Pyrethroid-sprayed tents for malaria control: an entomological evaluation in Pakistan

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Abstract. Field trials were undertaken in the North West Frontier Province of Pakistan to determine the effects of pyrethroid-sprayed tents on feeding success, mortality and biting-rates of wild mosquitoes attracted to bait cows confined within the tents. Under natural conditions, endophagic mosquitoes rested only briefly in untreated tents during the night, followed by complete exodus at dawn.

In tents sprayed on the interior surface with permethrin 0.5 mg/m² or with deltamethrin 0.03 g/m² the biting rate of Anopheles stephensi was reduced by about 40%; detergency against culicines and other anophelines was much less. Mortality-rates of bloodfed mosquitoes from the treated tents were 75% An.stephensi, 65% An.subpictus but only 10% of culicines.

Outer fly-sheets prolonged the effective life of the treatment; bioassays on the sprayed inner-sheets showed that insecticidal efficacy remained high for over a year, whereas on tents without fly-sheets permethrin residual efficacy declined rapidly 20–40 weeks post-treatment. It is concluded that tent-spraying with fast-acting photostable residual pyrethroid insecticide would probably provide effective protection against malaria transmission for the inhabitants of tents in any part of the world where the vector mosquitoes are endophilic and susceptible to pyrethroids.

Key words. Anopheles culicifacies, An.stephensi, An.subpictus, culicine mosquitoes, permethrin, deltamethrin, pyrethroid insecticide, tents, pyrethroid-sprayed tents, malaria control, Afghan refugees, Pakistan.

Introduction

In 1991 there were 3.2 million Afghan refugees living in Pakistan, concentrated in approximately 350 camps in Baluchistan and the North West Frontier Province (NWFP). Despite the provision of health care through a network of basic health units, diseases such as malaria remain major health problems. For example 130,000 cases of malaria (27% Plasmodium falciparum) were reported in 1991 amongst refugees in NWFP.

The majority of refugees have been living in Pakistan for about 10 years and, during this time, most have constructed mud houses. For these people, house-spraying with residual insecticide — usually malathion 50% wettable powder formulation — has been applied regularly for malaria control. A substantial minority of refugees however are nomadic, living in tents and migrating seasonally between low altitude areas of the Punjab and the cooler hill regions of Waziristan in NWFP. Prior to 1990, malaria control for this community had been neglected, owing to the unsuitability of insecticidal wettable powder as a residual treatment for tents.

Efforts to control malaria by spraying tents with DDT, dieldrin or γ-BHC were made in Iran (Motobar, 1974), but gave disappointing results because of the poor adhesion of wettable powder to canvas. Liquid formulations of photostable pyrethroid insecticide should be suitable for treating tents, as they adhere well to various fabrics, have long-lasting residual efficacy as insecticides and can withstand weathering or washing (Miller, 1990, 1995; Schreck, 1991;
Heal et al., 1995). Therefore, following favourable reports on the use of pyrethroid-impregnated bednets for protection against malaria (Curtis et al., 1989; Alonso et al., 1991) staff of the United Nations High Commission for Refugees (UNHCR) sprayed 6000 of the Waziristan tents with aqueous emulsion of the pyrethroid permethrin. A malarial evaluation of the impact followed. (Parvez & Bouma, 1991) without entomological evaluation. Ethics and politics required all tents of the community to be treated, so comparison with an untreated control group was not possible. In tented refugee children from one district, where the spray programme had been strictly supervised, the falciparum rate fell from 44 to 18 per thousand during the post-spray year whereas, in local Pakistani children whose mud-brick homes had not been sprayed, it rose from 12 to 39 per thousand during the same period.

Many Afghan refugees return to a tent-dwelling lifestyle after repatriation. Elsewhere, millions of refugees or victims of natural disasters have to use tents, often in malaria areas. If the UNHCR and aid agencies are to continue to provide tents for these people, further evaluation of pyrethroid treatment is necessary before malaria policy control can be decided. This paper describes a series of investigations designed to assess the impact of sprayed tents on malaria vector behaviour (feeding success, deterrence, repellency and mortality) and yield data, similar to that from experimental hut studies of insecticide treatments (Smith & Webley, 1969; Curtis et al., 1992).

Material and Methods

Study area. Field experiments were carried out at Azakhel refugee camp, near Peshawar in NWFP, during August and September 1992. As is the case with most refugee settlements in Pakistan, Azakhel is situated in a marginal zone, on the banks of the Kabul River. The camp was established in 1981 and, at the time of our investigation, had a population of 25,000. The numerous wet borrow pits and elevated water table, with spring and autumn flooding account for high mosquito densities throughout the summer and autumn. Transmission of malaria in NWFP is seasonal, mainly between April and December. P. vivax cases peak in September and October. P. falciparum in November.

Test sites and procedures. To avoid the threat of interruption from seasonal flooding, three raised platforms of gravel, covered with mud plaster, were constructed above the upper flood level c. 10 m apart in a large mud-walled compound. Each platform (6 x 5 m) had a central wooden-frame cow pen (2 x 1 m), designed to keep the bait cow secure without hindering host-seeking mosquitoes, and was surrounded by a water-filled channel to deny access to scavenging ants. As a precaution to detect ants, a shallow paper dish holding 20 dead mosquitoes was placed on each platform and checked periodically to see if any had been removed, but none were. Support poles were erected at the four corners of each platform for attachment of rectangular trap nets, similar to those described by Service (1976).

For each experiment, a tent was erected over the cow pen on each platform. Doors of the tent were tied open constantly, as is customary in Pakistan during summer.

The ridge-pole tents consisted of a double sheet of cotton (30 m² surface area: 3.5 m wide, 4.0 m long, 2.1 m high) with a canvas outer fly-sheet waterproofed with paraffin wax and aluminium sulphate. Over each tent, a trap net (made of 100 denier white polyester mosquito netting, 2.5 m high) was hung from the support poles.

Preliminary counts of mosquitoes attracted to various types of host revealed that cows and goats were both considerably more attractive than men. Per kilogram body-weight the relative attractiveness of goats:men for anophelines was 9:6:1. No significant differences were detected in the mosquito species attracted to each host. Cows, being more attractive than men and less troublesome than large numbers of goats, were chosen as the bait animals for these trials. The same cows were used in their respective pens throughout. To facilitate the collection of dead and knocked-down mosquitoes, white sheets were spread over the mud-plastered platform floors.

An hour before sunset a cow was tethered in the pen within each tent and the sides of the trap nets were lowered around them, leaving a gap of 0.3 m from the ground to allow entry of naturally host-seeking mosquitoes. One hour before sunrise the nets were closed and the dead and knocked-down mosquitoes were collected from the platform floors. Live mosquitoes were then collected from the trap nets and tents. All specimens were taken to the laboratory for identification. Mosquito females collected from all three tents were classified as culicines or anophelines. The latter were identified to species, sorted according to their abdominal condition (unfed or blood-fed) and counted. To ensure consistency, the same entomologist (S.H.) undertook all mosquito identifications. Each tent was evaluated at each of the three sites, over three consecutive nights, using a Latin square design over treatments, platforms and nights.

Statistical analysis. For each type of mosquito, the proportions of blood-fed females alive (bfL) or dead knocked-down (bfD) and of unfed females alive (ufL) or dead/knocked down (ufD) were ARC SIN transformed and then subjected to analysis of variance (ANOVA) using UNISTAT (Toker, 1991). Numbers of mosquitoes collected were LOG₁₀ transformed and then subjected to ANOVA as above.

Experiment 1. Distribution of mosquitoes resting in/on untreated tents. In order to assess mosquito preferences for resting on different parts of the tent, collections were made from each section (Table 1) of tents 1 and 2, and from their trap nets, every 3 h during three consecutive nights. To investigate the dawn exodus of mosquitoes, tent 3 was left uncovered until 30 min before the first light of dawn, when the net sides were dropped and weighted down to the platform in order to prevent mosquitoes escaping. Mosquitoes resting on the inner surface of the net were then collected so that, by 10 min before first
light, the mosquitoes remaining within were only those resting on the tent or on the ground. From first light, net collections were made every 10 min until no more mosquitoes could be found within the net.

**Experiment 2. Effects of pyrethroid treated tents on mosquito survival after blood-feeding: ‘open net’ trial.** Inner surfaces of the cotton inner nets were treated with pyrethroid insecticide applied by compression sprayer (Hudson 'X-pert'), one week before the experiment started. One tent was sprayed with permethrin ('Imperator' 25% EC, from Zeneca), another with deltamethrin ('K-Othrine' 2.5% EC, from Roussel-Uclaf), to give target doses of 0.5 g/m² and 0.03 g/m² respectively. The third tent was left unsprayed for comparison. Mosquitoes were collected and data interpreted according to the general procedures described above.

**Experiment 3. Effect of pyrethroid-treated tents on mosquito biting rates.** The same three tents were used as for Experiment 2: one treated with permethrin 0.5 g/m², one treated with deltamethrin 0.03 g/m² and the third tent untreated. The same general evaluation procedures were followed and, in addition, all-night biting catches were made from each of the cows. In each tent, mosquitoes biting the tethered cow were collected by one collector from 19:00 until 24:00 hours, and by another collector from 24:00 until 05:00 hours. To minimize any effects of personal variation, individual collectors worked the same shifts at the same times throughout the experiment, while tents rotated between sites.

**Experiment 4. Effects of pyrethroid treated tents on captive mosquito feeding and survival rates: ‘closed net’ trial.** Tents, nets and cows were set up on each platform, as before, but this time the sides of the nets were lowered and sealed to the ground. At dusk, large numbers of wild-caught unfed female mosquitoes were released into each closed trap net and left there overnight. These mosquitoes had been collected nearby from the outside of three untreated bednets, each baited with a cow, during the previous night. During the intervening day they were held in uncrowded cages in a cool, dark room and were provided with food of sugar solution on cotton-wool pads. Humidity was kept high by wrapping the mesh cages in damp towels and plastic sheeting. Half an hour before sunrise, mosquitoes were collected from the sheets, tents and nets, then taken to the laboratory for identification. Each tent was evaluated twice at each site.

**Experiment 5. Effects of weathering on the residual efficacy of pyrethroid treatments.** From the date of spraying in August 1992 the tents used in these trials were left erected to weather in the open air until August 1993. Additional canvas tents (similar to the outer-fly of the double sheet tents) were also sprayed with the same target dosages of permethrin 0.5 g/m² and deltamethrin 0.03 g/m² and left to weather outdoors.

Every 6 weeks, two 0.2 m² samples were cut from the treated sheet of each tent and used for contact bioassays (30 min exposure) of laboratory-reared females of *Anopheles stephensi* (DUB-S strain). Unfortunately the single-sheet tent that had been sprayed with deltamethrin was stolen soon after the trial began, so no bioassay data for this permutation were obtained.

**Results**

**Experiment 1. Distribution of mosquitoes resting in untreated tents (Table 1)**

Totals of 1619 culicines and 790 anophelines were collected resting in untreated tents, of which 664 were *Anopheles stephensi* and 80 were *An. culicifacies*. Smaller numbers of four other anopheline species collected have been excluded from the analysis. Of the tent-resting mosquitoes, all the culicines, 98% of *An. stephensi* and 96% of *An. culicifacies* were bloodfed, showing that host-seeking mosquitoes settled on the tent only briefly-if at all-before feeding, whereas they rest on the inner surfaces of tents for some time after feeding. The proportions collected from the tent were 74% of *An. culicifacies* and 45% of *An. stephensi*, with the remainder caught in the net trap. Apparently *An. culicifacies* rests for longer, or possibly escapes from the trap net more efficiently, than *An. stephensi*. Anophelines preferred the apex, while culicines preferred the wall panels of the tent. However, relatively few (4%) culicines were collected on the tent, since they mostly rested on the ground beneath the cow after blood-feeding, and the majority (96%) were found in the net trap, showing that they exited from the tent soon after feeding.

Trap net collections carried out at 10 min intervals from first light revealed a pre-down exodus of mosquitoes from the tents, mostly 20–30 min before sunrise. By 10 min after sunrise all had left (Table 2).

The results demonstrate the relatively short resting time of anophelines in tents. As so few mosquitoes occurred on the fly-sheet or outer surface of the inner tent, it was decided that treatment would involve spraying only the inner surface of the inner sheet of the tents.

**Experiment 2: ‘open net’ trial. Mortality-rates of blood-fed mosquitoses from treated tents (Fig. 1A; Table 3)**

2778 culicines and 876 anophelines were collected, including 116 *An. stephensi*, 618 *An. subpictus* and five other anopheline species in smaller numbers. The deltamethrin-sprayed tent caused higher mortality than the permethrin-sprayed tent, overall, but this difference was significant only for *An. subpictus* (\( P < 0.05 \), Student-Newman-Kuels multiple range test).

Proportions of bloodfed mosquitoes killed by the treated tents were about 75% of *An. stephensi*, 65% of *An. subpictus* but only 10% of culicines (Table 3).

**Experiment 3: ‘biting net’ trial. Mosquito biting-rates on cows in treated tents (Fig. 2)**

Totals of 2716 culicines and 1929 anophelines were collected, including 285 *An. stephensi*, 1536 *An. subpictus*
Table 1. Mosquito collections from five sections of tent interior surface and from net traps over the tents: pooled data from three nights of collection in two untreated tents.

<table>
<thead>
<tr>
<th>Tent section</th>
<th>Culicines</th>
<th></th>
<th>An.stephens</th>
<th></th>
<th>An.culicifacies</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total</td>
<td>$\bar{x}$/m$^2$</td>
<td>Total</td>
<td>$\bar{x}$/m$^2$</td>
<td>Total</td>
<td>$\bar{x}$/m$^2$</td>
</tr>
<tr>
<td>Inside inner sheet</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Apex</td>
<td>6</td>
<td>2.5</td>
<td>73</td>
<td>30.4</td>
<td>11</td>
<td>4.6</td>
</tr>
<tr>
<td>Sloping roof panel</td>
<td>21</td>
<td>2.2</td>
<td>99</td>
<td>10.4</td>
<td>26</td>
<td>2.7</td>
</tr>
<tr>
<td>Vertical wall panel</td>
<td>23</td>
<td>5.8</td>
<td>35</td>
<td>8.8</td>
<td>6</td>
<td>1.5</td>
</tr>
<tr>
<td>Inter-sheet space</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Outer side of fly sheet</td>
<td>6</td>
<td>0.3</td>
<td>8</td>
<td>0.4</td>
<td>2</td>
<td>0.3</td>
</tr>
<tr>
<td>No. resting in tent</td>
<td>56 (4%)</td>
<td></td>
<td>216 (45%)</td>
<td></td>
<td>45 (74%)</td>
<td></td>
</tr>
<tr>
<td>No. in trap net</td>
<td>1230 (96%)</td>
<td></td>
<td>268 (55%)</td>
<td></td>
<td>16 (26%)</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>1286 (70%)</td>
<td></td>
<td>484 (26%)</td>
<td></td>
<td>61 (4%)</td>
<td></td>
</tr>
</tbody>
</table>

Table 2. Dawn exodus of mosquitoes from an untreated tent (pooled data from three nights). The trap net over the tent was pre-cleared of mosquitoes by first light. Mosquitoes trapped after leaving the tent were then collected from the net at 10 min intervals (* denotes time of sunrise).

<table>
<thead>
<tr>
<th>Minutes after first light</th>
<th>No. of mosquitoes caught in trap net:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Culicines</td>
</tr>
<tr>
<td>10</td>
<td>220</td>
</tr>
<tr>
<td>20</td>
<td>93</td>
</tr>
<tr>
<td>30</td>
<td>18</td>
</tr>
<tr>
<td>40</td>
<td>0</td>
</tr>
<tr>
<td>50*</td>
<td>2</td>
</tr>
<tr>
<td>60</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 3. Mortality-rates of blood-fed mosquitoes from the 'closed' and 'open net' collections (percentages corrected for control mortality using Abbott's formula). * Experiment 2; † Experiment 4.

<table>
<thead>
<tr>
<th></th>
<th>Permethrin</th>
<th></th>
<th>Deltamethrin</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>% Dead</td>
<td>Confidence interval</td>
<td>% Dead</td>
<td>Confidence interval</td>
</tr>
<tr>
<td>Culicines</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Closed net†</td>
<td>59.4</td>
<td>31–88</td>
<td>68.8</td>
<td>52–86</td>
</tr>
<tr>
<td>Open net*</td>
<td>9.3</td>
<td>−1–20</td>
<td>11.4</td>
<td>−4–27</td>
</tr>
<tr>
<td>An.stephensi</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Closed net†</td>
<td>88.3</td>
<td>80–97</td>
<td>95.3</td>
<td>89–102</td>
</tr>
<tr>
<td>Open net*</td>
<td>71.3</td>
<td>35–108</td>
<td>81.7</td>
<td>79–85</td>
</tr>
<tr>
<td>An.subpictus</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Closed net†</td>
<td>88.9</td>
<td>74–104</td>
<td>94.1</td>
<td>84–104</td>
</tr>
<tr>
<td>Open net*</td>
<td>56.0</td>
<td>37–75</td>
<td>77.7</td>
<td>50–105</td>
</tr>
</tbody>
</table>

and six other anopheline species in smaller numbers.

Comparing the numbers of blood-fed females collected in the treated tent with those collected in the untreated control tent gives an estimate of the deterrent (i.e. feeding prevented) effects of the tent treatments (Fig. 2). In both permethrin and deltamethrin tents there was a significant 40% reduction in the number of An.stephensi biting (F = 19.037, df 2, P < 0.05). Deterrence of culicines was also apparent, but not significant, whereas effects on An.subpictus were unclear. There was no indication of mosquito repellency (i.e. resting in the tent prevented after blood-feeding) caused by contact with either pyrethroid (Fig. 2).
Fig. 1. Physiological condition of mosquitoes collected from net traps over three tents: untreated control; permethrin treated; deltamethrin treated. (A) Wild mosquitoes from ‘open net’ trial (Experiment 2), (B) captive mosquitoes from ‘closed net’ trial (Experiment 4). Abbreviations: uf, unfed; bf, blood-fed; D, dead/knocked-down; L, live.

Trap net efficiency. With the untreated tent, numbers of mosquitoes caught actually biting versus those caught blood-fed in the ‘open net’ trial (Experiment 2) give a measure of the relative efficiency of the trap net as a capture technique.

Blood-fed mosquitoes were always found resting in the net at dawn in Experiment 3, showing that the catching efficiency of the collectors was less than 100%, since blood-fed mosquitoes rarely entered the net after feeding outside. To calculate the capture efficiency of the net, a formula was derived which takes catching efficiency of collectors into account.

For the ‘open net’ experiments, if \( a_o \), number attracted to the bait, \( d_o \), number collected from the net at dawn, and \( e_o \), number escaping from the net, then:

\[
a_o = d_o + e_o
\]  
(1)

If \( E \) is the constant ratio of escaped mosquitoes (\( e_o \)) to captured mosquitoes (\( d_o \)), then:

\[
e_o = E(d_o)
\]

thus, from equation (1):

\[
a_o = d_o + E(d_o)
\]  
(2)

For the biting collections, if \( b \), number caught feeding on the bait, \( a_b \), number attracted to the bait, and \( d_b \), number collected from the net at dawn, then:

\[
a_b = b + d_o + E(d_b)
\]  
(3)

If \( a_o \) and \( a_b \) are assumed to be the same, which is
Fig. 2. Deterrent and repellent effects of treated tents on mosquitoes: histograms represent mosquitoes collected from the deltamethrin-treated tent (stippled), the permethrin-treated tent (shaded) and the untreated comparison net (black histograms, taken as 100%). (D) mosquitoes captured biting the bait cow in the treated tents, as a proportion of those in the untreated control tent; giving a measure of deterency. (D&R) blood-fed mosquitoes captured from the nets enclosing the treated tents, as a proportion of those from the control net; giving a measure of deterency and repellency combined. Comparison of corresponding bars in series D and series D&R gives an estimation of the repellent effect of each treatment for each mosquito group.

Table 4. Relative efficiency of the trap net, using the dawn catch technique as a device for sampling different groups of mosquitoes.

<table>
<thead>
<tr>
<th></th>
<th>Culicines</th>
<th>An. stephensi</th>
<th>An. subpictus</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>$d_o$ (fed mosquitoes in net, open net trial)</td>
<td>638</td>
<td>45</td>
<td>156</td>
<td>839</td>
</tr>
<tr>
<td>$d_b$ (fed mosquitoes in net, biting net trial)</td>
<td>359</td>
<td>34</td>
<td>30</td>
<td>423</td>
</tr>
<tr>
<td>$b$ (biting collection, biting net trial)</td>
<td>330</td>
<td>101</td>
<td>483</td>
<td>914</td>
</tr>
<tr>
<td>$100 \frac{d_o - d_b}{b}$ = % capture efficiency of open net technique:</td>
<td>85%</td>
<td>11%</td>
<td>26%</td>
<td></td>
</tr>
</tbody>
</table>

reasonable since replicates of the ‘open net’ and biting collections were performed on consecutive nights, equations (2) and (3) may be rearranged thus:

$$d_o + E(d_o) = b + d_b + E(d_b)$$

hence:

$$E = \frac{b + d_b - d_o}{d_o - d_b}$$  (4)

The percentage capture efficiency ($%C$) may be derived from the ratio $E$ by the following equation:

$$%C = 1/(1 + E(100))$$

Hence, from equation (4):

$$%C = ((d_o - d_b)(100))/b$$

The results (Table 4) demonstrate the ease with which anophelines left the raised net after feeding. Capture efficiency of the net trap was estimated as only 11% for *An. stephensi* and 26% for *An. subpictus*. The majority of culicines, in contrast, tended to rest inside the net until dawn giving a capture efficiency of 85%.

Experiment 4: ‘closed net’ trial. Effects on blood-feeding and survival rates (Fig. 1B)

Because so many anophelines avoided capture by the trap net, the ‘closed net’ trial was designed to measure feeding success and mortality in mosquito populations that were unable to escape from the net after feeding or after resting on the tent.

Over the six nights of the closed net trial, 4154 culicines and 1494 anophelines were collected, of which 648 were *An. stephensi*, 570 were *An. subpictus* (smaller numbers of five other anopheline species were also captured). The proportions of blood-fed mosquitoes killed by the two treatments are shown in Table 3. In each case the deltamethrin-sprayed tent appeared to cause higher mortality than the permethrin-sprayed tent, although the differ-
ence between the two treatments was not significant. The mortality-rate of blood-fed culcines, usually lower than for anophelines, was very much higher than in the ‘open net’ experiment. Moreover, during this ‘closed net’ trial, large numbers of mosquitoes were found dead but unfed in the treated tents (Fig. 1B). This was surprising, because the behavioural data collected during Experiment 1 revealed that only a very small proportion of unfed mosquitoes rest in tents. Both anomalies can be explained by the fact that certain aspects of the design of this experiment were unrealistic. Many of the mosquitoes would have refrained from host-seeking at the time of release and, atypically, many probably rested on the tent for some time before feeding. The high mortality-rate of blood-fed culcines and the abnormally large proportion of dead culcines found to be unfed in the treated tents were artefacts of the experimental design. Although anopheline mortality may also have been increased as a result of their resting on the tent before feeding, anophelines tend to rest naturally on the tent after feeding so there was less distortion of the mortality-rate (Fig. 3).

Experiment 5. Persistence of insecticidal activity.

Pyrethroid-treated tents with fly-sheets remained effective for well over a year whereas, without a fly-sheet, the insecticidal activity of the permethrin sprayed tent declined rapidly during 20–40 weeks post-treatment (Fig. 4).

Discussion

Whereas pyrethroid-impregnated bednets kill or repel mosquitoes before they bite, pyrethroid-sprayed tents are anal-

![Fig. 3](image-url) Relative effects of pyrethroid-treated tents on blood-feeding success and survival of *An. stephensi* (*n* = 648) and *An. subpictus* (*n* = 570). Corrected results of ‘closed net’ experiment, i.e. after the removal of any dead specimens that had not fed.

![Fig. 4](image-url) Residual activity of pyrethroid treatments on tents with and without fly-sheets. Mortality-rates of 100 *An. stephensi* females (laboratory-reared DUB susceptible strain) scored 24 h following 30 min exposure in contact bioassay cones. Abbreviations: P, permethrin; D, deltamethrin; C, untreated control; s, single-sheeted tent; d, double-sheeted tent.
ogous to sprayed houses for killing freshly blood-fed endophagic female mosquitoes which rest post-gradually on treated interior surfaces. In our study area of Pakistan NWFP, Anopheles females were found to be very vulnerable to residual pyrethroid treatment inside tents because, after taking a bloodmeal, they tended to rest within the tent until first light. Culicines were less vulnerable because they rested on the tent only briefly, if at all, and tended to exit immediately after feeding or to rest on the ground within the tent until leaving at dawn. The dawn exodus of mosquitoes from tents was complete, presumably due to photophobia, since it preceded the daytime rise in temperature. Fast-acting phototactic pyrethroids, such as permethrin and deltamethrin, with their long residual efficacy, are the most appropriate insecticides for killing mosquitoes during their brief rest in treated tents.

The parallel with house-spraying implies that tent spraying should have the same aim: community protection from malaria, for which compliance from householders and good spray coverage are essential. As a method of self-protection against mosquitoes and malaria in NWFP, treated tents are less effective than impregnated bednets because inhabitants prefer to keep the door flaps open in the warm summer months. Malaria transmission in Pakistan continues into the autumn when tent flaps are more likely to be closed for warmth at night, giving better protection against endophilic mosquitoes.

The methodology for evaluating insecticidal house treatment is well established (Fontaine, 1983; Pant, 1988). Experimental huts fitted with veranda or window traps facilitate observations on various responses, such as mortality, deterrence, repellency and inhibited feeding of mosquitoes (Smith & Webley, 1969). Entomological evaluation of sprayed tents is more problematic because points of entry and exit are numerous and because traps cannot be fitted readily. We hoped that the giant net traps used to cover the tents in our experiments would overcome these problems and allow mosquitoes to enter freely while trapping those that attempted to leave. Three types of experimental design were devised to measure the spectrum of possible responses.

The ‘closed net’ experiment gave accurate mortality estimates because mosquitoes were unable to escape from the site after contacting the bait or tent. Its main limitation was the disproportionate killing of unfed mosquitoes after their release within the net.

The ‘open net’ experiment required no human interference and measurements were made on freely-entering, naturally host-seeking mosquitoes. It was particularly successful for culicines since the majority were trapped by the net. The estimate of c. 10% culicine mortality is therefore considered accurate. The ‘open net’ gave a distorted view of anopheline mortality because 75–90% escaped after feeding.

Biting collections (Experiment 3) in sprayed and unsprayed tents gave the best estimate of deterrence: approximately 20% of culicines and 40% of An. stephensi were deterred from biting in the pyrethroid-treated tents, but An. subpictus was apparently not affected. Evidence for deterrence was also apparent in the ‘open net’ (Experiment 2), where the proportion of unfed An. stephensi and culicines resting on the net trap was considerably larger in the case of pyrethroid treated tents than the untreated tent. Perhaps the deterred mosquitoes found it difficult to resist the attractive stimuli emanating from the cow and hence rested in the vicinity of the tent, i.e. in the trap net, because there was little or no evidence of repellency post-feeding.

Of the anophelines that escaped from the ‘open net’ site after feeding (89% of An. stephensi and 74% of An. subpictus), the important question is: how many had acquired a lethal dose of insecticide? The most optimistic model is provided by the ‘closed net’ experiment, showing mortality-rates of 88–95%, whereas the ‘open net’ experiment gave estimates of 56–82% mortality. The most pessimistic model assumes that all mosquitoes which escaped from the open net would have survived, implying mortality-rates of only 15–20% for An. subpictus and 8% or 9% for An. stephensi, probably gross under-estimates, judging by the high proportion found on the tent versus the net (Experiment 1).

Comparing the two pyrethroids used for treatment of tents, deltamethrin killed a slightly greater proportion of all mosquito species than permethrin. However, toxicity is a function of dosage and, in our experiments, permethrin was applied at the rate of 0.5 g/m² used operationally for bednets (Alonso et al., 1991; Miller, 1995) whereas deltamethrin was sprayed at 0.03 g/m², twice the rate of 0.015 g/m² recommended for bednets (Dartigues, 1987). Permethrin has the advantage of being less irritant to the skin than deltamethrin, an important factor when spraying in the close confines of a tent. Heal et al. (1995) recently reported that tents treated with oil-based 1% formulation of permethrin applied at 1.1 gai/m² gave significant protection against pest Aedes in Canadian woodland for at least 6 weeks. To avoid the smell, fire hazard and oiliness of this formulation, they recommended the use of aqueous pyrethroid for tent treatment, such as we employed in Pakistan. Permethrin’s main disadvantage is its relatively shorter residual life, although this should not be a serious limitation when tents have fly-sheets. It can be concluded that where large tented settlements, in malarious districts such as NWFP, are sprayed with either permethrin or deltamethrin there will be a resultant reduction in malaria transmission, as from residual house-spraying in this situation (Rowland et al., 1995). A strategy of tent-spraying would probably be effective against malaria in any part of the world where the vectors are endophilic and susceptible to pyrethroids.

Acknowledgments

We thank the field staff of MSF HealthNet for all their hard work. This research was supported by Medecins Sans Frontières (Holland) and the European Union.
References


Miller, J.E., Lindsay, S.W., Armstrong Schellenberg, J., Adiamah J., Jawara, M. & Curtis, C.F. (1995) Village trial of bednets impregnated with wash-resistant permethrin compared with other pyrethroid formulations. Medical and Veterinary Entomology, 9, 43–49.


Accepted 17 June, revised December 1994