Preliminary Field Testing of a Long-Lasting Insecticide-Treated Hammock Against Anopheles gambiae and Mansonia spp. (Diptera: Culicidae) in West Africa

J.-M. HOUGARD,1,2 T. MARTIN,1,3 P. F. GUILLET,4 M. COOSEMANS,5 T. ITOH,6 M. AKOGBÉTO,7 AND F. CHANDRE1


ABSTRACT The efficacy of an experimental long-lasting insecticide-treated hammock (LLIH) with a long-lasting treated net used as a blanket and made of the same fabric (polyethylene) was tested in a concrete block experimental hut, against the malaria vector Anopheles gambiae s.l. and the arbovirus vectors and nuisance mosquitoes Mansonia africana (Theobald) and Mansonia uniformis (Theobald). The LLIH was treated with the pyrethroid insecticide permethrin. It was evaluated concurrently with ignited mosquito coils over 20 successive weeks. In total, 2,227 mosquitoes (130 An. gambiae and 2,097 Mansonia spp.) corresponding to 27.8 mosquitoes per trap-night were collected in the untreated hut (control). The repellent effect of both coils and LLIH significantly reduced the number of mosquitoes entering the huts (35–60%). There was no significant difference between LLIH and mosquito coils in blood-feeding inhibition (93–97%) or in mortality (88–98%). The LLIH is more cost-effective and user-friendly than mosquito coils, which need to be replaced nightly to protect people sleeping indoors from mosquito bites. The effects of LLIH on exophagic vectors also need to be investigated because most people that sleep in hammocks are outdoors.

KEY WORDS insecticide-treated hammock, permethrin, field testing, malaria vectors, pest mosquitoes

The large-scale use of insecticide-treated nets is a major component of the overall strategy of malaria control (Lines 1996). Over the last decade, long-last-
exophagic and endophagic malaria vectors. We report the mosquitocidal efficacy of a long-lasting insecticide-treated hammock (LLIH) for use against the arbovirus vectors and nuisance mosquitoes *Mansonia* spp. and the malaria vector *Anopheles gambiae* s.l. under well-controlled conditions in experimental huts.

**Materials and Methods**

The LLIH used for this study was an experimental hammock made of open mesh high-density polyethylene multilayer film containing 2% (wt:wt) permethrin (pyrethroid insecticide) at a *cis-trans* ratio ranging from 50:50 to 30:70 (Sumitomo Chemical Co. Tokyo, Japan). It included a net, also treated with permethrin and made of the same fabric used as a blanket. At this dosage and isomer ratio, permethrin incorporated into polyethylene filaments follows the interim specifications recently proposed by the World Health Organization (WHO 2006).

The test was conducted in a savannah environment in Malanville, northern Benin, West Africa, near irrigated rice fields and ponds. Biting mosquitoes morphologically identified to species were *An. gambiae*, the major malaria vector in Africa (Hougard et al. 2002), and *Mansonina africana* (Theobald) and *Mansonia uniformis* (Theobald) (Laurence 1960), both major nuisance mosquitoes and vectors of arboviruses. Ninety-five percent of *An. gambiae*, identified to species and molecular forms by polymerase chain reaction (PCR) analysis, were *Anopheles gambiae* s.s. (M molecular form) and 5% were *Anopheles arabiensis* (Patton). The very few mosquito species collected other than *An. gambiae* s.l., *Ma. africana*, and *Ma. uniformis* were discarded.

Construction of experimental huts (Fig. 1) consisted of concrete brick walls, with a corrugated metal roof, a ceiling of thick polyethylene sheeting, and a light gray concrete floor (to facilitate mosquito collection) surrounded by a water-filled channel to prevent entry of ants (Darriet et al. 2002). Mosquito access was via four window slits on three sides and a large screened veranda on the fourth side (the back wall of each hut). The window entry slits were constructed from sheets of metal, fixed at an angle to create a funnel with a 1-cm-wide gap. Mosquitoes flew upward to enter through the gap and downward to exit; this greatly limited the number of mosquitoes exiting from the aperture, and it enabled the majority of entering mosquitoes to be accounted for. The veranda trap was 2 m in length, 1.5 m in width, and 1.5 m in height, and it was made of polyethylene sheeting and screening mesh. Mosquitoes could only exit into the verandah, which was shut at dawn by lowering a curtain separating the sleeping room from the veranda.

Before the trial, differences in hut attractiveness to mosquitoes were tested by putting one volunteer in each hut during 12 nights without any treatment. Three huts were treated as follows: 1) an untreated control, 2) one commercially available mosquito
coating containing 0.2% d-allethin burning from 2000 to 0600 hours, and 3) a LLIH hung from opposing walls at 50 cm above the floor. Human volunteers in the control and mosquito coil huts slept on a cotton mattress with a bed sheet. This trial received the ethical clearance of the national authority of Benin (through the Ministry of Health). The volunteers were informed on the objectives of this study and signed an informed consent form including a detailed human use protocol. The three volunteers entered the hut at 2000 hours and left 0600 hours. To avoid contamination with d-allethin, the treatments were not rotated among huts.

Adult volunteers slept in the huts during the entire night, four nights per week. They were rotated randomly among each of the huts nightly during the study. In the morning, dead mosquitoes were collected from the floor of the hut and the veranda trap; resting mosquitoes were collected from the walls and roof of the hut and the veranda trap by using mouth aspirators. Mosquitoes in each hut were scored as dead or alive and fed or unfed. Live mosquitoes were put into small cups, and they were provided with a sugar solution for 24 h to assess delayed mortality.

Blood-feeding inhibition (BFI) and mortality resulting from the use of LLIH and mosquito coils were compared. Mortality was assessed during the morning collection (immediate mortality) and 24 h later (delayed mortality). The percentage of mortality is derived from the number of dead mosquitoes compared with the total number in the hut. The percentage of blood feeding is derived from the number of blood-fed female mosquitoes compared with the total number in the hut. The BFI is the reduction of blood feeding between a treated hut and the control hut.

The ability of the treatment to deter mosquito entry (deterrence/repellency) and to induce mosquito exiting into the veranda (exophily) was also assessed because these parameters may contribute to a better interpretation of the two main outcomes. The entry rate is the total number of female mosquitoes found in the hut and veranda trap. The difference of entry rates between a treated hut and a control hut allowed an estimation of deterrence/repellency due to treatment. The percentage of exophily is the proportion of female mosquitoes found in the veranda compared with the total number present in the hut. The reduction in exit rates between the treated huts and the control hut (representing natural exophily) allowed an estimation of the induced exophily.

The proportional data were analyzed using logistic regression (XLSTAT 2006 software, Addinsoft, Paris, France). Because of the non-normality of the numbers of mosquitoes collected from each hut, these data were analyzed using the nonparametric Mann–Whitney U-test for each pair of huts. The differences in hut attractiveness were globally compared using a Kruskal–Wallis nonparametric test.

Results

Interhut Attractiveness to Mosquitoes. Preliminary catches without any treatment were conducted for 12 trap-nights in the three experimental huts, 2 wk before starting the trial. In total, 763,679, and 719 An. gambiae and Mansonia spp. were collected in huts 1, 2, and 3, respectively, corresponding to an average of 63.6, 56.6, and 59.9 mosquitoes per trap-night. There was no significant difference in hut-to-hut attractiveness (K = 0.68, df = 2, P = 0.71 [> 0.05]; n = 36).

Efficacy of LLIH in Experimental Huts. Untreated huts and huts with a LLIH or mosquito coils were evaluated for 20 wk (50 trap-nights), from November 2005 to March 2006 (Table 1). During this period, 2,227 mosquitoes in total (130 An. gambiae and 2,097 Mansonia spp.), corresponding to 27.8 mosquitoes per trap-night, were collected in the untreated hut (control). Both coils and LLIH repelled a significant percentage of mosquitoes from entering the hut (35% with An. gambiae [P < 0.05], 60% with Mansonia spp. [P < 0.0001]). Coils were significantly more repellent than LLIH with Mansonia spp. (P < 0.0001) but not with An. gambiae, although a higher percentage was repelled (P = 0.201). For An. gambiae, exophily did not differ significantly between huts, including the control hut (P > 0.8). For Mansonia spp., a slight but significant induced exophily (P < 0.0001) was observed with coils (20.4%). Blood feeding in the treated huts was significantly lower than in the control hut (P < 0.0001), but it did not differ between the two treatments or with mosquito genus (P > 0.05). The percentage of BFI was high with the two treatments for both mosquito genera (93–97%). Immediate and delayed mortality in the treated huts was significantly higher than in the control hut (P < 0.0001). No significant difference between treatment and species was noted, excepted for Mansonia spp. between coils and LLIH (P < 0.0001). Percentages of immediate mortality, which varied from 82 to 94% according to treatments and mosquito species, were slightly lower than those of delayed mortality (88–98%).

Discussion

The lower number of mosquitoes caught per trap-night in the control hut during the trial (28.3, i.e., half the average number recorded during the preliminary catches) is a confirmation of the importance of the presence of stagnant water on mosquito densities. In late October 2005, in the absence of irrigation and sufficient rains, larval numbers dropped rapidly in the surrounding rice fields and ponds, resulting in a progressive decline in adult mosquito population until reaching a plateau due to the maintenance of perennial breeding sites.

Because deterrence was relatively high, the values given by proportions of blood-fed mosquitoes in the treatment huts may underestimate the full personal protective effect. Thus, it is better to assess this parameter relative to the control, according to the following formula: 100 × (Bc – Bt) / Bc, where Bc is the
<table>
<thead>
<tr>
<th>Species</th>
<th>Control (%)</th>
<th>LLIH (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>An. gambiae</td>
<td>13.0b</td>
<td>2.097a</td>
</tr>
<tr>
<td>M. uniformis</td>
<td>30.0b</td>
<td>5.95c</td>
</tr>
</tbody>
</table>

In each row, values (confidence intervals) followed by the same lowercase letter are not significantly different from each other (P > 0.05). Values in parentheses are the number of the adult mosquitoes analyzed. N.S., value for the same mosquito species not significantly different from the control (P > 0.05).

Acknowledgments

We are grateful to the manufacturer Sumitomo Chemical Co., Inc. (Tokyo, Japan) for providing the experimental long-lasting insecticide-treated hammock and 0.2% d-allethrin mosquito coils. We are also grateful to Seth Irish for reading the final version of the manuscript and to the three volunteers who agreed to participate in this study for the entire duration of the trial.

References Cited


Received 13 September 2006; accepted 16 April 2007.