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PROCEDURES FOR THE EVALUATION OF FIELD EFFICACY OF SLOW-RELEASE FORMULATIONS OF LARVICIDES AGAINST *AEDES AEGYPTI* IN WATER-STORAGE CONTAINERS

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ABSTRACT. In Thailand, water-storage jars, barrels, drums, pails, and tanks constitute vast developmental sites for *Aedes aegypti* in urban, semiurban, and rural areas. Earthen water jars, cement jars, and concrete tanks constitute the greatest proportion of artificial containers where *Ae. aegypti* breed. This species is a major vector of the causal agents of dengue and dengue hemorrhagic fever, and vector control by larviciding is one of the main approaches to disease control. At present, temephos sand granules (SG) (1%) are used in large-scale community-based larviciding programs. Because of the use of this larvicide over the past 30 years, the likelihood exists that *Ae. aegypti* already has become resistant to this larvicide. To develop more options for control and make them available for use, we evaluated VectoBac tablets (*Bacillus thuringiensis* var. *israelensis* [*Bti*] 5%) and a new formulation of zeolite granules (ZG) of temephos (1%) and compared these formulations for efficacy with temephos SG (1%) in water-storage jars. In these tests, we used 48 identical glazed earthen water-storage jars (200-liter capacity) and developed quantitative sampling procedures for larvae, pupae, and pupal skins. Pupal skins were the easiest to count and this technique was used for the 1st time for assessing emergence of adults in water-storage containers. Three water regimens were used: full jars, half-full jars, and full jars emptied half way and refilled weekly. The 3 formulations with 3 regimens of water were assessed over a period of 6 months. VectoBac tablets at the dosage of 1 tablet or 0.37 g per 50 liters of water provided excellent control for about 112 days in full water jars. In the other 2 water regimens, VectoBac gave excellent control for 90 days. The 2 temephos formulations at the operational rate of 5 g per 50 liters of water were equal in efficacy, yielding almost 100% control for more than 6 months. Unlike temephos SG, the temephos ZG had no objectionable odor. Both the temephos ZG and *Bti* tablets increased clarity of the water, a feature desired by the users. Lack of odor and depression of turbidity are important attributes of *Bti* tablets and temephos ZG.

KEY WORDS *Aedes aegypti*, control, *Bacillus thuringiensis* var. *israelensis* tablets, temephos formulations, water-storage containers

INTRODUCTION

Aedes aegypti (L.), with its cosmopolitan distribution, is an important human pest and serves as the primary vector of dengue viruses in tropical and subtropical regions (Halstead 1966, Russell et al. 1969, Gubler and Casta-Velez 1991, Thavara et al. 1996). To prevent epidemics of dengue and dengue hemorrhagic fever, greater reliance is placed on area-wide control of this vector, by using larvicidal formulations that possess long-lasting residual activity. In Thailand, temephos sand granules (SG) (1%) have been used since the early 1970s (Bang and Tonn 1969a, 1969b; Bang et al. 1972) in operational community-based control programs at the rate of 5 g per 50 liters of water in water-storage jars, barrels, concrete water tanks, metal and plastic drums, and other large artificial containers. The efficacy of temephos emulsifiable concentrate and temephos SG 1% in water-storage containers and jars was studied in late 1960s (Bang and Tonn 1969a, 1969b), and they found that temephos SG (1%) (1 ppm active ingredient) provided complete control of *Ae. aegypti* for about 2 months in one study and for 13 weeks in another under simulated water-use conditions. Soon after these findings, temephos SG (1%) were used in operational control programs. It is now believed that temephos resistance might already be present, or will soon emerge in some areas subjected to temephos treatments for many years. Resistance in *Ae. aegypti* to temephos has been reported in the field from Malaysia (Lee et al. 1984, Lee and Lime 1989), the Caribbean (Georghiou et al. 1987), Dominican Republic (Mekuria et al. 1991), British Virgin Islands (Wirth and Georghiou 1999), and Brazil (Campos and Andrade 2001, Lima et al. 2003). In view of the high probability of resistance development to larvicides, it is necessary to evaluate and develop new modes of actions and formulations for use as substitutes in case the use of temephos SG becomes unacceptable and impractical.

In determining longevity of slow-release formulations of larvicides against *Ae. aegypti* in large-capacity (200- to 2,000-liter) water-storage containers, no precise quantitative evaluation techniques are available. In most cases, estimates of living larvae and pupae are made visually by scoring their abundance or assigning positive or negative values. In the latter case, presence of a few larvae in containers will have the same weight as a container with hundreds or thousands of larvae. Such sampling tactics have little or no relationship to the yield of adult mosquitoes that are vectors of pathogens. Also, such estimates can vary greatly depending on the sampler, size, and color of the container and water-use practices and water-quality

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Fig. 1. Arrangement of glazed earthen jars (capacity 200 liters) for investigating the long-term efficacy of slow-release formulations of larvicides against *Aedes aegypti* at Bang Bua Thong, Nonthaburi Province, Thailand, in 2002.

parameters. Larvae and pupae of *Ae. aegypti* generally are distributed throughout the column (100 cm deep or more) of water and most dive down on disturbance and remain down in the container for a long time. Netting and dipping of the larvae and pupae are time consuming and cause significant disturbance of water and deposits in the jars, which will alter the release characteristics of the formulations tested as well as influence the absorption profile of the active ingredients on sediments at the bottom and sides of the jars. To overcome these problems, we focused on developing methods for the precise visual assessment of live larvae, pupae, and pupal skins (indicating successful emergence of adults) without disturbing the water, as well as removing pupal skins from the water surface by syringe or fish net for counting. These assessment techniques, especially that of counting the total number of pupal skins either visually or by removal, will provide for accurate estimate of the populations and the assessment of the efficacy and longevity of 3 slow-release formulations of larvicides, temephos SG (1%), an experimental temephos zeolite granules (ZG) (1%) formulation, and *Bacillus thuringiensis* var. *israelensis* tablets, studied for their longevity in water-storage jars.

MATERIALS AND METHODS

Study site: A field research facility for the evaluation of mosquitocidal products and other agents was constructed in 2001 in Bang Bua Thong District, Nonthaburi Province, Thailand, and studies were initiated in February 2002. The facility consists of a concrete slab slanted toward the center with a 25-cm-wide, 3- to 4-cm-deep gutter to drain off water during emptying and washing of water-storage jars. The slab was shaded with a roof of flatsheet made from cement and Chrysotile fibers (Siam Fibre-Cement Co., Siam Cement Road, Bang-Sue, Bangkok, Thailand) (4 m high at the eaves level), with the eaves extending about 30 cm beyond the edges of the slab.

Test units and sampling: One hundred new, glazed earthen jars (200-liter capacity) were placed in 4 rows (25 jars/row) with 2 rows on each side of the gutter (Fig. 1). This type of jar is the most commonly used by homeowners in Thailand (Kittyapong and Strickman 1993), and is the primary habitat of *Ae. aegypti.* The jars have a capacity of 200 liters, when fully filled the depth is 62 cm, and when half full (100 liters) the water depth is 32 cm. Lids for covering the mouth of the jars were cut from 5-mm-thick Celocrete sheets. Covers were in place all the time except during addition of larvae and assessment of efficacy twice a week for 2–3 h each time. Placement of covers prevented light (especially ultraviolet light) and wind-borne debris from entering the jars.

For developing sampling methods, several jars (dark-brown color on the inside) were filled with water from the domestic water supply and 50 4th-stage larvae of *Ae. aegypti* were added. To count the larvae visually without disturbing them, 3 or 4 samplers independently counted the larvae in the same jars visually with the aid of a flashlight. Sev-

Table 1. Assessment of the efficacy of controlled-release formulations of VectoBac tablets, temephos sand granules (SG), and temephos zeolite granules (ZG) in water-storage jars (200 liters) against 3rd and 5th cohorts of 3rd-instar *Aedes aegypti*, treated February 8, 2002.

Materials	Row	Water level in jars	Larvae (48 h)[1]		Emergence (1 wk)	
			Larvae (*n*)	Reduction (%)	Pupal skins (*n*)	IE[2] (%)
			3rd cohort, added 14 days after treatment (Feb. 22, 2002)			
VectoBac tablets	A	Full	16	84	9	91
	B	Full, half removed	3	97	0	100
	C	Half full	6	94	1	99
Temephos SG 1%	E	Full	0	100	0	100
	F	Full, half removed	0	100	0	100
	G	Half full	0	100	0	100
Temephos ZG 1%	H	Full	9	91	3	100
	I	Full, half removed	39	61	7	93
	J	Half full	19	81	3	97
Control	D	Full	100	0	99	1
	K	Full, half removed	96	4	98	2
	M	Half full	100	0	98	2
			5th cohort, added 28 days after treatment (March 8, 2002)			
VectoBac tablets	A	Full	33	67	24	76
	B	Full, half removed	0	100	0	100
	C	Half full	23	77	18	82
Temephos SG 1%	E	Full	2	98	0	100
	F	Full, half removed	0	100	0	100
	G	Half full	0	100	0	100
Temephos ZG 1%	H	Full	12	88	2	98
	I	Full, half removed	8	92	3	97
	J	Half full	21	79	6	94
Control	D	Full	96	4	98	2
	K	Full, half removed	92	8	92	8
	M	Half full	99	1	99	1

[1] In cohorts 1 and 2, 100% mortality of larvae occurred 48 h after exposure.
[2] IE, inhibition of emergence.

eral attempts were made to count the larvae in full and half-full jars. The counts between individuals were quite variable and it was not possible to count all the larvae that were added. The inside of the jars were dark brown in color and this made it difficult to spot and count all still or moving larvae and pupae visually. Therefore, this method of assessment was considered inappropriate. One reason for this difficulty was that larvae and pupae move around actively throughout the water column, and after disturbance dive down and remain at the bottom for a long time.

We also considered the dipping technique but this technique also was considered undesirable because it would disturb the water too much, influencing the release and absorption patterns of formulations tested. In addition, dipping disturbed the water and caused larvae and pupae to dive, and then take a long time to resurface. We then tried netting (by using fish nets) larvae and pupae from the jars, but this technique was time consuming (because they remain at the bottom for a long time) and netting caused disturbance of the water, which could influence the release and absorption–adsorption characteristics of the insecticides subjected to testing. We also decided against using sentinel cages or bioassaying field water against larvae in laboratory, because these techniques are time consuming. Finally, we designed a method of painting the inside of the jars with white paint to facilitate visual counting. A white alkyd-resin paint (Glipton G 100, synthetic resin high-gloss enamel, TOA Paint Co., Bangkok, Thailand) was tested in a few jars. Survival of 4th-stage larvae in the painted jars was excellent (92–98%) for 5–7 days of observation. The white background inside the jars made it easy to spot and count all larvae, pupae, and pupal skins in the jars visually with or without the aid of flashlights. Therefore, all 100 jars were painted on the inside, by applying 2 coats a day apart. After allowing the paint to dry for 1–2 days, the jars were filled with water from a domestic water supply. Additionally, we developed a technique for precise assessment of adult emergence by visually counting pupal skins in the painted jars, or by removing pupal skins from painted or unpainted jars with a small fish net or a syringe. Pupal skins float on the surface, mostly at the meniscus level, and can be removed with a net or syringe without disturbing the water. The netted or syringed pupal skins were placed in water in white plastic trays and counted.

To determine the disintegration and sinking of pupal skins over time, we added 50 pupae of *Ae. aegypti* to each of 11 unpainted jars and counted

Table 2. Assessment of the efficacy of controlled-release formulations of VectoBac tablets, temephos sand granules (SG), and temephos zeolite granules (ZG) in water-storage jars (200 liters) against 6th and 9th cohorts of 3rd-instar *Aedes aegypti*, treated February 8, 2002.

Materials	Row	Water level in jars	Larvae (48 h)		Emergence (1 wk)	
			Larvae (*n*)	Reduction (%)	Pupal skins (*n*)	IE[1] (%)
			6th cohort, added 35 days after treatment (March 15, 2002)			
VectoBac tablets	A	Full	27	73	19	81
	B	Full, half removed	7	93	3	96
	C	Half full	24	76	14	86
Temephos SG 1%	E	Full	0	100	0	100
	F	Full, half removed	0	100	0	100
	G	Half full	0	100	0	100
Temephos ZG 1%	H	Full	6	94	0	100
	I	Full, half removed	3	97	0	100
	J	Half full	14	86	0	100
Control	D	Full	2	98	99	1
	K	Full, half removed	7	93	94	6
	M	Half full	3	97	98	2
			9th cohort, added 56 days after treatment (April 5, 2002)			
VectoBac tablets	A	Full	21	79	11	89
	B	Full, half removed	21	79	18	82
	C	Half full	37	63	21	79
Temephos SG 1%	E	Full	0	100	0	100
	F	Full, half removed	0	100	0	100
	G	Half full	0	100	0	100
Temephos ZG 1%	H	Full	16	84	0	100
	I	Full, half removed	4	96	0	100
	J	Half full	14	86	0	100
Control	D	Full	98	2	98	2
	K	Full, half removed	98	2	98	2
	M	Half full	94	6	95	5

[1] IE, inhibition of emergence.

pupal skins visually and by netting or syringing them over a 16-day period, by sampling 3 jars at each interval. The retrieved pupal skins were counted 48 h and 7 and 16 days after the addition of pupae to the jars. This test was done to determine the length of the period during which pupal skins remain intact and floating at the surface of water.

Assessment of efficacy: On February 3–5, 2002, the 100 jars were washed, dried, and painted with white synthetic resin high-gloss enamel paint. The paint was allowed to dry for 1–2 days before filling the jars with water. The jars were arranged in 4 rows (25 jars/row) on the concrete slab.

On February 6–7, 2002, some jars were filled with tap water and some were filled half way as required by the experimental design. About 1 g of ground mouse food was added to each fully filled jar and 0.5 g was added to each half-filled jar. The jars were covered and treated on February 8, 2002, after the addition of the 1st cohort of larvae. Additional larval food was added at the rate of 1 g/200 liters and 0.5 g/100 liters and any water lost was replenished monthly.

The treatments were challenged weekly with a fresh cohort of laboratory-reared larvae, where 25 3rd-stage larvae of *Ae. aegypti* were added per jar. Live larvae were counted 48 h after exposure, whereas live pupae and pupal skins were counted 1 wk after placement.

Materials and treatments: VectoBac tablets (*Bti*, 2,700 International Toxic Units/mg; lot 64-164-BD-XR-10, Valent BioSciences Corporation, Libertyville, IL) were used at the rate of 1 tablet per 50 liters of water. The average weight of a tablet was 0.37 g. Temephos SG (1%) (BASF Thai Limited, Bangkok, Thailand) were applied at the operational rate of 5 g of the formulation per 50 liters of water, yielding 1 ppm temephos. A new experimental temephos ZG (1%) formulation (Ikari Trading Co., Ltd., Bangkok, Thailand) was applied at the dosage of 5 g per 50 liters of water, yielding 1 ppm temephos. This formulation is made on zeolite green granules (clinoptilolite mineral, a sodium aluminum silicate) that are commercially available and used for water filtration, as animal feed filler, and for pharmaceutical purposes. Each treatment and control was replicated 4 times.

Twelve treatments were conducted in this experiment, with 3 larvicides and 1 control in 3 water regimens (fully filled, half-filled, and fully filled jars with half of the water removed and refilled weekly). Each treatment consisted of 4 jars, set in

Table 3. Assessment of the efficacy of controlled-release formulations of VectoBac tablets, temephos sand granules (SG), and temephos zeolite granules (ZG) in water-storage jars (200 liters) against 10th and 13th cohorts of 3rd-instar *Aedes aegypti*, treated February 8, 2002.

Materials	Row	Water level in jars	Larvae (48 h)		Emergence (1 wk)	
			Larvae (*n*)	Reduction (%)	Pupal skins (*n*)	IE[1] (%)
			10th cohort, added 63 days after treatment (April 12, 2002)			
VectoBac tablets	A	Full	23	77	17	83
	B	Full, half removed	23	77	16	84
	C	Half full	38	62	12	88
Temephos SG 1%	E	Full	0	100	0	100
	F	Full, half removed	0	100	0	100
	G	Half full	0	100	0	100
Temephos ZG 1%	H	Full	1	99	0	100
	I	Full, half removed	1	99	0	100
	J	Half full	9	91	1	99
Control	D	Full	95	5	98	2
	K	Full, half removed	96	4	97	3
	M	Half full	98	2	98	2
			13th cohort, added 84 days after treatment (May 3, 2002)			
VectoBac tablets	A	Full	—	—	2	98
	B	Full, half removed	—	—	3	97
	C	Half full	—	—	11	89
Temephos SG 1%	E	Full	—	—	0	100
	F	Full, half removed	—	—	0	100
	G	Half full	—	—	0	100
Temephos ZG 1%	H	Full	—	—	0	100
	I	Full, half removed	—	—	0	100
	J	Half full	—	—	0	100
Control	D	Full	—	—	90	10
	K	Full, half removed	—	—	97	3
	M	Half full	—	—	87	13

[1] IE, inhibition of emergence.

a row from east to west. The larvae were added to the jars longitudinally, to spread larval variability over treatments. Treatments were arranged in block design and made on February 8, 2002. Before the treatment, larvae were placed in the containers and then the required amount of the formulation was applied. All applied materials sank to the bottom of the jars.

Reduction and inhibition of emergence: The magnitude of reduction (%) and inhibition of emergence (IE, %) were calculated on the basis of larval mortality (48 h after addition and based on total number added) and on the number of pupal skins (indicating adult emergence, 1 wk after addition) as compared to the initial number of larvae added. Mortality in the checks was not considered in the calculations, because in general it was very low, which will not change the results. Although treatments were challenged weekly with new cohorts of larvae, we report here part of the data for brevity. From the 26 cohorts used during this experiment over a period of 6 months, we present data on 13 cohorts only. Presentation of all the data will be voluminous and not necessary for elucidating the longevity of the treatments and drawing meaningful inferences.

RESULTS AND DISCUSSION

Water-storage containers

Artificial containers holding or storing water constitute major habitats for the development of *Ae. aegypti*. These containers include water jars, concrete tanks, pails, barrels, drums (plastic and metal), uncapped empty beverage bottles, tires, ant guards, and potted plant saucers. Among these water containers, water-storage jars and cement tanks and basins constitute by far the largest proportion of water volume producing *Ae. aegypti* (Kittayapong and Strickman 1993, Thavara et al. 2004). For this reason we selected the 200-liter-capacity earthen jars for the evaluation of the larvicides.

Sampling methods

For determining the comparative efficacy and longevity of larvicidal formulations over time, we found that visual counting of live larvae, pupae, and pupal skins was relatively accurate, with little or no variation among samplers in jars (200 liters) painted with white paint. The paint coat lasted for the entire duration of the test period (more than 6 months) and the paint had no adverse effects on

Table 4. Assessment of the efficacy of controlled-release formulations of VectoBac tablets, temephos sand granules (SG), and temephos zeolite granules (ZG) in water-storage jars (200 liters) against 14th and 17th cohorts of 3rd-instar *Aedes aegypti*, treated February 8, 2002.

Materials	Row	Water level in jars	Larvae (48 h)		Emergence (1 wk)	
			Larvae (*n*)	Reduction (%)	Pupal skins (*n*)	IE[1] (%)
			14th cohort, added 91 days after treatment (May 10, 2002)			
VectoBac tablets	A	Full	2	98	2	98
	B	Full, half removed	21	79	4	96
	C	Half full	31	69	25	75
Temephos SG 1%	E	Full	0	100	0	100
	F	Full, half removed	0	100	0	100
	G	Half full	0	100	0	100
Temephos ZG 1%	H	Full	0	100	1	99
	I	Full, half removed	2	98	2	98
	J	Half full	13	87	5	95
Control	D	Full	91	9	92	8
	K	Full, half removed	94	6	95	5
	M	Half full	76	24	93	7
			17th cohort, added 112 days after treatment (May 31, 2002)			
VectoBac tablets	A	Full	12	88	4	96
	B	Full, half removed	56	44	33	67
	C	Half full	47	53	26	74
Temephos SG 1%	E	Full	0	100	0	100
	F	Full, half removed	0	100	0	100
	G	Half full	0	100	0	100
Temephos ZG 1%	H	Full	0	100	0	100
	I	Full, half removed	0	100	0	100
	J	Half full	98	2	0	100
Control	D	Full	93	7	98	2
	K	Full, half removed	98	2	98	2
	M	Half full	97	3	97	3

[1] IE, inhibition of emergence.

larval and pupal survival. It should be pointed out that painting of jars is practical for use in experimental research units and not in operational research or evaluation of area-wide control programs for *Ae. aegypti*. In the latter situations, estimates of larval or pupal populations or the simple assignment of positivity–negativity scores (Bang and Tonn 1969a, Bang et al. 1972) are deemed adequate. We also employed small fish nets or syringes for removing pupal skins floating on the water surface in nonpainted jars. The pupal skins were easily netted or syringed out and counted by inverting the nets containing pupal skins into water in plastic trays. Two or 3 nettings removed all the pupal skins from the water surface. This sampling technique provides a sensitive means and is the least disruptive for assessing efficacy in both small and large jars. Both visual counting and removal of pupal skins in white-painted jars yielded essentially the same numbers. Visual counting and syringing of pupal skins in unpainted jars is difficult in large water-storage jars, but netting of pupal skins provides rapid and accurate sampling for determining the magnitude of emergence of adults, which reflects the extent of overall control. Netting of live larvae and pupae was not deemed desirable and practical in large jars. Pupal skin counts by netting from unpainted jars over a 16-day period, where 50 pupae were stocked per jar, yielded data on their persistence. The number of countable pupal skins, either visually or by netting, decreased over the 16-day period, with the counts being essentially the same for 2 days (94% by each method with 6% mortality in the pupae) and after 7 days (72 and 78% by the 2 methods) after addition of pupae. The pupal skins began to disintegrate or sink after 16 days, at which time only 46% (visually) and 32% (netting) were noted and those netted were broken and difficult to count. Persistence of pupal skins for up to 1 wk is adequate for most assessment purposes. We noted that netting of pupal skins changed their floating ability, the number counted or netted after putting the netted pupal skins back into the jars became smaller as compared to the 1st visual or netting count. Once the pupal skins are netted out, they should not be put back into the containers, and this procedure is not necessary for assessing the efficacy of larvicides or determining the extent of adult emergence.

Larvicidal efficacy

During the experiment, larvae and pupae of *Ae. aegypti* developed rapidly because water tempera-

Table 5. Assessment of the efficacy of controlled-release formulations of VectoBac tablets, temephos sand granules (SG), and temephos zeolite granules (ZG) in water-storage jars (200 liters) against 18th and 20th cohorts of 3rd-instar *Aedes aegypti*, treated February 8, 2002.

Materials	Row	Water level in jars	Larvae (48 h)		Emergence (1 wk)	
			Larvae (*n*)	Reduction (%)	Pupal skins (*n*)	IE[1] (%)
			18th cohort, added 119 days after treatment (June 7, 2002)			
VectoBac tablets	A	Full	46	54	29	71
	B	Full, half removed	72	28	57	33
	C	Half full	65	35	38	62
Temephos SG 1%	E	Full	0	100	0	100
	F	Full, half removed	0	100	0	100
	G	Half full	0	100	0	100
Temephos ZG 1%	H	Full	0	100	0	100
	I	Full, half removed	2	98	0	100
	J	Half full	19	81	3	97
Control	D	Full	94	6	96	4
	K	Full, half removed	95	5	96	4
	M	Half full	98	2	98	2
			20th cohort, added 133 days after treatment (June 21, 2002)			
VectoBac tablets	A	Full	62	38	46	54
	B	Full, half removed	72	28	47	53
	C	Half full	50	50	50	50
Temephos SG 1%	E	Full	0	100	0	100
	F	Full, half removed	90	10	0	100
	G	Half full	96	4	0	100
Temephos ZG 1%	H	Full	23	77	2	98
	I	Full, half removed	22	78	8	92
	J	Half full	23	77	3	97
Control	D	Full	96	4	97	3
	K	Full, half removed	98	2	99	1
	M	Half full	97	3	97	3

[1] IE, inhibition of emergence.

ture in the jars was relatively high and constant (maximum 31–34°C, minimum 28–30°C). By day 7 after exposure, adult emergence was complete and no larvae or pupae were noted. The assessment of the 1st cohort (added on the day of treatment) and the 2nd cohort (added 7 days after treatment) showed that all treatments yielded 100% mortality of larvae in 48 h, resulting in no pupae or adult emergence as indicated by the absence of pupal skins (data not present in table). The 3rd cohort larvae showed some larval survivorship in treatments with VectoBac tablets, but further mortality beyond the 48-h assessment resulted in high IE (91–100%) at the 1 wk assessment (Table 1). A slight emergence of 7 and 3% (93 and 97% IE) also occurred in 2 temephos ZG treatments; however, the full-jar treatment had an IE of 100%, despite the fact that some larvae survived (see Table 1). For the 4th cohort larvae (data omitted because they were similar to the previous and immediately following cohorts), slight emergence occurred in the full jars treated with VectoBac tablets and temephos ZG. The 5th cohort larvae were added to the jars 28 days after treatment. In the treatments with VectoBac tablets in full jars with half of the water removed and then refilled, the IE was 100%, whereas in the other 2 VectoBac water regimen treatments, the IE was lower but still ranged between 76 and 82% (see Table 1). Temephos SG, on the other hand, yielded close to 100% IE, whereas temephos ZG yielded 2–6% emergence in cohort 5. In cohort 6 (added 35 days after treatment), the 3 water regimens with VectoBac tablets yielded larval mortality and adult emergence (Table 2) values similar to those in cohort 5, and both temephos granules produced 100% IE in cohort 6. Assessment of cohorts 7 and 8 yielded results (data omitted) similar to those of the previous 2 cohorts. The results obtained with cohort 9 (added 56 days after treatment), showed 100% IE in all temephos granule treatments (see Table 2). The level of IE was 79–89% in the various VectoBac tablet treatments.

In cohort 10 (added 63 days after treatment) and cohort 13th (added 84 days after treatment), IE was approximately 100% in all temephos granules treatments (Table 3). However, some emergence (2–17%) occurred in VectoBac tablet treatments, with the results being similar to those for cohorts 6 and 9. In cohorts 11 and 12 (70 and 77 days after treatment, respectively, data omitted) temephos granules yielded about 100% IE, whereas VectoBac tablets yielded 87–98% IE. Assessment of cohort 13 pro-

Table 6. Assessment of the efficacy of controlled-release formulations of VectoBac tablets, temephos sand granules (SG), and temephos zeolite granules (ZG) in water-storage jars (200 liters) against 22nd, 24th, and 26th cohorts of 3rd-instar *Aedes aegypti*, treated February 8, 2002.

Materials	Row	Water level in jars	Larvae (48 h)		Emergence (1 wk)	
			Larvae (*n*)	Reduction (%)	Pupal skins (*n*)	IE[1] (%)
			22nd cohort, added 153 days after treatment (July 12, 2002)			
Temephos SG 1%	E	Full	4	96	0	100
	F	Full, half removed	16	84	0	100
	G	Half full	21	79	0	100
Temephos ZG 1%	H	Full	18	82	0	100
	I	Full, half removed	14	86	1	99
	J	Half full	34	66	1	99
Control	D	Full	99	1	99	1
	K	Full, half removed	97	3	97	3
	M	Half full	98	2	98	2
			24th cohort, added 167 days after treatment (July 26, 2002)			
Temephos SG 1%	E	Full	1	99	0	100
	F	Full, half removed	3	97	0	100
	G	Half full	11	89	1	99
Temephos ZG 1%	H	Full	19	81	1	99
	I	Full, half removed	6	94	1	99
	J	Half full	12	88	0	100
Control	D	Full	98	2	99	1
	K	Full, half removed	97	3	97	3
	M	Half full	98	2	99	1
			26th cohort, added 185 days after treatment (August 13, 2002)[2]			
Temephos SG 1%	E	Full	0	100	0	100
	F	Full, half removed	0	100	0	100
	G	Half full	0	100	0	100
Temephos ZG 1%	H	Full	0	100	0	100
	I	Full, half removed	0	100	0	100
	J	Half full	1	99	0	100
Control	D	Full	95	5	95	5
	K	Full, half removed	93	7	93	7
	M	Half full	96	4	96	4

[1] IE, inhibition of emergence.
[2] Experiment terminated on August 19, 2002, 197 days after treatment.

duced results (see Table 3) that showed similar trends as cohorts 11 and 12. The IE in temephos granules was 100% in cohort 13 and slight emergence (2–11%) was noted in VectoBac tablet treatments (see Table 3). In cohort 14, all treatments of VectoBac tablets and temephos granules and regimens, except VectoBac tablets in half-full jars, yielded a high IE of 95–100% (Table 4). The IE for VectoBac tablets in half-full jars was 75%, showing declining efficacy. In cohorts 15 and 16 (data omitted), the IE, especially in half-full jars, was mediocre with VectoBac, but was almost 100% in all temephos treatments. In cohort 17 (added 112 days after treatment), some of the VectoBac treatments began to decline and showed some emergence. In cohort 17, the full jar tablet treatment still yielded 96% IE, whereas the other 2 jar treatments yielded 67 and 74% IE (Table 4). This level of decline in efficacy in these 2 treatments also was noted in cohorts 15 and 16 (data not presented). All the temephos treatments provided 100% IE in cohort 17.

To confirm the decline in efficacy of VectoBac tablets, 3 additional cohorts were used: 18 (added 119 days after treatment), 19 (added 126 days after treatment), and 20 (added 133 days after treatment). VectoBac treatments showed low IE values in all of these 3 cohorts (Table 5, data for cohort 19 omitted). In all 3 cohorts, the IE in VectoBac treatments declined to 33–71%, further confirming the breakdown of all VectoBac treatments after 112 days. From the data of cohort 17, the conclusion can be made that VectoBac tablets (full jars) showed excellent efficacy for 112 days, whereas the full jars with half of the water removed and the half-full jars were efficacious for 91 days (cohort 14). It is further concluded that VectoBac tablets give satisfactory control for about 100 days (in full jars) at the very low dosage of 1 tablet (0.37 g) per 50 liters of water. Therefore, use of VectoBac tablets is an-

other option for achieving long-lasting control of larval *Ae. aegypti* in water-storage jars. After 133 days of treatment, the temephos SG provided 100% IE in cohort 20 in all treatments, whereas temephos ZG provided a high IE (92–98%; see Table 5). After assessment of the 19th and 20th cohorts, we discontinued assessing VectoBac tablets but continued assessing the efficacy of temephos treatments by challenging them with successive cohorts of larvae.

In cohort 22 (added 153 days after treatment), both temephos granules yielded almost 100% IE (Table 6). However, in cohort 23 (added 160 days after treatment, data omitted, see above and Materials and Methods), the IE was still high with temephos SG, but with temephos ZG the IE declined to 81% in full jars, but was still high in the other 2 regimens (data omitted). In cohorts 24 (167 days), 25 (175 days, data omitted, see above and Materials and Methods) and 26 (185 days), the IE was 99–100%, indicating that both temephos granules are highly efficacious. The last assessment of IE was made on August 19, 2002, 197 days after treatment. At this point, both temephos granules yielded 100% IE, with most mortality occurring in the larvae.

In conclusion, these long-term studies show that the 3 larvicidal formulations tested provide long-lasting control of *Ae. aegypti* in water-storage jars under the conditions of the experiment. Vectobac tablets at the low dosage of 1 tablet (0.37 g) per 50 liters of water yielded IE greater than 90% for 112 days in full jars. The IE was lower in the other 2 water regimens, but satisfactory control of greater than 80% was obtained for about 91 days in both.

Both temephos granular formulations at the dosage of 5 g per 50 liters of water consistently yielded high IEs (95–100%) for more than 190 days after treatment. In some of the cohorts, the IE may have reached 80%, but subsequent cohorts exhibited high IEs, indicating that the previous low IE reading on occasions may have been due to experimental errors. Where the IE in any 2 or 3 consecutive cohorts reached below 80%, the assessment of efficacy was terminated. Note that under controlled experimental conditions, it is possible to get this kind of residual activity. Under real-world conditions, such a long-term activity is not to be expected (Bang and Tonn 1969a, 1969b). As a sequel to this study, we investigated temephos ZG (1%) at 5 g per 50 liters of water in village trials and found the residual activity to last for only 3 months (Thavara et al. 2004). The longevity of temephos SG (1%) in early field trials also was reported to be for 3 months (Bang and Ton 1969a, 1969b). On the basis of these field studies and those of Thavara et al. (2004), longevity of control for 2–3 months in water-use containers is all that can be expected under normal water-use practices. Many factors, especially water-use practices (adding, removing, draining, cleaning, and washing), influence the longevity of control, and in practice, even a long-lasting formulation will be lost due to removal or dilution. However, we have noted that jars treated with temephos and emptied, washed and refilled with fresh water 5–6 months after treatment were still contaminated and killed the exposed larvae. This indicates that temephos released in water is adsorbed and absorbed into the wall of the jars. However, in large jars, where water is removed through a faucet and added at the top and that are infrequently emptied and washed, the applied materials will persist for longer periods, and can provide control for 6 months or longer.

From these studies, all 3 larvicidal formulations tested clearly have a good potential for the control of *Ae. aegypti* in water-storage containers, which constitute a major habitat for this mosquito in tropical regions. With the potential of resistance to temephos because of its use in control programs for many years in Thailand and reported from other areas of the world, *Bti* tablets offer a good and acceptable substitute. The temephos SG formulation is known to have 2 drawbacks, objectionable odors and turning water turbid, which discourage its use by homeowners. The temephos ZG formulation does not have these drawbacks. We noted that water in jars treated with temephos ZG was consistently clear. The *Bti* tablets also possess these advantages because *Bti* has no odor problem and has been shown to reduce water turbidity and algal growth (Su and Mulla 1999).

The assessment techniques developed here offer quantitative sampling methods for larvae, pupae, and pupal skins of mosquitoes in water containers. Pupal skin sampling either by counting visually in white-painted jars or netting from the water surface in small to large water containers (painted or unpainted) was suitable for estimating adult yield. Assessment of pupal skins, either visually or by netting, provides critical data on the efficacy of larvicides, and yields useful information on the magnitude of emergence of adult mosquitoes from many types of water-storage containers, which constitute major sources of *Ae. aegypti.*

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