

Sequential release and residual activity of temephos applied as sand granules to water-storage jars for the control of *Aedes aegypti* larvae (Diptera: Culicidae)

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ABSTRACT: Two long-term experiments were carried out on the release profile and efficacy of temephos 1% GR (sand granules) against *Aedes aegypti* larvae in water-storage containers. In the first experiment, the efficacy of temephos 1% GR enclosed and tied in a muslin cloth and placed in water at the bottom of 200 L earthen water-storage jars was studied by exposing the packets for four to nine wk in one set of jars and then transferring them sequentially to new sets of jars four times successively. Temephos released slowly from the granules, the magnitude of release being adequate in the initial period of two to three wk after treatment. Following this period, the efficacy of the granules increased substantially where 92 – 100% inhibition of emergence even at the lowest dosage of 1g/100 L (0.05 mg/L AI) was obtained for about another five mo or longer. On removal of the packets from a given set of jars, the released residues remaining in the jars and water lasted a maximum of one to six wk post-removal depending on the magnitude of prior release into the jars. This experiment provided clear evidence that temephos is released slowly over a long period of time in water-storage jars. In the second experiment, we compared the efficacy of temephos 1% GR at 1 and 10 g (0.05 and 0.5 mg/L AI) per 200-L water in jars painted and unpainted on the inside. The efficacy in the painted jars, although high, was consistently lower than that in the unpainted jars, where 99 - 100 % control of larvae was achieved at both rates for a minimum of five mo after treatment. On the basis of this experimental evidence, it is desirable to study the efficacy of lower dosages of temephos than those currently used in *Ae. aegypti* control programs. The use of controlled release formulations or sachets that are retrievable during cleaning and washing will be more practical and desirable. Both of these interventions will make the program more cost effective. *Journal of Vector Ecology* 30 (1): 62-72. 2005.

Keyword Index: *Aedes aegypti*, control, temephos, granules, controlled release.

INTRODUCTION

Temephos 1% GR (sand granules) has been used for the control of *Aedes aegypti* (L.) larvae for many years in Thailand and elsewhere (Bang et al. 1972, Carvalho and da Silva 2000, Weber et al. 1987). The efficacy of this formulation was studied in detail in Thailand in the late 1960s and early 1970s (Bang and Tonn 1969ab, Bang et al. 1972). On the basis of their findings, temephos sand granules (1%) were recommended for use in *Ae. aegypti* control programs and are currently used at the standard dosage of 10 g/100 L water (1 mg/L AI). Recently, Mulla et al. (2004) studied the efficacy of 1% sand granules and a new granular formulation of temephos (1% Zeolite) at the dosage of 10 g/100 L each in 200-L capacity earthen water-storage jars painted on the inside for ease of counting live larvae, pupae, and pupal skins. Both formulations yielded close to 100% control (inhibition of emergence) for over six mo under three different water-use conditions. Even though temephos 1% GR has been used for over 30 years in Thailand, there is no published information

on the release pattern of temephos from granules applied to water-storage jars. Although temephos has low solubility in water (0.03 mg/L at 25° C), it is not clear whether temephos release occurs in a short time or is it released over a long period of time from granular formulations under field conditions. This question is often posed and still remains unanswered. To shed light on the efficacy and continuous release of temephos over time, we carried out the present study where temephos release, as reflected in the level of larval control and adult emergence over time, was determined by sequential transfers of temephos granules in packets from one set of jars to another. Efficacy was determined in each sequence both in the presence of temephos granule packets in a given set of jars as well as after removal and transfer of the packets to the succeeding sets of jars. A second experiment was carried out to compare efficacy of temephos sand granules (1%) added to water in painted and unpainted water-storage jars. In our previous study (Mulla et al. 2004), we tested two temephos granular formulations in painted jars only, using three water-use regimens. At that time, we were not able to determine the effect of the alkyd resin paint used on the release

and efficacy of temephos as compared to unpainted jars. In order to compare the release of temephos and efficacy of sand granules, we did a comparative study of painted versus unpainted jars into which sand granules were sprinkled loosely where they sank to the bottom.

MATERIALS AND METHODS

Two experiments were carried out in the current study. In the first experiment, sequential release of temephos from 1% sand granules contained in a packet and applied to water in earthen water-storage jars was determined. In the second experiment, activity and long-term efficacy of temephos GR applied loosely (not in packets) to the jars was compared in earthen water-storage jars painted and unpainted on the inside. Since we got an unexpectedly low release of temephos from granules contained in packets during the first two mo after application, we ran a third experiment using the same amounts of GR in packets to re-evaluate the release pattern over 56 d period post-application. Packets in this experiment remained in the initially treated jars without transfer to other sets. Experimental set-up and design and evaluation procedures developed in our previous studies (Mulla et al. 2004) were closely followed.

Sequential release

Earthen jars (200-L capacity) were filled with domestic water and set for a wk before treatment. The depth of water in the jars was 62 cm. The jars were treated with packets of 1, 5, and 10 g temephos 1% GR (Larvifos 15/30 mesh, lot 5B030822-2, Ikari Trading Co. Thailand) on August 27, 2003. The treatments were challenged weekly by adding cohorts of 25 third-instar laboratory-reared larvae of *Ae. aegypti*. Prior to the addition of larvae, 0.5 g larval food (ground-up mouse pellets) was added to each jar. Additional food was given on a monthly basis. Water level in the jars was replenished monthly. The jars were covered with sheets of celocrete protecting the jars from intrusion of extraneous material and penetration by UV light. The jars were set on a concrete slab under a roof, but the area was completely open on all sides. Water temperature was monitored throughout the test by submerging a minimum-maximum thermometer in one of the jars. To determine sequential release and longevity of temephos from the sand granules, quantities of 1, 5, and 10g per jar to yield three concentrations (0.05, 0.25, and 0.50 mg/L AI) were confined in muslin cloth and the pack of granules was tied with cotton string and lowered to the bottom of the jars. A small pebble was enclosed in the packets to facilitate sinking of the granule packets to the bottom of the jars. Sand granules without temephos were placed in control jars. A long string of cotton was attached to the knotted end of the packets to facilitate lowering and lifting the packets during treatment and transfer to a new set of jars. The treatments and control were replicated four times. In total, 80 identical jars were used in this experiment.

For assessing the activity and longevity of the treatments, 25 third-instar laboratory-reared larvae of *Ae. aegypti* were introduced into each jar, usually on weekly basis. Assessment

of inhibition of emergence (IE %) was carried out by counting (five-seven d post-addition of larvae) pupal skins floating on the surface of water. Due to the high temperature of water in the jars, third-instar larvae completed development to adult emergence in five d. Pupal skins were removed by syringes to white plastic trays with water, where the skins were readily visible and counted. The pupal skins were removed in totality for each cohort before placement of larvae of the next cohort. The inhibition of emergence (IE %) was calculated by comparing the number of pupal skins to the original number of larvae added. Correction for control mortality was not made as the mortality in the controls was usually 10% or less while that in most of the treatments and at most intervals was 100%. The data are presented in tables and, to reduce the tabular data, we have omitted some cohorts where the results of the omitted cohorts were identical to either the immediately preceding or succeeding cohorts or both. This omission does not impair the strength of the data presented and the trends noted.

Two types of assessment of the efficacy of temephos were made in this experiment. The first assessment involved determining the efficacy of temephos 1% GR when the granules packets were in the jars. This was carried out for 28 – 67 d in jars with granule packets present in the jars in the 5 sequences. The packets were transferred (four transfers) from one set of jars to another, but the efficacy of residual temephos was also determined in jars without the packets for three to seven wk in each sequence. This second type of assessment was made on the longevity of released and absorbed or adsorbed temephos in the jars after removal of the packets. This assessment will provide valuable information following removal of the source of applied temephos granules due to washing or cleaning of the jars.

Longevity in painted and unpainted jars

A total of 24 glazed-clay jars (200 L capacity) was used in this experiment of which 12 jars were painted with alkyd resin paint (Glipton G100, synthetic resin high gloss enamel, TOA Paint Co., Bangkok) as in our previous study (Mulla et al. 2004) and 12 jars were left unpainted. The jars were arranged on a concrete slab under a roof. The jars were covered with celocrete sheets, preventing extraneous materials and UV light from entering the jars. Water temperature was measured throughout the test by submerging a minimum-maximum thermometer in one of the jars. Temephos 1% GR at two rates (1 and 10 g/jar (0.05 and 0.5 mg/L AI) were sprinkled loosely on the top of water in each treated jar where the granules sank to the bottom. The same granules (Larvifos) as used in the previous experiment were used here. Control jars (both painted and unpainted) were treated with blank sand granules. Each treatment was replicated four times.

After filling the jars from a domestic water supply, they were set for a week before treatment. At the outset, each jar was provided with 0.5 g powdered larval food. Prior to treatment, each jar was stocked with 25 third-instar laboratory-reared larvae of *Ae. aegypti*. Subsequent larval cohorts were added at weekly intervals. Assessment of activity was made by counting pupal skins (indication of successful emergence)

five to seven d post-addition of larvae. Due to the high temperature of water, third-instars were able to complete development to emergence of adults in five d. The overall efficacy and longevity of temephos were determined by calculating the inhibition of emergence (IE %) on the basis of pupal skins in treatments versus the number of larvae added. Mortality in controls was not considered in these calculations, as the control mortality was usually 10% or less while in the treatments it was generally 95-100%. In total, 21 cohorts of larvae were used over a period of five mo after treatment. For reducing the tabular data, information on some cohorts is not presented where the deleted results were similar to the immediately preceding or succeeding cohorts or both.

Re-evaluation of temephos GR in packets

To determine the release of temephos 1% GR enclosed in packets, a third experiment was run for 56 d, using 1, 5, and 10 g packets per jar, where the packets remained in the treated jars over the entire period. The experiment was run for 48 d. Procedures of assessment were the same as in the previous two experiments.

RESULTS AND DISCUSSION

Temephos, an organophosphate larvicide, has been used for the control of *Ae. aegypti* in Thailand for over 30 y, since it was first evaluated in water-storage containers (Bang and Tonn 1969ab, Bang et al. 1972). The current dosage used in the *Ae. aegypti* control program in Thailand is 10 g of 1% sand granules per 100 L water in water-storage containers. It is not clear whether temephos releases quickly in water-storage containers and is absorbed or adsorbed to particles and sides of the containers and then released back slowly or is it released slowly over time. To answer this crucial question, the current study was carried out.

Sequential release

In this study we wanted to provide evidence for the release pattern of temephos from sand granules and its longevity after release into the water jars, by sequential transfers of the same packets of temephos granules to new sets of jars four times over a period of about eight mo. Efficacy of granule packets transferred sequentially and the longevity and residual activity of released temephos into the jars after removal of packets were studied over time. Minimum-maximum water temperatures during the test period were 28-30° and 31-34° C, respectively.

In the first sequence, the granule packets were left in the first set of jars for 28 d, where temephos release was moderate, yielding low to moderate level of efficacy (IE %) at the two lower dosages (Table 1). The highest level of control was 88% at the 10 g/jar dosage. As a whole, during the initial period of two mo, the magnitude of release, although adequate, was not high as compared to the release later on in the experiment as reflected in almost complete control of emergence. This low level of initial release during the first two mo is in contrast to the high level of release (giving IE 100% within a wk or two of application of loose granules) as

found in the second experiment here (see Table 6) and in our previous studies (Mulla et al. 2004). The low level of initial release in the present experiment could be due to uncontrolled experimental conditions. We therefore ran another experiment using the same three dosages of granules enclosed in packets and placed continuously in the jars for 56 d. The release of temephos was assessed in the same manner as in the previous experiment. In this experiment, the release was moderate for 19 d post-treatment, but the treatment gave 89 to 100% IE at the two high dosages during the 26 to 56 d post-treatment (tabular data omitted). This clearly indicates the low level of release in the previous experiment was due to experimental error. The results of the second experiment are in agreement with those in the following experiment and those of Mulla et al. (2004).

Since the release of temephos was not high in the first sequence jars of the first experiment, the residual activity of released temephos was also not adequate and only short-lived. The maximum IE% was 70% at the highest dosage for one wk (data not shown) after removal of the packets, declining precipitously during the second and third wk (Table 1).

After transfer of the granule packets into sequence II jars, moderate to high level of control (IE 86 - 97%) at the highest dosage was achieved for 28 d of observations post-transfer of packets into these jars (Table 2). However, on and after the 43rd day post-transfer into sequence II jars, the extent of release and control increased markedly in all three dosages (IE 90-100%) on day 43 and 50 post-transfer into sequence II jars (Table 2), where the lowest dosage (1 g/jar) yielded IE 92 - 99%. On removal of granule packets from these jars, the residual activity of released temephos was higher than before in the jars (IE 95-100%) at all three dosages for one wk only (Table 2). The activity of temephos, however, was very low (IE 12-43%) during the next two wk of evaluation. These results show that adequate release in sequence II jars occurred six wk after placement of the packets in the first sequence jars.

After transfer of granule packets to sequence III jars, the magnitude of release and efficacy (IE 96-100%) in these jars was studied for 53 d, where the IE% ranged from 91-100% in the presence of granule packets during the whole period (Table 3). After removal of packets (after 53 d) from sequence III jars, the residual activity of released temephos remained very high (IE 92-100%) at the two higher dosages but lower (75%) at the lowest rate for a period of 39 d post-removal and IE 88-95% at the two high dosages and 66% at the lowest dosage, 46 d post-removal (Table 3). We did not test for residual activity after this period, but it appears from the low as well as the two higher dosages that activity was declining.

The transfer of granule packets from sequence III to sequence IV jars still exhibited a very high level of quick release and activity of temephos after the transfer (Table 4). The IE% ranged from 97 to 100% in the three dosages for a period of 38 d or longer, when the packets were removed and transferred to sequence V jars (Table 4). After removal of granule packets from sequence IV jars, the residual activity of released temephos remained very high (IE 94-100%) at all three dosages for up to two w post-removal (Table 4).

Table 1. Sequential release and residual activity of temephos when temephos sand granules (1%) were applied as packets to water in 200 L earthen jars and challenged with cohorts of 3rd instar larvae of *Ae. Aegypti*. Bang Bua Thong, Nonthaburi, Thailand. Sequence I treated Aug 27, 2003.

Sequence I jars	Granules g/jar	mg/L AI	Pupal skins	EI* (%)
<u>1st cohort assessed 7 d post-treatment (Sept 3, 2003)</u>				
1A	1	0.05	4.75	81
1B	5	0.25	5.00	80
1D	10	0.50	5.50	78
1C	0	0.00	24.00	4
<u>3rd cohort assessed 21 d post-treatment (Sept 17, 2003)</u>				
1A	1	0.05	7.00	72
1B	5	0.25	7.00	72
1D	10	0.50	3.00	88
1C	0	0.00	22.75	9
<u>4th cohort assessed 28 d post-treatment (Sept 24, 2003)**</u>				
1A	1	0.05	16.50	34
1B	5	0.25	16.75	33
1D	10	0.50	5.50	78
1C	0	0.00	22.50	10
<u>2nd cohort assessed 14 d post 1st removal (Oct 8, 2003)</u>				
1A	1	0.05	22.50	10
1B	5	0.25	22.00	12
1D	10	0.50	14.75	41
1C	0	0.00	23.25	7
<u>3rd cohort assessed 21 d post 1st removal (Oct 15, 2003)</u>				
1A	1	0.05	21.00	16
1B	5	0.25	20.75	17
1D	10	0.50	18.00	28
1C	0	0.00	23.50	6

*Data for cohort 2 with packets and cohort 1 without packets omitted. For explanation see text.

**Packets of temephos granules removed from sequence I (A-D jars) and placed in sequence II (2A-2D jars) on Sept 24, 2003 when the pupal skin count was taken. Both A-D (without packets) and 2A-2D (with transferred packets see Table 2) were evaluated.

Table 2. Sequential release and residual activity of temephos when temephos sand granules (1%) were applied as packets to water in 200 L earthen water storage jars and challenged with cohorts of 3rd instar larvae of *Ae. aegypti*. Bang Bua Thong, Nonthaburi, Thailand. Second sequence. (Sequence I treated Aug 27, 2003).

Sequence II jars*	Granules g/jar	mg/L AI	Pupal skins	EI** (%)
<u>1st cohort assessed 7 d post 1st transfer (Oct 1, 2003)</u>				
2A	1	0.05	14.00	44
2B	5	0.25	13.50	46
2D	10	0.50	3.50	86
2C	0	0.00	23.50	6
<u>4th cohort assessed 28 d post 1st transfer (Oct 23, 2003)</u>				
2A	1	0.05	11.50	54
2B	5	0.25	5.50	78
2D	10	0.50	0.75	97
2C	0	0.00	22.50	10
<u>5th cohort assessed 43 d post 1st transfer (Nov 5, 2003)</u>				
2A	1	0.05	0.25	99
2B	5	0.25	0.25	99
2D	10	0.50	1.00	96
2C	0	0.00	22.50	10
<u>6th cohort assessed 50 d post 1st transfer (Nov. 12, 2003) ***</u>				
2A	1	0.05	2.00	92
2B	5	0.25	0.00	100
2D	10	0.50	2.50	90
2C	0	0.00	23.00	8
<u>1st cohort assessed 7 d post 2nd removal (Nov 19, 2003)</u>				
2A	1	0.05	1.25	95
2B	5	0.25	0.00	100
2D	10	0.50	0.75	97
2C	0	0.00	22.50	10
<u>3rd cohort assess 28 d post 2nd removal (Dec 8, 2003)</u>				
2A	1	0.05	22.00	12
2B	5	0.25	18.75	21
2D	10	0.50	15.75	35
2C	0	0.00	22.50	10

*Packets of granules from sequence I (A-D jars) transferred to sequence II (2A-2D jars) on Sept 24, 2003.

**Data for cohort 2 and 3 before and cohort 2 after removal of packets omitted. For explanation see text.

***Packets of granules from Sequence II (2A-2D jars) transferred to Sequence III (3A-3D jars) on Nov 12, 2003. Both sequence II jars (Table 2) and sequence III jars (Table 3) were evaluated.

Table 3. Sequential release and residual activity of temephos sand granules (1%) when applied as packets in 200 L earthen water storage jars and challenged with cohorts of 3rd instar larvae of *Ae. aegypti*. Bang Bua Thong, Nonthaburi, Thailand. Third sequence (1st sequence treated Aug 27, 2003).

Sequence III jars*	Granules g/jar	mg/L AI	Pupal skins	EI** (%)
<u>1st cohort assessed 7 d post 2nd transfer (Nov 19, 2003)</u>				
3A	1	0.05	0.00	100
3B	5	0.25	0.00	100
3D	10	0.50	1.00	96
3C	0	0.00	23.25	7
<u>3rd cohort assessed 26 d post 2nd transfer (Dec 8, 2003)</u>				
3A	1	0.05	3.00	88
3B	5	0.25	0.00	100
3D	10	0.50	2.25	91
3C	0	0.00	24.00	4
<u>6th cohort assess 53 d post 2nd transfer (Jan 5, 2004)</u>				
3A	1	0.05	0.25	99
3B	5	0.25	0.00	100
3D	10	0.50	0.00	100
3C	0	0.00	23.50	6
<u>1st cohort assessed 12 d post 3rd removal (Jan 12, 2004) ***</u>				
3A	1	0.05	0.75	97
3B	5	0.25	0.00	100
3D	10	0.50	0.00	100
3C	0	0.00	23.00	8
<u>5th cohort assessed 39 d post 3rd removal (Feb 9, 2004)</u>				
3A	1	0.05	5.75	77
3B	5	0.25	0.00	100
3D	10	0.50	2.00	92
3C	0	0.00	23.75	5
<u>6th cohort assessed 46 d post 3rd removal (Feb 16, 2004)</u>				
3A	1	0.05	8.50	66
3B	5	0.25	1.25	95
3D	10	0.50	4.50	82
3C	0	0.00	24.00	4

*Packets from sequence II (2A-2D jars) transferred to sequence III (3A-3D jars) on November 12, 2003.

**Data for cohort 2, 4 and 5 before removal and cohorts 2, 3 and 4 post-removal are omitted. For explanation see text.

*** Packet transferred to sequence IV (4A-4D jars) on Dec 31, 2003.

Table 4. Sequential release and residual activity of temephos granules (1%) when applied as packets in 200 L capacity earthen jars and challenged with cohorts of 3rd instar larvae of *Ae. aegypti*. Bang Bua Thong, Nonthaburi, Thailand. Fourth sequence (1st sequence treated Aug 27, 2003).

Sequence IV jars*	Granules g/jar	mg/L AI	Pupal skins	EI** (%)
<u>1st cohort assessed 5 d post 3rd transfer (Jan 5, 2004)</u>				
4A	1	0.05	0.50	98
4B	5	0.25	0.50	98
4D	10	0.50	0.75	97
4C	0	0.00	23.00	8
<u>6th cohort assessed 38 d post 3rd transfer (Feb 9, 2004)</u>				
4A	1	0.05	0.25	99
4B	5	0.25	0.00	100
4D	10	0.50	0.00	100
4C	0	0.00	24.00	4
<u>1st cohort assessed 7 d post 4th removal (Mar 10, 2004)***</u>				
4A	1	0.05	0.50	98
4B	5	0.25	0.00	100
4D	10	0.50	0.00	100
4C	0	0.00	24.00	4
<u>3rd cohort assessed 14 d post 4th removal (March 22, 2004)</u>				
4A	1	0.05	1.50	94
4B	5	0.25	0.25	99
4D	10	0.50	0.25	99
4C	0	0.00	24.75	1
<u>6th cohort assessed 35 d post 4th removal (April 12, 2004)</u>				
4A	1	0.05	14.00	44
4B	5	0.25	6.75	73
4D	10	0.50	1.50	94
4C	0	0.00	23.00	8

*Packets transferred to sequence IV on December 31, 2003.

**Data for cohorts 2, 3, 4 and 5 pre-removal, and 2, 4 and 5 post-removal are omitted. For explanation see text.

***Packets transferred to sequence V on March 3, 2004.

Table 5. Sequential release and residual activity of temephos granules (1%) when applied as packets to 200 L earthen water-storage jars and challenged with cohorts of 3rd instar larvae of *Ae. aegypti*. Bang Bua Thong, Nonthaburi, Thailand. Fifth sequence (First sequence treated Aug 27, 2003).

Sequence V jars*	Granules g/jar	mg/L AI	Pupal skins	EI** (%)
<u>1st cohort assessed 7 d post 4th transfer (Mar 10, 2004)</u>				
5A	1	0.05	0.00	100
5B	5	0.25	0.00	100
5D	10	0.50	0.00	100
5C	0	0.00	24.50	2
<u>5th cohort assessed 33 d post 4th transfer (April 5, 2004)***</u>				
5A	1	0.05	1.00	96
5B	5	0.25	0.00	100
5D	10	0.50	0.00	100
5C	0	0.00	23.00	8
<u>1st cohort assessed 7 d post 5th removal (April 12, 2004)</u>				
5A	1	0.05	1.25	95
5B	5	0.25	0.00	100
5D	10	0.50	0.00	100
5C	0	0.00	22.75	9
<u>3rd cohort assessed 21 d post-5th removal (April 26, 2004)***</u>				
5A	1	0.05	4.75	81
5B	5	0.25	2.25	91
5D	10	0.50	1.50	94
5C	0	0.00	22.50	10

*Packets transferred to sequence V jars on March 3, 2004.

**Data for cohorts 2, 3, 4 before removal of packets and cohort 2 after removal omitted. For explanation see the text.

***Packets removed from sequence Von April 5, 2004 and disposed.

Table 6. Residual activity of temephos (1%) sand granules applied to painted and unpainted earthen water-storage jars, challenged with cohorts of 3rd instar larvae of *Ae. aegypti* at intervals post-treatment. (Treated November 12, 2003).

Granules g/jar	mg/L AI	Jars	Painted jars		Unpainted jars		
			Pupal skins	EI (%)	Jars	Pupal skins	EI (%)
<u>1st cohort assessed 7 d post-treatment (Nov 19, 2003)*</u>							
1	0.05	G	0.50	98	L	0.25	99
10	0.50	I	0.25	99	K	0.25	99
0 (control)		H	22.50	10	J	24.00	4
<u>5th cohort assessed 40 d post-treatment (Dec 29, 2003)*</u>							
1	0.05	G	4.75	81	L	0.00	100
10	0.50	I	2.00	92	K	0.00	100
0 (control)		H	24.25	3	J	24.00	4
<u>11th cohort assessed 82 d post-treatment (Feb 9, 2004)*</u>							
1	0.05	G	5.75	77	L	0.00	100
10	0.50	I	4.25	83	K	0.00	100
0 (control)		H	22.75	9	J	24.00	4
<u>19th cohort assessed 145 d post-treatment (April 12, 2004)*</u>							
1	0.05	G	7.75	69	L	0.50	98
10	0.50	I	1.50	94	K	0.00	100
0 (control)		H	23.00	8	J	22.50	10
<u>21st cohort assessed 161 d post-treatment (April 26, 2004)*</u>							
1	0.05	G	8.25	67	L	1.00	99
10	0.50	I	4.25	83	K	0.00	100
0 (control)		H	23.50	6	J	23.50	6

*Cohorts 2, 3, 4, 6, 7, 8, 9, 10, 12, 13, 14, 15, 16, 17, 18, and 20 are omitted as the results of these were similar to those immediately preceding or succeeding these cohorts.

However, IE% declined precipitously to 62% and 49% after three and four wk (data not shown) at the low dosage, but remained high at the other two dosages. Likewise, the activity declined to IE 73% and 94% at the 5 and 10 g/L dosages 35 d post-removal (Table 4). It is evident that even in the absence of temephos granules, the previously released temephos persisted in the jars providing excellent control for an additional five - six wk at the highest dosage. It should be noted, however, that in actual practice under constant water use conditions, the residues may be diluted and not expected to yield adequate control for five - six wk post-removal or loss of granules.

The granule packets when removed and transferred for the fourth time to sequence V jars, yielded 96-100% IE for several weeks of assessment (Table 5). After about five wk in this sequence, the granule packets were removed and disposed of and the experiment terminated. Activity of the released temephos in sequence V jars after removal of packets was monitored for three wk only, and the residues were high enough to yield 91% to 100% IE at the two high dosages. The test was then discontinued (April 26, 2004) eight mo from the start (August 27, 2003) of the experiment.

From this and the third experiment, it is clear that temephos is released slowly from sand granules over a long period of time. It is equally important to note that once released in adequate quantities, it remains in the jars and yields excellent control of larvae for three - six wk after removal of the granule packets depending on the extent of prior release. These findings point out the possibility of employing temephos granules in retrievable formulations which can be recovered during washing and cleaning of water-storage containers with temephos dosages lower than those currently used in operational control programs. Removable and retrievable formulations in packets or sachets can be reused and will have decided advantages over placement of granules as loose particles in the jars. During the process of cleaning and washing water-storage jars, loose granules are washed out and thus are lost, while granules in a retrievable form can be removed and transferred to the same jars after washing or to other untreated jars. This scheme will diminish the waste of scarce and costly formulations and will extend control program capacity to treat large numbers of jars which often go untreated due to insufficient amounts of the formulation being available to users.

Residual activity in painted versus unpainted jars

In this experiment, temephos 1% GR was added to water-storage jars at the dosages of 1 g and 10 g per 200 L of water equaling 0.05 and 0.5 mg/L AI respectively and challenged with 21 weekly cohorts of larvae over a period of five mo. Here, we present results for 10 cohorts only, omitting the remaining 11, where the results were similar to those of the immediately preceding and succeeding cohorts to the omitted ones. The minimum-maximum temperatures of water during the test period were 28-30° C and 31-33° C respectively. At both dosages, release of temephos in painted jars was moderate to high throughout the duration of the experiment, which lasted for about five mo. The inhibition of emergence in the painted

jars ranged from 51% to 98% (mostly in the 80th percentile) at the 1 g/jar dosage in the 21 cohorts of larvae added during this period (Table 6). The inhibition of emergence was consistently higher (IE 83% to 99%) at the higher dosage than at the low dosage in the painted jars. The inhibition of emergence overall was lower in the painted jars as compared to the unpainted jars. It seems that temephos released in painted jars is absorbed and bound in the paint layers, releasing into the water slowly. In contrast, the activity of temephos granules in our previous study, when we used 2x (20 g/jar) the current high dosage, was much higher in jars painted than here (Mulla et al. 2004). The difference is probably caused by the higher dosage used in that earlier study.

The magnitude of release in the unpainted jars was consistently very high in both dosages. In every cohort (a total of 21 cohorts) of assessment, the inhibition of emergence was 98% to 100%, most of the times the IE was 100% in both dosages (Table 6). It seems that even at the 1 g / 200 L water, which is 1/20 of the recommended dosage used in the control program, the level of control was about 100% for up to five mo or longer. In view of these findings, it will be desirable to consider studies in the field on reducing the currently used dosage to 2-5 g/200 L of water, providing material to treat many other water-storage containers than is currently possible.

The longevity and efficacy of temephos 1% GR in unpainted jars found here are identical (over five mo) to what was found in our earlier studies in painted jars (Mulla et al. 2004). However, this level of activity and longevity are not to be expected in community-wide *Ae. aegypti* control programs. As pointed out by Thavara et al. (2004) many factors, especially water use patterns, influence the longevity and efficacy of chemical control agents in water-storage jars.

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