Evaluation of the residual effect of temephos on *Aedes aegypti* (Diptera, Culicidae) larvae in artificial containers in Manaus, Amazonas State, Brazil

Avaliação do efeito residual do temefós em larvas de *Aedes aegypti* (Diptera, Culicidae) em recipientes artificiais em Manaus, Amazonas, Brasil

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Abstract *Trial tests and container observations were conducted in households to verify the residual effect of temephos in Manaus, Amazonas State, Brazil. Three plastic buckets, three tin cans, and three tires filled with water from an artesian well and larvicide were used in the experiment, with twenty-five third-instar larvae, which remained exposed for 24h, followed by mortality readings. The same types of containers were selected from common households. Collection and counts followed by chemical treatment were carried out on the larvae that were found. Follow-up was performed weekly to verify recolonization by* Aedes aegypti. *The experiment showed 100% mortality in the plastic buckets until day 90, and 80% in the tin cans until day 30, decreasing from day 45 onwards. Mortality in the tires decreased to 35% in the first month. Household results showed 100% mortality for all containers after 24h and differentiated values in the subsequent readings. Larvae were observed on day 35 in a tin can and on day 21 in a gallon can. There was a large diversity of results in the tires, with recolonization observed from day 7 onwards.*
Key words *Temephos;* Aedes aegypti*; Vector Control*

Resumo *Foram realizados testes experimentais e observações de recipientes nas residências para verificar o efeito residual do temefós em Manaus, Amazonas, Brasil. Na forma experimental, utilizou-se três baldes plásticos, três latas e três pneus, os quais receberam água de poço artesiano e o larvicida. Usou-se 25 larvas de 3º estádio que ficavam em exposição por 24 horas, seguindo-se a leitura da mortalidade. Selecionou-se os mesmos tipos de recipientes nas residências, realizou-se a coleta e contagem das larvas encontradas, realizando-se o tratamento químico. Semanalmente fazia-se o acompanhamento para se verificar a recolonização por* Aedes aegypti. *Em condições experimentais, constatou-se 100% de mortalidade no balde plástico até 90 dias do experimento, na lata 80% até o 30º dia, reduzindo-se a partir do 45º dia. Os pneus apresentaram queda na mortalidade para 35% no primeiro mês. Nas residências, observou-se 100% de mortalidade em todos os recipientes após 24 horas e resultados diferenciados nas leituras seguintes. Na lata, verificou-se larvas no 35º dia e no galão no 21º dia. Nos pneus, ocorreu grande diversidade de resultados observando-se recolonização a partir de sete dias.*
Palavras-chave *Temefós;* Aedes aegypti*; Controle de Vetores*

Introduction

Dengue is an arboviral disease caused by four virus serotypes of the genus *Flavivirus* (DEN-1, DEN-2, DEN-3, and DEN-4), with *Aedes (Stegomyia) aegypti* (L.) as the mosquito vector. Since the 1980s and 90s this disease has caused major epidemics in Latin America, Asia, and Africa.

Presently the basis for *Aedes aegypti* control programs focuses on combating the immature forms with focal treatment using an organophosphate called temephos (commercial name: Abate). Periodicity of focal treatment is based on the residual larvicidal effect, which is approximately three months according to the manufacturer.

According to the literature, specimens from sites where there has already been intensive use of the larvicide in dengue control programs are more likely to show resistance to the larvicide. There are reports of resistance in Asia and the Caribbean (Chiong et al., 1985; Georghiou et al., 1987; Mazzarri & Georghiou, 1995; Rawlins, 1998; Rawlins & Ragoonansingh, 1990; Rawlins & Wan, 1995; WHO, 1980), as well as in some South American countries like Bolivia (Schofield et al., 1984) and Venezuela (Mazzarri & Georghiou, 1995). The first signs of resistance are appearing in Brazil, as reported by Macoris et al. (1995c; 1999), Carvalho & Silva (1999), and the Brazilian Ministry of Health (MS, 1999).

The main objective of this study was to measure the residual effect of temephos in *A. aegypti* control activities in Manaus. The larvicide has been used systematically for focal treatment since 1996, when the vector became widely established in Manaus.

Methods

Temephos was used in the 1% a.i. (active ingredient) formulation at a 1 ppm (one part per million) concentration to evaluate its effectiveness in controlling immature forms of *A. aegypti*. This dose is harmless to humans but lethal to mosquito vectors.

There were two kinds of tests: (1) experimental, at the campus of the National Institute for Amazonian Research (INPA) and (2) direct observation of containers treated in households in the District of Coroado, situated near the INPA campus.

Experiment on the INPA campus

Three ordinary household containers were used for the experiment: plastic buckets, tin cans, and tires. The test was set up amidst the vegetation in the area behind the main entrance to the Health Sciences Research Coordination building (CPCS).

Three containers of each kind were used, containing water from the INPA's own artesian well system, mixed with the larvicide. On the day the test was set up, and every two weeks thereafter, 25 third-instar larvae from the INPA's Malaria and Dengue Laboratory insectary were placed directly in the container water for exposure. Mortality readings were conducted after 24h. Larvae were recaptured with the aid of a larvae net.

Mosquitoes used in the tests came from a colony formed from larvae collected from artificial containers found in households. After emergence of the adults, the females were fed and placed for oviposition. Following eclosion, larvae were kept until the third instar and used in the experiments.

One control was kept for each kind of container, which also received 25 larvae.

Experiment in households

The same containers, buckets, tin cans, and tires, were used in the household experiment. Larval and pupal collection and counts were performed in each selected container. Next, larvae were replaced in the containers that had received the chemical treatment according to National Health Foundation (FUNASA) guidelines (MS, 1994). Readings were performed after 24h and every seven days until recolonization by *Aedes* was verified. Water was replaced in the containers, allowing observations to be extended up to 90 days.

The observed containers were kept in the yards of houses, in aired places with cover or shaded by bushes. At every visit for readings, residents were instructed as to the importance of maintaining the containers for performing the research.

The Abbott formula (WHO, 1970) was used to correct mortality results in the control group, where they varied from 5% to 20%.

Considering that salinity can be a limiting factor for *Aedes* larval development and that a pH of more than 9.0 can lead to hydrolytic degradation of temephos, these two parameters were evaluated at baseline and at the end of the experiment. Electric conductivity was measured using a Jenway 4010 conductivity-meter. A WDW 391 pHmeter was used to measure pH. Water temperature was measured using an ordinary mercury thermometer.

Total and dissolved iron analysis was also performed, using spectrophotometers, using a

512-nm wavelength. Titration according to Golterman et al. (1978) determined the chemical oxygen demand.

Results

Results of temperature, pH, and conductivity measurements were recorded at the beginning (September 1, 1999) and end (December 15, 1999) of the experiment at the INPA campus. The difference between the initial and final temperature was only 1ºC. The pH fluctuated between 4.40 and 4.65, not presenting great modifications following the addition of temephos. Variation remained between 4.50 and 6.80, with the highest pH values recorded in tires in which temephos was applied (6.60 and 6.80). The control tire had a pH of 4.90.

Conductivity data (Table 1) showed higher initial and final variation. At the time of the assembly, µS/cm for the water used in all the containers was 14.4. There was a variation from 35.5 to 69µS/cm in the plastic buckets at the end of the experiment. Values between 21.2 and 35.2µS/cm were recorded in the tin cans, and the largest difference was observed in the three replicate tires that had received temephos (123.4 to 149.1µS/cm). A lower value (83.6µS/cm) was recorded in the control tire.

Data in Table 2 refer to the whole and dissolved iron analysis and chemical oxygen demand (COD) values. They also include data on household containers: a gallon can and two tires. Considering the INPA campus experimental containers, for whole and dissolved iron, the highest COD values were found in the tires. Values in the plastic buckets were relatively high as compared to the tin cans. However, tires again showed the highest values.

The pH values in the three types of household containers were quite high, but the electrical conductivity values varied considerably. For whole and dissolved iron, the variations were again in the tires, while COD values were high in all three containers.

Table 3 shows total mortality in each replicate, the number of larvae per container, and reading periods. Mortality percentiles observed in all three types of containers are shown graphically in Figure 1. The results in the plastic bucket remained near 100% during the 90 days of the experiment, dropping to 79% at day 105. Mortality in the tin cans remained above 80% until day 30, dropping to 77% at day 45 and to 68% at day 60. A steep increase to 93% was observed at day 65, decreasing to 55% at the end of three months.

The highest decreases in the mortality rate were observed in the tires. Values were high until day 15, dropping to 35% at the end of the first month, increasing to 53% at day 45. At days 60 and 75, the mortality rate was down considerably, to 13 and 7% respectively. An increase was recorded at the end of three months, with mortality at 55%. The latter value was ruled out, since this increase was attributed to predators that were found together with *Aedes* larvae remaining from the tests.

Table 1

Temperature, pH, and conductivity in test containers treated with temephos (Abate) at the campus of the National Institute for Amazonian Research (INPA) from September 1 to December 15, 1999.

	Replicates	Temperature (°C)		pH		Conductivity (µS/cm)	
		I	F	I	F	I	F
Plastic bucket	1	27.5	27	4.65/4.61*	5.6	14.4	35.5
	2	27.5	27	4.62/4.61*	5.7		69.6
	3		27	5.0			37.3
	4C		28	5.6			47.8
Tin can	1	28	27	4.62/4.21*	4.5	14.4	31.3
	2	28	27	4.40/4.18*	5.2		23.7
	3		27	5.4			21.2
	4C		27	5.7			35.2
Tire	1	27.5	27	4.58/4.55*	6.6	14.4	123.4
	2	27.5	27	4.56/4.54*	6.8		149.1
	3		27		6.8		142.0
	4C		27		4.9		83.6

I = Initial; F = Final; C = Control; µS/cm = Microsiemens.
* pH after adding temephos to the container.

Table 2

Results for pH, conductivity, whole and dissolved iron, and chemical oxygen demand (COD) in test containers at the National Institute for Amazonian Research (INPA).

Container	Replicates	pH	Electric conductivity (μS/cm)	Whole iron (mg/L)	Dissolved iron (mg/L)	COD (mg/L)
INPA						
Plastic bucket	1			0.006	0.006	33.91
	2			0.043	0.03	59.2
	3			0.006	< 0.005	46.39
	4C			0.092	0.055	40.23
Tin can	1			< 0.005	< 0.005	11.83
	2			< 0.005	< 0.005	11.36
	3			< 0.005	< 0.005	23.85
	4C			< 0.005	< 0.005	14.12
Tire	1			0.055	0.043	75.43
	2			0.128	0.092	85.65
	3			0.948	0.838	73.00
	4C			0.496	0.434	77.22
Households						
Gallon can		7.5	187.0	0.3	< 0.005	53.53
Tire 1		7.6	366.0	0.018	< 0.005	45.42
Tire 2		6.8	71.7	0.067	0.018	74.62

μS/cm = Microsiemens.

Data in Table 4 refer to the trials for observing containers that were treated directly in households. Unfortunately, observations were very limited, since the residents destroyed most of the containers. One-gallon paint cans (3.6L), large cans (18L), automobile tires, plastic buckets, and water storage tanks were observed in both neighborhoods. Readings performed 24 hours after treatment showed 100% mortality in all gradients.

Readings to record recolonization varied considerably. In the large 18L tin can, the larvicide proved effective up to 28 days, with larvae recorded on day 35 (containers 9 and 10). The gallon can (container 3) had larvae and pupae recorded at day 21. Leftover food was found in this container on day 7.

The greatest variation was observed in tires. Larvae were recorded from days 7 and 14 (containers 11 and 7) until days 35 and 42 (container 4). Only one indoor container was observed to be recolonized after 21 days.

Discussion

In Manaus, *A. aegypti* control measures are based on control of immature forms using temephos. Tests were thus conducted to verify this larvicide's effectiveness in different containers.

The most common household containers in the Coroado district were used to conduct the tests (Pinheiro, 2000). According to FUNASA guidelines, such containers are classified as non-serviceable and should be destroyed, and therefore not receive any treatment (FUNASA, 2001). However, they currently provide an enormous amount of breeding sites, especially during the rainy season, the period of greatest proliferation. Several authors (Gubler, 1989; Melo, 1997; Moore, 1978; Tauil, 2001) have discussed the difficulty in eliminating these containers, which are kept by residents for future use, making health agents' work more difficult.

For example, in Manaus all types of tin cans and similar recipients are used to store cement, paint, and water around construction sites. Buckets are used for the same purpose, and also serve to store water in the household whenever running water is cut off. Tires are also commonly used as vases for plants or even as makeshift playground equipment (swings, etc.) for children.

Results under test conditions showed differentiated residual effects, perhaps due to the basic physical and chemical composition of the

Table 3

Mortality observed in three kinds of containers treated with temephos (Abate) used at the campus of the National Institute for Amazonian Research (INPA).

Containers	**Replicates**	**Number of tested larvae**	**Readings (days)**							
			24h	15	30	45	60	75	90	105
Plastic bucket (10 liters)	B1	25	25	25	25	25	25	25	24	15
	B2	25	25	25	25	23	22	25	25	20
	B3	25	25	25	25	25	22	25	25	24
	Total	75	75 (100%)	75 (100%)	75 (100%)	73 (97%)	69 (92%)	75 (100%)	74 (99%)	59 (79%)
	Control	25	0	0/2	0	0	0/2	0	0	
Tin can	L1	25	25	25	15	13	10	23	15	
	L2	25	25	25	25	20	20	22	11	
	L3	25	25	25	25	25	21	25	15	
	Total	75	75 (100%)	75 (100%)	65 (87%)	58 (77%)	51 (68%)	70 (93%)	41 (55%)	
	Control	25	0	0/2	1	0	0	0	0	
Tire (4 liters)	P1	25	25	25	6	12	3	2	16	
	P2	25	25	16	1	14	4	2	7	
	P3	25	25	25	19	14	3	1	18	
	Total	75	75 (100%)	66 (88%)	26 (35%)	40 (53%)	10 (13%)	5 (7%)	41 (55%)	
	Control	25	0	0	0	0/1	0/2	0	0	

0/0 = The second number (to the right of the slash) refers to pupae.

Figure 1

Mortality percentage observed in the three kinds of containers treated with temephos (Abate) at the campus of the National Institute for Amazonian Research (INPA).

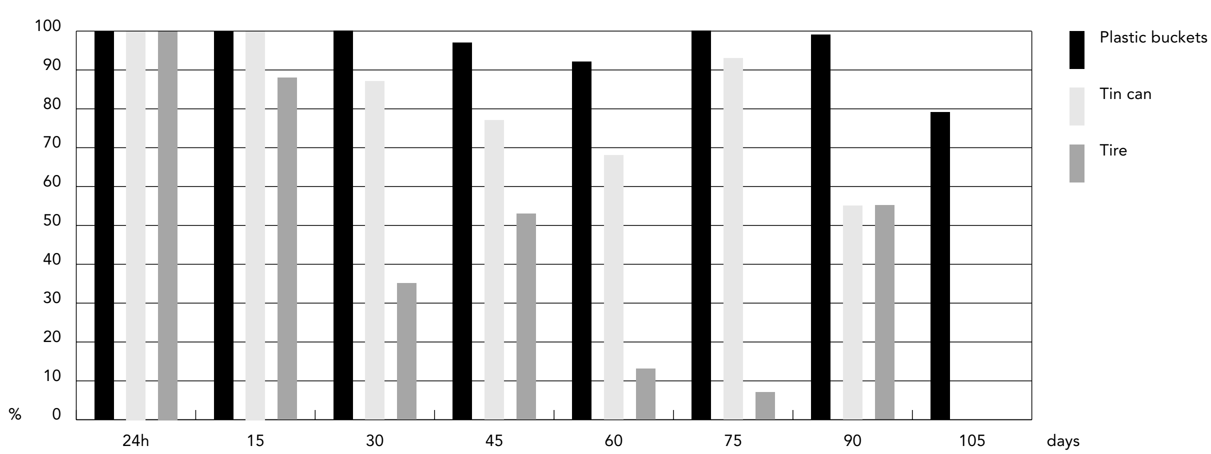

Table 4

Recolonization of household containers treated with temephos (Abate).

Containers studied	Number larvae	Mortality	Larval presence following treatment (days)					
		24h	7	14	21	28	35	42
01 Tin can	157	157	0	0	0	0	*	
02 Gallon can	7	7	0	0	0	*		
03 Gallon can	61	61	0		18/17**			
04 Tire	120	120	0	0	0	0	0	67
05 Tire	65	65	0	0	*			
06 Tire	83	83	0	0	0	0	28/3	
07 Tire	98	98	0	0	0	0	13	
08 Plastic buckets	300	300	0	*				
09 Tin can	72	72	0	0	0	0	67	
10 Tin can	258	258	0	0	0	0	98	
11 Tire	320	320	0	36***				
12 Tin can	36	36	0	0	*			
13 Water storage tanks	536	536	*					
14 Tire	48	48	00	0	0	3		
15 Tin can	63	63	0	*				
16 Gallon can	105	105	0	0	0	*		
17 Tire	225	225	0	32				
18 Tire	323	323	0	0	0	78		
19 Tin can	325	325	*					
20 Tin can	63	63	0	0	0	0	0	*
21 Gallon can	382	382	*					

* Container destroyed by residents.
** Container altered by the addition of leftover food.
*** Container retreated and recolonized after 21 days (inside the household).
0/0 = Second number (to the right of the slash) refers to pupae.

materials used to manufacture the containers. There are ion exchanges between the water and materials, altering the pH and conductivity, as shown in Table 1. According to our study, the reduction detected in tires probably results from changes in the initial and final conductivity. Alterations were not as great in the control groups as in the treated tires.

Whole iron, dissolved iron, and COD (Table 2) were also measured to analyze other water parameters in the containers. Based on these three parameters, the greatest changes also occurred in tires. According to these results, alterations in the water in tires are the result of ion exchanges between the water and the material used to make the tires. All these factors contribute to larvicide degradation.

Temephos can undergo hydrolytic decomposition in an alkaline pH, above 9.0. In this study's tests, both on controls and experimental replicates, pH variations did not surpass 6.8. Considering that salinity at concentrations above 20,000ppm are lethal to *A. aegypti* larvae, it is possible to approach this parameter, taking the conductivity data measured in this study into account. Conductivity values (µS/cm) were remarkably higher in the treated tires. These data signal changes in water conditions in the containers which contribute to degradation of the larvicide. Macoris et al. (1995a) report that salinity also interferes with the *A. aegypti* oviposition site. The authors verified that females avoid laying eggs in water with high NaCl concentrations (2.5% and 3%).

Similar results were obtained by Macoris et al. (1995b), who found a minimum residual effect of 90 days for outdoor containers and 120 to 240 days for indoor containers. Outdoor results were similar to those observed in the INPA campus experiments, in which mortality was high in plastic buckets until day 90 (Figure 1). Camargo et al. (1998) also observed a reduction in larval mortality in tires after the fifth and seventh weeks, verifying 100% efficiency in the other types of containers. The authors attributed this difference in tires to a possible alteration in the larvicide as a result of modifications in tire components, also observed in the current study.

In households, as shown in Table 4, the recolonization period in containers changes considerably as compared to observations from laboratory tests. This probably results from drastic reductions in the water in containers, undergoing the influence of ion exchanges with the gradient's component material, in addition to debris inside the deposits.

Table 2 also shows data from a gallon can and two tires in households. Remarkable differences occurred as compared to the INPA campus experimental containers (Table 1). The values were considerably higher for both pH and conductivity. Whole iron, dissolved iron, and OCD were quite similar. These factors probably interfere in temephos degradation by reducing its residual effectiveness. Data in Table 4 actually show a remarkable reduction in temephos effective time in households.

These results are important for dengue control campaigns. While temephos is the basis for controlling immature forms of *A. aegypti*, data analysis shows that efficacy is hindered in field treatment. Containers undergo alterations in conductivity, a factor that appears to interfere in degradation of the larvicide. There is a need to monitor the scheduling of visits, currently conducted every three months, since the residual effect of larvicide in the field does not last as long as in laboratory experiments. It is thus essential to consider that focal treatment is the main strategy for maintaining a reduced *Aedes* population density, compatible with interruption of transmission of the dengue virus. Biological control measures such as the use of *Bacillus* var. *israelensis thuringiensis*, shown to be effective in the control of immature forms of *Aedes*, are mentioned as an alternative to temephos (Barjac & Sutherland, 1990).

Acknowledgments

We thank the Manaus office of the National Health Foundation, the Amazonas State Health Department, and the Manaus Municipal Health Department for providing health services personnel to support the field activities. We also thank INPA's dengue and malaria vector laboratories for identifying the specimens, together with the Lymnology Research Coordinating Department (CPGC) for the water chemical analysis. We also wish to thank Jorge Antunes for helping draft the original English version of the text. Funding: MCT/INPA-PPI 3110 – MS/FUNASA.

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Submitted on 13 June 2001
Final version resubmitted on 19 September 2001
Approved on 4 February 2002