 1969; Bang et al. 1970, Lofgren et al. 1970, Pipitgool et al. 1991, Bang and Tonn 1993, Rojanapithayakorn 1998). For routine disease control, space spraying has been intensively

used once dengue outbreak was reported. Organophosphorous compounds such as malathion, fenitrothion, and pirimiphos methyl were heavily used in the past before being replaced with pyrethroids (Chareonviriyaphap et al. 1999). Deltamethrin, cypermethrin, and permethrin are now the main pyrethroids used to control adult *Aedes* mosquitoes through mass spraying (Vector Borne Disease Annual report 2002–2003). According to the yearly report by Division of General Communicable Diseases, CDC Department, Ministry of Public Health of Thailand, deltamethrin has been the major adulticide used in the national *Aedes* control program over the last two decades until present. Space spraying techniques, either with ultralow volume or thermal fog generators, are widely used for deltamethrin treatments in all areas of the country. Additionally, household insecticide products (aerosols, mosquito coils, mats, and liquid forms) containing pyrethroids such as permethrin, *d*-tetramethrin, and esbiothrin have been widely used (Paeporn et al. 1996). Some information on vector susceptibility/resistance patterns to insecticides and resistance mechanisms is available for some areas of Thailand, mostly for focal points (Neely 1964, Yasuno and Kerdpibule 1967, Bang et al. 1971, Gratz 1993, Chareonviriyaphap et al. 1999, Prapanthadara et al.

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2002, Ponlawat et al. 2005). A control program cannot succeed without adequate information on insecticide susceptibility in the vector. Moreover, insecticide susceptibility of *Ae. albopictus* in Thailand has been rarely evaluated.

Diagnostic doses of various insecticides have been established according to World Health Organization (WHO) criteria mainly for monitoring *Anopheles* mosquitoes, the malaria vectors. Although it has been suggested that the same diagnostic doses can be applied to *Aedes* mosquitoes, we still lack conclusive information on susceptible baselines and diagnostic doses of various insecticides for *Aedes* mosquitoes. Susceptibility baselines and diagnostic doses of four adulticides (deltamethrin, fenitrothion, permethrin, and propoxur) were therefore established in the current study. Comprehensive approach on dengue vector susceptibility using standard WHO susceptibility testing protocols (WHO 1970, 1980, 1981) were carried out on *Aedes* populations from different collection sites across Thailand.

Materials and Methods

Mosquitoes. Bangkok and four provinces of each region with high incidence of dengue cases, based on the epidemiological data in 2002, were selected as mosquito-collecting sites. They were Bangkok, Chonburi, Nakhon Sawan, Nakhon Ratchasima, and Songkhla, which represent central, eastern, north central, northeastern, and southern part of the country, respectively. Mosquito larvae and pupae collections were performed in four to five districts of each province to assess their distribution in response to insecticide. An additional three collection sites from Chiang Rai (northern), Chanthaburi (eastern), and Kanchanaburi (western), also were performed to cover all parts of the country. Pooled samples of *Ae. aegypti* and *Ae. albopictus* larvae and pupae from each district were identified and colonized under laboratory conditions at 28°C and 70–80% RH. Larvae were fed on dog food (Alpo), and adults were provided with 10% sugar solution. Adult were fed on blood from Swiss mice twice a week. After optimum numbers of eggs were obtained, the F₁ progeny were prepared for bioassay testing according to WHO standard procedures (WHO 1981). However, some bioassays of *Ae. albopictus* were performed on F₂ or F₃ generations due to insufficient numbers of F₁ progeny.

Insecticides. Technical grade insecticides, fenitrothion (PS-678, Supelco, Bellefonte, PA), permethrin (4-42748, Supelco), deltamethrin (PS-2071, Supelco), and propoxur (994806, Bayer, Bangkok, Thailand) were used in the present work.

Susceptibility Baseline. Susceptibility baselines for fenitrothion, permethrin, deltamethrin, and propoxur were established using *Ae. aegypti*, Bora (French Polynesia), a reference susceptible strain. Bioassays were performed using Whatman 12- by 15-cm filter papers evenly treated with 2-ml series of technical grade insecticide concentration in acetone solution with silicone oil. The papers were left to air dry over-

night, and then they inserted into standard tubes (purchased from WHO, Malaysia). Nonblood-fed, 2–5-d-old female mosquitoes were introduced into each holding tube, 25 mosquitoes per tube, and observed for viability after 1 h. Each set of four replicate holding tubes per concentration was then connected with the test tubes, in which the mosquitoes were exposed to the impregnated papers for 1 h. After returning to the holding tubes, the mosquitoes were provided with sugar pad and kept at 27–28°C and 80% RH to determine the mortality rate after 24 h. The 50 and 99% lethal concentrations (LC₅₀ and LC₉₉) were calculated using log-probit analysis (SPSS version 11.5, SPSS Inc., Chicago, IL). The tests that yielded straight-line relationship between the logarithm of the concentration and probit mortality were selected. The final estimations of LC₅₀ and LC₉₉ were calculated from five replicate tests performed on different batches of mosquitoes. Double concentration of LC₉₉ was then used as the diagnostic dose for bioassay of field samples.

Susceptibility Test on Field Samples. Bioassays were performed in the same manner with the diagnostic doses for each insecticide (Table 1). Sets of four replicate tubes with 25 nonblood fed female mosquitoes per tube were tested with each insecticide and control (impregnated with acetone and silicone oil used as diluent). Mortality rates were determined after incubation at 27–28°C incubator with 80% RH for 24 h.

Interpretation of results of the bioassay tests were based on WHO recommendations (WHO 1998). Mosquitoes were considered susceptible if the percentage of mortality was 98–100%, resistant if mortality was <80%, and possibility of incipient resistance if mortality was 80–98%. Percentage of mortality was adjusted by Abbott's formula if control mortality exceeded 4%.

Results

Susceptibility baselines for each insecticide were established from five experiments that exhibited a straight-line relationship between log dose and probit mortality. LC₅₀ and LC₉₉ were presented in Table 1 with lower and upper 95% CL (Pearson goodness-of-fit chi-square test). According to WHO guidelines (WHO 1981), double of the extrapolated LC₉₉ from the probit line could be used as the discriminating concentration. Therefore, these lethal concentrations were selected as diagnostic doses (Table 1) for susceptibility tests on field-collected samples.

Ae. aegypti collected from different localities of Thailand (Table 2 and 3) exhibited various levels of susceptible/resistance to each insecticide. Mosquitoes from Bangkok and Chonburi, located in central and eastern parts of Thailand, were still susceptible to fenitrothion, whereas most of them were resistant to deltamethrin and permethrin. However, they were sensitive to propoxur, except that a trend of resistance was observed in mosquitoes from Muang, Panusnikom, and Banglamung in Chonburi province. For mos-

Table 1. Diagnostic dose for *Aedes* mosquitoes based on Bora, a reference susceptible strain

Insecticide	Statistical test (Pearson goodness-of-fit)	LC ₅₀ (95% upper-lower limit)	LC ₉₉ (95% upper-lower limit)	Diagnostic dose (%)
Fenitrothion (PS-678, Supelco)	$\chi^2 = 19.67, df = 5, P = 0.001$	0.11 (0.10-0.12)	0.24 (0.22-0.29)	0.5
Deltamethrin (PS-2071, Supelco)	$\chi^2 = 39.45, df = 5, P = 0.000$	0.004 (0.003-0.004)	0.03 (0.02-0.53)	0.06
Permethrin (442748, Supelco)	$\chi^2 = 20.59, df = 5, P = 0.001$	0.16 (0.15-0.17)	0.43 (0.38-0.50)	0.9
Propoxur (994806, Bayer)	$\chi^2 = 151.19, df = 5, P = 0.000$	0.04 (0.03-0.06)	0.16 (0.11-0.38)	0.3

quitoes from north central and northeastern areas, Nakhon Sawan and Nakhon Ratchasima provinces, various levels of resistance to fenitrothion, deltamethrin, and permethrin have been developed. However, they also were sensitive to propoxur, except those from Muang, Mae Wong, and Krok Pra in Nakhon Sawan province. Most *Ae. aegypti* from Songkhla province in the south were susceptible to all tested insecticides, except those from Muang, which were resistant to permethrin, whereas others were incipient resistance. Mosquitoes from Muang, Bangklam, and Had Yai showed a trend of resistance to deltamethrin. Although, most of them were susceptible to propoxur, incipient resistance was suspected in mosquito from Muang in Songkhla province. *Ae. aegypti* from an additional three collection sites, Chiang Rai, Chanthaburi, and Kanchanaburi, also were resistant or incipient resistant to deltamethrin and permethrin except those from Chiang Rai, which were sensitive to permethrin. They also were susceptible to fenitrothion and propoxur, except those from Chantaburi, which were incipient resistant to fenitrothion.

It was interesting to observe that *Ae. albopictus* from Muang, Nakhon Sawan, and Seekhew, Nakhon Ratchasima, were sensitive to all insecticides tested in the current study although *Ae. aegypti* from the same areas showed various levels of resistance. Almost all

Ae. albopictus from Songkhla were susceptible to tested insecticides except those from Chana, which were incipient resistant to fenitrothion.

Discussion

During the past 40 yr, commonly used insecticides such as temephos, malathion, permethrin, propoxur, and fenitrothion have led to development of resistance in dengue vectors in many countries (Brogdon and McAllister 1998, Hemingway and Ranson 2000). It was clearly shown in the present work that almost all *Ae. aegypti* populations exhibit various levels of resistance to pyrethroid, permethrin, and deltamethrin, the commonly used insecticide in Thailand (Table 3). Fortunately, most of the samples were still susceptible to organophosphate (fenitrothion) and carbamate (propoxur), except those of Nakhon Sawan, Nakhon Ratchasima, and Chanthaburi provinces were incipient resistant or resistant to either fenitrothion or propoxur. Previous studies have found insecticide resistance in various areas of Thailand (Chareonviriyaphap et al. 1999, Somboon et al. 2003, Paeporn et al. 2004, Ponlawat et al. 2005). We also found a high level of DDT resistance in *Aedes* population from Bangkok and Nakhon Ratchasima, whereas those from Songkhla were susceptible (data not shown). Application of

Table 2. Collection site with global positioning system coordinate and altitude in Thailand during 2003–2005

Collection site/province	District	Subdistrict	GPS coordinates		Altitude (m)
Bangkok	Bangkoknoi		13° 45' 40" N	100° 2' 1.9" E	7
	Huaykwang		13° 4' 47.4" N	100° 3' 52.3" E	3
	Laksi		13° 5' 28.2" N	100° 3' 41.2" E	4
	Ladkrabang		13° 4' 47.5" N	100° 4' 23.6" E	10
Chonburi	Rasburana		13° 3' 59.2" N	100° 3' 58.8" E	8
	Muang	Ban saun	13° 2' 10.7" N	101° ' 18.1" E	22
	Panusnikom		13° 2' 2.9" N	101° 1' 5.3" E	28
	Banglamung	Nong pruo	12° 5' 42.2" N	100° 5' 32.2" E	41
Nakhon Sawan	Sriracha	Bor win	13° ' 19" N	101° ' 11.8" E	121
	Muang	Nong grod	15° 4' 47" N	100° ' 34.7" E	31
	Mae Wong		15° 4' 35" N	99° 3' 7.4" E	109
	Mae Pern	Mae Pern	15° 3' 21" N	99° 2' 48" E	116
Nakhon Ratchasima	Krok Pra	Krok Pra	15° 3' 12" N	100° ' 33.7" E	16
	Taklee		15° 1' 53" N	100° 1' 48.2" E	21
	Prathai	Wang Mai Dang	15° 3' 56" N	102° 3' 45.7" E	196
	Kornburi	Bann Mai	14° 3' 50" N	102° 1' 45.5" E	218
Songkhla	Kangsannamngang	Boung Sum rong	15° 4' 14" N	102° 1' 36.9" E	187
	Serngsang	Serngsang	14° 2' 35.2" N	102° 2' 45.6" E	216
	Seekhew	Bann Han	14° 5' 19.5" N	101° 4' 28.8" E	233
	Muang	Bor Yang	7° 1' 41.8" N	100° 3' 54.6" E	10
Songkhla	Singhanakhon	Hoa Kao	7° 1' 31.8" N	100° 3' 4.7" E	15
	Bangklum	Ta Chang	7° ' 42.7" N	100° 2' 34.6" E	21
	Chana	Na tub	7° ' 19.4" N	100° 4' 40.4" E	9
	Had Yai	Kor Hong	7° ' 55.4" N	100° 3' 28.4" E	11
	Tungtumsao		6° 5' 38.8" N	100° 1' 26.0" E	22

Table 3. Mortality rates of *Ae. aegypti* and *Ae. albopictus* after exposure to each insecticide at diagnostic doses

Collection site	Mortality rate (%)							
	Fenitrothion		Deltamethrin		Permethrin		Propoxur	
	<i>Ae. Aegypti</i>	<i>Ae. albopictus</i>						
Bangkok (central)								
Bangkoknoi	100.0		87.9		18.5		99.0	
Hauykwang	100.0		52.0		29.0		99.5	
Laksi	100.0		75.7		26.1		98.6	
Ladkrabang	100.0		52.1		51.3		99.5	
Rasburana	99.5		73.0		48.8		100.0	
Chonburi (eastern)								
Muang	100.0		86.6		85.4		97.1	
Panusnikom	100.0		52.9		11.2		82.4	
Banglamung	99.0		43.4		5.0		88.3	
Sriracha	100.0		80.5		77.6		100.0	-
Nakhon Sawan (north central)								
Muang	60.0	100.0	85.09	98.87	53.1	98.01	82.0	100.00
Mae Wong	50.5		54.5		47.9		69.1	
Mae Pern	18.4		52.0		37.8		99.0	
Krok Pra	59.6		85.3		72.1		86.0	
Taklee	98.0		74.3		36.3		100.0	
Nakhon Ratchasima (northeastern)								
Prathai	41.75		81.63		89.9		100.0	
Kornburi	93.68		78.79		80.0		100.0	
Kangsanamnang	68.25		89.59		94.73		100.0	
Serngsang	61.62		60.6		69.3		100.0	
Seekhew	58.82	98.59	96.94	100.00	96.0	100.00	100.0	100.00
Songkhla (southern)								
Muang	100.00		81.44	100.00	61.39		95.88	
Singhanakorn	100.00		99.02	100.00	94.18	100.00	100.00	100.00
Bangklum	100.00	100.00	96.81	100.00	97.98	100.00	100.00	100.00
Chana	100.00	96.00	99.02	100.00	94.06	100.00	100.00	100.00
Had Yai (Tambon Kor Hong)	100.00	98.00	88.89	100.00	84.00	100.00	99.0	100.00
(Tambon Tungtumsao)		100.00		100.00		100.00		100.00
Chiang Rai (northern)								
Phan	100.00		96.97		100.00		100.00	
Kanchanaburi (western)								
Tamaka	100.00	91.34	86.36	100.00	73.26	100.00	100.00	98.13
Chanthaburi (eastern)								
Muang	95.00		61.46		80.39		99.05	

No value indicates no samples (*Ae. albopictus* could be collected in some areas).

DDT for controlling dengue vector in the past resulted in resistance to this insecticide, even after spraying for only 2 yr (Gratz 1993). Cross-resistance to DDT and pyrethroids has been reported in most species of mosquitoes of public health importance resulting from knockdown resistance (kdr) gene (Hemingway and Ranson 2000, Brengues et al. 2003). Resistance to pyrethroids generally confers cross-resistance to other insecticides that limits the alternative choices of effective insecticides. The use of pyrethroid for impregnated bed-net and for intradomicillary spraying for malaria control in many parts of the country (Malaria Division Annual Malaria Report 1995–2000) could result in promoting development of resistance in dengue vector. In addition, contamination of breeding places around the house with agricultural insecticide also may influence development of resistance.

In favorable environments for *Ae. aegypti*, it is not surprising that proliferation throughout the year under the exposure of survivors to insecticide over decades has led to insecticide resistance. With sporadic use of insecticides, especially in dengue outbreak situations, there has been consistent selection pressure for the emergence of resistant populations, especially if there were inappropriate application schemes. However, the resistance levels and spatial distribution

of resistant populations vary across Thailand. The lowest level of resistance to pyrethroid was found in mosquito samples from the south. They also were susceptible to fenitrothion, propoxur, as well as DDT, except those from Muang.

It is also of interest that the spread of resistance from different areas of the same province has different resistance profiles (Table 3). The dispersal of mosquitoes driven either by bloodmeal or oviposition needs as well as the spread of these vectors due to urbanization may not be significant; thus, *Aedes* populations in the same province may have different insecticide resistance characteristics. Our results also confirm previous findings that insecticide resistance was focal (Canyon and Hii 1999); thus, selection of the right control measures is crucial. Other factors could be the use of different insecticides for agricultural purposes in each area, which could result in resistance in insects of medical importance.

Low levels of resistance to either temephos, permethrin, or malathion have been reported in *Ae. albopictus* from various areas of Thailand such as Nakhon Sawan (Phayuhakhiri), Mae Sot, and Phatthalung (Ponlawat et al. 2005). *Ae. albopictus* populations in the present work, including those from the same province but different areas (Muang of Nakhon Sawan),

were susceptible to all insecticides tested, similar to what has been reported in *Ae. albopictus* from northern Thailand (Somboon et al. 2003). This could possibly be due to their different natural habitats, which were in less contact with insecticides used in the past. However, our collection team found *Ae. albopictus* in the same containers as *Ae. aegypti*, mostly outside the house (our unpublished observations). Their natural breeding places, usually in tree holes or natural containers, might have decreased and put pressure on them to breed in any possible containers, even those with *Ae. aegypti*, which are close to humans. This is crucial because they could become an important dengue vector as well. Although *Ae. albopictus* in Asia is less susceptible to dengue virus than *Ae. aegypti* (Vazeille et al. 2003), *Ae. albopictus* could nevertheless become an important vector once their habitat has been changed, placing them in close contact with human. It also is thought that they could be dengue virus reservoir in nature. It seems that we have to consider controlling *Ae. albopictus* as well to effectively control dengue disease.

It is important to understand local vector prevalence and to determine whether resistance to insecticides currently in use has already occurred. Our results provided baseline information on insecticide susceptibility that showed relevant status of insecticide resistance in the country. Based on knowledge of insecticide resistance status of the various geographic areas of Thailand, a greater variety of insecticides and frequency of applications are needed, along with a system for monitoring their effectiveness by local communities. Rotation systems for switching from one insecticide to another should be designed so that selection of resistant populations can be prevented. Continuous spraying of insecticide for dengue vector control could put us in danger if monitoring of insecticide resistance in this vector could not be vigorously and regularly done in all parts of the country. Cross-resistance or resistance to agricultural insecticides also should be considered in vector control.

It can be concluded that resistance of *Ae. aegypti* in Thailand was focal with locations near each other showing variable resistance levels. Biochemical methods allow detailed comparison of resistance levels over a large geographic area. Control failure also can result from many factors other than resistance, and the resistance problem should be mapped spatially. Based on each area's insecticide resistance profile, the use of physiological or biological controls, less expensive methods, should be considered as an alternative to the use of insecticides.

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