Asia Journal of Public Health
Journal homepage:http://www.ASIAPH.org

Original Articles

Bioassay and Effective Concentration of Temephos Against *Aedes aegypti* Larvae and the Adverse Effect Upon Indigenous Predators: *Toxorhynchites splendens* and *Micronecta* sp.

Usa Lek-Uthai [*] Potchara Rattanapreechachai [**] Laojana Chowanadisai[***]

**Department of Parasitology and Entomology, Faculty of Public Health, Mahidol University, Bangkok, Thailand*
*** Communicable Diseases Section, Klongloung District Health Office, Pathumthani province, Thailand*
**** Biological Control Section, National Institute of Health, Department of Medical Sciences, Ministry of Public Health, Thailand*

ARTICLE INFO

Article history :
Received May 2011
Received in revised form June 2011
Accepted July 2011
Available online August 2011

Keywords:
Temephos
Effective concentration
Adverse effect
Toxorhynchites splendens
Micronecta sp
Aedes aegypti
Predators

Corresponding Author:
Lek-Uthai U,
Department of Parasitology and Entomology,
Faculty of Public Health,
Mahidol University,
420/1 Ratchavithi Road,
Bangkok 10400, Thailand.
Email : phulu@mahidol.ac.th

Asia J Public Health 2011; 2(2): 67-77

ABSTRACT

Objective: The WHO recommended that the dosage of Temephos to eliminate mosquito larvae is 1 ppm. The toxicity of Temephos on non-target organisms has been shown in many ecological systems, especially in aquatic ecology, which is the breeding habitat of many insect predators of *Ae. aegypti* larvae. However the toxicity of Temephos against *Ae. aegypti* larvae, *Toxorhynchites splendens* larvae, and *Micronecta* sp adults under simulated daily water consumptions were observed. **Materials and Methods:** Bioassay method was determined the toxicity of Temephos against *Ae. aegypti* larvae, *Toxorhynchites splendens* larvae, and *Micronecta* sp adults in terms of the median lethal concentration of Temephos (LC50) after 24 hours of exposure. This study was tested under the natural conditions in the enamel-coated jars. **Results:** LC50 of *Ae. aegypti* and *Tx. splendens* larvae, and *Micronecta* sp adults were 0.006, 0.1310 and 0.0028 ppm, respectively. The selected Temephos effective concentration was simulated to determine its continued effectiveness by using small enamel coated jars (50 litres), for which the average temperature and relative humidity were 27.71 ± 1.12 and 71.06 ± 2.88%. The average pH of tap water was 6.8; Temephos concentrations of 0.06, 0.12, 0.18 and 0.24 ppm in 40 litres of tap water were used. Continued effectiveness was observed after the removal and replacement of 25%, 50% and 90% of the water, which indicated that 0.12 ppm of Temephos, with removal and replacement of 90% of the water was the effective concentration yielding over a 90% mortality rate for *Ae. aegypti* larvae. The same results were obtained with a concentration of 0.18 and 0.24 ppm levels on day 7. When the concentration of 0.12 ppm was tested with the *Tx. splendens* and *Micronecta* sp, results indicated that in 100% of the trials, the time of exposure needed to deliver lethal results was 2 hours and 1 hour, respectively. **Conclusion:** The use of less than 1 ppm of Temephos in three common types of water containers and three water usage practices were recommended. Removal of the total volume of water at one time had greater effect than continuous removal of small water volume. This study shows beyond any doubt that much lower concentrations of Temephos and it should be used in conjunction with other natural methods of control, depending on the direction of the health policy and the global environmental considerations.which are much safer and, perhaps most importantly, effective.

INTRODUCTION

Dengue Haemorrhagic Fever (DHF) is an important health problem in the South-East Asia region, the Western Pacific, the Caribbean and in Thailand. *Aedes aegypti* has become the primary mosquito problem. A breeding container for mosquitos can be prevented by proper treatment of such water containers by either covering them or emptying them of water. *Aedes* is usually produced in this habitat and also breeds in naturally occurring areas where still water lies. Effective mosquito control includes periodic drainage of low lying areas, providing sanitary deepwater for larvivorous fish and maintaining a steep bank along rivers, streams and ponds. *Culex*, *Mansonia* and *Anopheles* mosquitoes are also frequently produced in these habitats. The three main strategies for mosquito control are 1) to reduce the breeding habitat 2)biological control, (such as using larvivorous fish, *Bacillus thuringiensis* var israelensis H-14, viruses, nematode worms, fungi and insect predators, and *Toxorhynchites* spp larvae and *Micronecta* sp. , commonly referred to as Water Boatman), and 3) using insecticide in breeding areas. Using the first two strategies can virtually eliminate insecticide usage and noticeable environmental impact was observed from this implementation. Maintaining water quality can be managed by proper preventive methods. However, biological control has limited potential to the overall control program. It can be used as an alternative or supplemental method for mosquito elimination[1]. The larvicide, Temephos is an organophosphate insecticide that has been used for controlling vector–borne diseases, especially Dengue Haemorrhagic Fever (DHF) and Japanese Encephalitis (JE), in Thailand since 1970. The sand granule (SG) formulation of Temephos is an effective larvicidal[2]; trade name: Abate 1% SG added to water containers indoors and around the house effectively prevents breeding in traditional places, such as water jars, cement tanks in bathrooms, ant guards, tires and flower pots, etc.[3]. The World Health Organization (WHO) has recommended the dosage of Abate at 1% SG to eliminate larvae population. A solution of 1 gram per10 litres of water gives an effective control period of 2.5 to 5 months, with an average of 3 months[4] and at a lower cost than using Larvitab when the two insecticides were compared. The cost of one usage for Abate and Larvitab were 0.09 and 0.15 USD respectively[5]. In the beginning, using Temephos for larvae control was rejected by users because it had a bad smell and people were uncertain of its safety[1]. The Office of Dengue Control, Department of Communicable Disease Control, and the Ministry of Public Health has paid about 5,461,104 USD to exterminate Temephos each year. A study of the toxicity of Temephos was assessed[6]. This study also concluded that volunteer workers could tolerate 64 mg per man, per day, for four weeks, without clinical symptoms, or the appearance of side effects attributable to Temephos, and there was no effect detected in the users' red blood cells or plasma cholinesterase. Areas such as Singapore, Malaysia, New Caledonia and South America have reported that mosquito larvae have developed resistance to Temephos[1], but there have been no such reports in Thailand[7]. The study of the impact of Temephos and Abate, demonstrated that small Western toads experienced a reduction in the tolerance to these chemical substances[8], and they further reported that it was highly toxic to many forms of wildlife. The reduction in the growth rate of gray tree frogs, (*Halavericolor*) tadpoles, etc. has also been reported[9]. Similarly, the impact of Temephos upon macro-invertebrate and amphibian larvae in freshwater macrocosms has been observed, which found that an acute toxicity dosage (LC50) was 4.24 ppm[10]. Moreover, it has been reported that the acute toxic dosage (LC50) of Abate for Chinese carp developed at 96 hours with 0.6 ppm. The acute toxicity of organophosphate (OP) to fish, which resulted in the observation of abnormal, quick swimming and convulsions, etc., has been reported[11]. Malathion, Abate and Pyrethrum are insecticides which have less toxicity to man, but may pollute the environment and may have adverse effects upon aquatic insects, such as predators that usually have been controlled in the natural ecological balance.

Yap[12] showed that bacteria, fungi, nematode worms, protozoans and predator insects were controlled by mosquito larvae in a natural state. Water Boatman, Backswimmers, Pygmy Backswimmers, Water Scorpions, Giant Water Bugs (Belostomatid), Creeping Water Bugs, Predaceous Diving Beetles, Whirligig Beetles, Burrowing Water Beetles, Skimmers, Clubtails, and Damselflies could feed on *Aedes*, *Anopheles* and *Culex* larvae.

Selection of good aquatic predators, such as *Toxorhynchites* larvae and Water Boatman that could be established in an ecosystem is one of the best methods to control malaria vectors[13]. Giant Water Bugs[14] and Water Scorpions[15] were not appropriate in controlling *Aedes* mosquitos, but it was also reported that *Anopheles* larvae were eaten as prey by many species of Backswimmers[16].

Temephos has a low toxicity to man. Thus, the proper management of insecticide concentrations among the larval vectors should be verified. At present, we cannot avoid using insecticide and have not yet found the best effective method for mosquito control. This research proved significant differences in effectiveness when using 1% Temephos and lower concentrations for mosquito larvae, and the adverse effect to indigenous predators, such as *Toxorhynchites*spp and Water Boatman was also noted. The acute toxicity (LC50) of 1 % Temephos for both insects, and the resistance of the *Aedes* mosquito to Temephos, has not yet been reported. An average of 73.7% of the *Aedes* larvae was killed using *Micronecta* sp as a predator in the control program[17]. Thus, this study of effective concentrations of Temephos upon mosquito larva in natural conditions was considered, along with the toxicity of Temephos upon predator insects, such as *Toxorhynchites* sp and Water Boatman.

MATERIALS AND METHODS

The laboratory bioassay of Temephos was carried out with indigenous predators such as *Tx. splendens* larvae, *Micronecta* sp adults and the early 4th instar of *Ae. aegypti* larvae, and the determination of the lethal concentration of Temephos to kill 50% of the tested insects (LC50), was under natural conditions. Early 4th instar of *Ae.aegypti* larvae were LC50 tested against the persistence of 10, 20, 30 and 40 fold dilutions of three rates of changing water: Those amounts were 25%, 50% and 90% of 40 litres. Finally, the early 4th instar of *Tx. splendens* larvae and *Micronecta* sp adult were tested to determine the adverse effect on mortality at the effective concentration of Temephos. Three phases were determined 1) Larvicidal activity of Temephos against *Ae. aegypti* and *Tx. splendens* and *Micronecta* sp 2) Persistence of Temephos against *Ae. aegypti* under simulated field conditions and 3) Temephos used at a concentration to effectively insure 100% mortality of *Tx. splendens* and *Micronecta* sp. The persistence was carried out in the tested cups for 24 hours at the Biological Control Laboratory of the National Institute of Health (NIH), Ministry of Public Health, Thailand.

Preparation of Temephos solution

Temephos in an emulsified concentrate containing 44.6% of the active ingredient was selected for the study. The dilution of Temephos to the desired concentrations for all bioassays was carried out by using deionized water.

Rearing of Aedes larvae for laboratory bioassay

Preparation of mosquito larvae for the bioassay was modified from those described by Chowanadisai (1987)[18]. Colonization of mosquitos was maintained at 25±1^{o}C and 70±2 % Room Humidity (RH). Egg batch of *Ae. aegypti* was kept dried on filter paper. The eggs were carefully soaked in the filter water. The newly hatched larvae appeared within 30 minutes after soaking and were then transferred to an aluminium pan containing 1,500 ml of water which was used for rearing 1,000 larvae. Approximately 0.5 gram of mouse pellet was offered to larvae in each pan. Water in the rearing pan would not be changed but evaporated water was replaced with fresh water. The early 4th instar or 4 day-old larvae were tested.

Colonization and preparation of Tx. splendens for laboratory bioassay.

The adult stage of *Tx. splendens* was colonized in a cage with the dimensions of 75 x 75x75 cm. A stick of cotton pad soaked with 10% honey was hanged in the cage and served as a nectar source. A black enamelled bowl, 10 cm in diameter was ¾ filled with water and served as the oviposition site. Soon after oviposition, the eggs were individually transferred to a plastic tube 2.8 cm in diameter and 5.5 cm in height. About ¾ of the tube was filled with water. The 2nd instar of *Ae. aegypti* was provided to the *Toxorhynchites* larvae in adequate number. The predaceous larvae were reared under room temperature (RT) (29±0.90^{o}C, 79±4.66 %RH) until they became early 4th instar or 12 days old when they were tested.

Colonization and preparation of Micronecta sp for laboratory bioassay.

The colony of *Micronecta* sp was supported

from the Vector Borne Disease Control Office, Region 3, Khon-Khaen Province. The mentioned species was reared in an earthen jar containing 40 litres of dechlorinated water. A water plant was provided as a resting and oviposition site. The 2nd instar *Ae. aegypti* larvae were offered daily to the bugs in sufficient numbers. The rearing jars were covered with a fine mesh cloth and placed outdoors in the shade. The adults of *Micronecta* sp, or 28 day old organisms, were tested.

Observations on larvicidal activities of Temephos against Ae. aegypti

A duplicate of an appropriate 10-fold concentration series of Temephos, ranging from 0.000078 to 1.0 ppm was performed. Ten 4th instar *Ae. aegypti* larvae were then exposed to the Temephos solution. Mortality of the larvae was recorded at 24 hours after exposure. Then four replicates of a concentration series of 0.0025, 0.005, 0.01, 0.02 and 0.04 ppm of Temephos were prepared. Ten 4th instar *Ae. aegypti* larvae were then released in a test cup containing 100 ml of tested solution. Mortality of larvae was also recorded at 24 hours after exposure and determined by counting the number of surviving larvae. Larvae that did not respond to the agitation of the test cups as well as needle touch were counted as dead. The mortality rate was plotted on logarithmic–probability paper. Linearity of the test as well as median lethal concentrations and correlation coefficients were calculated via computer programs for probit analysis. The bioassay was repeated 4 times, then the average median lethal concentration, standard error and correlation coefficient were calculated.

Affect of Temephos to local natural enemies of Ae. aegypti larvae, Tx. splendens and Micronecta sp.

An appropriate concentration series of 0.0187, 0.0375, 0.075, 0.15 and 0.3 ppm of Temephos was prepared in the test cups containing 100 ml of test solution. The early 4th instar *Tx. splendens* larvae were individually released in the test cups. Twenty larvae were used for each concentration. Mortality of the larvae was recorded at every hour after exposure. Accumulated mortality was totally summarized at 24 hours after exposure. The observation was repeated 4 times then the average median lethal concentrations as well as the mortality rate were calculated. For the observation on the larvicidal activity of Temephos against *Micronecta* sp a concentration series of 0.000625, 0.00125, 0.0025, 0.005 and 0.01 ppm of Temephos was also prepared in the test cups containing 100 ml of test solution. Twenty individual of the adult stage or 28 day old *Micronecta* sp were infested in the test cups. Mortality of tested species was recorded at 24 hours after exposure. Total mortality was summarized at 24 hours after exposure. The observation was also repeated 4 times then the average median lethal concentration and mortality rate were calculated

Persistence of Temephos toward Aedes larvae under simulated field conditions

The observation was conducted by preparing three replicates of a concentration series of 0.06, 0.12, 0.18 and 0.24 ppm of Temephos in the enamel-coated test jars each containing 40 litres of tested solution. To simulate the loss or consumption of water resulting in a loss of active ingredient; the tested jars were therefore divided into 3 groups. The tested solution in each group was changed daily and replaced by fresh water in amounts of 25, 50 and 90 % of the tested volume. The remaining larvicidal activity was followed up on day 0, 1, 3, 5, 7, 10 and then every 5 days thereafter and at these intervals, 25 early 4th instar larvae were added into the test jars. Mortality rate was recorded at 24 hours after exposure. The concentrations provided more than 90% mortality for at least 4 weeks. The observation was continued until larval mortality could not be recorded.

The concentrations equivalent to 10, 20, 30 and 40 folds of LC50 of Temephos to *Aedes* larvae in the test jars were prepared. Simulation of water consuming habit of people was by daily changing and replacing with water for 25%, 50% and 90% of tested volume. The 25 early 4thinstar of *Ae.aegypti* larvae were released in the jar on day 0, 1, 3, 5, 7, 15 and then every 5 days thereafter until larval mortality could not be observed. The mortality rate of early 4th instar larvae of *Ae. Aegypti*, in addition to *Tx. splendens* and *Micronecta* sp adults was calculated by mean and standard deviation (SD). The LC50 value of tested insects resulted from the adjusted mortality rate by Abbott's formula and plotted on the probit-log scale over computer program, while means of the mortality of *Ae. aegypti*, *Tx. splendens* and *Micronecta* sp in laboratory were analyzed by Analysis of Variance (ANOVA), and the mean difference

of the mortality of the early 4th instar larvae of *Ae. aegypti* in the simulated trial was analyzed by Duncan's New Multiple Range Test.

RESULTS

The results and discussion were divided as follows:

Larvicidal activity of Temephos toward Ae. aegypti

The laboratory bioassay to determine the larvicidal activity of Temephos against larvae of *Ae.aegypti*, indicated that the mortality rate of *Ae. aegypti* larvae, when exposed to different concentrations of Temephos, completely varied among the 4 observations. At the concentrations of 0.0025, 0.005, 0.01, 0.02 and 0.04 ppm of Temephos, the mortality rate range were 0-75, 0-55, 17.5-95, 57.5-100 and 90-100, respectively, which indicated that the average mortality rate became 1.88, 16.88, 50.63, 84.38 and 96.25, respectively. Temephos also exhibited larvicidal activity against local predaceous insects, *Tx. splendens* and *Micronecta* sp. At the concentrations of 0.0375, 0.075, 0.15 and 0.3 ppm of Temephos, the mortality rate found from the observations, varied relatively. The variances were 0-5, 10-35, 45-75 and 85-95, respectively. However, the average mortality percentages of 3.75, 23.75, 57.50 and 91.25, respectively, were recorded. *Micronecta* sp was much more susceptible to Temephos than *Ae. aegypti* and *Tx. splendens*. The Mortality rate obtained from 4 observations also varied relatively. It was found that mortality rate ranged from 5-10, 25-55, 75-90 and 95-100 when exposed to the concentrations of 0.00125, 0.0025, 0.005 and 0.01 ppm of Temephos, respectively. The average mortality rates were 7.50, 41.25, 86.25 and 98.75, respectively (Table1).

The larvicidal activity of Temephos toward *Ae. aegypti*, *Tx. splendens* and *Micronecta* sp was summarized. The median lethal concentrations of Temephos against *Ae. aegypti*, *Tx. splendens* and *Micronecta* sp. were 0.0060 ± 0.0020, 0.1310 ± 0.0090, and 0.0028 ± 0.0002 ppm, respectively (Table 1a). The correlation coefficient of the 3 target species were 4.1823 ± 0.5175, 3.7322 ± 0.3848 and 4.3669 ± 0.4739, respectively (Table 2).

Among the 3 candidate species, *Micronecta* sp was the most susceptible to Temephos. The susceptible ratio between *Tx. splendens* and *Ae .aegypti* [LC50(ppm)=0.1310:0.0060] was 21.83 while that between *Micronecta* sp. and *Ae. Aegypti* [LC50(ppm)=0.0028:0.0060] was-0.47.

Table 1 Mortality rates of the 4th instar *Ae.aegypti* larvae, *Tx. Splendens* larvae, *Micronecta* sp exposed to different concentrations of Temephos (ppm) in 4 replicates.

Concentration	% Mortality range[a]	Average % mortality[b] (ppm) (mean±SE)
***Ae. aegypti* larvae** (n=40)		
0.04	90-100	96.25
0.02	57.5-10	84.38
0.01	17.5-95	50.63
0.005	0-55	16.88
0.0025	0-7.5	1.88
control	0	0
***Tx. splendens* larvae** (n=20)		
0.3	85-95	91.25
0.15	45-75	57.50
0.075	10-35	23.75
0.0375	0-5	3.75
0.01875	0	0
control	0	0
***Micronecta* sp** (n=20)		
0.01	95-100	98.75
0.005	75-90	86.25
0.0025	25-55	41.25
0.00125	5-10	7.50
0.000625	0	0
control	0	0

a = mortality recorded after 24 hours of exposure
b = average from 4 observations

Table 2 The median lethal concentration of Temephos against *Ae. aegypti*, *Tx. splendens* and *Micronecta* sp and the correlation coefficient of the 3 target species.

Species	N	95%CI (ppm)	LC50±SE (ppm)[a]	b±SE[b]
Ae. aegypti	40	0.0025-0.0095	0.0060±0.0020	4.1823±0.5175
Tx. splendens	20	0.0961-0.1572	0.1310±0.0090	3.7322±0.3848
Micronecta sp.	20	0.0021-0.0035	0.0028±0.0002	4.3669±0.4739

a= average from 4 observations
b= correlation coefficient

Persistence of Temephos toward Ae. Aegypti under simulated field conditions

Results from this study indicated that Temephos at the concentration of 0.06, 0.12, 0.18 and 0.24 ppm could demonstrate more than 90% larval mortality for 5 days, followed by 7 days, then another 7 days and finally a 5 day period, respectively with daily removal and replacement of 90% of the test solution from the test jar, while 50% larval mortality could be observed after 7 days. The larvicidal activity ceased after 15, 20, 15 and 15 days of observations, respectively. The jars contained 50% of the test solution which was removed daily and replaced, and more than 90% larval mortality could be detected during the first 7, 15, 15 and 15 days of observations, respectively. The larvicidal activity rapidly dropped and no activity was found after 15, 30, 25 and 20 days, respectively. The larvicidal activity in the group of 25% daily removal and replacement exhibited longer persistence. More than 90% larval mortality could be recorded during the first 15, 20, 30 and 30 days of observations, respectively. More than 50% larval mortality was found during 20, 25, 40 and 40 days and no activity was found after 35, 40, 50 and 60 days of observations, respectively (Figure 1 a-d)

Figure 1d shows the relationship between the 4 concentrations of Temephos and the mortality of *Ae. aegypti* larvae with 90% removal and replacement water that simulated daily water consumption. The remaining activity of Temephos still exhibited larval dead, in the tested jars for all 4 concentrations of Temephos, although the death rate was not much different. It could be suggested that if no significance on the mortality rate was found among the different concentrations of Temephos, the lowest concentration should be selected to avoid the adverse effects that might occur to the aquatic insects living in the same breeding area of *Aedes*. Therefore, the values of impact to *Tx. splendens* and *Micronecta* sp showed the concentration of 0.12 ppm when compared to the duration time of 100% mortality at 0.006 and 1.0 ppm. The value of the duration time of 100% mortality was 2 and 1 hour for *Tx. splendens* and *Micronecta* sp, respectively.

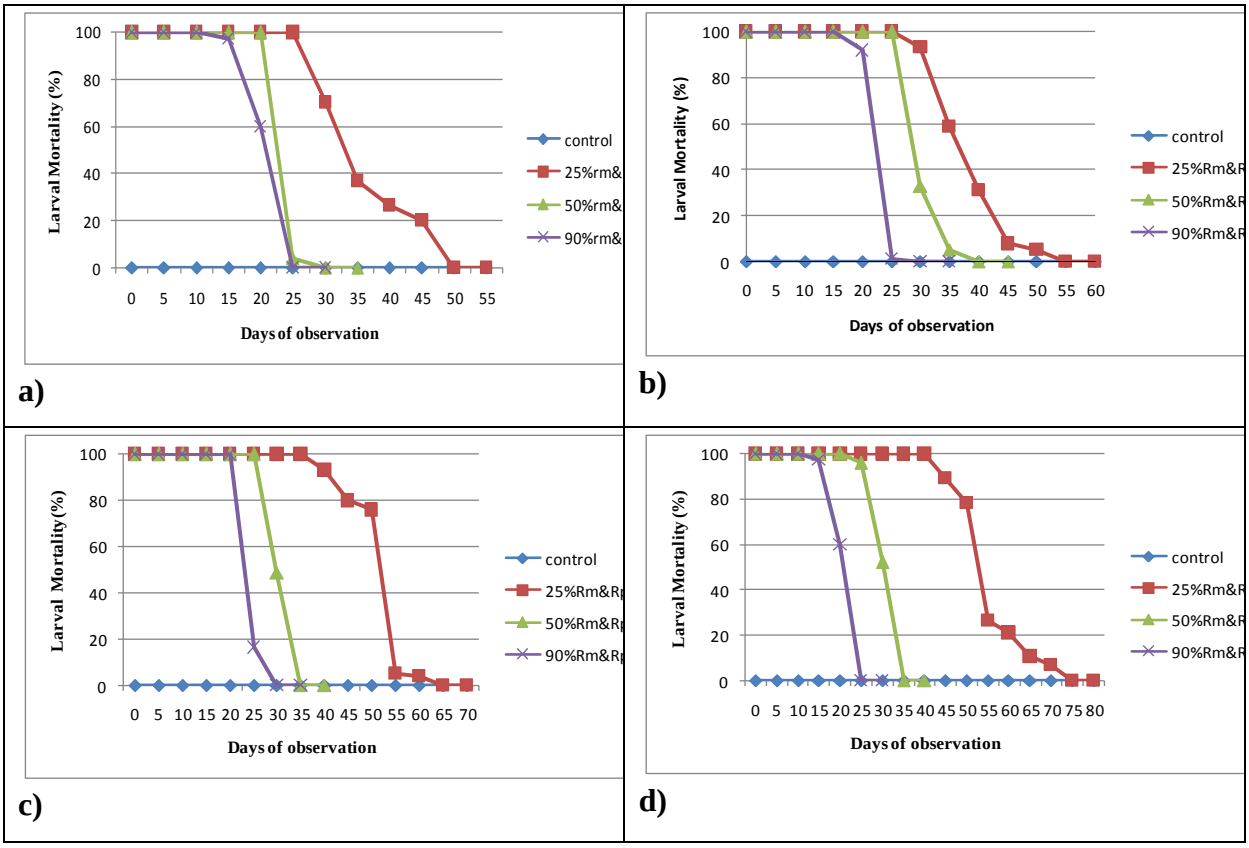

Figure 1 Persistence of a) 0.06 ppm (10 x LC50); b) 0.12 ppm (20 x LC50); c) 0.18 ppm (30 x LC50) and d) 0.24 ppm (40 x LC50) Temephos against the 4th instar larvae of *Ae. aegypti* under simulated field conditions.

At the level of 25% removal and replacement of tested solution

On day 20, the mortality of tested larvae of all concentrations did not differand on day 25, concentrations of 0.12 and 0.18 ppm showed that there were significant differences with the concentration of 0.06 ppm (F=3.60, p<0.05) (Table 3). On day 30, the mortality of tested larvae at the concentrations of 0.18 and 0.24 ppm were significantly different from the concentration of 0.06 and 0.12 ppm (F=4.78, p<0.05). On day 35, the mortality of tested larvae at the concentration of 0.24 ppm was not different from the concentration of 0.18 ppm and at the concentrationof 0.12 ppm, was not different from the concentration of 0.06 ppm. On day 40, the mortality of tested larvae at the concentration of 0.06 ppm was not different from the concentration of 0.12 ppm and at the concentration of 0.18 ppm, it was not different from the concentration of 0.24 ppm. The tested larvae did not die at the concentration of 0.06 ppm. On day 45, the mortality of tested larvae at the concentration of 0.18 ppm was significantly different from the concentration of 0.24 ppm (F=3.94, p<0.05). The tested larvae did not die at the concentrations of 0.06 and 0.12 ppm (Table 3).

Table 3 Larval mortality of *Ae. aegypti* in simulated conditions in different percentage of removal (Rm) and replacement (Rp) of tested solutions, by ANOVA.

Days of observation		SOV	df	SS	MS	F
25%RM&Rp	day 20	TRT	3	119.33	39.78	2.39
		ERR	8	133.33	16.67	
	day 25	TRT	3	550.33	183.44	3.61*
		ERR	8	407.33	50.92	
	day 30	TRT	3	843.00	281.00	4.78*
		ERR	8	470.00	58.75	
	day 35	TRT	3	958.00	319.33	11.68*
		ERR	8	218.67	27.30	
	day 40	TRT	3	1029.58	343.19	39.60*
		ERR	8	69.33	8.67	
	day 45	TRT	3	90.67	30.22	3.94*
		ERR	8	61.33	7.67	
50%RM&Rp	day 15	TRT	3	1262.25	420.75	420.75*
		ERR	8	8.00	1.00	
	day 20	TRT	3	288.67	96.22	4.89*
		ERR	8	157.33	19.67	
90%RM&Rp	day 7	TRT	3	204.00	68.00	12.36*
		ERR	8	44.00	5.50	

*significantly differences at p<0.05, ANOVA.
SOV=Sum square of Variance,
TRT=Sum square Treatment,
ERR= Sum square Error

At the level of 50% removal and replacement of tested solution

The results showed the mortality of tested larvae was 100% at the concentrations of 0.12, 0.18 and 0.24 ppm. There were significant differences at the concentrations of 0.06 ppm (F=420.75, p < 0.05). On day 20th, all tested larvae were not died. At the concentrations of 0.18 and 0.24 ppm, and the mortality of tested larvae were not different but they were significantly different from the concentration of 0.06 ppm (F=4.89, p < 0.05) (Table 3).

At the level of 90% removal and replacement of tested solution

The results showed the mortality of tested larvae was significantly different among the concentrations of 0.12, 0.18 and 0.24 ppm with the concentration of 0.06 ppm (F=12.36, p<0.05). On day 15, the mortality of tested larvae was lower than 20% and all concentrations were not different.

Affect of Temephos effective concentration to the duration of 100% mortality of Tx. splendens and Micronecta sp

Among the 3 target species, *Micronecta* sp seemed to be the most susceptible species to Temephos. Data shown in table 4 explained that Temephos at the concentration of 0.006, 0.12 and 1.0 ppm, accumulated the total mortality of *Tx. splendens* exposed to the three concentrations found within 4, 2 and 2 hours after exposure, respectively. While *Micronecta* sp exposed to the same concentrations of Temephos exhibited complete mortality with 2, 1 and 1 hour after exposure, respectively.

Table 4 Accumulated mortality rate of *Tx. splendens* larva at time interval when exposed to the 3 concentrations of Temephos

Hour of observation	Hour of Accumulated mortality rate at concentration (ppm)		
	0.006	0.12	1.0
***Tx. splendens* larva** (n = 20)			
1	15	30	80
2	47.5	100	100
3	87.5	100	100
4	100	100	100
***Micronecta* sp** (n = 20)			
1	90	100	100
2	100	100	100
3	100	100	100
4	100	100	100

DISCUSSION

Since Dengue Haemorrhagic Fever is among the communicable diseases that remains of public health importance in Thailand, as well as in many regions of the world [2] a control program for the vector is critical. According to the oral LD50 of 8,600 mg/kg rat [19], Temephos has been selected and implemented in the vector control program for the past 30 years as an effective larvicide with few choices of alternative control agents. The effective dosage recommended by WHO for application of Temephos in domestic containers is 1 ppm which was expected to persist against target larvae for at least 3 months [4]. The mentioned dosage seemed to be very high; about166 fold when compared to the median lethal concentration value obtained from this study. Due to the ecological impact and adverse effects to the local predaceous as well as the useful agricultural insects that breed in the same habitat of *Ae. aegypti* larvae, concern should be given. Considering the susceptibility ratio, *Tx. splendens* was the most tolerant species to Temephos while *Micronecta* sp seemed to be the most susceptible species (Table 1b).

the efficacy of Temephos toward *Ae. aegypti*. Phanthumachinda *et al.* [7] reported that the LC50 of Temephos against *Aedes* mosquito during 1976-1978 were 0.0004-0.0008, 0.0006-0.003 and 0.001-0.004 ppm, respectively. The LC50 of Temephos against *Aedes* mosquitos collected from Pathumthani province was 0.0037 ppm [20]. In addition, the susceptibility of the early 4th instar of *Tx. splendens* larvae to Temephos was 0.1310 ppm [21], and 0.2 ppm [10] while the LC50 of Temephos against *Micronecta* sp was 0.0028 ppm.

The time persistence of Temephos to kill tested larvae in simulated conditions were analysed by ANOVA and followed by method of Duncan's New Multiple Range test. The LC50 of *Ae. aegypti* larvae, *Tx. splendens* larvae and *Micronecta* sp adult were 0.006, 0.1310, and 0.0028 ppm, respectively. They were tested to determine the value of the Temephos effective concentration in phase II for 4 concentrations at 0.06, 0.12, 0.18 and 0.24 ppm, respectively, under simulated conditions.

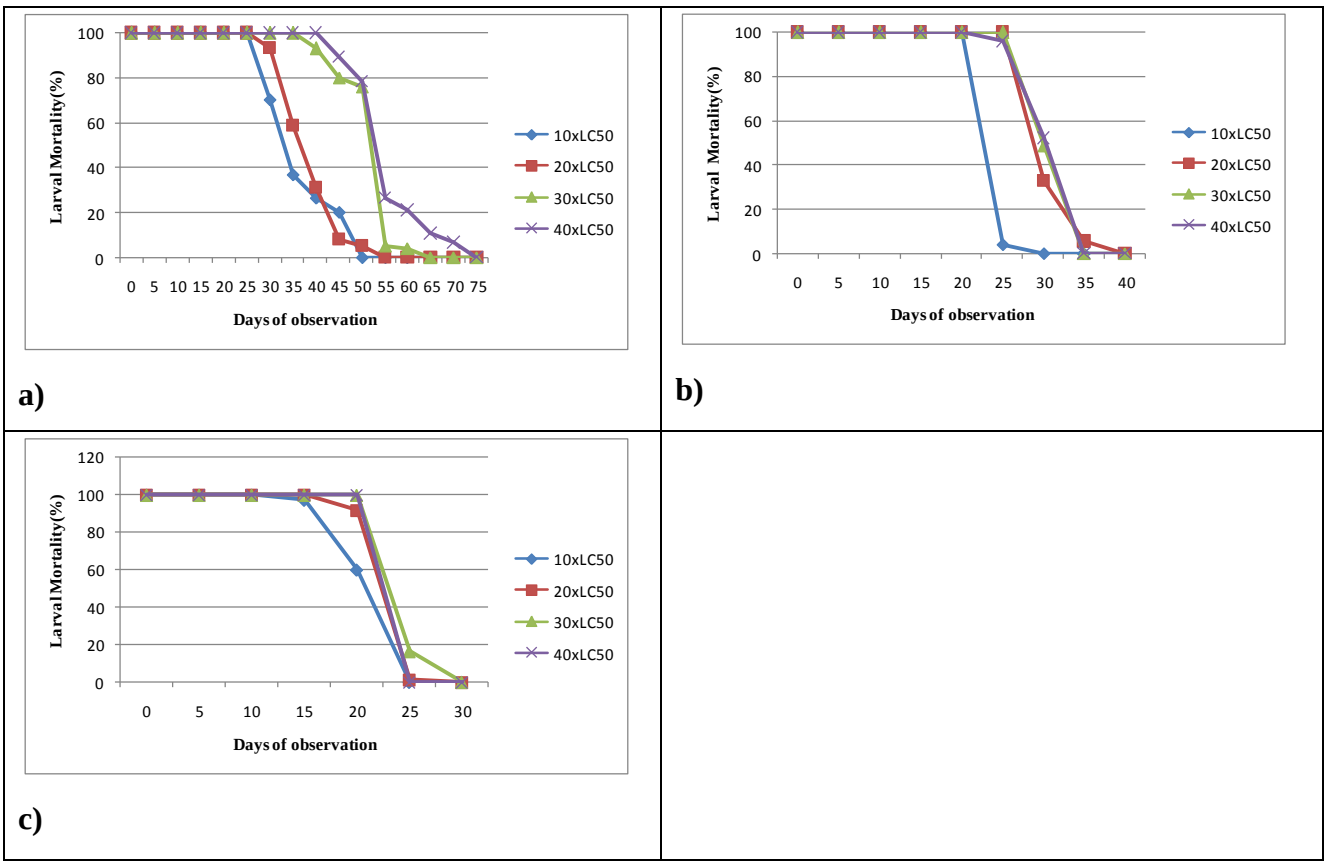

Figure 2.Residual effect of Temephos against the 4th instar larvae of *Ae.aegypti* under simulated field conditions when a) 25%, b)50%, and c) 90% removal and replacement of tested solution.

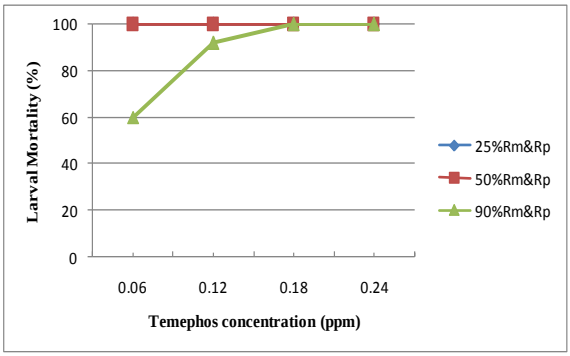

Figure 3 Temephos effective concentration at 7 days after 25%, 50% and 90% removal (Rm) and replacement (Rp) with fresh water.

In summary, the persistence of Temephos depended on the concentration as well as the remaining concentration of Temephos after removal and replacement of water at different volumes which resulted in the dilution of th active ingredient. The higher concentrations of Temephos persisted longer than those of lower concentrations (Figure 1a-d) whenever the same volume of water was removed and replaced (Figure 2a-c). Then, the effective concentration of Temephos was 0.12 ppm. It was the lowest concentration, but had a larval mortality of more than 90% (Macdonald, 1956) as well as this study which found the highest level concentration for 90% water removal and replacement (Figure 3). The Quantity of water consumed by people, as well as the replacement of fresh water completely influenced the remaining larvicidal activity of Temephos in the container. The use of 1 ppm of Abate in three common types of water containers and three water usage practices were reported [22]. It was found that the degree of efficacy varied according to the water management. Removal of the total volume of water at one time had greater effect than continuous removal of small water volume.

Temephos may have adverse effects upon aquatic life that breeds in the same habitat of *Aedes* larvae, such as *Tx. splendens* larvae [13] and *Micronecta* sp [17,23]. Both species were found in all regions of Thailand and live along with the *Aedes* breeding habitat. It was reported that an individual of *Micronecta* sp can ingest an average of 6-9 late 3rd or early 4th instars of *Ae. aegypti* larvae per day. This is a choice for *Ae. aegypti* control. The National Economy and Social Development Plan has directed the use of indigenous resources for local methods of mosquito control instead of insecticides, such as the use of herbs for killing insects, useful insect predators and bacteria for killing the larvae. These strategies could directly reduce the impact of insecticide upon man and the environment, and reduce costs. Using traditional methods without insecticides would be effective in controlling mosquitos. However, it is difficult to avoid using insecticide because it is necessary for vector control. Insecticides should be used responsibly using lower concentrations for greater effective control.

This study was tested under the natural conditions in the enamel-coated jars. It should be tested in other water containers such as cement baths and plastic tanks or other containers according to local usage. It should be done in a large field trial to confirm that the Temephos effective concentration of 0.12 ppm could kill *Ae. aegypti* larvae effectively. Because of the lower dosage of Temephos use and the residual time was decreased, the appropriate duration of effective concentrations of Temephos formosquito larval control should be studied. And because of the LC50 of *Ae. aegypti* was 14 times higher than *Micronecta* sp. In practice, mosquito larval control was not coordinated using Temephos and *Micronecta* sp. Because of the recommended dosage of Temephos by WHO was 1 ppm, which is 166.67 times higher than the dose in this study. It has certainly affected the mortality of both predators and *Aedes* larvae, so in the future it should be carefully used in the field. This study showed that LC50 of *Ae. aegypti* and *Tx. splendens* larvae, and *Micronecta* sp adult were 0.006, 0.1310 and 0.0028 ppm, respectively. The susceptible ratio between *Tx. splendens* and *Ae. aegypti* was 21.83 while the ratio between *Micronecta* sp and *Ae. aegypti* was -0.47. In the simulated conditions, Temephos concentrations of 0.06, 0.12, 0.18 and 0.24 ppm in 40 litres of tap water were prepared and the observed persistence from the residual effect of Temephos against *Ae. aegypti* after the removal and replacement were 25%, 50% and 90% of water. The result indicated the effective concentration of 0.12 ppm for Temephos with removal and replacement of 90% of the water. The concentrations of 0.18 and 0.24 ppm levels on the 7th day were observed. The concentration of 0.12 ppm was tested with the *Tx. splendens* and *Micronecta* sp, which showed 100% lethal time within 2 hours and 1 hour respectively.

The effect of Temephos concentrations upon 100% accumulated larval mortality of *Ae. aegypti* was confirmed. With respect to using the WHO recommendation of 1 ppm, the fact is it causes adverse effects upon other insect predators including *Tx. splendens* larvae and *Micronecta* sp. This study shows beyond any doubt that much lower concentrations of Temephos are much safer and, perhaps most importantly, effective. As used and implemented as outlined in this study, Temephos is appropriate for mosquito control programs, and should be used in conjunction with other natural methods of control, depending on the direction of the health policy and the global environmental considerations.

ACKNOWLEDGEMENTS

We wish to thank staff of the Biological Control Section, Department of Medical Sciences, Ministry of Public Health who help to provide the laboratory information support. We also wish to thank Miss Pankaew Rattanasingunchan, Vector-Borne Disease Control Unit 3rd, Khon-Kaen Province who supported the sample of water boatmen and the Taxonomy section, the Department of Education Agriculture, Ministry of Agriculture and cooperatives to classify the genus of water boatmen as *Micronecta* sp, that have enabled us to complete the study. UL, PR and LC provided constructive criticism and offered insightful ideas that helped guide the research methodology. This work was also partially supported by the Faculty of Graduate Studies (PR) and the China Medical Board, Faculty of Public Health, Mahidol University, Bangkok, Thailand (UL).

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