

Experimental hut trials of bednets impregnated with synthetic pyrethroid or organophosphate insecticide for mosquito control in The Gambia

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Abstract. 1. Nylon bednets impregnated with different insecticides were evaluated in 1988 against wild adult mosquito populations, mostly *Mansonia africana* (Theobald) and *Anopheles gambiae* Giles *sensu lato*, entering experimental verandah-trap huts in The Gambia. Each bednet had six 10 × 10 cm holes made in the walls to simulate torn conditions and permit female mosquitoes to enter and feed on sleepers.

2. Individual net treatments, determined by gas chromatography of net samples from before and after 12 weeks use of the bednets, were: permethrin 670 ± 159 and 405 ± 190 mg/m² (40% loss), cypermethrin 37 ± 8 and 16 ± 9 mg/m² (57% loss), deltamethrin 10 ± 7 and 10 ± 8 mg/m² (no loss), lambda-cyhalothrin 2.6 ± 0.9 and 1.6 ± 0.5 mg/m² (38% loss), pirimiphos-methyl 4017 ± 117 and 1160 ± 319 mg/m² (71% loss).

3. Washing three times in the traditional manner with local cow-fat soap reduced the initial dosages by about 85% of cypermethrin and lambda-cyhalothrin, 99.8% of pirimiphos-methyl and left no detectable residues of deltamethrin or permethrin.

4. The unwashed permethrin-treated bednet reduced the number of mosquitoes entering a hut by 60% of *An.gambiae s.l.* and 68% of *Mansonia* spp. This deterrence was less pronounced with the other insecticides and was lost by washing the bednets.

5. Each insecticide, especially lambda-cyhalothrin and pirimiphos-methyl, caused significant mortality rates of mosquitoes that entered huts with impregnated bednets, and prevented the majority of *An.gambiae s.l.* and *Mansonia* females from bloodfeeding. Washing completely removed the efficacy of deltamethrin and permethrin treated bednets, whereas nets treated with cypermethrin, lambda-cyhalothrin or pirimiphos-methyl remained significantly insecticidal after washing.

6. Aerial toxicity from the pirimiphos-methyl treated bednet killed 80% of *An.gambiae s.l.* confined overnight in the hut at the end of the trial, whereas the pyrethroid-treated bednets gave negligible mortality rates of mosquitoes.

7. Sleepers using the bednets had no medical symptoms significantly associated with any of the treatments. On the contrary, from 216 interviews, 4/10 complaints were associated with the use of untreated nets ($P \sim 0.05$), perhaps because sleepers were kept awake by mosquitoes and became more aware of any ailments.

8. It is concluded that permethrin tends mainly to deter mosquitoes from house-entry, enhancing personal protection, whereas the other insecticides kill higher proportions of the endophilic mosquitoes, which would give better community protection against malaria transmission.

Key words. *Anopheles gambiae*, *Mansonia*, cypermethrin, deltamethrin, lambda-cyhalothrin, permethrin, pyrethroids, pirimiphos-methyl, bednets, vector control, The Gambia.

Introduction

Bednets impregnated with insecticide are attracting increasing interest to control malaria, other vector-borne diseases and nuisance insects. Synthetic residual pyrethroids are the insecticides of choice for this purpose, because of their high insecticidal activity, low mammalian toxicity and rapid degradation in the environment (Elliot *et al.*, 1978; Leahey, 1985). The pyrethroids permethrin, deltamethrin and lambda-cyhalothrin have been evaluated in the field (Curtis, 1989; VCRC, 1990), but direct comparisons between the efficacy of different insecticides on bednets have seldom been made under standardized conditions. Long after the original use of DDT on bednets (e.g. Travis, 1946), organophosphates were compared by Brun & Sales (1976) and DDT was compared with deltamethrin by Li Zu-zi (1987).

Whereas in most studies the impregnated nets have remained unwashed, in The Gambia bednets are washed regularly in the rainy season, often at fortnightly intervals (Snow *et al.*, 1987). Vigorous lathering by hand in cold water, using locally made soap, greatly reduces the concentration of synthetic pyrethroid on fabric as assessed by bioassays and gas chromatography (Snow *et al.*, 1987; Lindsay *et al.*, 1991a). This study in The Gambia employed verandah-trap huts to investigate the effects of impregnated bednets on wild mosquitoes, principally *Mansonia* spp. and the malaria vector *Anopheles gambiae* Giles *sensu lato*. The pyrethroids

permethrin, cypermethrin, deltamethrin and lambda-cyhalothrin, and the organophosphate pirimiphos-methyl, were compared for their impact on mosquito populations, using nylon bednets treated with one or other of these insecticides. The four pyrethroids were selected from earlier studies comparing netting impregnated with different insecticides (Lindsay *et al.*, 1991b; Miller, 1990). The target concentrations of permethrin and deltamethrin were those used in The Gambia (Snow *et al.*, 1987) and China (Li Zu-zi, 1987) respectively. The target concentrations of cypermethrin and lambda-cyhalothrin were those estimated to be equitoxic with the other insecticides based on values given by Leahey (1985). The organophosphate was included in order to compare its performance with the pyrethroids and with a view to formulating mixtures of insecticides as a possible strategy to delay the evolution of pyrethroid resistance (Curtis, 1987). The second aim was to assess the effect of hand-washing on persistence and activity of these five insecticides on bednets.

Methods and Materials

Bednets. The bednets were made from nylon netting with a 1 mm mesh and weight of 31 g/m². They were 2.2 m long, 0.95 m wide and 1.8 m high, with a total area of 13.43 m². To simulate badly torn bednets, six 10 × 10 cm holes were cut from each net, two on either side and one at each end.

Insecticides and bednet treatments. Impregnation of bednets with insecticides followed procedures described by Lines *et al.* (1987). The target concentration of insecticide on each bednet was as follows: cypermethrin 100 mg/m², applied as aqueous dilution of 10% emulsifiable concentrate (10% EC 'Cymperator', ICI); deltamethrin 25 mg/m² (2.5% EC 'Decis', Roussel Uclaf); lambda-cyhalothrin 25 mg/m² (2.5% EC 'Icon', ICI), permethrin 500 mg/m² (25% EC, 'Imperator', ICI) and pirimiphos-methyl 1000 mg/m² (50% EC 'Actellic', ICI). Actual concentrations achieved were, respectively, 0.37 ×, 0.41 ×, 0.1 ×, 1.3 × and 4 × the target treatment rates (Table 1).

The amount of each insecticide required for treatment of a bednet was calculated using the following formula:

$$\text{amount of EC} = \frac{(\text{target dose} \times \text{area of fabric}) \times 100}{\% \text{ active ingredient in EC}}$$

Two nets were treated with each insecticide: each net was soaked for 5 min in dilute emulsion and then wrung out by hand in a standard way. Two nets were simply dipped in water and dried for use as untreated controls. Nets were laid out horizontally to dry indoors on plastic sheeting for 24 h and colour coded so that the trial could be carried out double-blind.

Washing of nets. At the start of the trial, one of each of the six pairs of nets was hand-washed three times. A Gambian woman washed each net separately in river water using local 'cow fat' soap. Each net was lathered thoroughly for about 7 min and then rinsed in the river. Nets

Table 1. Summary of gas chromatography assays of insecticides on samples cut from bednets.

Insecticide (mg/m ² target concentration)	Unwashed Washed (U/W)	Week	Mean dose (mg/m ²)	Standard deviation	Range	% reduction (weeks 0–12)
Cypermethrin (100)	U	0	37.2	8.3	28–48	57
		12	16.0	8.8	7–26	
	W	0	6.0	1.3	5–8	78
		12	1.3	0.5	1–2	
Deltamethrin (25)	U	0	10.2	7.2	5–24	0
		12	10.2	7.9	3–21	
	W	0	0	0	0	–
		12	0	0	0	
Lambda- cyhalothrin (25)	U	0	2.6	0.9	2–4	38
		12	1.6	0.5	1–2	
	W	0	0.4	0.1	0.3–0.6	0
		12	0.5	0.2	0.2–0.7	
Permethrin (500)	U	0	670	159	380–830	40
		12	405	190	170–170	
	W	0	0	0	0	0
		12	0	0	0	
Pirimiphos- methyl (1000)	U	0	4017	117	3900–4200	71
		12	1160	319	800–1600	
	W	0	10.2	5.5	7–21	48
		12	5.3	4.5	2–14	

were dried indoors to avoid possible ultra-violet degradation of the insecticides by sunlight.

Chemical assay. To determine the amount and distribution of insecticide on each net, the pieces of material removed to make holes in the nets were eluted overnight in chloroform, samples of which were subjected to gas chromatography (GC) and assayed by standard procedures (ICI, 1987).

Sleepers. Six Gambian men, aged 18–55 years, slept in the huts each night for the duration of the trial. They were questioned every morning to determine any perceived side-effects of sleeping under the treated bednets. Sleepers were given chloroquine anti-malaria prophylaxis weekly and paid.

Study area. The study was carried out at Wali Kunda, a field station in an area of Sudan savanna on the south bank of the River Gambia, approximately 290 km from the coast. This site is bordered on the north by the fast-flowing freshwater river. The main mosquito breeding sites are rice fields and swampland to the south, covering approximately 610 ha. The huts were situated in an area of open grassland with scattered trees.

Huts. Six square experimental huts with open eaves were built in a line, 11.75 m apart, parallel to the edge of the large area of rice and swamp. Hut design and dimensions resembled those described by Rapley (1961) and Smith (1965), based on mud-walled and thatch-roofed African houses, with verandahs on all four sides and open eaves permitting mosquitoes to enter or leave the huts. For trapping 50% of the mosquitoes exiting via the eaves, the verandahs of each hut were screened on both sides perpendicular to the edge of the rice swamp. Each wall of the hut had a window. Windows within verandah traps were fitted with exit traps so that 50% of mosquitoes exiting via windows could be trapped. The other two windows were closed. Unfortunately it was not practicable to alternate the walls fitted with exit traps and verandah traps, which would have been desirable to offset any directional bias in mosquito behaviour.

Each hut was constructed upon concrete pillars, so that the floor was raised from the ground. Each pillar had a surrounding channel of water to prevent the entry of ants and termites which might remove the corpses of dead mosquitoes.

Collection of mosquitoes. Each hut, with its

enclosed verandahs and exit traps, was searched using aspirators and electric torches for 60 minutes every morning. The mosquitoes were later identified and scored as fed or unfed. In this part of The Gambia, during the rainy season, populations of the *An.gambiae* complex (*An.gambiae* s.l.) comprise a small proportion of *An.arabiensis* Patton with a predominance of *An.gambiae* Giles *sensu stricto* savanna cyto-type (Bryan *et al.*, 1982; Coluzzi *et al.*, 1985; Lindsay *et al.*, 1991c). Identifications of 425 *Mansonia* from the experimental huts revealed 7% *Mn.uniformis* (Theobald) with 93% *Mn.africana* (Theobald).

All live-caught *An. gambiae* s.l. females were provided with a diet of 10% glucose solution on cotton-wool pads and held in paper cups until mortality rates were recorded after 24 h. The exit traps and verandahs were also furnished with pads of 10% glucose solution to reduce the risk that unfed female mosquitoes exiting in the night would die of starvation.

The total numbers of mosquitoes entering the huts were estimated by doubling the verandah trap catches (Smith, 1965) and adding this to the numbers found inside the huts plus the exit traps.

Design of trial. The trial lasted 6 weeks, with a one-night break each Saturday. Each sleeper slept from 22.00 to 06.30 hours in the same hut for six consecutive nights. Three Latin Square designs (Cochran & Cox, 1957) were used: (1) to allocate the six sleepers to a different hut each week, (2) to allocate the five different insecticide treated nets and the untreated net to different sleepers on each of the six nights of the week, and (3) to allow for systematic variation between nights of the week. In addition, the washed and unwashed nets were allocated by imposing washing on the second Latin Square, so as to be as nearly balanced as possible in each week.

Statistical analysis. The data for counts of mosquitoes were log transformed as $\ln(x + 1)$ and proportions angular transformed ($\arcsin \sqrt{x}$) to stabilize the variance. Analysis of variance (ANOVA) was carried out using the statistical software package GLIM (Payne, 1986).

The ANOVA allowed for variation between huts, nights, sleepers, treatments and for the effect of washing. To assess whether washing affected the insecticides in different ways, an 'insecticide by washing' interaction term was

Table 2. Results of Analyses of Variance (ANOVA) for *Anopheles gambiae s.l.*

Response analysed	Difference between insecticides			Difference between washed and unwashed nets		
	<i>F</i>	df	<i>P</i>	<i>F</i>	df	<i>P</i>
Total no. entering hut	8.3	5, 159	< 0.001	0.1	1, 159	> 0.05
Percentage dead	16.1	5, 155	< 0.001	11.8	1, 159	< 0.001
Total no. dead	21.5	5, 159	< 0.001	1.5	1, 159	> 0.05
Percentage bloodfed	2.1	5, 155	~ 0.05	0.9	1, 155	< 0.05

also included in the model.

The following response variables were analysed. (i) Total numbers of mosquitoes entering huts. (ii) Mosquito mortality: (a) percentage of the total number entering which were found dead in the hut, exit traps and verandahs plus those which had died after 24 h; (b) total number of mosquitoes which were found dead in the hut, exit traps and verandahs and after 24 h. (iii) Percentage of the total number entering which were found bloodfed in hut, exit traps and verandahs. In the consolidated data (Figs 1–3) the means have been adjusted for each night of the study, since only six of the twelve treatments were tested on any one night. The approximate standard error of the difference between any two means (S.E.D.) is also shown.

Results

Assays of insecticides on netting

Amounts of active ingredient detected on the unwashed unused bednets were less than expected for cypermethrin, deltamethrin and lambda-cyhalothrin, but more than intended

for permethrin and pirimiphos-methyl (Table 1). After washing the alternative set of treated bednets had drastically reduced concentrations of all insecticides: 84% less cypermethrin, 85% less lambda-cyhalothrin, 99.8% less pirimiphos-methyl and no detectable residues of permethrin or deltamethrin.

Over the 12 weeks from initial impregnation to the end of the trial there were significant losses of cypermethrin, permethrin and pirimiphos-methyl from the unwashed nets (*t*-tests: $P < 0.05$). Deltamethrin showed no reduction and the loss of lambda-cyhalothrin was insignificant (Table 1).

Effects of impregnated bednets on mosquitoes

Results of the ANOVAs for *An. gambiae s.l.* and *Mansonia* spp. are shown in Tables 2 and 3 respectively. Where *F* values are significant, *t*-test comparisons between individual treatments are justifiable.

(i) *Total numbers of mosquitoes entering a hut.* Fig. 1 shows the effects of impregnated bednets on the numbers of mosquitoes entering

Table 3. Results of the Analyses of Variance (ANOVA) for *Mansonia* spp.

Response analysed	Difference between insecticides			Difference between washed and unwashed nets		
	<i>F</i>	df	<i>P</i>	<i>F</i>	df	<i>P</i>
Total no. entering hut	11.8	5, 159	< 0.001	20.0	1, 159	< 0.001
Percentage dead	30.5	5, 159	< 0.001	106.8	1, 159	< 0.001
Total no. dead	23.4	5, 159	< 0.001	1.4	1, 159	> 0.05
Percentage bloodfed	14.2	5, 159	< 0.001	11.4	1, 159	< 0.001

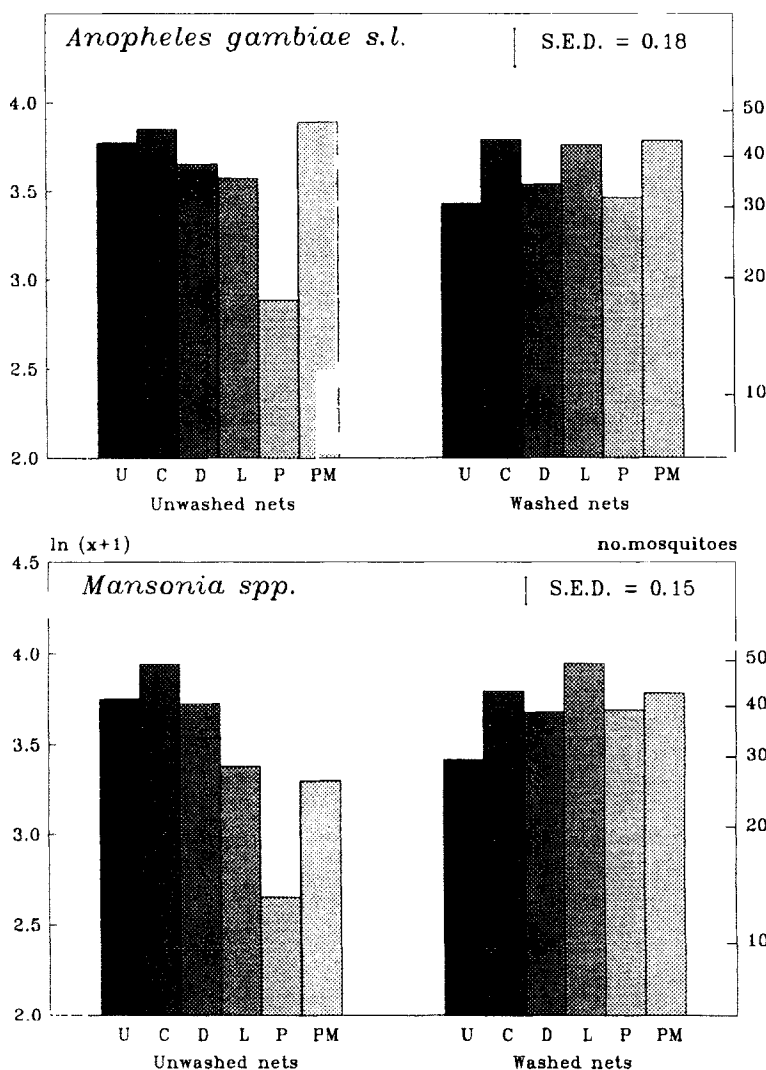


Fig. 1. Mosquito deterrence. Mean numbers of mosquitoes (x) per night entering huts where a sleeper used a bednet. U, untreated bednet; C, cypermethrin impregnated; D, deltamethrin impregnated; L, lambda-cyhalothrin impregnated; P, permethrin impregnated; PM, pirimiphos-methyl impregnated. S.E.D., standard error of difference between means.

huts. About 60% fewer *An.gambiae s.l.* entered the hut containing the unwashed permethrin treated net than the hut containing the unwashed untreated net ($t = 4.9$, $df = 30$, $P < 0.001$). There were no significant differences between the numbers of *An.gambiae s.l.* in the huts with the other nets. The deterrent effect of permethrin was lost after washing.

Significantly fewer *Mansonia* were found in

the presence of the unwashed permethrin net compared with other unwashed treated nets ($P < 0.001$) and 68% less than with the unwashed untreated net ($t = 7.3$, $df = 30$, $P < 0.001$). With the unwashed lambda-cyhalothrin and pirimiphos-methyl treated nets, significantly fewer *Mansonia* (32–37%) entered the huts than with cypermethrin or deltamethrin treated nets or unwashed untreated nets (t -tests: $P < 0.05$).

After washing, none of the insecticide treated nets reduced the mosquito entry rates.

(ii) *Mosquito mortality*. (a) *Percentages killed* (Fig. 2a). For *An.gambiae s.l.* with the unwashed nets, all the insecticides caused significantly greater mortality than the unwashed untreated net. After washing, all the nets, except the permethrin treated one, remained significantly more effective than the untreated net ($P < 0.05$).

For *Mansonia*, all the unwashed insecticide treated nets caused significantly higher mortality than the unwashed untreated net. The highest percentage of *Mansonia* entering the huts was killed with the pirimiphos-methyl net, and mortality was significantly higher with this organophosphate than with all the pyrethroids except lambda-cyhalothrin. The pirimiphos-methyl and lambda-cyhalothrin nets killed 64% and 62% more *Mansonia*, respectively, than the untreated net.

For the washed nets, only cypermethrin, pirimiphos-methyl and lambda-cyhalothrin caused significantly higher mortality than the untreated net (t -tests: $P < 0.05$), viz 29%, 35% and 40% respectively.

(b) *Total numbers of mosquitoes killed* (Fig. 2b). All the unwashed treated nets except that with permethrin killed a significantly higher number of *An.gambiae s.l.* than the untreated one. Fewer were killed with permethrin than the untreated net, although this was of borderline significance ($P \approx 0.05$). After washing the nets, fewer *An.gambiae s.l.* were killed – but all treated nets killed more mosquitoes than the untreated net.

For *Mansonia* also, all the insecticide treated nets, except permethrin, killed a higher number of mosquitoes than the untreated net. The cypermethrin treated net killed the most *Mansonia* (adjusted mean 45.6 per night), but this number was not significantly different from the deltamethrin, lambda-cyhalothrin or pirimiphos-methyl treated nets which killed, on average, 36.5, 30.7 and 30.6 *Mansonia* per night respectively.

After washing, all the treated nets still killed more mosquitoes than the untreated net; the lambda-cyhalothrin treated net killed the greatest number (adjusted mean 40.9 per night), but this was not significantly different from the number killed by the pirimiphos-methyl treated net (adjusted mean 29.2 per night).

(iii) *Percentages of bloodfed mosquitoes*

(Fig. 3). With unwashed nets, there were no significant differences in the proportions of bloodfed *An.gambiae s.l.* females in the huts + verandahs + exit traps, although the highest percentage was with the untreated net and the lowest was with the lambda-cyhalothrin treated net.

For *Mansonia*, the hut with the unwashed untreated net had a significantly higher percentage of bloodfed females than any of the unwashed treated nets ($P < 0.01$). The lambda-cyhalothrin and permethrin treated nets were associated with the lowest percentages of bloodfed mosquitoes, respectively 86% and 91% less than the untreated net. The pirimiphos-methyl treated net had least impact on *Mansonia* bloodfeeding rates. In huts with washed nets, that with pirimiphos-methyl had 46% more bloodfed *Mansonia* than that with the untreated net ($P < 0.05$), but no other significant differences were observed in this parameter.

Bloodmeal identification

ELISA tests of 705 *An.gambiae s.l.* blood-meals showed that 59.1% of the mosquitoes collected in the experimental huts had fed on humans and 40.9% fed on cattle or goats. All of the latter (and presumably some of the former) category must have entered the huts to rest after feeding elsewhere.

Aerial toxicity

At the end of the trial, the aerial toxicity of insecticide from the treated nets was investigated. Paper cups of *An.gambiae s.l.* females (mixed abdominal conditions) were placed overnight on the floor in the corner of each hut containing an unwashed net. The pirimiphos-methyl treated net caused approximately 80% mortality, whereas the other nets caused negligible mortality-rates of the mosquitoes.

Side-effects of sleeping under treated bednets

In ten out of 216 interviews (4.5%), sleepers reported some sort of adverse health experiences concurrent with using a bednet. These comprised two chest pains, four headaches and four stomach pains. No correlation between insecticide and symptoms was found. Four of the ten complaints came after a night sleeping under the untreated net and it is tempting to suggest that, in these cases, the sleepers were

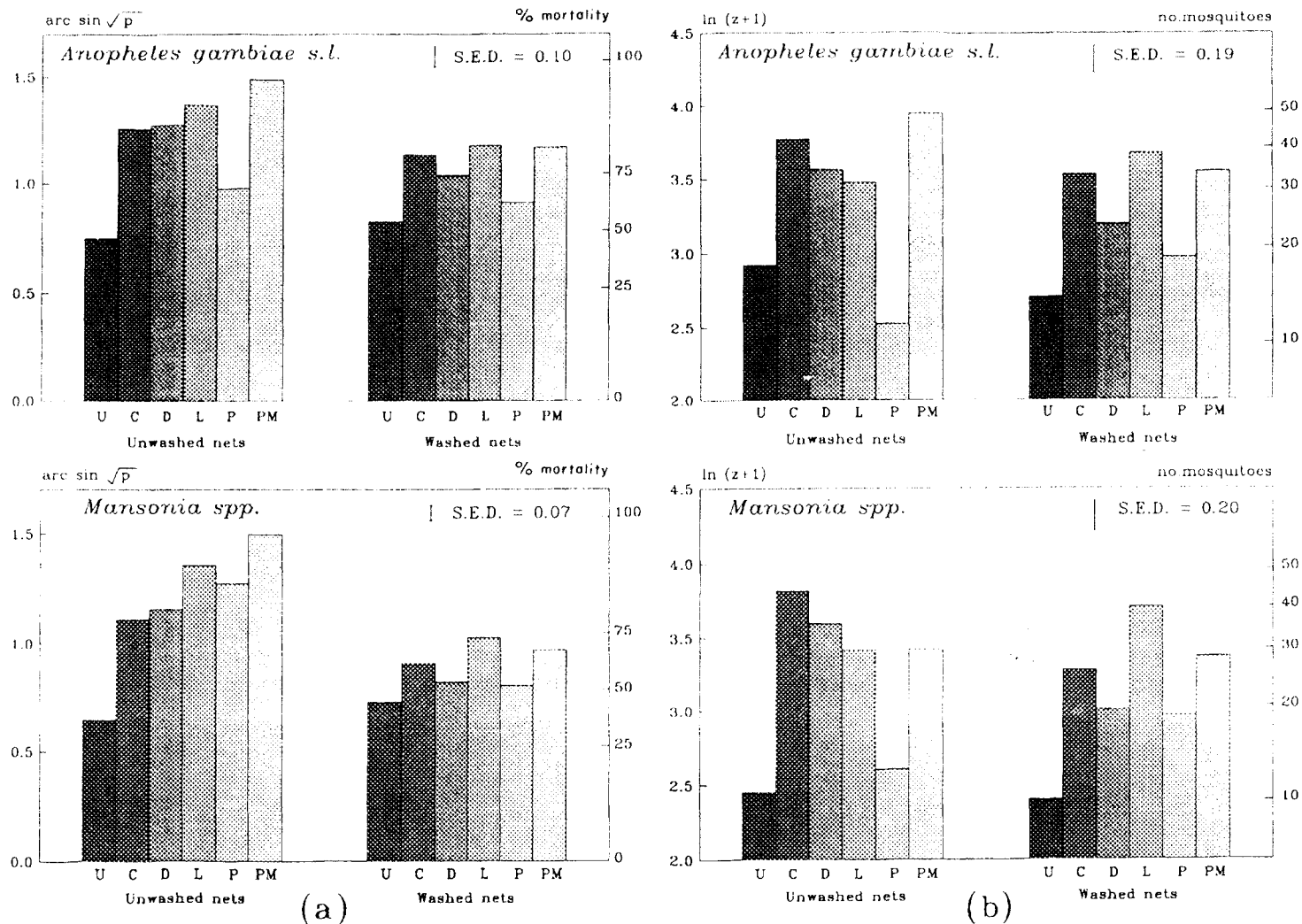


Fig. 2. Mosquito mortality: (a) percentage of mosquitoes which died after entering huts where a sleeper used a bednet (p), (b) numbers of mosquitoes killed (z). U, untreated bednet; C, cypermethrin impregnated; D, deltamethrin impregnated; L, lambda-cyhalothrin impregnated; P, permethrin impregnated; PM, pirimiphos-methyl impregnated. S.E.D., standard error of difference between means.

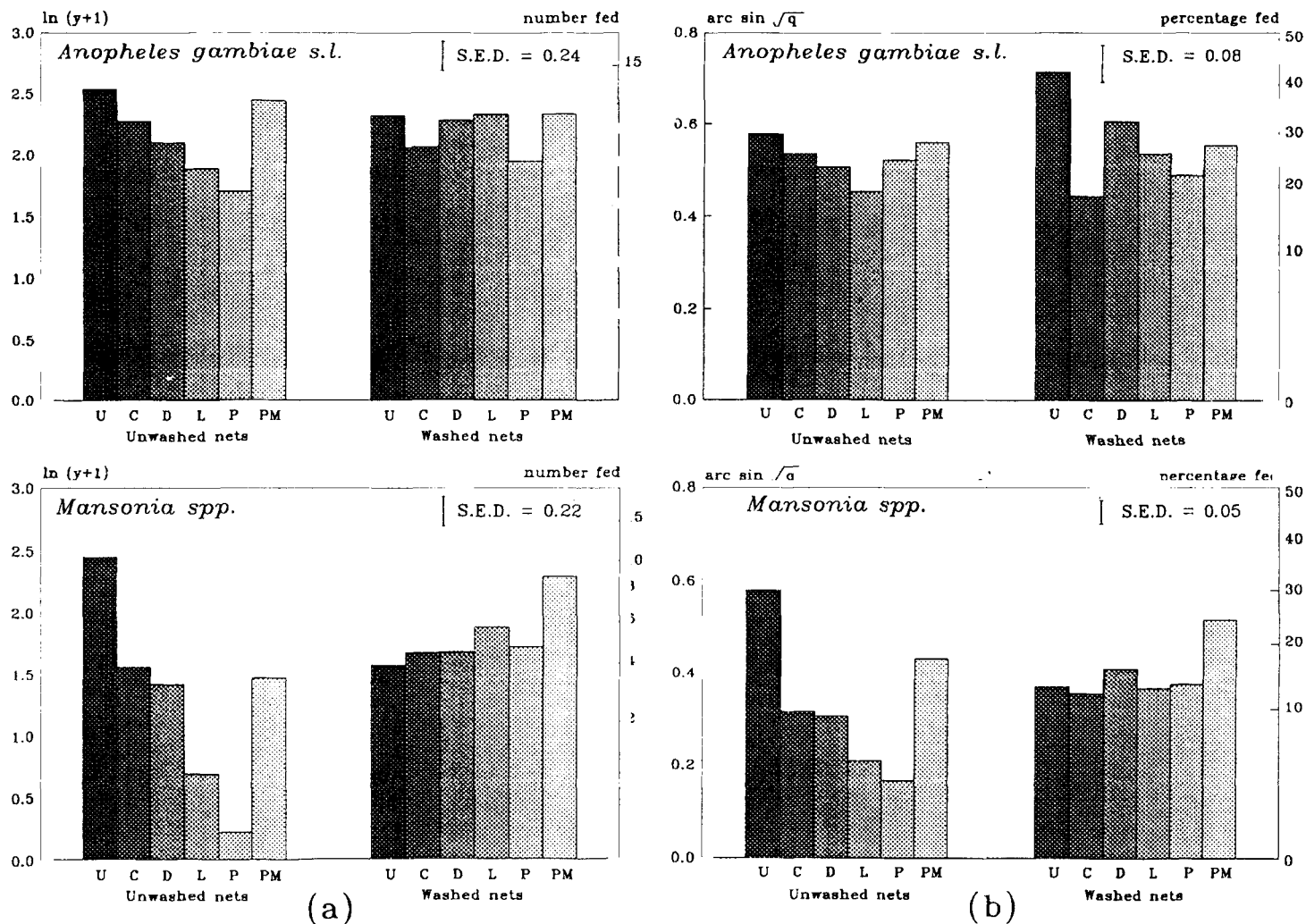


Fig. 3. Mosquito feeding: (a) mean total numbers of blood-fed mosquitoes (y), (b) percentage of blood-fed female mosquitoes collected from huts where a bednet had been used by a sleeper overnight (q). U, untreated bednet; C, cypermethrin impregnated; D, deltamethrin impregnated; L, lambda-cyhalothrin impregnated; P, permethrin impregnated; PM, pirimiphos-methyl impregnated. S.E.D., standard error of difference between means.

kept awake by mosquitoes and were consequently more aware of any ailments ($P \sim 0.05$).

Discussion

Despite careful impregnation of the nylon bednets by standard procedures, the resultant rates of insecticide treatment varied considerably from the target dosages, ranging from a minimum of only $0.1 \times$ the intended rate of lambda-cyhalothrin to $4 \times$ the intended rate of pirimiphos-methyl. Permethrin came closest ($1.3 \times$) to the target rate, indicating that uptake of this insecticide is neither selective nor inhibited. Pirimiphos-methyl treated bednets were more difficult to wring out and took much longer to dry than for any of the pyrethroid treated or untreated bednets. Nets treated with pirimiphos-methyl also had a pungent odour, felt slightly oily and became dirtiest, characteristics likely to increase their washing if used routinely under normal household conditions. Further use of each insecticide should ensure more accurate application of the target treatment rates and clarify the reasons for underdosage or overdosage, not encountered with permethrin (Hossain *et al.*, 1989).

The unwashed permethrin 670 mg/m^2 treated net exerted a deterrent effect against endophilic mosquitoes, whereby about 60% fewer *An.gambiae s.l.* and *Mansonia* spp. entered huts compared to those with an untreated net. Darriet *et al.* (1984) reported a similar reduction in the numbers of *An.gambiae s.l.* and *An.funestus* Giles entering huts containing cotton bednets treated with permethrin 80 mg/m^2 . This deterrent action of permethrin should be distinguished from an excito-repellent effect which occurs after mosquitoes have entered a hut and touched a treated surface (Davidson, 1953). Deterrence implies action at a distance and is difficult to explain with the very low vapour pressure of pyrethroids (Wells *et al.*, 1986; Miller, 1990), whereas the vapour toxicity (Gerolt, 1959) observed with pirimiphos-methyl is commensurate with its relatively high vapour pressure*.

* Vapour pressures expressed as mmHg: cypermethrin 1.4×10^{-9} at 20°C ; deltamethrin 1.5×10^{-8} at 25°C ; lambda-cyhalothrin 1.6×10^{-9} at 20°C ; permethrin 1.0×10^{-8} at 20°C ; pirimiphos-methyl 1.5×10^{-5} at 20°C .

Using the same experimental huts in The Gambia, Lindsay *et al.* (1991a) provided evidence that deterrence may be caused by other ingredients of EC formulations (non-ionic aromatic hydrocarbon solvents plus emulsifiers), not permethrin alone. For people using treated bednets, deterrence should be beneficial – particularly when the nets are damaged or used carelessly, but mosquitoes might be diverted to people sleeping without bednets. Lines *et al.* (1987) found that a child sleeping near someone protected by a bednet treated with permethrin 200 mg/m^2 received fewer bites than if no net was used by either of them: evidently permethrin-treated bednets, and curtains (Majori *et al.*, 1987), confer appreciable degrees of protection to everyone in the same room.

Considering the risk of mosquito populations being selected for resistance to pyrethroid insecticides used for community protection through mass bednet impregnation programmes, the deterrence of permethrin-treatment would cause less selection pressure than exerted by the other pyrethroids which were more insecticidal and less deterrent.

Prevention of mosquitoes probing and blood-feeding is a doubly important function of treated bednets, directly reducing transmission risks and allowing a good night's sleep. Thus our sleepers seemed to be less aware of medical ailments when using treated bednets. With untreated bednets, mosquitoes can probe through the net or enter through torn holes to bite the sleeper; engorged mosquitoes were invariably found resting inside untreated nets, not elsewhere in the hut or traps. With treated bednets, mosquitoes seldom remain in contact for long enough to probe successfully (Hossain & Curtis, 1989). Even so, Snow *et al.* (1987) found the proportions of blood-fed *An.gambiae s.l.* were not significantly different in rooms with and without permethrin-treated bednets. In our study also, insecticide-treated bednets did not decrease the percentages of bloodfed *An.gambiae s.l.* found in the experimental huts, although 41% of them were shown to have fed on animals outside. Previous studies in The Gambia by Boreham & Port (1982) highlighted three salient points: female mosquitoes may enter houses for shelter after bloodfeeding outdoors; bloodfed mosquitoes may move from house to house, but a large proportion become trapped inside torn bednets after bloodfeeding on people sleeping

therein. The first two points indicate that indoor-resting bloodfed female mosquitoes have not necessarily fed on the occupants of that house, as confirmed by our data.

Survival of bloodfed mosquitoes in the huts or traps indicates their vector potential. All unwashed impregnated bednets killed significant proportions of mosquitoes that entered the huts, but only pirimiphos-methyl killed all the bloodfed females. Of the four pyrethroids tested, lambda-cyhalothrin gave the highest observed mortality with both *Mansonia* and *An. gambiae s.l.* Washing significantly decreased the insecticidal efficacy of all the treatments, especially for permethrin and deltamethrin. The total number of *An.gambiae s.l.* killed by the permethrin-treated bednet did not differ significantly from the untreated bednet, presumably because mosquitoes were deterred from entering the hut with the permethrin impregnated net. As washing reduced the efficacy of all treatments, it is necessary to reimpregnate bednets after washing, unless a wash-resistant and effective treatment can be devised.

As the unwashed permethrin-treated net was associated with the lowest number of bloodfed mosquitoes, its deterrent effect seemed to provide the most personal protection. For community protection, however, mosquitoes must be killed before they become infective. So which effect of impregnated bednets is preferable? For individuals sleeping in rooms with permethrin-impregnated bednets or curtains, the deterrence of permethrin should be most advantageous. Since the other four insecticide treatments appeared to have more impact on mosquito survival, and hence on vectorial capacity, their potential advantages for the community would increase with the proportion of people protected by impregnated bednets.

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References

- Borcham, P.F.L. & Port, G.R. (1982) The distribution and movement of engorged *Anopheles gambiae* Giles in a Gambian village. *Bulletin of Entomological Research*, **71**, 489–495.
- Brun, L.O. & Sales, S. (1976) Stage IV evaluation of four organophosphate insecticides. Unpublished document WHO/VBC/76.630 19pp. World Health Organization, Geneva.
- Bryan, J.H., Di Deco, M.A., Petrarca, V. & Coluzzi, M. (1982) Inversion polymorphism and incipient speciation in *Anopheles gambiae* s.str. in The Gambia, West Africa. *Genetica*, **59**, 167–176.
- Cochran, W.G. & Cox, G. (1957) *Experimental Designs*. Wiley, New York.
- Coluzzi, M., Petrarca, V. & Di Deco, M.A. (1985) Chromosomal inversion intergradation and incipient speciation in *Anopheles gambiae*. *Bolletino di Zoologia*, **52**, 45–63.
- Curtis, C.F. (1987) Genetic aspects of selection for resistance. *Combating Resistance to Xenobiotics* (ed. by M. G. Ford *et al.*), pp. 150–161. Ellis Norwood, Chichester.
- Curtis, C.F. (ed.) (1989) *Appropriate Technology for Vector Control*. CRC Press, Boca Raton, Florida.
- Darriet, F., Robert, V., Tho Vien, N. & Carnevale, P. (1984) Evaluation of the efficacy of permethrin impregnated intact and perforated mosquito nets against vectors of malaria. Unpublished document WHO/VBC/84.99. 19pp. World Health Organization, Geneva.
- Davidson, G. (1953) Experiments on the effect of residual insecticides in houses against *Anopheles gambiae* and *Anopheles funestus*. *Bulletin of Entomological Research*, **44**, 231–254.
- Elliot, M., Jones, M.F. & Potter, C. (1978) The future of pyrethroids in insect control. *Annual Review of Entomology*, **23**, 443–469.
- Gerolt, P. (1959) 'Vapour toxicity' of solid insecticides. *Nature*, **183**, 1121–1122.
- Hossain, M.I. & Curtis, C.F. (1989) Permethrin impregnated bednets: behavioural and killing effects on mosquitoes. *Medical and Veterinary Entomology*, **3**, 367–376.
- Hossain, M.I., Curtis, C.F. & Heekin, J.P. (1989) Assays and bioassays of permethrin impregnated fabrics. *Bulletin of Entomological Research*, **79**, 299–308.
- ICI (1987) The determination of active ingredients on pyrethroid impregnated mosquito bednets. Analytical Method PAM 722, ICI Jealott's Hill Research Station, Berkshire RG12 6EY. 9pp.

- Leahey, J.P. (ed.) (1985) *The Pyrethroid Insecticides*. Taylor and Francis, London.
- Lindsay, S.W., Adiamah, J.H., Miller, J.E. & Armstrong, J.R.M. (1991a) Pyrethroid-treated bed-net effects on mosquitoes of the *Anopheles gambiae* complex in The Gambia. *Medical and Veterinary Entomology*, **5**, 477–483.
- Lindsay, S.W., Hossain, M.I., Bennett, S. & Curtis, C.F. (1991b) Preliminary studies on the insecticidal activity and wash-fastness of twelve pyrethroid treatments impregnated into bednetting assayed against mosquitoes. *Pesticide Science*, **32**, in press.
- Lindsay, S.W., Wilkins, H.A., Zieler, H.A., Daly, R.A., Petrarca, V. & Byass, P. (1991c) Ability of *Anopheles gambiae* mosquitoes to transmit malaria during the dry and wet seasons in an area of irrigated rice cultivation in The Gambia. *Journal of Tropical Medicine and Hygiene*, in press.
- Lines, J.D., Myamba, J. & Curtis, C.F. (1987) Experimental hut trials of permethrin impregnated mosquito nets and eave curtains against malaria vectors in Tanzania. *Medical and Veterinary Entomology*, **1**, 37–51.
- Li Zu-zi (1987) Practical evaluation of deltamethrin treated mosquito nets against *Anopheles sinensis* populations and malaria prevention. *IV Congrès sur la protection de la santé humaine et des cultures en milieu tropical*, p. 627.
- Majori, G., Sabatinelli, G. & Coluzzi, M. (1987) Efficacy of permethrin-impregnated curtains for malaria vector control. *Medical and Veterinary Entomology*, **1**, 185–192.
- Miller, J.E. (1990) Laboratory and field studies of insecticide impregnated fibres for mosquito control. Ph.D. thesis, University of London.
- Payne, C.D. (ed.) (1986) *The GLIM System Release 3.77 Manual*. NAG, Oxford.
- Rapley, R.E. (1961) Notes on the construction of experimental huts. *Bulletin of the World Health Organization*, **24**, 659–663.
- Smith, A. (1965) A verandah-trap hut for studying the house frequenting habits of mosquitoes and for assessing insecticides. I. A description of the verandah-trap hut and of studies of the egress of *Anopheles gambiae* Giles and *Mansonia uniformis* (Theo.) from an untreated hut. *Bulletin of Entomological Research*, **56**, 161–165.
- Snow, R.W., Jawara, M. & Curtis, C.F. (1987) Observations on *Anopheles gambiae* Giles s.l. (Diptera: Culicidae) during a trial of permethrin treated nets in The Gambia. *Bulletin of Entomological Research*, **77**, 279–286.
- Travis, B.V. (1946) Effectiveness of DDT as a residual treatment of bednets. *Mosquito News*, **6**, 25–30.
- VCRC (1990) Village scale trial with lambdacyhalothrin impregnated bed-nets. *Annual Report 1990, Vector Control Research Centre, Pondicherry 605006, India*, pp. 35–37.
- Wells, D., Grayson, B.T. & Lagnier, E. (1986) Vapour pressure of permethrin. *Pesticide Science*, **17**, 473–476.
- WHO (1985) *Safe Use of Pesticides*. Technical Report Series No. 720. World Health Organization, Geneva.

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