SIX MONTHS OF *AEDES AEGYPTI* CONTROL WITH A NOVEL CONTROLLED-RELEASE FORMULATION OF PYRIPROXYFEN IN DOMESTIC WATER STORAGE CONTAINERS IN CAMBODIA

Chang Moh Seng[1], To Setha[2], Joshua Nealon[1], Duong Socheat[2] and Michael B Nathan[3]

[1]World Health Organization, Phnom Penh; [2]Center of Parasitology, Entomology and Malaria Control, Ministry of Health, Phnom Penh, Cambodia; [3]Department of Control of Neglected Tropical Diseases, World Health Organization, Geneva, Switzerland

Abstract. A field evaluation of a novel, 5% controlled-release formulation of pyriproxyfen was carried out in 400-500 liter concrete water storage jars, the most common and important larval habitat of the dengue vector, *Aedes aegypti,* in Cambodia. The formulation consisted of cylindrical resin strands, 3 mm in diameter and 40 mm in length. Pyriproxyfen was applied to 100 jars at a target dose of 0.03-0.04 mg of active ingredient (a.i.) per liter (30-40 ppb) in households in Phum Thmei, a village near the capital city of Phnom Penh, in April 2005. Inhibition of adult emergence (IE) in field populations of *Ae. aegypti* was measured every 2 weeks for 34 weeks. IE in treated jars exceeded 90% for 20 weeks, and remained above 80% until the end of the study. In 25 untreated jars, failure of pupae to metamorphose into viable adults remained below 4%. No alteration of taste or other undesirable effects of the treatment were reported by householders. This single treatment provided control of *Ae. aegypti* in water jars for the length of the main dengue transmission season in Cambodia that normally extends from May to November.

INTRODUCTION

Dengue fever (DF) and the more serious clinical manifestation, dengue hemorrhagic fever (DHF), are endemic in Cambodia with year-round transmission. Nearly all cases occur in children under 15 years of age, and during the peak transmission season, from May to November, it is the leading cause of mortality at the National Pediatric Hospital (DeRoeck *et al*, 2003). Between 2003 and 2006 hospitalization rates ranged between 70.3 and 130 per 100,000 population. In 2007 a major nationwide epidemic occurred, with over 38,000 hospitalizations and approximately 400 deaths.

In Cambodia, dengue viruses are transmitted primarily by *Aedes aegypti* mosquitoes. Over 80% of larval foci for this species occur in ubiquitous, concrete jars filled with rain, river, well or tap water, which are used for domestic purposes. The capacity of the jars varies from 150 to 500 liters, the most common size being 400 liters. The vector is extremely abundant, with houses in endemic areas harboring an average of 3 or more infested water jars (Socheat *et al*, 2004; unpublished data). Control measures targeting these highly productive containers is thus a priority of the

Correspondence: Chang Moh Seng, World Health Organization, 177-179 Pasteur Street (51), Phnom Penh, Cambodia.
Fax: (855) 23-216211
E-mail: changm@cam.wpro.who.int

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National Dengue Prevention and Control Program. This is currently limited to seasonal larviciding in high-risk urban areas, mainly with the organophosphate temephos (Suaya *et al*, 2007).

The effectiveness of the insect growth regulator, pyriproxyfen, for controlling container-breeding mosquitoes is well documented (Estrada and Mulla, 1986; Adames and Rovira, 1993; WHO, 2001; Sihuincha *et al*, 2005; Vythilingam *et al*, 2005). It is one of several formulations which, at recommended application rates, can prevent *Aedes* breeding and are approved for use in drinking water (WHO, 2002).

Previous testing of an experimental controlled-release "chip" formulation of pyriproxyfen under simulated field conditions in Cambodia resulted in nearly 90% inhibition of adult emergence (IE) of *Ae. aegypti* for more than 6 months in 200 liter concrete water storage jars (Chang *et al*, 2006). The field study described herein was carried out with an experimental "second generation" strand formulation of pyriproxyfen placed in naturally infested concrete water storage jars in a village setting, between April and December 2005.

MATERIALS AND METHODS

Study site and selection of water jars

Phum Thmei, the study village, is 16 km from Phnom Penh, located in the suburbs of the city. At the time of the study it was comprised of 366 households, with a population of 1,875 people. Many houses are built on stilts, and are of wooden or concrete construction, with a variety of livestock living in close proximity. Typical inhabitants engage in commerce or factory work. There is no piped water supply or sanitation, but most households have mains electricity. All households in the village stored well, rain or river water for domestic use in concrete jars outdoors. There were approximately 4 jars per house. The most recent treatment with larvicide (temephos) was in April 2003, two years prior to the study. It was assumed that there would be no residual effect of the chemical from this earlier intervention. Monthly rainfall between April and December in Phnom Penh typically varies between approximately 80 mm (in the early and later months) and 300 mm (June-September), with between 6 and 20 rainy days per month. The temperature fluctuates between 22 and 35°C. Prior to the study, a meeting was convened with the village chief and elders to explain the purpose of the study and to seek their consent. Along one side of the road that passes through the village, houses were visited sequentially. The purpose and procedures of the study were explained to the heads of households and they were invited to participate. Their written consent was a prerequisite for inclusion in the study. Only water storage jars of 400-500 liters capacity that contained *Aedes* immatures at the time of the initial visit and which did not harbor larval predators, *eg*, fish or aquatic predatory insects such as corixid bugs (Family: Corixidae) or water scavenger beetles (Family: Hydrophilidae) were recruited for the study. Each jar was assigned a unique identifying number, and successive houses were visited until a total of 100 jars were identified for inclusion. Along the other side of the road, an additional 25 jars were similarly identified as controls.

The formulation of pyriproxyfen 5% comprised of resin strands 3 mm in diameter and 40 mm in length (135 mg w/w containing 6 mg a.i. per strand). Pyriproxyfen was incorporated into an inner core and released into the water through an outer sheath. Based on the capacity of the jars the estimated target dosage was 0.03 mg a.i. per liter (30 ppb) for 400 liter jars (2 strands) and 0.036 mg a.i. per liter (36 ppb) for 500 liter jars (3 strands), consistent with the optimal dosage of 0.036 mg a.i. per liter previously reported for the "chip" formulation (Chang *et al*, 2006). Householders were

encouraged to keep the strands in the jars. When emptying and cleaning the jars, they were advised to remove the strands and replace them before refilling. In households with control jars, there was no intervention.

Determining the duration of efficacy against field populations of *Ae. aegypti*

Inspections of jars and collection of mosquito pupae were carried out at two-week intervals. The first such assessment was performed immediately prior to treatment, at week "0"; this provided baseline entomological measurements. At each assessment, intervention jars were first examined for the presence of pyriproxyfen strands. Jars which no longer contained any strands were subsequently excluded from the study. All pupae in each of the intervention and control jars were collected from the surface of the water with the aid of torchlight into a white enamel bowl into which water from the same jar had been added. Pupae were pipetted from the bowl into a plastic bag partially filled with the same water, tied, labeled with the identifying number of the water jar and the date of collection and transported to the laboratory.

In the laboratory, pupae from each jar were counted and transferred to labeled, clear plastic drinking cups half-filled with water from the corresponding jar. Cups were covered with fine mesh netting held in place with elastic bands. The number of viable adults emerging, and the number of dead pupae and non-viable adults were recorded on a daily basis. This procedure was repeated until the last adults had emerged or all remaining pupae had died. The IE was calculated by dividing the number of pupae failing to metamorphose into viable adults by the total number of pupae collected and multiplying by 100. The differences between the IE in intervention and control jars were assessed using the *Z* test of significance. The numbers of pupae per jar in the two groups were compared using an unpaired *t*-test.

The study was designed to end when the IE fell below 80% for two successive sampling rounds, or after 34 weeks of the study, the latter being chosen to reflect the extreme limits of the main dengue transmission season in Cambodia.

Assessing acceptability among householders

At the time of the initial visit to each household, and thereafter on alternate visits, the head of household was invited to comment on the activity through a series of open-ended questions related to water quality and taste, specific actions taken, and the handling, loss or disappearance of strands. Findings were reported on a standard form.

RESULTS

Pyriproxyfen strands were applied to 100 water storage jars, of which 96 were of 400 liters capacity; the remaining jars were of 500 liters capacity. Retention of strands in the jars was high. Twelve weeks after application, only 5 jars (5%) had lost all pyriproxyfen strands and by 34 weeks, they had disappeared from a total of 8 jars (8%) (Table 1).

Over the 34 weeks of follow-up, a total of 6,553 pupae were recovered from treated water jars, an average of 3.8 pupae per jar (Table 1). This compared with 1,746 pupae collected from the control jars, with an average of 3.9 pupae per jar. Over the period of the study there was no statistical difference in the average number of pupae per jar in the intervention and control jars (difference = 0.08, p=0.93).

At baseline the "IE" was 0% and 2.0% in the intervention and control areas, respectively. Two weeks after treatment the intervention area the IE was 99.8% and remained above 97% until week 22. From week 22 until the end of the study (week 34), the IE fluctuated between 100% and 80.4%. In the con-

Table 1
Inhibition of emergence (IE) of adult *Aedes aegypti* in domestic water storage jars, Phum Thmei, Cambodia, April-December, 2005.

	Weeks after treatment with pyriproxyfen[a]																		
	0	2	4	6	8	10	12	14	16	18	20	22	24	26	28	30	32	34	Total
Treated jars																			
No. of water jars	100	100	99	99	96	96	95	95	95	95	94	94	94	94	94	93	92	92	
No. of pupae collected	291	414	225	654	337	555	219	763	722	364	499	376	250	124	246	299	77	138	6,553
No. of viable adults emerged	291	1	0	5	1	0	0	2	13	11	13	48	0	22	14	30	10	27	488
Inhibition of emergence (%)	0.0	99.8	100.0	99.2	99.7	100.0	100.0	99.7	98.2	97.0	97.4	87.2	100.0	82.3	94.3	90.0	87.0	80.4	92.6
Control jars																			
No. of water jars	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	
No. of pupae collected	51	206	36	129	47	137	53	135	225	231	18	86	36	35	149	86	39	47	1,746
No. of viable adults emerged	50	199	36	126	46	135	53	132	223	231	18	83	35	35	146	85	39	47	1,719
Inhibition of emergence (%)	2	3.4	0	2.3	2.1	1.5	0	2.2	0.8	0	0	3.5	3.5	0	2	1.1	0	0	1.5

[a]Week 0 was a pre-treatment assessment.

trol jars the IE never exceeded 3.5%. The differences in the IE between the intervention and control jars was highly significant at all sampling intervals (Z<-5; p<0.001).

Householder perceptions

Although there was initial concern among some householders about safety of the treated water for drinking purposes, this apprehension abated after the first post-treatment inspection. Thereafter, perceptions were overwhelmingly positive. No complaints about changes in the taste or appearance of the water were received. Reported behaviors, included tying the strands together to preserve them, and requests for continued government support for the project, were common.

DISCUSSION

At a target dosage of 0.03-0.04 mg a.i. per liter (30-40 ppb), the experimental controlled-release pyriproxyfen formulation was effective against *Ae. aegypti* (>80% IE) in domestic concrete water storage jars for the duration of the 34 week study. This was despite the high turnover of water in these domestic containers due to normal household drawdown and replenishment from various sources, including roof rainwater collection during the wettest months of the year. The results are comparable to an earlier study under controlled conditions, in which monthly removal and replacement of two-thirds of the volume of water did not reduce the duration of efficacy (Chang *et al*, 2006). The prospect of providing *Ae. aegypti* control for the entire high-risk dengue transmission season following a single, timely larvicide application targeted to these important and accessible container habitats in Cambodia would appear to offer significant benefits to a program challenged by major operational constraints. As mentioned in the previous study, ease of handling, dosing and acceptability by householders may also be advantageous.

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