Trend of Temephos Resistance in *Aedes* (*Stegomyia*) Mosquitoes in Thailand During 2003–2005

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ABSTRACT The diagnostic dose for temephos susceptibility test was established based on *Aedes aegypti*, the susceptible Bora (French Polynesia) strain, for practical and routine use. The diagnostic dose was subsequently used to evaluate the susceptibility/resistance status in F1 progenies of field-collected samples from Bangkok and various parts of Thailand. It appeared that *Ae. aegypti* mosquitoes of one collection site each in Bangkok, Nakhon Sawan (northcentral), and Nakhon Ratchasrima (northeast) were resistant to temephos, with mortality ranging from 50.5 to 71.4%. Moreover, there was a trend of resistance to temephos among *Ae. aegypti* populations of all studied districts of Nakorn Ratchasima and most areas of Nakhon Sawan, of which those in one area were susceptible. However, various levels of temephos susceptibility were found in Bangkok populations, including resistance and incipient resistance. In Chonburi Province (eastern), all mosquitoes were susceptible to temephos with an indication of tolerance in one sample. Additionally, mosquitoes from Songkhla (south), Chiang Rai (north), Kanchanaburi (west), and Chanthaburi (east) remained susceptible to temephos during the sample collecting period. Bioassay tests on *Aedes albopictus* populations collected in this study from Nakhon Sawan, Nakorn Ratchasima, Songkhla, and Kanchanaburi revealed high susceptibility to temephos. Although the use of temephos seems to be potentially effective in many areas of the country, a noticeable trend of resistance indicated that alternative vector control methods should be periodically applied.

KEY WORDS temephos, diagnostic dose, resistance, *Aedes* (*Stegomyia*), Thailand

Temephos, an organophosphate larvicide, has been used for dengue vector control in Thailand since 1950 (Chareonviriyaphap et al. 1999). The efficacy test of temephos sand granules was performed in 1972, and this larvicide has since been recommended for use in *Aedes aegypti* control (Bang et al. 1972). This larvicide is acceptable for public use and believed to be an effective larval control agent at present. However, a high incidence of dengue cases has been reported yearly (Epidemiology Division, Ministry of Public Health 2005). A rapid diagnostic test on susceptibility to temephos is necessary for monitoring and effective planning vector control programs after long-term use. Moreover, it is important to detect the presence of resistant individuals in mosquito populations as soon as possible so that alternative control plans could be implemented. Establishment of diagnostic dose is therefore necessary for effective practical use. Although tentative diagnostic doses of temephos for larval mosquitoes have been recommended for *Anophelines, Culex quinquefasciatus,* and *Ae. aegypti* (WHO 1981), there is a lack of conclusive information on susceptible base line and diagnostic dose for *Aedes* mosquitoes. Before evaluating insecticide susceptibility/resistance in field populations, a susceptibility baseline on the relationship of insecticide concentration and mortality must be established in a normal population for reference (WHO 2005). Selection of proper baseline population is very important, and the *Ae. aegypti*, Bora (French Polynesia) susceptible strain, was used to establish the baseline and the diagnostic dose in this study.

Although vector control seems to be the only means for controlling dengue disease at present, it is essential to know the current susceptibility status of its vector to insecticides. After long periods of temephos application, there have been reports on decreasing susceptibility in *Ae. aegypti* populations worldwide; i.e., Caribbean (Georghiou et al. 1987, Mekuria et al. 1991, Rawlins and Wan 1995), French Polynesia (Failloux et al. 1994), Cuba and Venezuela (Rodriguez et al. 2001), and Brazil (Lima et al. 2003),

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Table 1. Dengue case rates in Thailand in 2002 and average house index observed at each collection site

Collection sites		Case rates in 2002[a]	House index[b]
Bangkok	Bangkoknoi	108.80	58.40
	Huaykwang	272.00	37.90
	Laksi	208.10	26.60
	Ladkrabang	299.80	32.60
	Rasburana	183.10	44.80
Chonburi	Muang	210.51	40.83
	Panusnikom	55.44	68.45
	Banglamung	216.14	76.00
	Sriracha	192.49	74.78
Nakhon Sawan	Muang	264.74	45.80
	Mae Wong	197.91	29.40
	Mae Pern	425.42	23.10
	Krok Pra	187.37	66.70
	Taklee	158.33	31.60
Nakorn Ratchasima	Prathai	349.95	40.00
	Kornburi	273.58	53.30
	Kangsanamnang	463.54	34.70
	Serngsang	155.85	46.00
	Seekhew	409.26	59.50
Songkhla	Muang	541.36	91.70
	Singhanakorn	321.28	45.10
	Bangklum	350.18	50.00
	Chana	533.46	44.80
	Had Yai	344.29	65.20

[a] Case rate means no. cases per 100,000 populations.
[b] House index = percentage of houses infested with *Ae. aegypti* larvae or pupae.

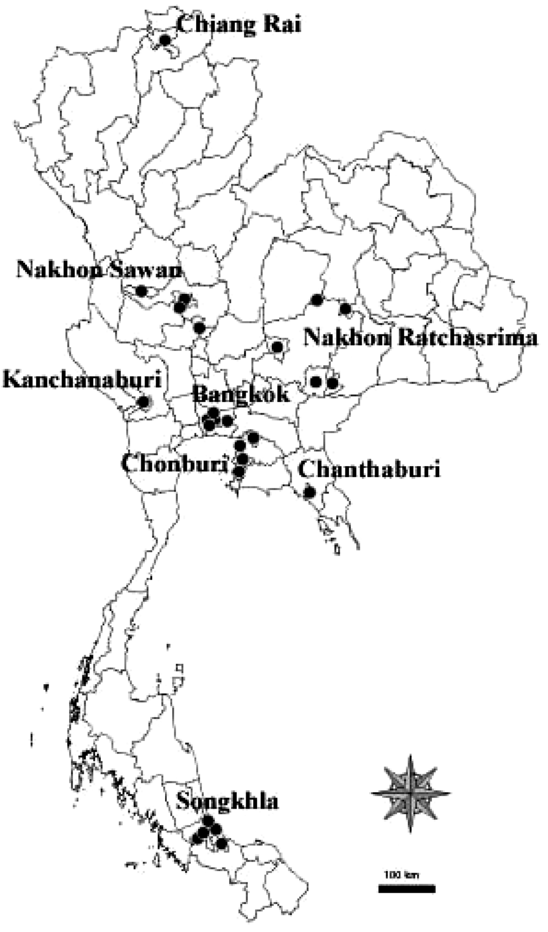


Fig. 1. Collection site for *Ae. aegypti* and *Ae. albopictus* mosquitoes cover all part of Thailand.

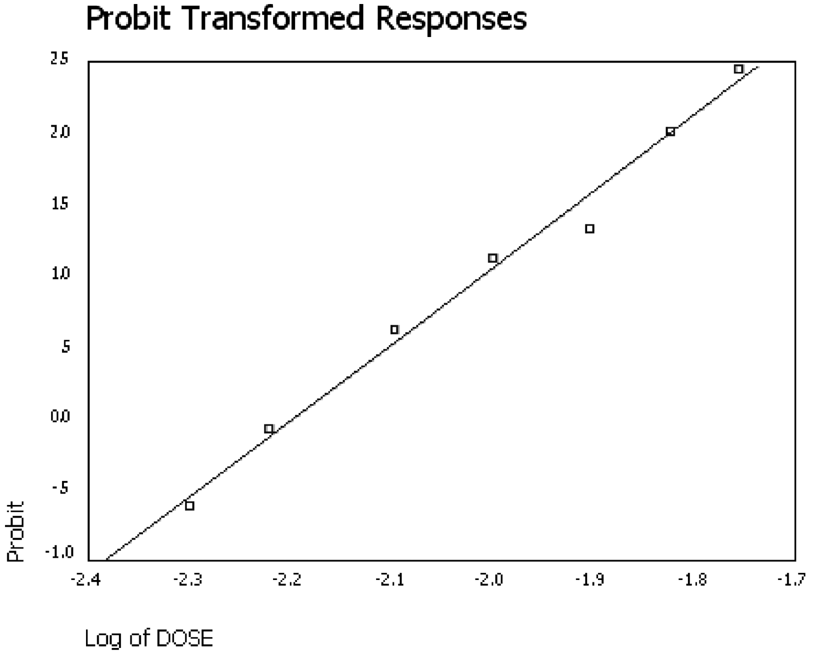
including countries in Southeast Asia (Lee and Lime 1989, Liew et al. 1994). High levels of temephos resistance were found in *Ae. aegypti* in the British Virgin Islands, whereas there was relatively low resistance to organophosphate and carbamate insecticides (Wirth and Georghiou 1999). In Thailand, there were few reports on the responses of this vector to temephos in samples from single location in the country or single collection site within some areas (Chareonviriyaphap et al. 1999, Ponlawat et al. 2005). Besides, there was only one report on low level of temephos resistance in *Ae. albopictus,* a secondary dengue vector, from the areas of southern-north and southern Thailand (Ponlawat et al. 2005). We report herein the evaluation of susceptibility/resistance status to temephos, based on the established diagnostic dosage, in *Ae. aegypti* and *Aedes albopictus* mosquitoes collected during 2003–2005 spanning the areas of Bangkok, northcentral, northern, northeastern, eastern, western, and southern Thailand.

Materials and Methods

Mosquitoes

Susceptible Strain. *Aedes aegypti,* Bora (French Polynesia) strain, obtained from Laboratoire de lutte Contre les Insectes Nuisibles (LIN/IRD), WHO Collaborating Center for Vector Control, Montpellier, France, was used. The eggs laid on filter paper were hatched in dechlorinated water. After hatching for 24 h, first-instar larvae were transferred to 20 by 30 plastic trays with 2 liters of water to obtain 300–400 larvae per container. Dog food (Alpo low fat, to avoid buildup of oil film on water surface; Nutrix Public Company Ltd., Chachoensao, Thailand) was given daily until they become pupae. The pupae were transferred to 30 by 30-cm cages, and the emerging adults were provided with 10% sugar solution. Blood from Swiss mice was also given twice a week for colonization.

Field Samples. Based on the annual epidemiological data in 2002 (Table 1), Bangkok and one province in each of four regions of Thailand known to have high incidence of dengue cases were selected as mosquito collecting sites (Fig. 1). These provinces were Bangkok (central), Chonburi (east), Nakhon Ratchasrima (northeast), Nakhon Sawan (northcentral), and Songkhla (south). Mosquito larvae and pupae collections were made in four to five districts of each province during 2003–2005 to assess their distribution in response to larvicide use. Additional three collection sites from Chiang Rai (north), Chanthaburi (east), and Kanchanaburi (west) were also done to cover all parts of the country. Pooled samples of *Ae. aegypti* and *Ae. albopictus* larvae and pupae from each



Pearson Goodness-of-fit Chi square = 12.820, DF = 6, P = 0.046

Fig. 2. Susceptibility baseline of *Ae. aegypti*, Bora bora reference strain to temephos.

district were marked and brought back to the laboratory for identification and colonization. Each mosquito sample was raised in the laboratory at 28°C, 70–80% RH. Larvae were fed on dog food (Alpo), and adults were provided with 10% sugar solution. Blood meals from Swiss mice were also given twice a week. After an optimum number of eggs were obtained, their F1 progenies were prepared for bioassay tests according to standard procedure from WHO (1981). However, some bioassays of *Ae. albopictus* were performed on the F2 generation because of insufficient numbers of F1 progenies.

Larvicide (Technical Grade): Temephos (SUPELCO)

Susceptibility Baseline Test. Batches of 20 fourth-instar *Ae. aegypti*, Bora (French Polynesia) strain, larvae were distributed into disposable plastic cups filled with 99 ml of water. After an observation for mortality or any abnormalities for 1 h, 1 ml each of a series of five insecticide concentrations was dispensed in five replicate cups. In the control cups, 1 ml of ethanol used as diluent was added. The larvae were exposed to the different concentrations of insecticide for 24 h at 28°C, during which no food was added. Moribund and dead larvae were combined in the mortality counts. The larvae that pupated during the test were discarded, and this should not exceed 10% in the controls. The test was also discarded if mortality in the control was >4%.

Preliminary tests were conducted using technical grade temephos concentrations ranging from 0.001 to 1 mg/liter to determine the general level of susceptibility. Selected concentrations of 0.005–0.02 mg/liter were used for the test, and the susceptibility base line was established based on the insecticide concentration giving 10–95% larval mortality. The 50 and 99% lethal concentrations (LC_{50} and LC_{99}) were calculated using log-probit analysis (SPSS version 11.5; SPSS, Chicago, IL). The tests that yielded a straight line relationship between the logarithm of concentration and probit mortalities were selected. The final estimation of LC_{50} and LC_{99} was determined from four replicate tests performed on different batches of larvae. Double concentration of LC_{99} was used as the diagnostic dose for tests on field samples.

Field Sample Tests. Bioassays were performed in the same manner on late third and early fourth instars. Five replicates of 20 larvae per test were used for each bioassay with insecticide solution at the diagnostic dose of 0.04 mg/liter temephos. Mortality was recorded after exposure for 24 h.

Results

Sets of insecticide concentration ranging from 0.005 to 0.02 mg/liter yielded 10–100% mortality of *Ae. aegypti*, Bora (French Polynesia), a susceptible strain. Four tests that yielded a straight line relationship between the logarithm of the concentration and probit mortalities were selected to analyze for the susceptibility baseline (Fig. 2). LC_{50} and LC_{99} were 0.0063 and 0.01737, with lower and upper limits at 0.00597–0.00667 and 0.01570–0.01973, respectively (Pearson Goodness-of-fit χ^2 = 12.820, df = 6,

Table 2. Resistance status to temephos in *Aedes* mosquitoes collected during 2003–2005 from all part of Thailand

Collection site		Mortality rate (%)	
		Aedes aegypti	*Aedes albopictus*
Bangkok	Bangkoknoi	90.6	—
	Hauykwang	68.8	—
	Laksi	98.3	—
	Ladkrabang	100.0	—
	Rasburana	99.8	—
Chonburi	Muang	99.0	—
	Panusnikom	98.5	—
	Banglamung	96.0	—
	Sriracha	100.0	—
Nakhon Sawan	Muang	90.1	99.00
	Mae Wong	50.5	—
	Mae Pern	92.2	—
	Krok Pra	87.0	—
	Taklee	100.0	—
Nakhon Ratchasrima	Prathai	71.43	—
	Kornburi	91.41	—
	Kangsanamnang	85.85	—
	Serngsang	84.85	—
	Seekhew	89.89	100.00
Songkhla	Muang	100.00	—
	Singhanakorn	100.00	100.00
	Bangklum	100.00	100.00
	Chana	100.00	100.00
	Had Yai (Tambon Kor Hong)	100.00	100.00
	(Tambon Tungtumsao)	—	100.00
Chiang Rai	Muang	100.00	—
Kanchanaburi	Tamaka	100.00	100.00
Chanthaburi	Muang	100.00	—

Mortality rate 98–100% = susceptible, 80–97% = incipient resistance, <80% = resistance (WHO 1998).

—, not done (*Ae. albopictus* could not be collected in the areas).

$P = 0.046$). According to WHO criteria, double the extrapolated LC_{99} (0.01737 mg/liter) from the probit line could be used as the discriminating concentration. Therefore, lethal concentration at 0.04 mg/liter was selected as a diagnostic dose for the larval test on F1 progenies of the field-collected samples.

Dengue case rates in 2002, used as a database for sample collection from all parts of the country (Fig. 1), showed no correlation with either the house index in each area or with the level of susceptibility to temephos (Tables 1 and 2). In Bangkok, of five populations tested, the Huaykwang population showed resistance to temephos, whereas the Bangkok noi population had developed an incipient resistance (Table 2). In eastern Thailand, including Chonburi and Chanthaburi provinces, most populations were susceptible to temephos except for specimens from Bang-la-mung district, which showed incipient resistance. A larva test of samples from northcentral and northeast Thailand, Nakhon Sawan and Nakhon Ratchasrima provinces, mostly revealed incipient resistance, except for population in Taklee, Nakhon Sawan. One population tested in each province had developed temephos resistance: Mae Wong in Nakhon Sawan and Prathai in Nakhon Ratchasrima. *Ae. aegypti* populations from Songkhla (southern), Chiang Rai (north), Kanchanaburi (west), and Chanthaburi (east) provinces remained susceptible to temephos.

Aedes albopictus mosquitoes are most prevalent in the southern part of Thailand. In many areas, only a small number of *Ae. albopictus* could be collected, and their progeny were insufficient for the test. All populations tested, based on the diagnostic dose used for *Ae. aegypti*, were highly susceptible to temephos during the collection period (Table 2).

Discussion

A susceptibility baseline based on the susceptible Bora (French Polynesia) strain of *Ae. aegypti* was established in this study to facilitate determination of susceptibility/resistance in dengue vector. A linear relationship between insecticide concentration and probit mortality confirmed that the test on susceptible mosquito strain was correctly done (Fig. 2). Double the concentration at LC_{99} (0.0174 mg/liter) was taken to be the diagnostic concentration that would normally produce a complete kill of insecticide susceptible mosquitoes (WHO 1981). A concentration of 0.04 mg/liter temephos was selected as the diagnostic dose to use in further laboratory tests for resistance on field-collected samples. Persistent survivors at this dose in field populations would be indicative that resistance to temephos has been developed.

In general, tendency of resistance to temephos in *Ae. aegypti* was most prevalent in Nakhon Sawan and Nakhon Ratchasima, located in the northcentral and northeastern parts of Thailand (Table 2; Fig. 2). Although temephos has been widely used throughout the country, naturally resistance was focal. For instance, only the population in the Hauykwang area of Bangkok was found to have temephos resistance similar to that reported during 1986 and 1992 in unspecified areas of Bangkok (Chareonviriyaphap et al. 1999). Recently, resistance to temephos in *Ae. aegypti* in Surat Thani, Nakhon Sawan, and Tak provinces during July 2003 to April 2004 was also documented (Ponlawat et al. 2005). In the same study, susceptibility to temephos was reported in the Nong Suang district of Nakhon Ratchasima province, whereas we found incipient resistance and resistance in samples collected from different areas of the same province. There were also varying levels of resistance (Mae Wong, Krok Pra, Muang, and Mae Pern) and susceptible to temephos (Taklee) in populations from the Nakhon Sawan province. Such findings indicate the necessity for careful and complete monitoring of resistance in all areas. Evaluation of susceptibility status should take this variability into account, and a plan must be made to collect samples from a range of sites within the areas. In other words, one or few sample points may not provide sufficient information for determining the efficacy of temephos in the field. Canyon and Hii (1999) came to a similar view after they found varying levels of temephos susceptibility among *Ae. aegypti* populations in Townsville, Australia. Differences in control effort and frequency of the insecticide application in public health and agriculture might account for different levels of response to insecticide over the landscape.

Susceptibility testing of *Ae. albopictus* collected during the study period revealed that they were susceptible to temephos, although they co-habitated in some areas with *Ae. aegypti.* This could be because of the fact that *Ae. aegypti* breed and mainly lay their eggs in household containers inside the house, whereas *Ae. albopictus* prefer outside. In addition, no control action has been directed against *Ae. albopictus* because they are not the main dengue vector in Thailand. Ponlawat et al. (2005) also reported lower levels and fewer cases of resistance to temephos in *Ae. albopictus* than in *Ae. aegypti.* However, monitoring for insecticide resistance in *Ae. albopictus* is necessary because this species could serve as reservoir of dengue viruses in nature.

Unlike adulticide, the use of nontoxic to human larvicide like temephos leads to unawareness of resistance development because of improper application schemes. The slow release pattern of temephos sand granules over time has been noted under field conditions (Thavara et al. 2005) because of its low solubility in water (0.03 mg/liter at 25°C). This character could facilitate selection for tolerance up to resistance levels in nature on exposure to insecticide at below-mortality doses. We found that susceptibility/resistance levels were not related to either dengue case rates or house index in the study areas. We also observed larvae positive containers with temephos sand granules in some collection sites during our collection period (unpublished observations). Level of effective activity and longevity of temephos in the fields should therefore be evaluated for the most appropriate use.

In conclusion, the data obtained in this study could serve as a useful baseline response for the known normal susceptible population of *Ae. aegypti,* Bora (French Polynesia) strain. This baseline concentration can be used for future comparison and monitoring tests for resistance in the field populations. The discriminate concentration (dosage) can also be easily and routinely used to detect and monitor insecticide resistance in this vector. Continuing field surveillance for development of temephos resistance is necessary, so that any other control measures or tactics to delay insect resistance can be suitably applied at the right time. Source reduction is the most effective, direct, and simple method of intervention in *Aedes* populations to prevent or delay development of massive levels of resistance.

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