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Experimental hut trials of permethrin-impregnated mosquito nets and eave curtains against malaria vectors in Tanzania

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ABSTRACT. Permethrin impregnated netting was tested against Tanzanian populations of *Anopheles arabiensis* Patton, *An.gambiae* Giles and *An.funestus* Giles in experimental huts fitted with traps to catch samples of the mosquitoes exiting during the night. Treated bednets killed some mosquitoes and increased the tendency of survivors to exit during the night. Treated cotton did not perform so well as treated nylon bednets. An impregnated bednet in which holes had been cut, to simulate a torn net, reduced the number of mosquitoes which fed and survived approximately as well as an intact untreated net. Treated curtains around the eaves of experimental huts did not perform so well as bednets but caused considerable reductions in the number of mosquitoes which fed and survived. However, there was no such effect when treated netting was placed around the eaves of a dwelling house. When one child slept under a treated net and another slept outside the net in the same hut, the number of bites on the latter child was less than if neither child had been under a net. Various aspects of the applicability of permethrin impregnated nets on a community basis are discussed.

Key words. *Anopheles,* mosquitoes, bednets, permethrin, malaria control.

Introduction

Bednets and window screening have long been used as protection against mosquitoes and mosquito-borne disease. However, as emphasized by Gillett (1985), mosquitoes can find their way through even small holes or tears in netting, and into badly tucked-in bednets. Impregnation of clothing with the insecticide permethrin, a fast-acting excito-repellent pyrethroid, has been successfully used against outdoor nuisance mosquitoes in North America (Schreck *et al.*, 1978, 1984; Lindsay & McAndless, 1978). There is now much interest in application of this compound, or deltamethrin, to bednets, to improve their effectiveness against malaria mosquitoes. We know of studies of this type in Burkina Faso (Darriet *et al.*, 1984), China (Li Zu-Zi, 1986), The Gambia (Snow *et al.*, 1987), Malaysia (Luong *et al.*, 1985), Mali (Ranque *et al.*, 1984), Papua New Guinea (D. Charlwood and P. M. Graves, pers. comm.) and Suriname (J. Rozendaal, pers. comm.).

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Several of these studies have proceeded directly to a test of the effect of using impregnated netting on the incidence of disease. In our own work in Tanzania we have followed Darriet *et al.* (1984) by initially making detailed entomological studies in experimental huts. We used huts of the type designed by Smith (1964) and Smith *et al.* (1972), which allow one to measure separately the effect of different treatments on the entry, feeding success, and survival of *Anopheles* females seeking a meal. In this way, we have compared nets with or without holes, both with and without permethrin. We have gone on to compare cotton with nylon nets, and to investigate the possibility of using, instead of bednets, curtains of netting hung loosely inside windows or eaves. We have also studied how much, when one person is sleeping under a bednet, hungry mosquitoes divert to another person without a net in the same room.

Methods and Materials

Permethrin impregnation of nets

The dimensions of each bednet were approximately 2 m long, 1 m wide and 1.15 m high. With two exceptions, we used nylon netting. We treated it with permethrin E.C. containing 10% active ingredient, supplied by Wellcome Research Laboratories, Berkhamsted, U.K. The volume of concentrate required to give the required dosage (generally 0.2 g/m^2 of net) was mixed with an amount of water known to be enough to just wet the whole net. We rubbed and squeezed the net in a plastic bowl, then laid it on a polythene sheet and repeatedly turned it over as it dried. The permethrin content of areas of netting treated in this way was checked using gas–liquid chromatography by Wellcome Research Laboratories. We also did bioassays in the field, using freshly fed *Anopheles gambiae s.l.* confined in W.H.O. bioassay cones attached to different parts of the nets. After 1–15 min exposure, the mosquitoes were kept in cardboard cups with access to sugar solution for 24 h, after which mortality was scored.

The verandah trap huts

Most of the work was carried out in the verandah trap huts of the Tropical Pesticides Research Institute outstation at Magugu, Arusha Region, Tanzania. The huts were designed by Smith (1964; Smith & Webley, 1969). At night, two children slept in each hut and the protection they gained, either from a net or from the presence of impregnated curtains, was tested. Each morning collections were made, with torches and aspirators in the huts and the verandah traps, for both live resting mosquitoes and mosquito corpses on the white hardboard floors. The collections were sorted by species and gonotrophic stage, and counted. In the second and third experiments, the live catches were kept in small cages with access to sugar solution for 24 h, to check for delayed mortality.

In these huts, two eaves on opposite sides of the hut are open for mosquitoes to enter and leave freely. On the other two sides the eaves open into screened verandahs, so that mosquitoes exiting this way are trapped. The screened and unscreened sides are switched each week to correct for any bias in the direction of exit. It can therefore be assumed that, on average, half the mosquitoes leaving the hut are caught in the traps. The huts are built on concrete supports surrounded by gulleys filled with water as ant traps, to try to ensure that mosquitoes which die during the night are not scavenged by ants and so can be found in the morning. Estimates of the total number of mosquitoes entering the hut are made by doubling the numbers dead and alive found in the verandah traps and adding the numbers inside the hut.

Five or six such huts were used in each experiment. The different 'treatments' with nets or curtains were rotated daily from hut to hut to compensate for differences in the attractiveness to mosquitoes between the huts. Each hut–treatment combination thus appeared once in each weekly cycle, as in a latin square, which was repeated each week. The same two children slept in each hut throughout each experiment, so that differences in their attractiveness would have been confounded with those of the huts.

Three separate trials, each lasting 4–6 weeks, were carried out in these huts. In the first, we measured the effect of permethrin on the efficacy of both intact bednets and bednets into which eight holes of 10×20 cm had been cut to simulate a badly torn net. In the second trial we measured the degree to which mosquitoes were diverted from one child to the other, when only one of the two was protected by an intact net, and in this case the children took turns on successive occasions to sleep under the net or to be unprotected. This was done with both untreated and permethrin-impregnated nets. At the same time we tested an impregnated cotton net, for comparison with the nylon nets we otherwise used. In the third trial we studied the effect of increasing the dose of permethrin from our standard one of 0.2 g/m^2 to 1 g/m^2. We also tried curtains treated with the same two doses. The curtains consisted of strips of netting 60 cm wide hung loosely with nails and string inside the eaves with the 60 cm dimension vertical. A gap of a few centimetres was left between the curtain and the wall, so that a mosquito was not physically prevented from entering the hut, but on the way it would be likely to encounter the permethrin-treated curtain.

The window-trap hut

This hut, near Muheza, Tanga region, Tanzania, was fitted on one side with a wooden louvre window, so as to admit mosquitoes but not light, and on the opposite side, facing east, with a window trap. The intention of this design is that most mosquitoes leaving the hut should go into the well-lit trap, and not escape through the louvre (Smith *et al.*, 1972). The eaves were constructed with a wooden frame so that they could be left open or sealed with heavy cloth blinds. A cloth ceiling was fitted beneath the thatched roof to facilitate collection of resting mosquitoes. One man slept in the hut each night, and each morning the mosquitoes in the trap and hut were collected, sorted and counted. There was no satisfactory ant-trap, so that counts of mosquito corpses were probably much less than the number of mosquitoes which died.

The hut was used to measured the protection afforded by the use of the net or by curtains. In two experiments the eaves were closed, and the louvre window opened: in one of these experiments a curtain was hung loosely inside the louvre. In the third experiment the louvre was sealed with black cloth, and the eaves were opened so that mosquitoes could enter and leave freely, and the window trap would catch an unknown fraction of those leaving the hut. Curtains were then hung loosely inside the eaves on the appropriate nights. The curtains measured 60 cm vertically.

The different nets and curtains could not be tested simultaneously in this one hut but, so as to control for day-to-day fluctuations in the local mosquito population, parallel daily collections lasting 1½ man-hours were made in a bedroom of a nearby house.

The dwelling house

Impregnated curtains were also tested in a semi-detached staff house at Muheza made of concrete bricks with a corrugated iron roof. There were two rooms with several windows. The eave gap was about 5 cm wide and broken by rafters about 1.5 m apart. A similar gap existed above the single wall shared by this house and the adjacent one. Mosquitoes were monitored during the night with a battery-operated CDC light-trap. The occupants of the houses slept under untreated bednets. The effect was tested of placing pieces of permethrin-treated nylon netting over the eave gaps, the gap leading to the adjacent house, and those windows which were not already thoroughly screened. The netting pieces were about 7.5 cm wide, and were pinned to sticks for ease of fixing in place. Pieces of untreated fibreglass netting were tacked around the edges of the door. In no case could the roughly fixed netting be expected to form a mosquito-proof physical barrier. After an assessment of the effect of introducing untreated netting curtains in this way, the netting was impregnated at a target dosage of about 0.7 g/m^2.

Results

Bioassays and permethrin assays

Table 1 shows the results of bioassays with *An.gambiae s.l.* on several of the nets used in the experimental huts. With the nylon nets treated with 0.2 g permethrin/m^2 exposure for 1–3 min was sufficient to give high mortality, but using 0.08 g permethrin/m^2 cotton net (as used by Darriet *et al.*, 1984) much lower killing power was noted. Many months of storage and several weeks of usage did not greatly reduce the killing power of the treated nylon nets, except in the case of the bednet at Muheza which showed consistently less kill than the eave curtain, perhaps because of more handling and body contact with the former. For unknown reasons the tests

TABLE 1. Permethrin assays and bioassays with fed *An.gambiae s.l.* on treated nets.

Serial no. of net or curtain	Test site	Cotton/nylon, net/curtain	Months of use	Permethrin content (mg/m²)		Bioassay		
				Impreg. target	Assay*	Exposure (min)	% mort.	No. tested
1	Magugu	Nylon net	0	0	–	15	7	103
2	Magugu	Cotton net	1	80	52; 46	1	0	20
			1			15	40	20
3	Magugu	Nylon net	0	200	–	1	82	77
			1		317; 184	1	100	10
			2		–	1	55	80
4	Magugu	Nylon net	1	200	–	1	100	60
5	Magugu	Nylon net	1†	200	84; 114	1	100	11
			2		–	1	83	60
6	Magugu	Nylon curtain	0	1000	853	1	100	10
7	Muheza	Nylon net	0	0	–	3	4	25
8	Muheza	Nylon net	0	200	185; 82	–	–	–
			1		31	1	12	8
			1			2	25	20
			1			3	46	12
9	Muheza	Nylon curtain	1	200	–	1	27	22
			1			2	74	35
			1			3	84	25

*Permethrin assays from two samples cut from the net in most cases.
†11 months of storage.

at Magugu showed higher mortalities than those at Muheza.

The chemical analyses generally agreed fairly well with the intended permethrin dosages in each of the nets except in the case of the heavily used net at Muheza.

Effects of permethrin on nets with and without holes

The majority of the mosquitoes caught in the verandah trap huts were of the *An.gambiae* complex and in the experimental area at Magugu they have been shown to consist almost entirely of *An.arabiensis* Patton (F. Mosha and A. Mnzava, pers. comm.).

Summaries of the daily counts of dead and live, and fed and unfed *An.arabiensis* in the huts and the verandah traps are given by Lines *et al.* (1985) and these were used to calculate estimates of the total numbers entering the hut, feeding, surviving until the morning of collection and exiting as shown in Table 2. Semi-gravids were included in the total fed during the preceding night but fully gravids were omitted, assuming that they had fed elsewhere and entered the experimental huts later. In a retrospective test of the reliability of the counts of dead mosquitoes, known numbers of corpses were left on the hut floors. Often some or all of these had disappeared overnight, so it appears that the ant traps did not exclude all scavengers. In all the experiments, therefore, data on the numbers of dead mosquitoes should be regarded as minimum estimates.

A log $(x+1)$ transformation of the daily counts was found to give an acceptable approximation to a normal distribution; they were therefore subject to three-way analysis of variance for the factors treatment, hut and day. Interactions between factors were included in the error variance. This was used to calculate a standard error for the treatment means, and hence to detect significant differences between individual treatments, which are shown in Table 2. While we were mainly interested in isolating treatment differences, both hut and day were also shown to have significant effects on the numbers caught in each category. When the measures were re-analysed as proportions (e.g. % feeding), the hut and day effects disappeared, as would be expected if the huts (together with the children inside) differed independently of treatment in their attractiveness to the entry of mosquitoes, but not in the subsequent feeding and survival of those which entered.

TABLE 2. Numbers, feeding rates and survival rates of *Anopheles* females in verandah trap huts used for trials of nylon nets with or without holes and with or without 0.2 g permethrin/m² impregnation. Numbers in the same column followed by different letters differ significantly at the 5% level.

Nature of net		A		B		C	D	E		F	
		Estimated total* *Anopheles* females entering each hut to feed	% of control	No. which fed	% of control	% which fed	% survival of those which fed	No. which fed and survived	% of control	% survived of the unfed	% of control
None (control)		403	(100%)	347[a]	(100%)	86%	93%	342[a]	(100%)	78%	(100%)
Holes	Permethrin										
No	No	223	(55%)	62[b]	(17%)	27%	98%	61[b]	(19%)	34%	(44%)
Yes	No	408	(105%)	324[a]	(93%)	79%	96%	311[a]	(97%)	47%	(60%)
Yes	Yes†	320	(79%)	124[b]	(36%)	39%	44%	54[b]	(17%)	38%	(49%)
No	Yes	267	(66%)	75[b]	(22%)	28%	47%	36[b]	(11%)	35%	(45%)

*Totals estimated from 2× no. found in verandah traps plus no. found in the hut (gravid mosquitoes were omitted from these counts).
†Net serial no. 5 in Table 1.

From Table 2, column A, it may be seen that some of the nets reduced the number of females entering the huts to feed. This apparently depended more on whether the net was intact than on the presence of permethrin. The results suggest that intact nets but not those with holes were restricting the drift of attractive host odours out of the huts, and that the permethrin itself had little deterrent effect. In later experiments, however, nets with holes and permethrin did have a deterrent effect.

The numbers and proportions feeding (Table 2, columns B and C) provide a measure of the success of the nets in protecting the sleepers. The intact nets both allowed only about 27% of those entering the hut to feed, a reduction of 70% compared to the controls with no net. Those mosquitoes which did feed in the presence of an intact net could have done so (i) by finding a way under the net, (ii) by feeding through it on a part of the body pressed close to the net, or (iii) by feeding while the sleepers were preparing for bed or getting up. We found eight dead mosquitoes among the bedclothes or on the sleeping mat following use of the impregnated intact net, and a similar number of live fed females in the untreated intact net, suggesting that sometimes the nets were not properly tucked in. Feeding through the permethrin-impregnated net seems quite likely, since Hossain *et al.* (1986) showed that this could occur in the laboratory, but the mosquitoes often died later. In the case of those which fed in the presence of the treated nets about 50% did not survive, whereas there was very little mortality of fed females in the absence of a treated net (Table 2, column D).

Permethrin treatment had a much greater effect on the nets with holes. Compared with the control, the untreated net with holes reduced the number feeding only very slightly, but the permethrin-treated net with holes reduced it by 64% (Table 2, column B). For the sleepers, therefore, an untreated net with holes gave no significant protection, while a permethrin treated one was almost as effective as an intact net.

As well as reducing feeding, permethrin treatment caused considerable mortality – about 50% – among the mosquitoes which did feed (Table 2, column D). A reduction in the number both feeding and surviving decreases the chance that a malaria infection in one of the sleepers would be passed on to someone else, and thus provides a measure of the epidemiological impact of the net (Garrett-Jones, in Hudson & Esozed, 1971). The intact untreated net, and the two treated nets, all showed highly significant reductions of 80–90% in the number of feeder-survivors, but the untreated net with holes had no appreciable effect (Table 2, column E).

Column F of Table 2 shows that the survival of unfed females was reduced with all types of net compared with the control. However, many of these deaths occurred in the verandah traps, and it may be that unfed mosquitoes starved to death there because they were prevented from finding a meal elsewhere. Bioassays showed that the deaths were not due to toxic contamination of the verandah trap screens.

Cotton v. nylon nets

At the same time as five of the verandah-trap huts were used for another experiment (see Table 5 below), an impregnated intact cotton net was tried in the sixth hut. It was treated with 0.08 g/m^2 permethrin, to match the treatment of cotton nets by Darriet *et al.* (1984) in Burkina Faso. These workers had found such nets very effective in experimental huts but, in contrast to ours, their nets seemed to work more by deterring the mosquitoes from entering than by inhibiting feeding or killing those which came in.

Because our cotton net was not rotated among the verandah trap huts, the children slept alternatively with and without the net. The results are shown in Table 3. Use of the net caused only a slight decrease in the number entering, but a significant reduction in those feeding. Like the nylon nets in this but not the other two verandah-trap hut experiments, there was little mortality but greatly increased exophily among those which fed. Thus, although the bioassays indicated that permethrin is much less potent on cotton than on nylon (Table 1), neither the difference in fabric nor the change of dosage can explain the contrast between the way the nets were seen to work in our studies and those in Burkina Faso. Darriet *et al.* mainly used large 'group' nets, which may have hung close to the walls, and therefore perhaps resembled in the pattern of their action not so much the bednets as the eave curtains in our studies described below.

For a more direct comparison of cotton and nylon nets, a net of each fabric was treated with 0.2 g/m^2 permethrin. These nets, and the control without a net, were used in turn on successive days in the window trap hut. The predominant species caught in the hut were *An.funestus* Giles and *An.gambiae s.l.* The latter has been shown to consist of more than 90% *An.gambiae* Giles *s.s.* in the neighbourhood of the hut (I. Amri and A. Mnzava, pers. comm.). The results are shown in Table 4. The window trap caught an unknown proportion of those leaving the hut, and since the treated nets caused almost complete exodus of fed females while in the control feds tended to remain inside, the apparent reduction in biting caused by the nets may have been exaggerated. With this qualification it may be noted that both nets reduced biting to a very low level, even more, apparently, than in the verandah trap huts. With both *An.gambiae* and *An.funestus* the protection was more complete with the nylon than the cotton net.

Diversion of biting from a protected to an unprotected person

In this experiment we compared the number of bites occurring when both, neither, or just one of the two children in each hut slept under an intact net, and we did this with both permethrin impregnated and untreated nets. The summarized results are shown in Table 5.

This time the deterrent effect of the nets was

TABLE 3. Effect of no net compared with an intact cotton net treated with 0.08 g permethrin/m^2 against *An.arabiensis* in a verandah-trap hut.

Net	No. entered (%)*	No. fed (%)*	No. fed and survived (%)*	% exiting among survivors		% survival of unfed
				Fed	Unfed	
None	653 (100%)	421 (100%)	416 (100%)	64%	95%	83%
Permethrin†	584 (89%)	168 (40%)	163 (39%)	98%	99%	76%

*% of no. with no net.
†Net serial no. 2 in Table 1.

TABLE 4. Comparison in window-trap hut of cotton and nylon intact nets treated with 0.2 g/m² permethrin.

Anopheles species	No. days	No. in control house	Net in hut	No. fed in hut (%)* and trap	No. fed and survived (%)* in hut and trap	% of those fed and survived which were in trap	No. unfed (%)*
gambiae	9	76	None	68 (100%)	68 (100%)	30%	21 (100%)
	9	98	Cotton	2 (2%)	2 (2%)	100%	26 (49%)
	9	111	Nylon	0 (0%)	0 (0%)	–	24 (37%)
funestus	9	1383	None	447 (100%)	447 (100%)	44%	44 (100%)
	9	1563	Cotton	82 (16%)	67 (13%)	97%	78 (94%)
	9	1215	Nylon	27 (7%)	22 (6%)	100%	24 (24%)

*As percentage of those when there was no net, corrected for relative no. in control house.

TABLE 5. Diversion of mosquitoes from one host to another by untreated and treated (0.2 g/m² permethrin) intact bednets (a) entry, feeding and survival of *An. arabiensis* in verandah-trap huts when neither, both, or one of the two children slept under a net; (b) calculation of biting on each child.

(a) Net	No. children in net	A No. entered (%)*	B No. fed (%)*	C No. fed and survived (%)*	D % exited among fed and survived	E % exited among unfed and survived	F % survival of unfed
None	Neither	1574[a] (100%)	752[a] (100%)	742[a] (100%)	70%	96%	85%
Untreated	Both	1276[a] (81%)	158[b] (21%)	150[b] (20%)	55%	97%	82%
Untreated	One	1284[a] (82%)	527[a] (70%)	522[a] (70%)	59%	96%	84%
Permethrin†	Both	1079[a] (69%)	57[c] (8%)	47[c] (6%)	98%	98%	76%
Permethrin†	One	1204[a] (77%)	202[b] (27%)	185[b] (25%)	93%	99%	69%

(b)		Observed total no. bites	Calculated no. bites per protected child	Calculated no. bites per unprotected child
None	Neither	752	–	376
Untreated	Both	158	79	–
Untreated	One	527	0–79	448–527
Permethrin	Both	57	28.5	–
Permethrin	One	202	0–28.5	173.5–202

Different letters in the same column indicate differences significant at the 5% level by analysis of variance.
*As percentage of those when there was no net.
†Net serial no. 3 in Table 1.

slight. In contrast to the other two experiments in the verandah-trap huts, analysis of variance indicated that the intact nets did not significantly reduce the number of mosquitoes attracted into the huts, either with or without permethrin treatment (Table 5(a), column A). The differences between the huts themselves were also less strong than before and, overall, fewer of those entering fed, even in the control with no net.

As before, however, the nets had a great effect on the number of bites suffered by the sleepers. When both children slept in the intact nets, the protection was as good or better than in the first experiment (Table 5(a), column B). This time, the protection provided by the permethrin-impregnated net was significantly more complete (92%) than with the untreated net (79%). There was, however, little evidence of the insecticidal killing of fed females which had been seen before (Table 5(a), columns B and C), and unfed mortality was also slight (column F). In this experiment, unlike the preceding one, mosquitoes collected alive were retained for 24 h to allow detection of delayed mortality. However, on the few occasions when this occurred it was not associated with the treated nets but with large collections leading to crowding in the maintenance cages.

In the control, 30% of fed mosquitoes remained in the hut, but with both children under the permethrin-treated net, only one of the forty-seven females feeding failed to exit. With the untreated nets there was no significant change in the proportion of fed mosquitoes exiting, suggesting that this time the chemical did have a repellent effect (Table 5(a), column D) on fed females. Even when just one of the two children was under the treated net, only 7% of the much larger number of fed mosquitoes remained inside the hut – a significant reduction measured either as a simple proportion or compared with the control by a two-sample Mann-Whitney *U*-test.

We did not attempt to trace bloodmeals back to individual hosts, so we cannot give accurate counts of the bites suffered by each child when one had a net and the other did not. Nevertheless, we can calculate limits for the biting on each by reference to the results when both were protected. The availability of an unprotected host is likely to make hungry mosquitoes less, and certainly not more, insistent on feeding on a child under a net. We can therefore safely assume that the child under the net will receive no more bites than he or she did when both were protected. Thus if there were x bites when both were under the net, then when only one child is protected he or she may expect to receive on average somewhere between zero and $x/2$ bites, the remainder being suffered by his or her unprotected partner. Since each treatment was tried each day in a different hut, and the children in each hut took turns to sleep without a net, these calculations are independent of differences in the attractiveness of the children or the huts, and of random changes in the density of blood-seeking females.

The results of the calculations are shown in Table 5(b). The use of an untreated net by one child is seen to divert hungry mosquitoes and increase the biting on the other child from 376 to at least 448. In contrast, the biting on the unprotected child when a treated net is used by his or her partner was reduced to at most 202 bites. We can therefore conclude that when a treated net is acquired by one person, the rest of the family will not suffer more bites, and may well be better off. If the net is not treated, on the other hand, there is a real danger that the protecton for those under the net is gained at the expense of others sleeping close by, who may get bitten more.

Curtains v. nets

Preliminary trials of permethrin-impregnated curtains were carried out in the window trap hut, the curtains being hung across the louvre window through which mosquitoes could enter. The results (Lines *et al.*, 1985) showed that an untreated curtain had no effect and permethrin impregnation made the mosquitoes exophilic – almost all the mosquitoes which had entered the hut were found in the window trap and not in the hut itself. In the first trial an impregnation dose of 0.2 g/m^2 was used and no significant reduction was achieved in the number of fed mosquitoes. In a second trial with an impregnation dose of 1 g/m^2 a significant reduction was achieved.

In a more systematic experiment in the veran-

TABLE 6. Effect of bednets with holes, or curtains, impregnated with permethrin at two different doses, on the numbers of *An.arabiensis* entering, feeding and surviving in verandah trap huts.

Protective treatment	No. entering	% fed	No. fed	% fed surviving 24 h	No. feeding and surviving	% fed exiting	% unfed surviving 24 h
No net	497^a	91	451^a	95	429^a	36	52
Net 0.2 g/m^2 permethrin	213^b	25	53^d	33	17^d	63	15
Net 1 g/m^2 permethrin	166^b	37	62^{cd}	54	32^d	92	16
Curtain 0.2 g/m^2 permethrin	313^b	61	192^b	78	143^b	82	55
Curtain 1 g/m^2 permethrin*	212^b	57	120^c	72	82^c	71	44

Note: different letters in the same column indicate differences significant at the 5% level by analysis of variance.
*Curtain serial no. 6 in Table 1.

dah-trap huts, we compared the efficacy of eave curtains and nets with holes, both impregnated with permethrin at either 0.2 g/m^2 or 1 g/m^2. Table 6 shows the effect of these treatments, measured and subject to analysis of variance as before. All the treatments, including this time the treated nets with holes, produced a statistically significant reduction relative to the control in the number entering the huts. The degree of the reduction did not differ significantly between the nets and curtains, although in both cases it appeared to be greater when the higher dose was used.

The nets allowed only 25–37% of those entering to get a meal and this amounted, together with the reduction in the number entering, to an 86–88% reduction in biting compared to the control (Table 6). With the curtains, about 60% of those entering successfully fed, so that the total number of bites was reduced by 57–73%. Measured as the proportion feeding, application of the higher dose did not improve the efficacy of either the nets or the curtains – the high-dose curtains were associated with fewer bites than the low dose curtains, but this was mainly a result of fewer entering in the first place.

Of those feeding in the presence of the net, only 33–54% survived, a result comparable with the first experiment (Table 2). 72–78% of fed mosquitoes survived in the presence of curtains – significantly less than in the control, but more than with the nets. The improvement gained by the higher dose was again zero or negative with the nets, and negligible or slight with the curtains. As in the preceding experiment, delayed mortality was not significantly associated with the use of permethrin. Both nets and curtains caused increased exophily of fed mosquitoes.

Overall the results suggested that increasing the dose above 0.2 g/m^2 produces an improvement in deterrency, but not feeding inhibition or mortality. Curtains were not as efficient as nets, whether measured in terms of personal protection or epidemiological impact.

A further trial of permethrin-impregnated eave curtains was carried out in the window trap hut with the eaves opened to admit mosquitoes and the louvre closed. In this trial, a net with holes treated with the standard dose of 0.2 g/m^2 permethrin was compared with eave curtains treated with either the standard dose or the increased dose of 1 g/m^2. These treatments were used alternately in the hut. On a few occasions, the high-dose curtain was substituted with an untreated curtain.

The results are shown in Table 7. The net provided excellent protection against both *Anopheles* species, reducing by more than 90% the numbers of fed mosquitoes caught. All of these were in the window-trap, whereas with no net 80–90% were caught in the hut. As before, therefore, the net caused considerable exophily of fed female mosquitoes, many of which may have escaped unrecorded through the open eaves, exaggerating the apparent success of the net. This could also be why the net was associ-

TABLE 7. Numbers and survival-rates of *Anopheles* females in window-trap hut used for trials of bednet with holes, and curtains around eaves, treated with permethrin at two different doses.

Anopheles species	No. days	No. fed in control house	Net with holes or curtain (dose g/m^2)	No. fed in hut and trap (%)*	No. fed and survived in hut and trap (%)*	% of fed survivors which were in trap	No. unfed (%)*
gambiae	26	344	None	525 (100%)	525 (100%)	10.3%	68 (100%)
	21	425	Net (0.2)†	30 (5%)	19 (3%)	100%	38 (50%)
	5	130	Curtains (0)	157 (79%)	157 (79%)	4%	16 (84%)
	26	414	Curtains (0.2)‡	96 (15%)	84 (13%)	83%	56 (53%)
	20	376	Curtains (1.0)	93 (16%)	79 (14%)	97%	40 (34%)
funestus	26	2548	None	1987 (100%)	1987 (100%)	19%	288 (100%)
	21	2314	Net(0.2)†	53 (3%)	28 (2%)	100%	98 (34%)
	5	322	Curtains (0)	368 (147%)	339 (135%)	9%	19 (73%)
	26	2605	Curtains (0.2)‡	286 (15%)	246 (12%)	78%	126 (41%)
	20	1715	Curtains (1.0)	217 (16%)	161 (12%)	92%	69 (38%)

*As percentage of numbers with no net or curtain, corrected for relative numbers in control house.
†Net serial no. 8 in Table 1.
‡Curtain serial no. 9 in Table 1.

TABLE 8. Effect of eave curtains on light-trap catches in dwelling-house.

Type of curtain	No. days	Average no. per day of:			
		Culex quinquefaciatus		*Mansonia* spp.	
		Unfed	Fed	Unfed	Fed
Untreated	12	44.5	4.3	4.0	0.5
None	25	25.2	2.1	17.0	0.7
Permethrin treated 0.7 g/m^2	16	23.6	1.4	18.8	0.5

ated with a reduced catch of unfeds. Smith & Webley (1969) and Smith & Chabeda (1968) showed that irritant insecticides could influence the relative proportions leaving an experimental hut by the eaves rather than the windows.

The treated curtains also reduced the number of fed mosquito females by about 85%. Like the nets, the curtains caused much exophily but, with the curtains, exited fed mosquitoes are more likely to have been driven into the trap, causing a greater proportion to be caught. This figure may therefore underestimate the efficacy of the curtains in reducing biting, which was apparently much better than was observed in the verandah-trap huts. The untreated curtain was only tried on five occasions, so the results were more subject to daily fluctuations, but no substantial reduction was observed in the number of fed female mosquitoes, showing that it was the permethrin that was responsible for the effect of the treated curtains.

Only a few dead mosquitoes were found in the hut or trap, though some corpses may have been taken by ants. Both the net and treated curtains were associated with a slight increase in the proportion of fed mosquitoes known to have died.

Overall, therefore, the window-trap hut results indicate that both the net and curtains worked at least as well as they did in the different experimental and ecological situation of the verandah-trap huts, and that the net seemed to be more effective than the curtains in both experimental types of hut.

In the dwelling house, the treated eave curtains gave very disappointing results. Table 8 shows the average daily catch of unfed and fed mosquitoes for the preliminary period when untreated curtains were fitted, and for the subsequent 6 weeks when permethrin treated curtains were alternated (approximately weekly) with leaving the eaves open. The curtains had no appreciable effect on the numbers caught of either *Culex quinquefasciatus* Say or *Mansonia* spp. Very few anophelines were caught, but there was no indication that the curtains worked any better against these than the predominant culicines.

Discussion

Personal protection with nets and curtains

As a means of personal protection, the intact nets worked well with or without permethrin. A net with holes, in contrast, was useless if untreated, but almost as effective as an intact net if impregnated with permethrin (Table 2).

In the case of cotton net, the protection against bites was good but not as complete as with nylon (Table 4). The bioassay results suggested that a given dose of permethrin may be more available to the insect in non-porous synthetic fibre than in absorbent cotton (Table 1). In the huts, however, increasing the target permethrin dose above 0.2 g/m^2 did not increase the effectiveness of nylon nets (Tables 6 and 7).

Treated nylon nets reduced biting in the verandah-trap huts by 78–92% if intact (Tables 2 and 5), and by 64–88% if holes had been cut (Tables 2 and 6). In the window-trap hut the experimental set-up permitted less accurate measurement, but the nets apparently protected the sleeper even more completely (Tables 4 and 7) than in the verandah-trap huts.

Although the impregnated eave curtains were consistently less efficient than the nets with which they were compared, they still provided about as much protection (57–73%) in the verandah-trap huts (Table 6) as the pyrethrum mosquito coils tested in the same huts by Smith *et al.* (1972) and Hudson & Esozed (1971). Like the nets, they performed better in the window-trap hut, reducing biting by about 85% (Table 7). It was therefore surprising and disappointing that applying permethrin-treated eave curtains to an occupied house had no apparent effect on the density of mosquitoes inside (Table 8). The door of the house was closed for most of the day and night, but it may have been left open when the occupants were busy getting up in the morning, and preparing the evening meal, both times when mosquitoes are actively entering and leaving houses. We do not know, however, which points of entry the mosquitoes used. As pointed out by Gillett (1985), devices intended to keep mosquitoes out can, if they do not work properly, keep them inside instead. One possible reason, which requires investigation, for the discrepancy between the results in the experimental huts and the dwelling house is that, because of the different construction of the eaves, the vertical height of the curtains used in the former was 60 cm and in the latter was only 7.5 cm. If the curtains are encountered not so much at the time of entry but subsequently as a nocturnal resting place, it is understandable that the 60 cm curtains would have been far more effective.

Effects on mosquito behaviour

The permethrin-treated nets and curtains produced a range of behavioural effects – deterrency, feeding inhibition, repellency and mortality – typical of excito-repellent compounds such as DDT, and natural and synthetic pyrethroids. Table 9 shows a comparison of some of these effects from previous studies in the Magugu verandah-trap huts using such compounds in the form of residual sprays and smouldering repellent coils. The comparison is approximate, partly due to unexplained lack of repeatability between some of our measurements and partly because our method of collection differed from that of the previous work.

The nets had a relatively slight deterrent effect against hut entry, but strongly inhibited feeding, and many of the entering mosquitoes were killed. The curtains depended more on deterring entry, but still caused noticeable feeding inhibition. They killed about 20–30% of the mosquitoes that fed; some of those deterred from entering may have picked up a lethal dose and died outside. The observed mortality rate was less than with nets, but greater than with pyrethrum mosquito coils (Table 9). The permethrin-treated fabrics also shared with the excito-repellent residual sprays and coils a tendency to induce exophily in fed mosquitoes.

Some aspects of mosquito behaviour in relation to bednets were revealed in the experiment with one child sleeping under the net and the other outside it (Table 5). Compared to the con-

TABLE 9. Summary comparison of various insecticide application techniques from studies in the Magugu verandah-trap huts over the last 20 years.

Treatment	No. or proportion relative to untreated control					Reference
	Total entering	Proportion feeding	No. of bites	Overall survival	No. of fed-survivors	
DDT residual spray (first month)	+++	+++	++	+++	++	Smith & Webley (1969); summarized by Smith & Chabeda (1968)
Tetramethrin residual spray (first month)	++	+	+	++	+	Smith & Chabeda (1968)
Pyrethrum coils	++	+++	++	++++	++	Hudson & Esozed (1971); Smith *et al.* (1972)
DDT coils	+	++	+	++++	+	Hudson & Esozed (1971)
Net with holes and permethrin 0.2 g/m^2	+++	++	+	++	+	Present study
Curtains and permethrin 0.2 g/m^2	+++	+++	++	+++	++	Present study

Key: + = strong reduction (0–25% of the untreated control); ++ = medium reduction (25–50% of the untreated control); +++ = weak reduction (50–75% of the untreated control); ++++ = slight or no reduction (75–100% of the untreated control).

trol with no net, the exposed child suffered more bites if the net used by the other was untreated. This did not happen if the net was treated, indicating that the permethrin induced a specific change in behaviour – the mosquitoes stopped trying to feed, although an unprotected host was available, and for the most part they left the hut. C. Schreck (pers. comm.) has observed a similar reluctance to feed lasting several hours in caged *Aedes aegypti* after treatment with sub-lethal doses of permethrin. Action at a distance by the treated net on those mosquitoes which were first attracted to the exposed host cannot be ruled out, but is most unlikely, since permethrin has an extremely low vapour pressure and our nylon nets were virtually lint-free. Therefore many of the females going first to the exposed child must have contacted the net before feeding. They could have done so either accidentally on the way, or if they investigated both hosts before choosing one on which to feed, or else they may have been disturbed while attempting to feed on the exposed child and were then attracted to the child under the net. Even those which fed – presumably mainly on the exposed child – were apparently affected by permethrin picked up either before or after feeding: the increase in exophily was far greater than would be expected if only those feeding on the child under the net had been affected.

Benefits for the family and community

In the experimental huts, about as much fabric was needed to make a set of curtains as a bednet. Nevertheless, if curtains could be made to work on a household scale, more people could probably be protected than if the same amount of fabric were made into nets. This would be a substantial saving, since the netting is likely to cost much more than permethrin treatment. Curtains would benefit all the occupants of the house equally, and would also protect people sitting inside before they go to bed.

Bednets would not be so fair. Visitors, people with low family status, and those who dislike sleeping under a net, are all likely to remain unprotected. The burden of biting would fall more heavily on such people if the nets were untreated. It would be especially important if children suffer in this way, as they are not only more vulnerable to the effects of malaria, but are also more likely to be infective to mosquitoes. Treated nets, on the other hand, apparently confer partial protection even to exposed people sleeping nearby (Table 5). In fact, when one child was exposed and the other slept under a treated net, the reduction in the total number of bites in the verandah-trap hut was greater than when both were protected by curtains treated with 0.2 g/m^2 permethrin (Table 6). Further work, using techniques to trace bloodmeals to individual hosts, would be necessary to show whether treated nets or curtains can divert mosquitoes between households rather than individuals within a house.

The killing of mosquitoes coming to bite may be a crucial additional benefit for the whole community. In the verandah-trap huts, the treated nets killed only a few mosquitoes in one trial (Table 5) but 46–67% of fed mosquitoes in the other two (Tables 2 and 6). Overall, this compares favourably with a conventional DDT residual spray tested in the same huts (Table 9 and Smith & Webley, 1969), which killed only about 20% of fed mosquitoes, and 34% of the total entering, during the first month after spraying. With curtains, the observed mortality was less than with the nets, but, as mentioned above, some of those deterred from entering may also have died.

The killing of fed mosquitoes would reduce the chance that an infected host would pass on the infection and, as with house-spraying, a female would risk being killed each time she comes into a house to feed. Widespread use of treated nets in a community could therefore reduce the longevity, and hence the vectorial capacity, of the local mosquito population. Good coverage might even produce a noticeable reduction in the population density. A reduction in the local density of hungry nuisance mosquitoes has been detected with permethrin-impregnated jackets (Lindsay & McAndless, 1978). Such 'community' effects would be important as indirect protection not only for people without nets or curtains, but also for anyone sitting outside in the evening before going to bed. Biting in this situation could be further reduced by the use of strips of cloth worn on the ankles and impregnated with 'deet' (diethyl toluamide), which have been shown to give about 70% protection in such circumstances (Curtis *et al.*, 1987).

Two trials carried out so far with permethrin or deltamethrin impregnated nets (Snow *et al.*, 1987; Ranque *et al.*, 1984) have shown significant but not dramatic protection against malaria for people with nets. In both cases, however, the comparison was between people with and without treated nets in the same community, being bitten by the same mosquito population. Table 9 shows that, at least in our hut, the pattern of deterrency, feeding inhibition, and mortality produced by the treated nets was similar to that of residual sprays which are known to have little effect when only isolated houses in a village are treated, and to be effective only when good coverage is achieved. Perhaps the same will turn out to apply to permethrin-treated bednets and curtains. In collaboration with the staff of the Tanzanian National Institute for Medical Research, we hope to test for such effects during a study of community-wide use of permethrin impregnated bednets, in comparison with DDT house-spraying.

Side-effects

People tend to notice the side-effects of vector control operations as much as their intended benefits. One advantage of using pyrethroid-treated curtains rather than bednets might be that long-term skin contact would be avoided. This may be an important point with the α-cyano-pyrethroids which can irritate sensitive skin, eyes and mucous membranes. However, permethrin has a very low mammalian toxicity and its use in bednets has been sanctioned by the World Health Organisation (1985). The short-term studies so far carried out of the use of permethrin-treated bednets (Darriet *et al.*, 1984; Snow *et al.*, 1987), or skin application of permethrin (Yap, 1986), have reported no unpleasant side-effects.

Impregnated nets and curtains are unlikely to have the toxic side-effects on domestic animals that have sometimes been noted in, and interfered with, house-spraying operations, since general contamination with the insecticide will be greatly reduced. Other side-effects may be beneficial: we found several cockroach, bedbug and louse corpses in the experimental huts after the treated nets were introduced. Human infestation with scabies might also be reduced by the use of treated nets. Protection against such pests, and against nuisance mosquitoes, could have more immediate public appeal than an effect on vector-borne disease.

Impregnated fabrics in public health

Malaria vector control by conventional house spraying with residual insecticides requires a large work-force of spray-teams which usually has to be trained, supervised and transported to rural areas by a specialized central organization. The only two major house spraying projects currently operating in tropical Africa, in the Gezira area of Sudan and the islands of Zanzibar, Tanzania, depend on substantial subsidies in hard currency as foreign aid or loans, and are restricted to places with a relatively high population density. In these and other parts of the world, difficulties have been encountered from insecticide resistance, exophilic behaviour of the mosquitoes, and refusal by householders to the entry of spray-teams. We suggest that the use of impregnated fabrics may help to circumvent these problems.

Resistance to permethrin has been produced in *An.gambiae* by laboratory selection (Prasitissuk & Curtis, 1982). The *kdr* form of DDT resistance may give cross resistance to permethrin, but it remains to be seen whether this will be strong enough to protect mosquitoes against contact with impregnated fabrics. Widespread use of permethrin-treated fabrics would create a selection pressure for resistance, and this could cause problems in the future.

Mosquitoes which feed inside houses but then exit are notoriously hard to suppress with house spraying. They would be expected to be controlled with permethrin-treated nets or curtains, however, since these act before feeding by interposing an insecticide-laden barrier between the mosquito and the host.

The overall cost of impregnated fabrics will depend on how often they need to be re-treated with permethrin. Our bioassays suggest that the permethrin deposit has a very long shelf-life (net no. 5, Table 1) but frequent washing and constant handling are likely to reduce its effective life. The necessary frequency of re-impregnation will therefore have to be determined in practice, but we anticipate that treatment every few months will suffice to maintain an active deposit as we have obtained good performance from an impregnated net with holes after 5 months of use (Curtis & Lines, 1987). The cost of permethrin formulated for fabric impregnation has yet to be fixed, but it is likely to be of the order of U.S. $0.30 for treatment of a single net.

The local cost of nets varies widely. In the U.K. we paid £8 ($12) each for good quality rectangular nylon nets, with a polyester-cotton border, made to fit a single bed ($1\times2\times1.4$ m). If we assume that such a net treated twice a year could protect on average 1.5 people for 5 years, the overall cost of materials works out at about $2 per person-year. This is similar to the cost of materials – insecticides, equipment, vehicles and fuel – for an anti-malaria malathion-spraying campaign in Africa, as estimated by U.S.A.I.D. (1983). Cotton nets do not respond so well as nylon to permethrin impregnation (Tables 1 and 4), but in countries where cotton is grown and netting manufactured it may well be more economical to use locally produced netting and to impregnate with a higher dose or frequency.

A control scheme using permethrin-treated bednets could be largely organized and carried out by existing primary health care structures. Health authorities which cannot afford a house-spraying campaign would be equally unable to supply free bednets to all, but it would be much cheaper to make permethrin treatment available as a public service to net owners. Bioassays and chemical analysis showed that dipping, as described above, gave an effective and adequately evenly distributed deposit of permethrin. The method requires only the chemical and a bucket; it is easy and safe enough to be done with a minimum of training by village health workers, local dispensary staff, or even householders themselves.

In some places, such as the Gambia and parts of tropical South America, nets are already widely used. In such places, permethrin treatment would dramatically increase the effectiveness of torn or badly tucked-in nets. In other areas, including Tanzania, nets are scarce and relatively expensive, but permethrin treatment might so increase the effective life of a net as to make it a more attractive investment. Nets are sometimes regarded by governments as a luxury for the rich; lowering of import and sales duties could bring them within the reach of more of the population.

House-spraying must operate for efficiency on a reasonably large scale, and depends for its effectiveness on treatment of a high proportion of buildings. Good coverage may not be so essential for impregnated fabrics – their use could begin on a small scale, through local self-help initiatives. As long as people who acquire nets or curtains get noticeable protection, the idea that their purchase is worthwhile could spread gradually through a community. In Tanzania, such popular recognition of the efficacy of chloroquine treatment against malaria has led to a commercial demand for it, and distribution via small shops extends far beyond that which state or municipal organizations can manage at present (MacCormack, 1984).

Impregnated fabrics may also have an advantage in large-scale aid-financed development projects, such as irrigation and hydro-electric schemes, which may cause an increase in mosquito-borne disease. The cost of supplying the endangered inhabitants with subsidized bednets or curtains may be small compared to the cost of the project, and aid donors are likely to prefer the high capital but low maintenance cost of this method to a house spraying operation, for which the running costs would be too expensive for local authorities to keep going once the donors withdraw.

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