

Effects of Permethrin-impregnated Bed Nets on Malaria Vectors of Northern Guatemala¹

FRANK O. RICHARDS, JR.,² RODOLFO ZEA FLORES,³
JOHN D. SEXTON,² RAYMOND F. BEACH,² DWIGHT L. MOUNT,²
CELIA CORDÓN-ROSALES,³ MARIO GATICA,⁴
& ROBERT E. KLEIN²



The authors evaluated the effects on malaria vectors of bed nets impregnated with permethrin over the course of a 16-month controlled study in four communities of Northern Guatemala. Anopheles albimanus and An. vestitipennis were the known malaria vectors in the area. Households were allocated to one of three experimental groups: those receiving bed nets impregnated with 500 mg/m² of permethrin, those receiving untreated bed nets, and those where no intervention measures were taken. The impact of the treated and untreated bed nets on mosquito abundance, behavior, and mortality was determined by indoor/outdoor night-bite mosquito collections, morning pyrethrum spray collections, inspection of bed net surfaces for dead mosquitoes, and capture-release-recapture studies. The duration of the treated nets' residual insecticide effect was assessed by modified WHO cone field bioassays, and their pyrethrin content was estimated by gas-liquid chromatography analysis.

The most important observation was that fewer mosquitoes were found to be resting in the households with treated bed nets. The treated nets probably functioned by both repelling and killing vector mosquitoes. Capture-release-recapture studies showed exit rates from houses with treated nets were higher (94%) than those from control houses (72%), a finding that suggests repellency. However, no significant differences were noted between the indoor night-bite mosquito collections at houses with and without treated nets. The horizontal surfaces of treated bed nets were nearly 20 times more likely to contain dead anopheline mosquitoes than were the comparable surfaces of untreated nets. The bioassays indicated that unwashed permethrin-impregnated bed nets retained their insecticidal activity for 6 months after treatment.

There has been considerable interest in evaluating use of bed nets treated with synthetic pyrethroid for malaria control (1, 2). Studies in Africa and Asia

have found that besides serving as barriers to the vectors of human malaria, impregnated bed nets may alter mosquito behavior, mortality, and abun-

¹Financial support for the work reported here was provided by the Pan American Health Organization (Grant HPT/R/81/8) and by the U.S. Agency for International Development/Vector Biology and Control Project/CDC Participating Agency Service Agreement (AID/VBC/DTE-5948-P-H-C-6052). This article will also be published in Spanish in the *Boletín de la Oficina Sanitaria Panamericana*, Vol. 117, 1994. Reprint requests and other correspondence should be addressed to Dr. Frank Richards, Jr.; Division of Parasitic Diseases, MS F-22; National Center for Infectious Diseases, Centers for Disease Control and Prevention, Atlanta, Georgia 30333, USA. The copyright of the Pan American Health Organization does not apply to this article, which

was prepared as part of the principal author's official duties as an employee of the United States Government. Use of trade names in this article is for identification only and does not imply endorsement by the Public Health Service or by the U.S. Department of Health and Human Services.

²Division of Parasitic Diseases, National Center for Infectious Diseases, Centers for Disease Control and Prevention (CDC), Atlanta, Georgia, U.S.A.

³Health Research Center (Centro de Investigaciones en Salud), Universidad del Valle de Guatemala, AP 82, Guatemala City, Guatemala.

⁴Malaria Division (División de Malaria), Ministerio de Salud Pública y Asistencia Social, Guatemala City, Guatemala.

dance (1–9). These studies have also demonstrated that entomologic findings in one region do not necessarily hold true for different anopheline species encountered in other malaria-endemic regions (2). Up to now the effects of impregnated bed nets on the vectors of vivax and falciparum malaria in Guatemala, *Anopheles albimanus* (10) and *An. vestitipennis* (11), have not been well described. Accordingly, we report the findings of a controlled entomologic study of impregnated bed nets in northern Guatemala, a part of the country where pesticide resistance is relatively uncommon (12).

METHODOLOGY

The study was carried out near the town of Los Amates (15°23'N, 89°03'W) in Izabal Department, a region devoted principally to banana cultivation. Four rural communities (Nahua, Dakota, Palmilla, and Rio Blanco) referred to as communities A, B, C, and D, respectively, were included. These communities had a mean elevation of 350 m and were separated from each other by at least 2 km; their populations ranged from 500 to 1 500 residents.

Most of the families lived in simple homes with walls constructed of vertically placed bamboo sticks or mud, and roofs of laminated metal, thatch, or palm. Residents with higher socioeconomic status lived in houses with cement block walls. Irrespective of the type of construction, all of the homes had open and unscreened windows and eaves, permitting mosquitoes to enter and exit from almost any point.

The last intradomiciliary spraying in these communities, provided by the Guatemalan Ministry of Health, had occurred 14 months before initiation of the project and had employed deltamethrin. The Ministry's spraying program was suspended for the duration of the study.

Two months before field work began, *An. albimanus* specimens obtained from corals in the communities were found 95% susceptible to pyrethroids by the World Health Organization adult tube susceptibility test (13).

Participating households were divided among three study groups: those receiving impregnated bed nets, those receiving identical untreated nets, and those where no intervention measures were taken (controls). Ninety-eight percent of all the households in Community A received treated bed nets, and a similar percentage (95%) in Community B received untreated nets. In each of the other two communities (C and D), samples of 50 households were randomly selected; 25 received treated bed nets while the other 25 served as controls.

Bed Net Treatment and Distribution

The bed nets were made of polyester netting with an aperture diameter of 1.6 mm. They had an area of 14.9 m², and their specified dimensions when in place were 122 cm wide, 213 cm long, and 183 cm high. A concentrated permethrin solution (Peripel® emulsifiable concentrate, Rousel-Uclaf Laboratories, Paris) was diluted in 5-gallon plastic vats to a strength and volume deemed appropriate for impregnating 20 nets with 500 mg permethrin/m² (1). The nets were individually dipped into the solution for 2 minutes, wrung out, and then hung on ropes to dry. Residual volumes in the vats were measured and mean absorption (ml of solution per net) was calculated; the dose of permethrin delivered/m² was later determined by extrapolation.

Field work occurred over a period of 16 months (from July 1990 to October 1991). July, August, and September 1990 data were collected after household se-

lection but before bed net distribution (funding limitations precluded more than 3 months of baseline data collection). The treated and untreated nets were distributed during the last week of September 1990. Project staff members hung the nets over all beds in use, and the villagers were asked not to wash them until immediately before reimpregnation in March 1991. During that latter month, the washed treated nets were collected and reimpregnated in the same manner as described above.

Mosquito Collections

To measure the effect of the treated nets on the man-biting rate, night-bite mosquito collections were made in each community during two nights each month in houses selected for their high mosquito abundance. For a period of 5 hours, beginning at sunset, one technician seated near the house entrance and another seated 5 m from the house collected all anopheline mosquitoes landing on their exposed legs. The same houses were used to make these collections for the duration of the study in communities A and B. However, the houses used in communities C and D were changed several times in an effort to find higher mosquito densities. As a result, the night-bite collection data from those communities were variable and are not reported.

Pyrethrum spray mosquito collections were made twice a month to evaluate morning (7:00 a.m. to 9:00 a.m.) household resting densities in each experimental group. This work was done in randomly selected households as follows: A white canvas was placed on the floors and over furniture, and a 0.2% pyrethrum solution in kerosene was applied as a spray from inside and also from outside (around the eaves) of the house. After 30 minutes, fallen mosquitoes were col-

lected from the canvas and classified by genus (as culicine or anopheline).

Finally, every 2 weeks for the final 7 months of the study (from April 1991 to October 1991) project personnel examined the upper (horizontal) panels of all the treated and untreated bed nets for dead mosquitoes. The collected specimens were classified as anopheline or culicine.

Capture-Release-Recapture Studies

Rates of exit, feeding, and mortality were determined in capture-release-recapture experiments. Eight houses of similar construction (four with treated nets, two with untreated nets, and two controls) were employed. Two houses were selected from each community; in both Community C and Community D, one of the two selected houses belonged to the treated bed net group while the other was a control house.

At each house an external canvas/mesh curtain (the so-called "Colombian curtain"—14) was hung from the sides of the house, extending from the roof (above the eaves) to the ground. In addition, a polyester mesh was stretched out under the roof inside each house, starting at the top of the walls, to prevent mosquitoes in the house from flying upward and resting on inaccessible surfaces of the inner ceiling, rafters, and ridgepole.

After the curtain and ceiling screen were in place, all mosquitoes were removed from the house. Then a known number of anopheline mosquitoes was released inside the house during the evening. Mosquitoes attempting to exit from the interior were trapped by the net, recaptured, classified by feeding status, and observed for subsequent mortality. The house was reentered in the early morning to capture any remaining mosquitoes. Occupants of the house (who slept inside it during the experiments) were

offered chloroquine prophylaxis for the duration of the study.

These studies were done at each test house for two nights a month using the following routine: A 30-minute collection was made of the anopheline mosquitoes resting on the outer surface of the curtain (the first "entrance" capture). This first collection began at sunset. Unfed female mosquitoes were then released inside the house. Immediately after their release, a 30-minute inspection was made of the inner surface of the curtain facing the house (the first "exit" capture). The team then returned to entrance captures. This capture-release-recapture cycle was repeated four times, during which a number of mosquitoes (approximating 50 to the extent possible) was released. If necessary, entrance captures were supplemented with mosquitoes from night-bite collections made simultaneously in a nearby house. The final exit capture and an inside collection were made just before sunrise. Mortality among the recaptured mosquitoes was determined 16 to 21 hours (the average time being 18 hours) after their release into the house.

Residual Permethrin in the Impregnated Bed Nets

A modified WHO cone bioassay was used to assess the residual insecticidal effect of the impregnated bed nets' permethrin over time. Every 2 weeks all nets were tested in five houses randomly selected from the treated bed net group. With each net left hanging over the bed, three WHO cones were attached at standard positions (on the top panel, head panel, and entry panel). Five recently blood-fed, insectary-reared *An. albimanus* mosquitoes (of a colony originating from specimens captured in a Community A corral in May 1990) were placed into each cone for 30 minutes. The mosquitoes were then retrieved and held for 24 hours, at

which time mortality rates were determined. Control tests, done simultaneously at each house, were performed by exposing 15 mosquitoes (in three cones) to an untreated net.

Gas-liquid chromatography analysis for permethrin content was performed on a small sample of nets selected randomly from houses in the treated bed net group. Test samples were obtained twice: immediately after the first impregnation and 6 months later, just after the nets were washed by the villagers. A 10 cm × 10 cm square piece was cut out of each bed net and placed in a 10 ml volume of 0.3% didecylphthalate in chloroform. After 30 minutes of agitation, one microliter of the extract was injected into a gas-liquid chromatography system using a DB-17, megabore capillary column of fused-silica quartz with dimensions of 0.53 mm (interior diameter) × 30 cm (J & W Scientific, Folsom, California) and a flame ionization detector. For quantification, relative responses (the summed area of the permethrin peak over the area of the internal standard peak) were compared.

Statistical Analysis

All observations were considered valid and were used in the analysis except for some observations from the capture-release-recapture studies. In the latter case, only studies where approximately 50 mosquitoes were released (within a range of 49–53) were entered into the analysis. Comparisons between experimental groups were made using a z-test with a weighted variance about the mean (Survey Data Analysis Software Package [SUDAAN], Research Triangle Institute, Research Triangle Park, North Carolina). Normal probability tables were used to derive the corresponding p values.

In the capture-release-recapture studies, data obtained for all houses during the baseline data collection period were

analyzed as part of the control group data. Individual ratios for recapture, exit, feeding, and 18-hour mortality were calculated separately for each house and for the corresponding collection date. The recapture rate was defined as the total number of mosquitoes recaptured over the total number released; the exit rate was defined as the number recaptured outside of the house over the total number recaptured; the feeding rate was defined as the number fed over the total number recaptured; and mortality was defined as the number of deaths over the total number recaptured.

RESULTS

The Effect of Treated Bed Nets on Mosquito Abundance and Mortality

A total of 1 116 anophelines were obtained through night-bite collections; 761 (68%) were *An. albimanus*, 349 (31%) were *An. vestitipennis*, and 6 (<1%) were unidentified species. Thirty-nine percent of the *An. albimanus* were captured indoors, as compared to 45% of the *An. vestitipennis* ($z = 0.9$, $p = 0.39$). No significant differences were noted between the percentages of total captures obtained indoors by experimental group. That is, 41% of the total treated net night-bite collections were made inside, as compared to 39% of the combined untreated net and control house night-bite collections ($z = 0.2$, $p = 0.86$).

However, the night-bite collections yielded significantly fewer *An. vestitipennis* in the Community A households (where the entire community received treated bed nets) during the July–October 1991 rainy season than they did during the corresponding (mostly baseline) months of 1990. In contrast, as Figure 1 indicates, the *An. albimanus* abundance did not change significantly. Similar findings (a significant decrease in the *An. vestitipennis*

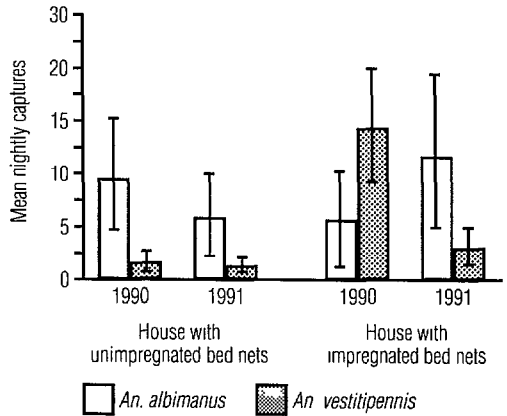


Figure 1. Anopheline abundances for the 1990 and 1991 rainy seasons, by species and study group. The mean night-bite collections of anopheline mosquitoes at one house with untreated bed nets in Community B (left bar grouping) and one house with treated bed nets in Community A (right bar grouping) are shown during the July–October rainy season months of 1990 and 1991. For 3 of the 4 months in 1990, collections were obtained prior to bed net distribution. The vertical lines show 95% confidence intervals. The abundance of *An. vestitipennis* in the house with treated nets declined significantly during the second transmission season, while that of *An. albimanus* did not change significantly.

and no significant change in the *An. albimanus* abundance) were obtained from the Community A entrance captures of the capture-release-recapture studies (data not shown). No significant variation in the rainy season abundances of either *An. vestitipennis* or *An. albimanus* was detected by the night-bite or entrance capture-release-recapture collections made in Community B, where the bed nets were untreated.

A total of 127 morning pyrethrum spray mosquito collections were carried out at 111 different houses (59 with treated bed nets, 36 with untreated nets, and 16 controls). Most houses (85%) were examined only once. As Table 1 indicates, significantly fewer culicine mosquitoes were

Table 1. Morning intradomestic mosquito resting (determined by pyrethrum spray collections), by study group.

	Treated bed nets	Untreated bed nets	Controls	Untreated bed nets & controls
<i>No. of collections</i>	64	42	21	63
<i>Anophelines:</i>				
Total recovered	10	30	4	34
Mean household density (range)	0.16 (0-6)	0.71* (0-8)	0.19 (0-2)	0.54* (0-8)
% households positive	2	9	4	7
<i>Culicines:</i>				
Total recovered	261	300	246	546
Mean household density (range)	4.08 (0-47)	7.14* (0-37)	11.71* (0-34)	8.66* (0-37)
% households positive	61	88	81	83

* $p < 0.03$ (z-test) compared with houses in the group with treated nets

collected at the houses with impregnated nets ($z = 4.1$, $p < 0.0001$). Compared to the number of culicine mosquitoes, the number of anophelines collected was small (34 *An. albimanus*, 7 *An. vestitipennis*, and 3 unidentified species). Still, anopheline mosquitoes were less common in houses with treated bed nets than in those with untreated nets ($z = -2.3$, $p = 0.02$).

The reverse pattern was observed in bed-net panel collections, where dead culicine and anopheline mosquitoes were significantly more abundant in the experimental group with treated bed nets (Table 2). Indeed, impregnated panels were 5 times more likely to contain dead culicines ($z = 8.5$, $p = 0.00001$) and nearly 20 times more likely to contain dead anophelines ($z = 7.3$, $p = 0.00001$).

Capture-Release-Recapture Studies

The results of 81 capture-release-recapture experiments are summarized in Table 3. Mean recapture rates of anophelines released into houses with treated bed nets (54%) were significantly

higher than the recapture rates in houses with untreated nets (42%, $z = 2.0$, $p = 0.045$) and control houses (42%, $z = 2.23$, $p = 0.03$). Exit capture rates from houses with treated bed nets (94%) were significantly higher than those from control houses (72%, $z = 3.8$, $p = 0.0001$); the exit capture rate from houses with untreated nets (83%) was intermediate, and

Table 2. Dead mosquitoes collected from the horizontal panels of bed nets, by study group.

	Treated bed nets	Untreated bed nets
<i>No. of examinations</i>	1 240	665
<i>Anophelines:</i>		
Total collected	145	4
Mean mosquitoes/net (range)	0.117 (0-9)	0.006* (0-1)
% nets with mosquitoes	7	0.6
<i>Culicines:</i>		
Total collected	1 071	106
Mean mosquitoes/net (range)	0.864 (0-34)	0.159* (0-12)
% nets with mosquitoes	25	8

* $p < 0.0001$ (z-test).

Table 3. Capture-release-recapture experiments: recapture, exit, feeding, and mortality rates, by study group.

	Treated bed nets	Untreated bed nets	Controls	Significance*
No. of experiments	41	15	25	—
Total mosquitoes released in houses	2 050	747	1 248	—
Mean recapture rate (%)	53.8	41.9	42.1	1, 3
Mean exit rate (%)	94.3	82.5	71.5	3
Mean blood-feeding rate (%)	7.0	6.8	31.0	2, 3
Mean 18-hour mortality (%)	16.8	14.3	13.0	NS

*Significance codes: 1 = $p < 0.05$, treated nets vs. untreated nets; 2 = $p < 0.05$, untreated nets vs. controls; 3 = $p < 0.05$, treated nets vs. controls; NS = not statistically significant.

not significantly different from either the treated or control group rates. Feeding success was greatest at the control group houses (31%) compared to both the treated group (7%, $z = 3.8$, $p = 0.0001$) and the untreated group (7%, $z = 3.6$, $p = 0.003$). No significant differences were observed in the 18-hour mortality among recaptured mosquitoes (the mortality range being 13%–17%).

Residual Effect of Permethrin in Treated Bed Nets

Over 900 cone bioassays were performed on bed nets hanging in 61 different houses. As Figure 2 shows, no appreciable reduction in insecticidal effect was noted over the 6-month periods following the first and second treatments. The mean dose of permethrin delivered by the first treatment (determined by extrapolation based on permethrin solution absorption) was 474 mg/m² (within a range of 450–492).

This value correlated well with that obtained from the chromatography analysis of netting samples ($n = 13$, mean = 467 mg/m², 95% confidence interval = 408–526). Samples obtained just before the 6-month reimpregnation, after a single washing by village women, yielded

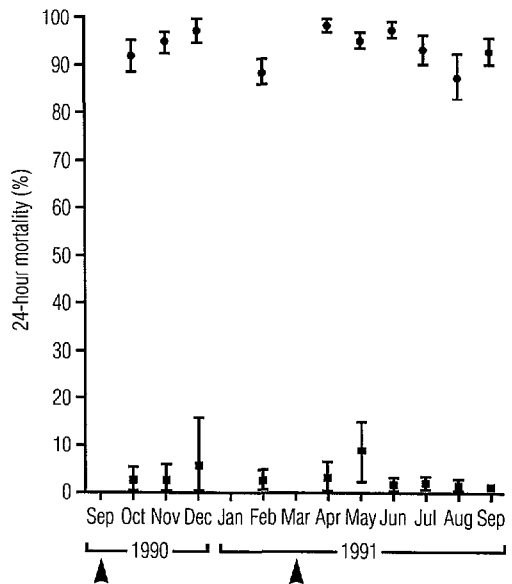


Figure 2. Mortality of colony-reared *An. albimanus* mosquitoes exposed to randomly selected impregnated bed nets (upper triangles) and untreated nets (squares), by month. A total of 4 578 mosquitoes were exposed to treated nets during the course of the study; 1 563 control mosquitoes were exposed to unimpregnated netting. Arrows beneath the months of September and March indicate the first and second impregnations of the treated bed nets. Vertical lines within the chart show 95% confidence intervals. No data were collected during the January and March holidays.

chromatography results indicating a concentration of 276 mg/m² (n = 9, 95% confidence interval = 205–348). This represented a 41% decrease in the insecticide concentration over the 6-month time period.

DISCUSSION AND CONCLUSIONS

Studies have shown that bed nets treated with synthetic pyrethroids not only provide a barrier to mosquitoes, but also repel and/or kill them (9). African and Asian studies have found that *An. gambiae*, *An. funestus*, *An. arabiensis*, *An. anthropophagus*, and *An. sinensis* were primarily repelled from houses with impregnated bed nets (3, 6–8, 15). In contrast, Ree's 1988 report from the Solomon Islands showed *An. farauti* had 80%–90% mortality after exposure to impregnated bed nets in experimental huts (5). These dissimilar observations may be explained by the use of different synthetic pyrethroids (permethrin vs. deltamethrin) and doses in these studies, and/or by differing mosquito behavior or insecticide susceptibility. It is interesting to note that the formulation of the synthetic pyrethroid applied could also have influenced the outcome of these studies; specifically, Lindsay suggested in 1991 that certain volatile hydrocarbons in emulsifiable concentrate carriers are repellents (9).

Perhaps our most significant observation in Guatemala was that houses with treated bed nets had significantly fewer resting mosquitoes than other houses (see Table 1). In other respects, the results of our different entomologic studies were ambiguous or in conflict with regard to the treated nets' method of action. The capture-release-recapture studies did not show higher mosquito mortality associated with the treated nets. The high rate of recapture in homes with treated nets indicated no rapid knockdown occurred

after mosquito liberation, nor were delayed insecticidal effects observed during the 18-hour holding period.

Although exit rates from houses with treated bed nets were substantial, similar exit rates were observed at houses with untreated bed nets. Considering this together with the poor feeding success observed in both bed net groups, we conclude that the high mosquito exit rate could not be solely attributed to a repellent effect, but was in part prompted by the search for a more accessible blood meal. In making this comparison between treated and untreated nets, however, we caution the reader that untreated bed nets prevent human-vector contact only so long as the net barrier is intact. In contrast, studies have shown that treated bed nets deter mosquitoes even after the netting has become old and is in disrepair (16).

Findings of other studies were also inconsistent with the hypothesis that the treated nets were repellent. One would have expected the night-bite collections to have yielded fewer indoor captures at the houses with treated nets if those nets were highly repellent. However, the proportion of anophelines attempting to bite inside the houses with treated nets was similar to that found at the houses with untreated nets. In addition, lethal contact with the treated surfaces (demonstrated in the panel collection study) would be unlikely in the face of high-grade repellency. To gain better understanding of the modus operandi of the treated nets, direct observation of dye-marked mosquitoes in houses is required and is underway (C. Cordón-Rosales, personal communication).

Zuzi (1989) reported reduced abundance of *An. anthropophagus* after community-wide distribution of impregnated bed nets in Guangdong Province, China, while the abundance of a less endophilic species (*An. sinensis*) was unchanged (7). Similarly, our

findings from the night-bite collections and capture-release-recapture studies suggest that a decrease in the abundance of *An. vestitipennis* occurred in Community A during the rainy season following community-wide distribution of the nets, while *An. albimanus* collections did not vary significantly over the course of the study. If distribution and use of the treated nets were responsible for this observation, one could postulate that (1) *An. vestitipennis* had behavior characteristics (anthropophilia, endophilia, etc.) resulting in relatively greater exposure to permethrin, or (2) *An. vestitipennis* was more susceptible to permethrin. Alternatively, changes in this mosquito's abundance could have been unrelated to the treated nets. Community A may have had an abnormally high population of *An. vestitipennis* during the first rainy season, or an unrecognized change in the local ecology could have reduced *An. vestitipennis* populations in the second year. Given the great limitations of the data, definite conclusions are impossible without further study.

We advised that the bed nets should not be washed for 6 months; but, because the bed nets became visibly soiled in this amount of time, the villagers stated that their preferred washing interval would be every 4–12 weeks (17). Therefore, prolonged potency of the treated nets (as indicated by the bioassay study) could not be guaranteed under nonstudy/operational conditions unless their use were accompanied by public education efforts. If one assumes that a malaria control program based on use of treated bed nets could afford only annual impregnations, we would recommend impregnation just before onset of the rainy season. A concerted educational effort directed at behavioral change would then be needed to persuade villagers not to wash their nets during the June–October malaria transmission season.

Acknowledgments. We would like to thank V.M. Molina Escobar, J.L. Guillen Rodríguez, and M.E. Gonzales Guerra for their laboratory and field work during the course of the study. We are also grateful to Drs. C. Padilla and R. Zeissig of the Malaria Division, who assisted in coordinating project activities with other Ministry of Health vector control work; to M.E. Chinchilla Solorzano of the Universidad del Valle de Guatemala and J. Roberts of the U.S. Centers for Disease Control and Prevention (statistical services section, Division of Parasitic Diseases), who helped with the statistical analysis; to Dr. T. Ruebush (U.S. Centers for Disease Control and Prevention), who helped in the early study design and reviewed the manuscript; and to Drs. K. Ogata, T. Umino, and T. Ikeda of the Japanese International Cooperation Agency, who provided advice during the early stages of the project. The permethrin used in the project was provided by the Rousel-Uclaf Laboratories in Paris.

REFERENCES

1. Schreck CE, Self LS. Bed nets that kill mosquitoes. *World Health Forum* 1985;6: 111–114.
2. Rozendaal JA. Impregnated mosquito nets and curtains for self-protection and vector control. *Trop Dis Bull* 1989;86:R1–41.
3. Darriet F, Robert V, Tho Vien N, Carnevale P. *Evaluation de l'efficacite sur les vecteurs du paludisme de la permethrine en impregnation sur des moustiquaires intactes et tronces*. Bobo-Dioulasso, Haute-Volta: OCCGE, ORSTOM; 1984. (Doc Tech 8.398/84.)
4. Lines JD, Myamba J, Curtis DF. Experimental hut trial of permethrin-impregnated mosquito nets and eave curtains against malaria vectors in Tanzania. *Med Vet Entomol* 1987;1:37–51.
5. Ree H. *Experimental hut studies on the effect of permethrin-treated mosquito-nets against Anopheles farauti in the Solomon Islands*. Geneva: World Health Organization; 1988. (Document WHO/VBC/88.963).

6. Snow RW, Jawara M. Observations on *Anopheles gambiae* Giles s.l. during a trial of permethrin-treated bednets in The Gambia. *Bull Entomol Res* 1987;77:279–286.
7. Zuzi L, Mancheng Z, Yuguang W, Binglin Z, Guangyu L, Hui H. Trial of deltamethrin impregnated bed nets for the control of malaria transmitted by *Anopheles sinensis* and *Anopheles anthropophagus*. *Am J Trop Med Hyg* 1989;40:356–359.
8. Sexton JD, Ruebush II TK, Brandling-Bennett AD, et al. Permethrin-impregnated curtains and bed-nets prevent malaria in Western Kenya. *Am J Trop Med Hyg* 1990;43:11–18.
9. Lindsay SW, Adiamah JH, Armstrong JRM. The effect of permethrin-impregnated bed nets on house entry by mosquitoes (Diptera: Culicidae) in The Gambia. *Bull Entomol Res* 1991;82:49–55.
10. Breeland SG. Studies on the ecology of *Anopheles albimanus*. *Am J Trop Med Hyg* 1972;21:751–754.
11. Loyola EG, Arredondo JI, Rodríguez MH, Bown DN, Vaca-Marin MA. *Anopheles vestitipennis*, the probable vector of *Plasmodium vivax* in the Lacandon forest of Chiapas, Mexico. *Trans R Soc Trop Med Hyg* 1991;85:171–174.
12. Brogdon WG, Beach RF, Stewart JM, Castanaza L. Microplate assay analysis of the distribution of organophosphate and carbamate resistance in Guatemalan *Anopheles albimanus*. *Bull World Health Organ* 1988;66:339–346.
13. World Health Organization. *Manual on practical entomology in malaria: part II, methods and techniques*. Geneva: WHO; 1975. (Offset publication 13).
14. Bown DN, Rios JR, Frederickson C, del Angel Cabanas G, Méndez JF. Use of an exterior curtain-net to evaluate insecticide/mosquito behavior in houses. *J Am Mosq Control Assoc* 1986;2:99–101.
15. Curtis CF, Lines JD. Impregnated fabrics against malaria mosquitoes. *Parasitol Today* 1985;1:147.
16. Port GR, Boreham PFL. The effect of bed-nets on feeding by *Anopheles gambiae* Giles (Diptera: Culicidae). *Bull Entomol Res* 1982;72:483–488.
17. Richards F, Klein RE, Zea Flores R, et al. Permethrin-impregnated bed nets for malaria control in Northern Guatemala: epidemiologic impact and community acceptance. *Am J Trop Med Hyg* 1993;49:410–418.