Medical and Veterinary Entomology (1991) **5**, 477–483

Pyrethroid-treated bednet effects on mosquitoes of the *Anopheles gambiae* complex in The Gambia

S. W. LINDSAY, J. H. ADIAMAH, J. E. MILLER* and J. R. M. ARMSTRONG Medical Research Council Laboratories, Fajara, The Gambia, and *London School of Hygiene and Tropical Medicine

Abstract. The response of *Anopheles gambiae* complex mosquitoes to men sleeping under insecticide-impregnated or untreated bednets in six verandah trap huts was studied during the dry season in The Gambia. With this type of hut it was possible to collect live and dead indoor-resting mosquitoes and estimate the number of wild mosquitoes which entered, bloodfed on man, and exited each night. Bednets were treated with emulsions targetted to leave deposits of 25 mg/m^2 lambda-cyhalothrin, or 5, 50 or 500 mg/m^2 permethrin, diluted from emulsifiable concentrates (EC), or a blank formulation similar to the EC except that the permethrin was omitted; the sixth net was left untreated. Nets and sleepers were rotated between huts on different nights, the design being based on a series of Latin squares and conducted double-blind.

Permethrin-impregnated bednets deterred mosquitoes from entering the huts. The degree of deterrency was proportional to the dosage of permethrin. This effect was also caused by the blank formulation and therefore attributed to other components of the formulation, rather than to the permethrin itself. The net impregnated with 500 mg permethrin per square metre gave the best individual protection, reducing mosquito bloodfeeding by 91% compared with untreated nets. However, lambda-cyhalothrin was proportionately more insecticidal than permethrin at doses of equivalent deterrency. At this stage of research, it remains conjectural whether chemical deterrency or killing of malaria vectors is better for community protection.

Key words. *Anopheles gambiae*, experimental huts, insecticide-treated bednets, lambda-cyhalothrin, mosquito bednets, mosquito behaviour, malaria vector control, permethrin, The Gambia.

Introduction

Insecticide-treated bednets are being used increasingly to control malaria mosquitoes (Rozendaal, 1989; W.H.O., 1989; Curtis, 1989). Permethrin is a pyrethroid insecticide used for impregnating nets and it has been demonstrated that, in addition to mosquitoes being killed by contact with permethrin-treated bednets, protection is due also to deterrency against

Correspondence: Dr S. W. Lindsay, Department of Zoology, University of Glasgow, Glasgow G12 8QQ, Scotland, U.K.

house-entry by mosquitoes. In Burkina Faso, for example, bednets treated to give a target dose of 80 mg permethrin per square metre of netting, using aqueous dilution of permethrin 20% emulsifiable concentrate (EC) supplied by the Wellcome Foundation, gave a 69% reduction in the numbers of female *Anopheles gambiae* Giles, *sensu lato*, entering experimental huts, compared with huts having untreated bednets (Darriet *et al.*, 1984). A similar result was found in The Gambia using permethrin (25% EC from ICI) at a treatment rate of $670 \pm 160\,mg/m^2$ which reduced house entry by 60% for *An.gambiae s.l.* and by 68% for *Mansonia* spp. (Miller *et al.*, 1991). However, a trial in Tanzania (Lines *et al.*, 1987) found little evidence of deterrency for *An.arabiensis* Patton due to bednets treated with permethrin (10% EC from Wellcome) at a target rate of $200\,mg/m^2$. That permethrin should have deterrent properties, putatively due to air-borne effects on the behaviour of potentially endophilic mosquitoes, may seem surprising since this chemical has a very low vapour pressure (Wells *et al.*, 1986) and therefore is not very volatile.

It has been suggested that the spectrum of insect responses to pyrethroids depends on the dose of insecticide (Ruscoe, 1977; Hervé, 1982; Lindsay *et al.*, 1989). At highest concentrations these chemicals deter insects from contact or serve as contact irritants. With decreasing doses, pyrethroids act as contact insecticides, knockdown agents and feeding inhibitors, with various other effects attributed to the lowest sub-lethal dosages. Nearly all this information comes from agricultural entomology and little work has been done on the reactions of mosquitoes to pyrethroids. We have therefore undertaken a study with three main objectives: to investigate the response of mosquitoes to a range of permethrin doses on bed-nets; to determine whether the solvents and emulsifiers used in permethrin EC formulations might be deterrent; and to find out whether a non-deterrent dose of permethrin (as determined empirically in this study) would be as insecticidal as the pyrethroid lambda-cyhalothrin, trademark 'Icon' (ICI, 1989). Treatment of bednets with lambda-cyhalothrin at a rate of $2.6\,mg/m^2$ was found to be non-deterrent and highly insecticidal against mosquitoes in an experimental hut trial by Miller *et al.* (1991) in The Gambia.

Materials and Methods

Experimental huts. The study was conducted at Wali Kunda in The Gambia, during the dry season of 1989, using the line of six experimental huts described by Miller *et al.* (1991) situated in an area of open grassland fringed by trees. Internally, each hut had a single room with one bed over which a bednet was hung to protect the sleeper at night. To prevent ants entering and foraging for dead mosquitoes, each hut had water-filled channels around concrete pillars supporting the raised floor. A large area of irrigated rice cultivation, about 1 km from the huts, was the main local breeding site for mosquitoes (Lindsay *et al.*, 1991). Adult mosquitoes entered the huts through open eaves on the sides parallel to the nearest edge of the irrigated area. Exiting mosquitoes were collected from window traps and enclosed verandahs on the other two sides.

Bednets and treatment. Six nylon bednets were used, each with an area of $14\,m^2$ approximately. To simulate badly torn nets and allow mosquitoes easier access to the sleeper, six squares $10 \times 10\,cm$ were cut from each treated bednet, as described by Miller *et al.* (1991). These six treated pieces of each unused net, plus six comparable samples taken from each net after 6 weeks of field usage, were assayed by gas chromatography (GC) to determine the actual insecticide dosage on each net at the start and end of the study (ICI, 1987).

Using impregnation procedures described by Lines *et al.* (1987), three bednets were treated with permethrin 25% EC (ICI trademark 'Imperator' 25EC) at target rates of 5, 50 and $500\,mg/m^2$. One net was treated with a permethrin-free 'base formulation' (supplied by ICI to comprise all the same ingredients as 'Imperator' except permethrin) at a rate equivalent to that applied to the net with $500\,mg/m^2$ permethrin. The fifth net was treated with lambda-cyhalothrin 2.5% EC (ICI trademark 'Icon' 2.5EC) targetted to give a concentration of $25\,mg/m^2$ on the netting. The sixth bednet was left untreated and used as the control.

Study design. Each bednet was used nightly by one sleeper in each experimental hut, following a protocol modified from that of Miller *et al.* (1991), with a one night break each Saturday during the 6-week trial. Sleepers went inside at 22.00 hours and came out at 07.00 hours the following morning. Mosquitoes were then collected from the room, both enclosed verandahs and the two exit traps of each hut. Each hut search lasted 45 min, starting soon after 07.00 hours. The numbers of unfed and bloodfed mosquitoes dead and alive, and their distribution within the huts, were recorded for each hut.

Mosquito bloodmeals of human origin were identified using an ELISA technique similar to that described by Burkot *et al.* (1981). The mean optical density of the negative controls (mosquitoes caught feeding on cattle) was determined and all bloodmeals giving readings greater than two standard deviations above this mean were assumed to be from humans.

The study design was based on Latin squares (Cochran & Cox, 1957) and was conducted double-blind. Each of the six sleepers was allocated to a different hut each week, the six nets to different huts on each of the six nights of the week.

Statistical analysis. Where appropriate, counts of mosquitoes were log transformed and proportions angular transformed to normalize the data. Analysis of variance was carried out using the statistical package GLIM (Payne, 1986), to partition variation into treatments, sleepers, huts and nights.

Results

Chemical analysis

Impregnations resulted in uneven distributions of insecticides on netting, with a wide range of concentrations between different parts of a treated net (Table 1).

Mosquito population

The principal vectors of malaria in the study area were freshwater members of the *An.gambiae* complex mainly *An.arabiensis* and *An.gambiae* Giles, *sensu stricto* (Lindsay *et al.*, 1991). The number of mosquitoes entering each hut was estimated by doubling the verandah trap catches and adding this to the numbers found in the room and exit traps for each night of the study. Estimated totals of 8462 *An.gambiae s.l.*, 3181 *An.rufipes* Gough, 1052 *An.pharoensis* Theobald and 818 culicines entered the huts during the 36 nights of the trial.

Deterrency

Similar levels of deterrency were shown by nets treated with 500 mg/m^2 permethrin and the equivalent rate of base (non-insecticidal) formulation. At lower doses of permethrin, the number of mosquitoes found in the huts increased (Fig. 1).

The proportion of exiting mosquitoes which were found in the verandah of huts with nets impregnated with 500 mg permethrin/m^2 (95% CI = 20.4–31.0%) was similar to that for huts with untreated nets (95% CI = 18.7–29.3%).

For huts with nets treated with 500 mg permethrin/m^2 and those with the base formulation, nightly deterrency was calculated from the ratio:

$$\frac{\text{No. mosquitoes in untreated hut}}{\begin{array}{c}\text{No. mosquitoes in}\\ \text{treated + untreated huts}\end{array}}$$

During the latter half of the trial, the deterrent effect was less than at the beginning, both for the net treated with the base formulation (t test = 2.97, 34 d.f., $P < 0.01$) and the 500 mg permethrin-impregnated net (t test = 1.98, 34 d.f., $P \sim 0.05$). The net treated with 25 mg/m^2

Table 1. Dosage of insecticide mg/m^2, determined by GC assay, on freshly treated netting and after 6 weeks use.

Insecticide	Target concentration	Geometric mean at start of trial (range)	Geometric mean at end of trial (range)
Permethrin	500	142 (51–550)	305 (130–630)
Permethrin	50	16 (11–60)	4 (3–7)
Permethrin	5	9 (4–36)	1 (0.2–4)
Lambda-cyhalothrin	25	14 (8–30)	10 (3–39)

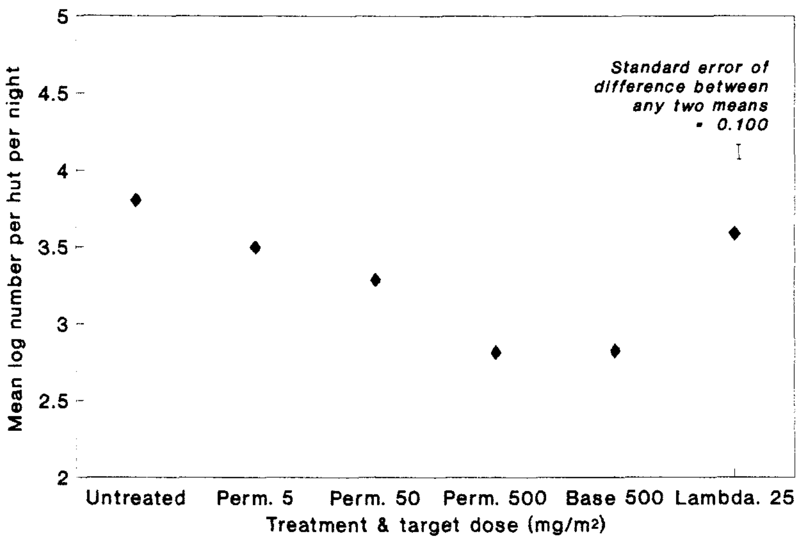

Fig. 1. Deterrency: responses of *An.gambiae s.l.* to treated bednets.

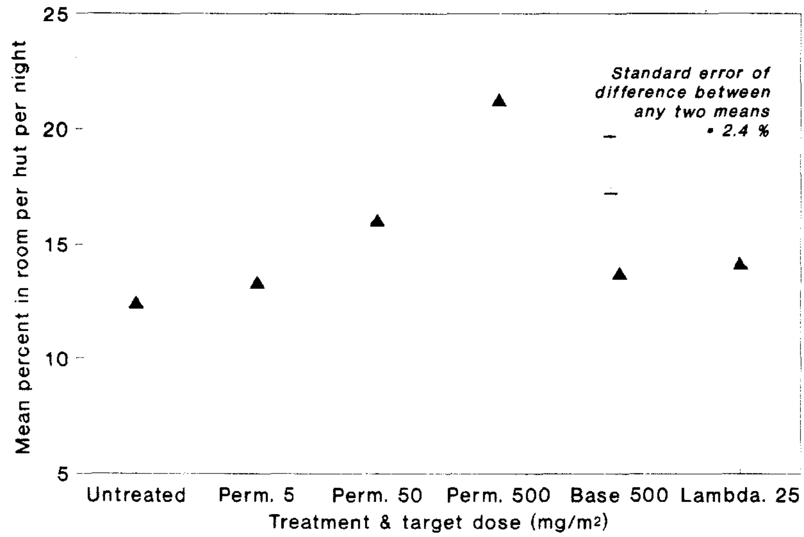

Fig. 2. Irritancy: responses of *An.gambiae s.l.* to treated bednets.

lambda-cyhalothrin/m^2 had a deterrency similar to the lowest dose of permethrin, 5 mg/m^2, as judged from the numbers of mosquitoes found in huts plus their verandahs and exit traps (Fig. 1).

Irritancy

At higher doses of permethrin there was no evidence that the insecticide acted as a contact irritant, increasing the rate at which mosquitoes fly out of a room (i.e. excito-repellency). The proportion of mosquitoes found indoors actually increased with permethrin dosage (Fig. 2). This effect was due mainly to the higher rates of mosquito mortality that occurred in huts having nets treated with high concentrations of permethrin. Nor was irritancy detected for nets treated with lambda-cyhalothrin or the base used for formulating the permethrin emulsion (Fig. 2).

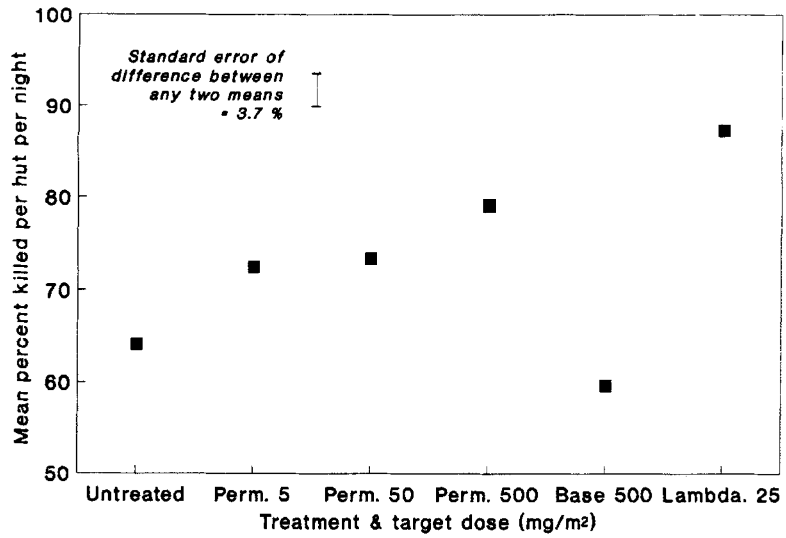

Fig. 3. Killing: reponses of *An.gambiae s.l.* to treated bednets.

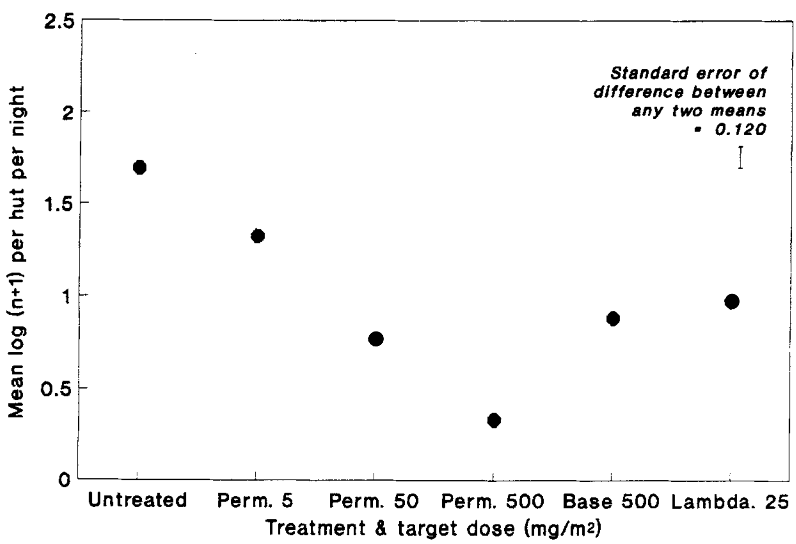

Fig. 4. The protective effects of treated bednets against *An.gambiae s.l.*: human bloodfed mosquitoes.

Killing

Lambda-cyhalothrin 25 mg/m^2 and permethrin 500 mg/m^2 proved to be the most effective treatments evaluated in this study (Fig. 3). This dose of lambda-cyhalothrin killed nearly 90% of *An.gambiae s.l.* entering the huts. However, the best personal protection was achieved with 500 mg/m^2 permethrin (Fig. 4), which reduced the number of human bloodfed mosquitoes by 91% compared with the untreated net.

Discussion

Impregnation of bednets with pyrethroid emulsion produced uneven distributions of the chemicals on netting, despite the care taken with these individual treatments. Variation in the dosage rate is inevitable when mass dipping and drying is carried out during vector control programmes.

Since the numbers of mosquitoes entering huts with nets treated with 500 mg permethrin per square metre and an equivalent rate of base emulsion (the same dilution of the same formulation except that it lacked the insecticide) were similarly reduced, as compared to huts with untreated nets or nets treated with lower doses of permethrin, deterrency seems to be due to components of the emulsifiable concentrate other than the insecticide itself.

Could apparent deterrency be caused by excito-repellency? If mosquitoes which come into contact with permethrin-treated netting become hyperactive they may fly into the enclosed verandahs, continue to search for an exit, and enter the room again before flying out through one of the open verandahs. If the deterrent effect was an artefact due to excito-repellency, and assuming that all mosquitoes entering window traps remained there, one would expect to find a smaller proportion of exiting mosquitoes in verandahs of huts with the permethrin-treated bednet than with the untreated net. This was not the case, however, indicating that deterrency was a real phenomenon and not due to excito-repellency.

This study demonstrates that mosquito behaviour can be affected by 'inert ingredients' of an insecticide formulation. In the ICI permethrin formulation ('Imperator' 25% EC) 50–60% of the emulsifiable concentrate consists of ionic and nonionic aromatic hydrocarbon solvents. Presumably it is these volatile chemicals which deter mosquitoes and explains why the deterrency decreases over time. This is an important finding since insecticide manufacturers may inadvertently produce an insecticide formulation with deterrent rather than lethal properties. It would seem better to kill mosquitoes rather than to deter them from entering a house with a treated bednet, since such deterred mosquitoes might later succeed in feeding on people outdoors or in untreated houses. However, this line of argument has not been investigated sufficiently in the field.

The hypothesis advanced by Ruscoe (1977), Hervé (1982) and Lindsay *et al.* (1989) that progressively decreasing the dosage of pyrethroids results in a spectrum of response ranging from deterrent, to contact irritant, to contact killer, to knock-down agent, to feeding inhibitor and lesser effects at lower sub-lethal dosages is not supported by the results of the present study on wild Afrotropical *Anopheles*. There is no evidence that a high concentration of permethrin acts only as a deterrent against mosquitoes. Moreover, deterrency and killing are both proportional to the concentration of permethrin and these effects decrease progressively with dose. The highest rate of permethrin 500 mg/m^2 on a bednet reduced the number of mosquitoes entering a house by 63%, compared with an untreated net, in agreement with our earlier findings (Miller *et al.*, 1991). Moreover, the net with this high dose of permethrin reduced the number of bites by 91% compared with the control net. Similar reductions in biting have been demonstrated in village trials of bednets treated with permethrin 500 mg/m^2 in The Gambia (Lindsay *et al.*, 1989). For personal protection, therefore, nets impregnated with a dose of 500 mg permethrin/m^2 should be preferred. However, for mass protection of a community using impregnated bednets against vectors the choice is between permethrin, which is a good deterrent and killer, and relatively low dosage of lambda-cyhalothrin having little deterrency and better killing effects on mosquitoes. The case for using an insecticide with little deterrency remains to be proven.

Acknowledgments

This study was financed largely by the Medical Research Council of the U.K. We would like to thank ICI Public Health for providing the chemicals, financing the huts and for the studentship for Jane Miller. We are also grateful for the fieldwork of Yusupha Sowe, the serology carried out by Fatou Ceesay and the constructive criticism of the manuscript offered by Drs Chris Curtis and Mark Rowland.

References

Burkot, T.R., Goodman, W.G. & DeFoliart, G.R. (1981) Identification of mosquito blood meals by enzyme linked immunosorbent assay. *American Journal of Tropical Medicine and Hygiene*, **30**, 1336–1341.

Cochran, W.G. & Cox, G.M. (1957) *Experimental Designs*. Wiley, New York.

Curtis, C.F. (1989) *Appropriate Technology for Vector Control*. CRC, Boca Raton.

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. Document No. 10/RAP/CM.ENT.84, Centre Muraz, Bobo-dioulasso, Burkina Faso and Document WHO/VBC 84.899 and WHO/MAL84.1008.

Hervé, J.J. (1982) Le mode d'action des pyrethrinoides et le probleme de la resistance a ces composes. *Monographie Deltamethrine (Roussel-Uclaf)*, 67–107.

ICI (1987) The determination of active ingredients on pyrethroid impregnated mosquito bednets. Unpublished document PAM/722/1. 9pp. ICI Jealott's Hill Research Station, Bracknell, Berkshire RG12 6EY.

ICI (1989) 'Icon' public health insecticide technical profile. 28pp. ICI Public Health, Haslemere, Surrey GU27 3JE.

Lindsay, S.W., Snow, R.W., Broomfield, G.L., Semega Janneh, M., Wirtz, R.A. & Greenwood, B.M. (1989) Impact of permethrin-treated bednets on malaria transmission by the *Anopheles gambiae* complex in The Gambia. *Medical and Veterinary Entomology*, **3**, 263–271.

Lindsay, S.W., Wilkins, H.A., Zieler, H.A., Daly, R.J., Petrarca, V. & Byass, P. (1991) 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 curtains against malaria vectors in Tanzania. *Medical and Veterinary Entomology*, **1**, 37–51.

Miller, J.E., Lindsay, S.W. & Armstrong, J.R.M. (1991) Experimental hut trials of bednets impregnated with synthetic pyrethroid and organophosphate insecticides for mosquito control in The Gambia. *Medical and Veterinary Entomology*, **5**, 465–476.

Payne, C.D. (1986) The GLIM system release 3.77 Manual. NAG, Oxford.

Rozendaal, J.A. (1989) Impregnated mosquito nets and curtains for self-protection and vector control. *Tropical Diseases Bulletin*, **86**, R1–41.

Ruscoe, C.N.E. (1977) The new NRDC pyrethroids as agricultural insecticides. *Pesticide Science*, **8**, 236–242.

Wells, D., Grayson, B.T. & Lagner, E. (1986) Vapour pressure of permethrin. *Pesticide Science*, **17**, 473–476.

W.H.O. (1989) The use of impregnated bednets and other materials for vector-borne disease control unpublished document WHO/VBC/89.981, 44 pp. World Health Organization, Geneva.

Accepted 10 October 1990