Susceptibility of Two Cambodian Populations of Aedes aegypti Mosquito Larvae to Temephos During 2001

by
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Abstract
Two populations of Aedes aegypti, i.e. Phnom Penh (temephos-treated area) and Kampong Cham (area untreated with temephos) were evaluated for their susceptibility to temephos. Larval bioassays were carried out in accordance with WHO standard methods.

Results showed that, when compared with the WHO diagnostic dosage of 0.02mg/l, the Phnom Penh population was resistant (LC95: 0.034mg/l) whereas that of Kampong Cham was susceptible (LC95: 0.015mg/l). 95% confidence intervals of 0.0298 – 0.0382 and 0.0115 – 0.0193, respectively, did not overlap, indicating that the difference in susceptibility between the two populations was significant.

Resistance of Ae. aegypti to temephos appears to be incipient in Cambodia. More studies are required with wider representations of localities.

Keywords: Aedes aegypti, temephos, susceptibility, Cambodia.

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Introduction

The most widely used larvicide for Ae. aegypti control, in potable water, is temephos (Abate®) 1% sand granules which, when applied at a dosage of 1ppm, has been proven to be effective for 8-12 weeks(1). Although temephos is used in many national dengue vector control programmes, insecticide resistance has been documented in Ae. aegypti in some countries in the Americas(2, 3) and in Malaysia(4).

The largest DHF epidemic recorded in Cambodia occurred in 1998(5). Based on the 3-4 year cyclical pattern(6), a major outbreak was anticipated for the year 2001. As part of a short-term, pre-emptive approach, pre-packaged 1% temephos sand granules were distributed for application in water storage jars, as a larviciding campaign, between April and August 2001, prior to and during the main transmission season in the capital city, Phnom Penh, and in other high human population density/transmission risk areas.

Recent field bioassay studies indicated the operational effectiveness of temephos against Ae. aegypti in Phnom Penh (Yeang Chang and Michael Nathan, unpublished data). The larval susceptibility studies herein described were undertaken at the National Centre for Parasitology, Entomology and Malaria Control, Cambodia, to further assess the susceptibility status of two geographically-distinct populations of Ae. aegypti.

Materials and methods

Two areas were selected for comparison based on their history of temephos use - Phnom Penh (temephos-treated area) and Kampong Cham (temephos-untreated area).

Ae. aegypti eggs were collected from both study areas using ovitraps as per the technique described by Polson et al(7). A total of 1,709 eggs were obtained from the two collection sites. Eggs were allowed to dry for three days and then flooded in batches to induce hatching.

Insecticide-susceptibility tests were carried out on late 3rd to early 4th stage larvae of the F1 generation, in accordance with WHO instructions for determining the susceptibility of mosquito larvae to temephos(8).

Larvae were initially screened at 0.02mg/l concentration (WHO diagnostic dosage) to determine whether to adjust tests to higher or lower dosages. Each test consisted of three replicates and one control, each with 20 larvae in plastic cups containing 250 ml. of distilled water with the required insecticide concentration. Mortality counts were made after 24 hours. Tests were done until repeatable results were obtained.

Data were analysed by the Probit analysis programme in SPSS version 9.0 to obtain LC50 and LC95 values. The obtained LC95 for both populations were compared with those of the WHO tentative diagnostic dosage of 0.02mg/l of temephos for larvae of Ae. aegypti in order to determine whether the populations tested were susceptible or resistant. [A population is considered to be susceptible to temephos if, when exposed to a concentration of 0.02mg/l, the per cent mortality is equal to or greater than 95%. A resistant population is one in which there is
less than 95% mortality when such a population is exposed to a concentration of 0.02mg/l of temephos.]

Results

The overall mortalities from five replicates of both populations tested are shown in Table 1. The pooled data from Phnom Penh and Kampong Cham showed significant heterogeneity about log dose/probit mortality regression lines ($X^2 = 14.10; df = 4; p = 0.007$ and $X^2 = 6.34; df 3; 0.05 < p < 0.1$, respectively). Therefore, confidence limits based on the pooled data were of doubtful validity. Instead, data collected on each of the five days on LC50 and LC95 were compared with SPSS 9.0. Standard errors and confidence limits were then calculated from the replicates and are shown in Table 2.

This data showed that when compared with the WHO recommended diagnostic dosage of temephos of 0.02mg/litre, the mean LC95 of the Phnom Penh Aedes aegypti population was 1.7 times higher, whereas the mean LC95 of Kampong Cham was markedly less than the WHO discriminating dose.

These results obtained showed that the 95% confidence intervals of the LC50 and LC95 in the Phnom Penh population did not overlap with those of the Kampong Cham population. An unpaired t-test on the pooled standard deviation of the samples further demonstrated significant difference in the susceptibility of the two populations to temephos ($t = 9.02; df 8; p < 0.001$).

### Table 1. Concentration of temephos and overall resulting mortalities in Aedes aegypti populations from all replicates

<table>
<thead>
<tr>
<th>Population</th>
<th>Phnom Penh</th>
<th></th>
<th></th>
<th></th>
<th>Kampong Cham</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Concentration of temephos (mg/l)</td>
<td>No. dead</td>
<td>No. tested</td>
<td>% Mortality</td>
<td>No. dead</td>
<td>No. tested</td>
<td>% Mortality</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.004</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>21</td>
<td>200</td>
<td>10.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.005</td>
<td>10</td>
<td>198</td>
<td>5.05</td>
<td>43</td>
<td>200</td>
<td>21.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.01</td>
<td>37</td>
<td>200</td>
<td>18.5</td>
<td>121</td>
<td>200</td>
<td>55.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.02</td>
<td>111</td>
<td>197</td>
<td>56.34</td>
<td>199</td>
<td>200</td>
<td>99.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.03</td>
<td>173</td>
<td>199</td>
<td>86.93</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.04</td>
<td>192</td>
<td>200</td>
<td>96.0</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 2. Susceptibility status of Aedes aegypti larvae to temephos

<table>
<thead>
<tr>
<th>Population</th>
<th>Phnom Penh</th>
<th>Kampong Cham</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. of tests</td>
<td>LC50 (mg/l)</td>
</tr>
<tr>
<td>1</td>
<td>0.0131</td>
<td>0.030</td>
</tr>
<tr>
<td>2</td>
<td>0.0178</td>
<td>0.033</td>
</tr>
<tr>
<td>3</td>
<td>0.020</td>
<td>0.032</td>
</tr>
<tr>
<td>4</td>
<td>0.023</td>
<td>0.037</td>
</tr>
<tr>
<td>5</td>
<td>0.024</td>
<td>0.038</td>
</tr>
<tr>
<td>Mean</td>
<td>0.0196</td>
<td>0.034</td>
</tr>
<tr>
<td>Standard deviation</td>
<td>0.0044</td>
<td>0.0034</td>
</tr>
<tr>
<td>95% confidence intervals</td>
<td>0.0141 - 0.025</td>
<td>0.0298 - 0.0382</td>
</tr>
</tbody>
</table>

Discussion

This study, the first in Cambodia on the susceptibility of Ae. aegypti to temephos, revealed a significant difference in the two Ae. aegypti larval populations. The Phnom Penh population exhibited some degree of resistance to temephos while the Kampong Cham population was susceptible. Exposure of the Phnom Penh population to temephos, since 1995(8) and later the increased frequency due to the extended transmission during the dry season as well, appears to have resulted in the development of resistance in that population. On the other hand, the absence of insecticidal pressure for at least the past 2-3 years may account for the continued susceptibility of the Kampong Cham population.

The emergence of resistance to temephos is important, as temephos is in widespread use in dengue prevention and control programmes and is one of only four larvicides approved by WHO for application to potable water. The recommended field dosage of 1.0mg/l far exceeds the calculated LC95 of 0.035mg/l of the larvae from Phnom Penh, so if properly applied it should remain very effective. Curtis et al.(9) found that in Tanzania where Culex quinquefasciatus is known to be resistant to chlorpyrifos, resistance did not prevent fresh insecticide applications killing larvae, but it did reduce the time before re-application was needed.

If resistant populations such as that of Phnom Penh are frequently exposed to temephos, higher levels of resistance appear inevitable, as is now the case in some countries in the Caribbean where 1mg/litre of temephos is ineffective against some strains of Ae. aegypti(2).

Acknowledgements

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References


