Medical and Veterinary Entomology (1996) **10**, 1–11

Comparison of different insecticides and fabrics for anti-mosquito bednets and curtains

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Abstract. Various formulations of six insecticides (a carbamate and five pyrethroids), were impregnated into bednets and curtains made from cotton, polyester, polyethylene or polypropylene fabric. For bioassays of insecticidal efficacy, female *Anopheles gambiae* mosquitoes were made to walk on the fabrics for 3 min and mortality was scored after 24 h. The main concentrations tested were: bendiocarb 400 mg/m^2, cyfluthrin 30–50 mg/m^2, deltamethrin 15–25 mg/m^2, etofenprox 200 mg/m^2, lambda-cyhalothrin 5–15 mg/m^2 and permethrin 200–500 mg/m^2. Field trials in Tanzania used experimental huts (fitted with verandah traps) entered by wild free-flying *Anopheles gambiae, An.funestus* and *Culex quinquefasciatus* mosquitoes.

Results of testing the impregnated fabrics in experimental huts showed better personal protection provided by bednets than by curtains. Permethrin *cis:trans* isomer ratios 25:75 and 40:60 were equally effective, and the permethrin rate of 200 mg/m^2 performed as well as 500 mg/m^2. Bioassay data emphasized the prolonged insecticidal efficacy of lambda-cyhalothrin deposits, except on polyethylene netting. Most of the impregnated nets (including the 'Olyset' net with permethrin incorporated during manufacture of the polyethylene fibre) and an untreated intact net performed well in preventing both *Anopheles* and *Culex* mosquitoes from feeding on people using them overnight in the experimental huts. *Anopheles* showed high mortality rates in response to pyrethroid-treated nets, but only bendiocarb treated curtains killed many *Culex*. Holed nets treated with either cyfluthrin (5 EW formulation applied at the rate of 50 mg a.i./m^2) or lambda-cyhalothrin (2.5 CS formulation at 10 mg a.i./m^2) performed well after 15 months of domestic use. Treatment with deltamethrin SC or lambda-cyhalothrin CS at the very low rate of 3 mg/m^2 gave good results, including after washing and re-treatment.

Key words. *Anopheles funestus, An.gambiae, Culex quinquefasciatus*, bednets, curtains, experimental huts, impregnated fabrics, malaria, personal protection, vector control, bendiocarb, cyfluthrin, deltamethrin, etofenprox, lambda-cyhalothrin, permethrin, cotton, polyester, polyethylene, Tanzania.

Introduction

In most malarious regions of the world, for enhanced personal and community protection against the vector *Anopheles* mosquitoes, increasing use is being made of insecticide-impregnated bednets or curtains (Curtis, 1991, 1992; Sexton, 1994). However, remarkably little attention has been given to comparative trials to determine which insecticides, doses and types of fabric are most effective. Some researchers consider that, in small houses, impregnated curtains – draped inside the walls, across doorways, windows and/or under the open eaves – are more appropriate than bednets (Majori *et al.*, 1987; Mutinga *et al.*, 1992). Of those using bednets, in China and francophone African countries deltamethrin-treated cotton nets are generally chosen (Chen *et al.*, 1995; Carnevale *et al.*, 1988). A few researchers have decided to use cyfluthrin (Yadav & Sharma, 1994), etofenprox (Le Dinh Cong, in Curtis, 1993) or lambda-cyhalothrin (Hii *et al.*, 1995). In most other cases the insecticide used has been permethrin at a target application rate of 500 mg active ingredient per square metre of treated fabric, though the basis of this choice of concentration is unclear: Lines *et al.* (1987) reported no better reduction of mosquito blood-feeding nor greater

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mortality by using more permethrin than 200 mg/m^2.

Curtains and bednets have been compared for their impact on malaria (Sexton *et al.*, 1990; Beach *et al.*, 1993). Also, in village-scale trials of impregnated bednets, deltamethrin was compared with permethrin (data from Jiangsu Province, China, quoted by Li Zuzi and Lu Baolin in Curtis, 1991) and lambda-cyhalothrin was compared with permethrin by Njunwa *et al.* (1991) and with deltamethrin by Njau *et al.* (1993). None of these studies conclusively demonstrated any difference between the efficacy of these compounds, but it should be noted that permethrin was used at about a 10 × higher dose than the other compounds.

The insecticidal effect of impregnated materials is commonly tested by bioassays in which mosquitoes are made to walk on nets for an arbitrarily chosen exposure time of 3 min (W.H.O., 1989). Miller (1994) reported a systematic comparison by bioassay of three different pyrethroids on each of three different types of netting. Testing at intervals, with or without washing of the material, enables a relative measure of the persistence of the insecticidal effect (Njunwa *et al.*, 1991). However, more realistic and complete assessments are obtainable with free-flying wild mosquitoes attracted into human-baited experimental huts (Fig. 1) fitted with window-traps and verandah traps on two of the four sides – to catch a known proportion (one half) of mosquitoes exiting through the eave gaps during the night, the huts being built on ant traps to exclude scavenging insects so that killed mosquitoes can be found and counted in the morning (Smith & Webley, 1969). Examination of the live and dead mosquitoes enables the proportion of blood-fed individuals (both surviving and dying after feeding), and hence the extent of personal protection, to be assessed.

We have reported several such trials in Tanzania (Lines *et al.*, 1987; Curtis *et al.*, 1992, 1994) and almost identical huts were built and used for more comparisons in The Gambia (Miller *et al.*, 1991; Lindsay *et al.*, 1991; Pleass *et al.*, 1993). Gokool *et al.* (1993) have established by a PCR-DNA profiling method that the majority of blood-fed *Anopheles gambiae* found in a hut had fed on the sleepers in that hut, even when intact impregnated nets are in use.

The present paper reports results of 4 years work at Ubwari Field Station, Muheza (lat. 5°11′5, long. 38°50′E) in Tanzania testing a range of different insecticides, doses and fabrics by bioassay and in experimental huts. Data from the huts are presented in terms of percentage blood-feeding – a measure of the failure of personal protection – and mortality which can be related to the likely mass effect on the vectorial capacity (*sensu* Garrett-Jones & Grab, 1964) of the mosquito population if the impregnated material was widely used in a community.

Methods and Materials

Bioassays. Transparent plastic bioassay cones (see Fig. 71, p. 154 in W.H.O., 1975) were attached with rubber bands to the tops and sides of impregnated nets used by local residents of villages near Ubwari Field Station, Tanzania. Test batches of ten to twenty blood-fed *An.gambiae* females, caught in a nearby village (where there were no treated nets), were put in the cones for a 3 min period (for most of which they were seen to walk on the netting) and then removed with an aspirator. Controls were exposed to untreated netting brought to the same houses where impregnated nets were in use. Each test batch of mosquitoes was held in a netting-covered paper cup and provided with a pad of glucose solution for nourishment. They were returned to the Field Station laboratory for scoring the number knocked down 1 h post-exposure and the mortality-rate after 24 h holding under ambient climatic conditions.

Experimental huts. Two experimental huts were fitted with verandah and window traps for exiting mosquitoes (Fig. 1, from Curtis *et al.*, 1992). Periodically we verified the effectiveness of the ant-proofing and of our ability to recover mosquitoes

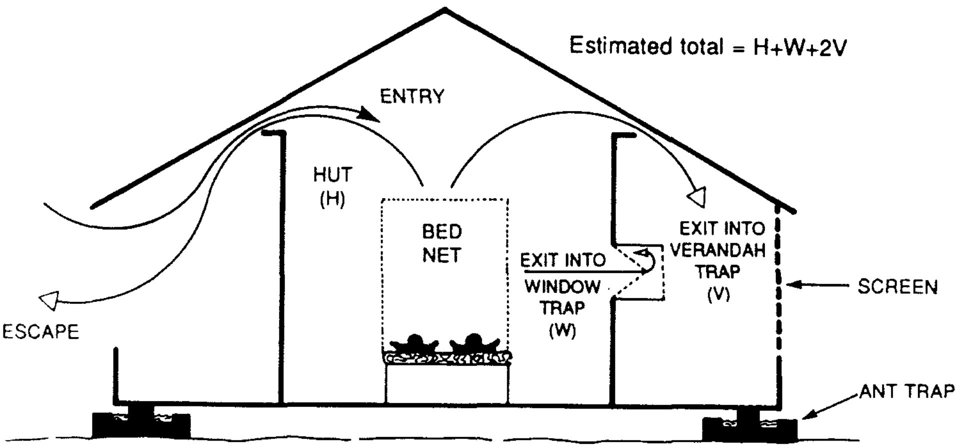

Fig. 1. Experimental hut: cross-section showing a pair of sleepers under a bednet within the single room: dark arrow shows how mosquitoes may enter the room via the open eave above the wall; open arrows show how mosquitoes may leave the room via the open eaves, either escaping completely (left side) or entering the window trap or verandah trap (right side of diagram). The number of mosquitoes entering a hut, on any night, is estimated by adding the number found in the hut (H), in the window trap (W) plus 2 × those in verandah traps (2V, mosquitoes trapped in verandahs on two sides of the hut). Diagram reproduced from Curtis *et al.* (1992) and Gokool *et al.* (1992) by courtesy of the publishers.

(dead or alive) which had been released in the huts during the night. Cotton pads of glucose solution were provided at various points in the huts and verandah and window traps to minimize deaths of mosquitoes from starvation. To attract mosquitoes into the experimental huts overnight, two boys slept on a bed in one hut while two girls slept in the other hut from 20.00 until 06.00 hours. As the homes of the sleepers are in houses neighbouring the experimental huts, they were not exposed to mosquito biting which they would not normally experience. Each sleeper had signed his/her prior informed consent to take part in the experiments. Our work and procedures received clearance from the ethical committees of the Tanzanian National Institute for Medical Research and the London School of Hygiene and Tropical Medicine.

After careful searches in the morning of the huts, window traps and verandah traps for live and dead mosquitoes, all specimens collected were returned to the Field Station, identified to species, classified by abdominal condition and held for 24 h to record any delayed mortality or revival of knocked down mosquitoes. Following each night's operation, collection data from each experimental hut were classified to give the nightly numbers of (i) unfed and (ii) fed+semi-gravid, live and dead mosquitoes of each species: *An.gambiae* Giles *sensu stricto* (this sibling species of the *An.gambiae* complex predominates in our study area, according to Mnzava & Kilama, 1988), *An.funestus* Giles and *Culex quinquefasciatus* Say. Occasional occurrence of other species and of male or gravid female mosquitoes was noted but they are not included in the results presented here. Each night's collections inside a hut plus its window traps were added to the collection in the two screened verandah traps, multiplied by 2. This multiplication was to allow for the inevitable unrecorded escapes through the other two verandahs which were left unscreened to allow routes for entry of wild mosquitoes via the 1 cm gaps under the eaves (Smith & Webley, 1989). Weekly in each of the two huts, five different treated materials were tested overnight. Also each experimental hut was evaluated with no net or curtain overnight once per week. At the end of each week the screening was moved from the north and south verandahs to the east and west ones, or vice versa, so as to compensate in the long run for possible biased sampling due to any tendency of mosquitoes to exit selectively via the eave gap to the verandah in one compass direction (e.g. towards the rising sun). For each 'experiment' the same set of treated materials was tested for a total of 6 weeks in both huts, totalling 12 replicate nights of each treatment. Before another experiment began, the huts were carefully cleaned to minimize the risk of insecticidal contamination of the huts, the occasional occurrence of which was signalled by unusual mortality of mosquitoes from nights when no net or curtain was used.

Fabrics tested. Most of the tests were with (i) bednets made of white 100 denier polyester fibre, with 156 meshes per square inch (i.e. mesh size of about 1.5 mm), made by SiamDutch Mosquito Netting Co. Ltd, Bangkok, Thailand. Also, tests were undertaken with (ii) bednets of 4 mm mesh, made by Swiss Net Co. Ltd, Nottingham, U.K. (supplied by Medical Advisory Service for Travellers Abroad, London, U.K.); (iii) polyethylene fibre bednets, blue-green colour, made by Unison Co., Manila, The Philippines; (iv) 'Olyset' nets (Sumitomo Chemical Co., Osaka, Japan), 60 mesh/inch^2 (i.e. 4 mm mesh), 220 denier, made from blue polyethylene with permethrin 2% (w/w) incorporated during fibre extrusion; (v) bednets formed from polypropylene sacking material available in Tanzania and stripped of its horizontal strands, except for three bands which were left to strengthen the material (see Fig. 1 in Curtis, 1990); (vi) eave curtains made of black 4 mm polyester netting, 50 cm deep, hung from strings under the eaves around all four walls of an experimental hut; (vii) curtains made of cotton sheeting, either black or white in colour, 1 m deep and 11 m wide, attached to the top or the bottom of the walls, or both.

One series of tests was made with the standard polyester bednets deliberately damaged by cutting six 4×4 cm holes. These were considerably smaller than the holes tested by Curtis *et al.* (1992), Miller *et al.* (1991) and Pleass *et al.* (1993) and are thought to be more representative of the kind of damage commonly seen in nets used in villages. In some cases nets were hand-washed with cold water and a local detergent, then rinsed carefully with clean water, and dried before testing in the experimental huts.

Insecticide formulations diluted for fabric treatment were as follows: (i) permethrin 50% emulsifiable concentrate (EC), with *cis:trans* ratio 40:60, 'Imperator' 50 EC, from Zeneca (formerly I.C.I.), U.K.; (ii) permethrin 20% emulsifiable concentrate, with *cis:trans* ratio 25:75, 'Peripel' 20EC, from AgrEvo (formerly Roussel Uclaf), U.K.; (iii) deltamethrin 2.5% suspension concentrate (SC), 'K-Othrine' 25 SC (aqueous flowable formulation), from AgrEvo (formerly Roussel Uclaf), U.K.; (iv) lambda-cyhalothrin 2.5% emulsifiable concentrate, 'Icon' 2.5 EC, from Zeneca (formerly I.C.I.), U.K.; (v) lambda-cyhalothrin 2.5% capsule suspension (CS), 'Icon' 2.5 CS (aqueous microencapsulated formulation), from Zeneca (formerly I.C.I.), U.K.; (vi) cyfluthrin 5% oil-in-water emulsion, 'Solfac' 5 EW, from Bayer, Germany; (vii) etofenprox 10% emulsifiable concentrate, 'Vectron' 10 EC, from Mitsui, Japan; (viii) bendiocarb 40% wettable powder WP, 'Ficam' 40 WP; (ix) bendiocarb 20% liquid formulation, 'Ficam' ULV, from Camco (now AgrEvo), U.K.

Liquid formulations (except the last) were diluted to an aqueous concentration calculated to give a specified target deposit on the netting, taking into account the absorbency per unit area of the fabric concerned after dipping and wringing by hand. The bendiocarb ULV formulation was diluted in 'Solvesso' (organic solvent from Esso) and impregnated into netting as described above. The bendiocarb WP was applied by spraying an aqueous suspension from a compression sprayer (Hudson 'X-pert'). For drying, treated nets were spread on polythene sheets in the shade.

Dried treated nets and curtains were stored in plastic bags before testing, except that in specified cases nets were lent to rural households for several months of domestic use between tests in the experimental huts. The households were asked not to wash these nets.

Statistical methods. Each night's data from the experimental huts were tabulated as numbers live fed, dead fed, dead unfed and live unfed for each species of mosquito. Histograms of such data were presented by Curtis *et al.* (1992) and Gokool *et al.* (1992). To compare the effectiveness of each type of material for (a) personal protection and (b) community impact on the vector population, each night's mosquito collection data were assessed with respect to the number blood-fed (whether alive or dead) and the number of dead mosquitoes (whether fed or unfed). For significance testing within each experiment involving five treatments and a control (each tested once weekly for 6 weeks in each hut), factorial analysis of variance of numbers of fed and dead mosquitoes by treatment, week and hut was used previously (Curtis *et al.*, 1992). However, we now have data from many different experiments to compare and, inevitably, not every treatment was represented in each experiment. Also, there were seasonal differences in the total number of mosquitoes in the village and entering the huts; and there is a tendency to find fewer mosquitoes (dead or alive) in huts with impregnated materials compared to those without (Lines *et al.*, 1987; Lindsay *et al.*, 1991). If the numbers of fed and dead mosquitoes in huts with impregnated materials were expressed as percentages of the totals found in those huts, this would underestimate the total impact on feeding and overestimate the total impact in killing members of the vector population in the village. We therefore decided to express the numbers fed and killed in a hut with impregnated materials as a percentage of the total number entering the same huts without a net or curtain during the same 2-week period. This makes allowance for differences between the numbers (a) entering each of the two huts and (b) over time, as mosquito populations densities changed seasonally. The central tendencies for each treatment were calculated as the geometric means of the percentages, as defined above.

Especially with treated intact bednets, there were many nights on which no mosquitoes fed. Also with *Cx quinquefasciatus* there were many nights on which no mosquitoes died. The distributions of numbers fed and numbers killed on different nights were therefore extremely skewed, in a way which could not be corrected by transformation. It was therefore decided to use Kruskal Wallis non-parametric tests for statistical significance, computed with 'Epi Info' (from C.D.C., Atlanta, Ga., U.S.A.).

Results and Discussion

Bioassays

Figs 2–4 show the mortality rates of *An.gambiae* following 3 min exposure to impregnated nets in domestic use in Tanzanian houses. Control exposures to untreated nets gave only 4.1% overall mortality ($n = 1315$).

Fig. 2 shows that nets made from any of the three fabrics and treated with permethrin 200 mg/m^2 initially caused high rates of mortality, but their efficacy declined sharply after a few months' use. There were some indications of a less severe decline with polyester netting than with the other two fabrics. Washing of all three fabrics, followed by re-treatment (indicated by black arrows in Fig. 2) every 6 months fully restored insecticidal power. These results resemble those reported by Njunwa *et al.* (1991) for polyester netting.

Fig. 3 shows similar steep declines in mortality over a 7–8-month period with permethrin at either 200 or 500 mg/m^2 or with lambda-cyhalothrin 10 mg/m^2 on nets made of polyethylene fibre. An Olyset net, made of polyethylene with 2% permethrin incorporated, never killed more than 50% of *An.gambiae* exposed for 3 min, but there was no consistent trend in insecticidal power over time.

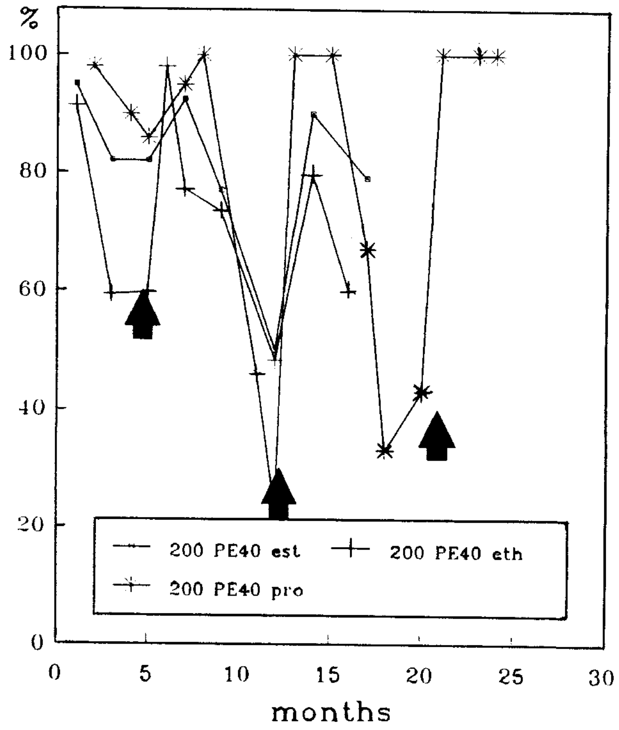

Fig. 2. Mortality of *An.gambiae* after 3 min exposure to nets impregnated with permethrin (40:60 *cis:trans*) 200 mg/m^2 (200 PE 40). The nets made of polyester (est) polyethylene (eth) or polypropylene (pro) were in domestic use and subject to washing and re-treatment (black arrows) at approximately 6-month intervals.

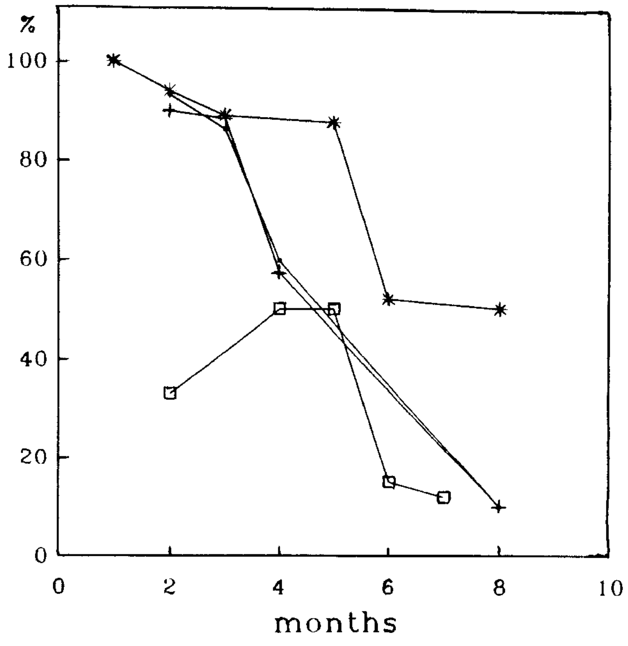

Fig. 3. Mortality of *An.gambiae* after 3 min exposure to polyethylene nets impregnated with permethrin (40:60, *cis:trans*), 200 mg/ m^2 (■) or 500 mg/m^2 (+), or lambda-cyhalothrin EC 10 mg/m^2 (✳), or an Olyset net with 2% permethrin incorporated (□).

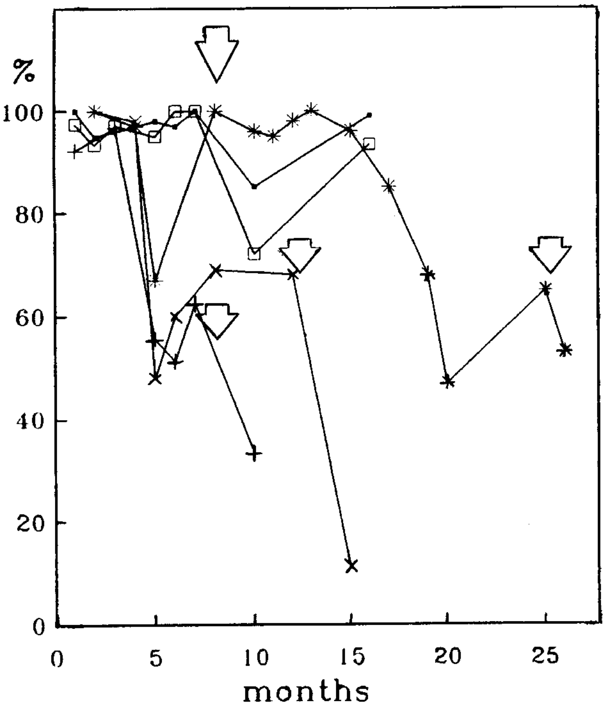

Fig. 4. Mortality of *An.gambiae* in bioassays on polyester nets impregnated with lambda-cyhalothrin (LA) made from EC or CS formulations, (■, 10 mg/m² LAec; +, 5 mg/m² LAec; ✳, 10 mg/m² LAcs), or deltamethrin 25 mg/m² SC (□) or etofenprox 200 mg/m² EC (×) formulations. White arrows indicate washing of nets without re-impregnation.

Fig. 4 shows results with polyester nets treated with lambda-cyhalothrin, deltamethrin or etofenprox, with washing of the nets as indicated by the white arrows, but no re-impregnation. An initital treatment rate of 10 mg/m² lambda-cyhalothrin killed 85–100% of *An.gambiae* tested up to 15 months later and still gave 50% mortality 2 years post-treatment. This very long persistence extends earlier results of Njunwa *et al.* (1991) and contrasts with much poorer persistence of the same treatment on polyethylene (Fig. 3). Efficacy of deltamethrin 25 mg/m² did not seem to be quite so resistant to washing as lambda-cyhalothrin 10 mg/m², although 5 mg/m² was distinctly less effective than the higher dose of lambda-cyhalothrin (Fig 3). Etofenprox at 200 mg/m² did not perform so well as deltamethrin or lambda-cyhalothrin at much lower doses and washing almost removed the insecticidal effect of etofenprox.

Limited numbers of bioassays with *Cx. quinquefasciatus* (data not shown) yielded very low mortality, which conformed with earlier laboratory indications of lower susceptibility of *Culex* than *Anopheles* to pyrethroids (Hossain *et al.*, 1989).

Experimental huts

Table 1 gives data for *Anopheles* in relation to intact bednets and curtains used overnight in the experimental huts. More than 90% of the data come from *An.gambiae s.s.*, with a limited admixture of *An.funestus* during some seasons. As results for the two malaria vector species seemed to be similar, they have been pooled in Tables 1 and 3.

From the tests of permethrin (nos. 1–6 in Table 1) there are no signs of any better performance with regard to prevention of blood-feeding or killing by a concentration of 500 mg/m² as compared with 200 mg/m². Nor was there any sign of better performance of the preparation with 40% of the more insecticidal *cis* isomer than with only 25% *cis*. Permethrin-treated polyester, polyethylene or polypropylene (nos. 4–6, 18 and 24 in Table 1) performed well in the hut tests after 4 months of domestic use, even though the bioassays in Figs 2 and 3 had shown a distinct decline in performance by that time.

All other treatments have been compared with the results with a fresh deposit of 200 mg permethrin (40:60, *cis:trans*) 200 mg/m² on polyester bednets (no.1 in Table 1) for which 59 replicate nights of data were available. This treatment gave significantly better prevention of blood-feeding by *Anopheles* than an untreated intact net (no. 16 in Table 1), though the latter showed that the physical barrier presented by finely-woven intact net reduced the feeding rate to 7%,which was very highly significantly different from the average of 69% when there was no net or curtain (no. 33 in Table 1). The *Anopheles* mortality rate with the untreated intact net was also significantly different from that with no net, probably because of deaths from starvation of mosquitoes prevented by the net from feeding – and unable to escape and find another host because of the verandah screening. [By providing glucose pads as nutrition for mosquitoes, this probably artefactual mortality – due to an untreated net in a verandah-trap hut – has been reduced well below the level reported by Curtis *et al.* (1992).]

The range of concentrations of different pyrethroids tested on polyester nets (nos. 7–15 in Table 1) gave results homogenous with each other and, for the most part, not significantly different from permethrin 200 mg/m². Exceptions were the EC formulations of lambda-cyhalothrin at only 5 mg/m² and etofenprox 200 mg/m² (nos. 9 and 15 in Table 1) which allowed more than 10% feeding. These treatments were shown to perform poorly in bioassays (Fig. 3), although they achieved mortalities of about 65% in the huts. Conversely, the Olyset net (no. 19 in Table 1) performed well at killing and preventing feeding in the huts, despite poor performance in bioassays (Fig. 3). There was some evidence that lambda-cyhalothrin was worse than permethrin at preventing feeding, but better at killing. In both cases, however, the significance level was 0.01–0.05 so, when so many comparisons have been made, this cannot be considered strong evidence that the differences were real.

The impregnated 4 mm mesh nets (nos. 20–21 in Table 1) and the polypropylene sacking material (nos. 23–24) were significantly worse at preventing feeding than permethrin-impregnated nets with a conventional 1.5 mm mesh, but significantly better at killing. All of the impregnated eave curtains (nos. 25–30) gave significantly worse prevention of feeding than the permethrin bednet. In thatched huts, curtains can be fixed more firmly to the roof (Majori *et al.*, 1987) and curtains may be more acceptable than nets to some people. However, we favour bednets, especially in areas of intense malaria transmission where anything but the best possible vector control may have no useful impact on malaria morbidity.

The liquid bendiocarb formulation was considered too hazardous to use on bednets but was used on curtains with good results when fresh (only 8.8% *Anopheles* blood-feeding, no. 29). This encouraged us to think that this carbamate insecticide, belonging to a distinct category from the pyrethroids, might be useful to prevent or counteract pyrethroid resistance (Curtis *et al.*, 1994). However, after 4 months there were strong indications of loss of effectiveness in preventing feeding and killing (though only four replicates were insufficient to establish this for *Anopheles* with statistical significance).

Deltamethrin-impregnated curtains, coloured black or white and positioned at the top or bottom of the wall, gave homogenous results which are pooled in Table 1, no. 32. With respect to *Anopheles* they provided no significant protection from biting (64.3% versus 69.1% with no curtain), but they killed a high proportion of the blood-fed mosquitoes. We consider that these data from verandah trap huts give a clearer picture of the effects of wall curtains than the methods of assessment adopted by Mutinga *et al.* (1992) and Poopathi & Rao (1995).

Table 2 shows data for *Cx quinquefasciatus* on the effects of intact bednets and curtains. The proportions of this species blood-feeding were very similar to those observed for *Anopheles* (Table 1). Most of the intact, fine-mesh nets (nos. 1–17 in Table 2) reduced feeding rates to less than 5%, and none differed significantly from the permethrin treated net – except for lambda-cyhalothrin at 15 mg/m^2 (no. 11 in Table 2) in the presence of which no *Culex* fed. The Olyset net (no. 19 in Table 2) reduced feeding highly significantly more effectively than the untreated net of a similar 4 mm mesh size (no. 22 in Table 2). The pyrethroid-impregnated eave and wall curtains (nos. 25–27 and 32 in Table 2) were significantly worse than the permethrin bednet in reducing feeding of *Culex*. The bendiocarb curtains (nos. 28–29) performed as well as pyrethroid-impregnated curtains when fresh but, after 4 months (no. 30), the power of bendiocarb against *Culex* had declined significantly.

The mortality of *Culex* was strikingly less than that of *Anopheles* with all the pyrethroid-treated nets and curtains. This conforms with the lack of reduction of *Culex* populations which has been observed in communities with widespread use of pyrethroid-treated nets, in contrast to the marked effects on endophagic *Anopheles* populations (Magesa *et al.*, 1991; Jana-Kara *et al.*, 1995). Presumably this is mainly associated with the general tolerance of *Cx quinquefasciatus* to tarsal contact with insecticides (Brown & Pal, 1971; Hossain *et al.*, 1989). It is possible that *Culex* are also more readily irritated than *Anopheles* and inclined to leave pyrethroid-treated surfaces. *Culex* mortalities greater than 12% were observed with fresh deposits of bendiocarb (nos. 28–29), but the observed mortality was only 4.5% after 4 months (no. 30). Recent trials of bendiocarb or pyrethroid-treated curtains against *Cx quinquefasciatus* in Sri Lanka showed that the pyrethroids performed significantly better (Weerasooriya *et al.*, 1996).

Tables 3 and 4 show results against *Anopheles* and *Culex* of a series of tests with holed polyester nets – some of which had been, for considerable periods, in domestic use and had been washed. The untreated, holed net (no. 57 in Table 3) showed no protection against *Anopheles* biting. At concentrations of 10 mg/m^2 or more on holed nets, all the pyrethroids gave much better protection than with untreated holed nets; except for the anomalously poor results with SC deltamethrin 25 mg/m^2 against *Anopheles* (no. 40 in Table 3) and with CS lambda-cyhalothrin 10 mg/m^2 against *Culex* (no. 53 in Table 4). The latter result was with a conical-shaped net where contact of the sleeper's body with the net is more likely than with rectangular nets. All the pyrethroid treatments caused high (not significantly heterogeneous) *Anopheles* mortality (Table 3) and low *Culex* mortality (Table 4).

After 6–9 months use and/or washing of permethrin-treated bednets the bioassay results (Fig. 2 and Njunwa *et al.*, 1991) suggest that their efficacy would be poor but, in fact, their performance did not differ significantly from that of the freshly impregnated permethrin net (nos. 34–37, Tables 3 and 4). Nor was there evidence for any different performance, after 6 months use and washing, of holed nets impregnated with permethrin 500 mg/m^2 (no. 39) compared with 200 mg/m^2 (no. 36). After 11 months use an etofenprox net (no. 56) performed satisfactorily against *Anopheles*, but was significantly worse than permethrin against *Culex*. After 15 months use holed nets impregnated with lambda-cyhalothrin 10 mg/m^2 (no. 52) or cyfluthrin 50 mg/m^2 (no. 55) were still effective. Hence insecticidal impregnation shows its greatest advantage by sustaining the efficacy of holed nets.

In view of the better results with the used and washed nets than might have been expected from bioassay data, we have started a series of tests of bednets impregnated with as little as 3 mg/m^2 of deltamethrin (nos. 42–44) or lambda-cyhalothrin (nos. 46–50). These have so far included up to two washes and, in some cases, re-treatment. *Anopheles* blood-feeding data gave significant indications that, after washing, nets treated with only 3 mg/m^2 (nos. 47–48) had lost effectiveness. However, re-impregnation with 3 mg/m^2, after washing, fully restored their efficacy to prevent feeding of both *Anopheles* and *Culex* (Tables 3 and 4, nos. 43–44 and 49–50). This procedure is being investigated further, since frequent washing and re-impregnation with a low dose to compensate for losses on washing could be an acceptable routine for householders to follow (Lines, 1996).

Acknowledgments

Clearance for this research was given by the Tanzanian National Institute for Medical Research and the Tanzanian Commission Science and Technology.

We are grateful to Charles Chambika, Mwanashaha Jumanne and Lucy George for conscientious technical assistance. The sleepers in the huts: Ali Sokoine, Selemani Jumanne, Fatuma Rashid and Asha Omari, are thanked for their excellent co-operation.

Financial support was provided by the British Medical Research Council with supplemetary grants from Bayer and Zeneca. Free samples of the insecticides were provided by the manufacturers.

Table 1. Data for *Anopheles gambiae s.s.* and *An.funestus* in relation to intact bednets and curtains. Results expressed as numbers collected blood-fed (alive or dead) and dead (fed or unfed) as percentage of the mean numbers entering the same hut in the same 2-week period. Geometric means of the percentages are shown, together with the results of non-parametric tests of significance (Kruskal-Wallis *H*, the significance levels being the same as for χ^2). These tests are made for each treatment on (i) heterogeneity between different dosages or months of prior usage of the net, (ii) difference from an unused polyester net impregnated with permethrin (40:60, *cis*: *trans*)/200 mg/m^2, (iii) the same type of net or curtain which had not been treated; the untreated nets and curtains are compared with no net or curtain.

Target concentration: expressed as milligrammes of insecticide active ingredient per square metre (mg a.i./m^2).

Insecticide formulations: BEwp, bendiocarb 40% wettable powder; BElv, bendiocarb 20% ULV liquid; CYew, cyfluthrin 5% oil-in-water emulsion; DEsc, deltamethrin 2.5% suspension concentrate; ETec, etofenprox 10% emulsifiable concentrate; LAcs, lambda-cyhalothrin 2.5% microencapsulated suspension; LAec, lambda-cyhalothrin 2.5% emulsifiable concentrate; PE25, permethrin (25:75 *cis*:*trans*) 20% emulsifiable concentrate; PE40, permethrin (40:60 *cis*:*trans*) 50% emulsifiable concentrate.

Fabrics: est, polyester bednet (1.5 mm mesh); eth, polyethylene bednet (1.5 mm mesh); Oly, permethrin 2% w/w incorporated into the polyethylene fibre; 4 m, 4 mm mesh polyester bednet; pro: polypropylene sacking material formed into a bednet; ecu, eaves curtains of 4 mm mesh; wcu, 11 × 1 m cotton wall curtains. *Prior use*: months for which the net had been in domestic use before the tests; *Replicates*: number of nights of testing in the verandah trap huts. ns = not significant; $*P < 0.05$; $**P < 0.01$; $***P < 0.001$.

						Anopheles (% fed)				*Anopheles* (% mortality)			
							Kruskal-Wallis *H*				Kruskal-Wallis *H*		
Treatment	Target concentration	Insecticide formulation	Fabric	Prior use	Replicates	Geom. mean	Hetero-geneity	*v*. 200 PE40 est (unused)	*v*. respective untreated	Geom. mean	Hetero-geneity	*v*. 200 PE40 est (unused)	*v*. respective untreated
1	200	PE40	est	0	59	2.3	–	–	7.9**	41.1	–	–	2.2ns
2	500	PE40	est	0	11	5.5	–	3.5ns	0.05ns	38.4	–	3.4ns	1.1ns
3	200	PE25	est	0	11	2.7	–	0.1ns	1.6ns	43.5	–	0.4ns	1.9ns
4	200	PE40	est	4	4	1.2				47.5			
5	500	PE40	est	4	6	1.4	0.2ns	1.9ns	9.2**	61.2	2.5ns	1.6ns	4.4*
6	200	PE25	est	4	6	0.9				46.4			
7	15	DEsc	est	0	12	2.0	3.2ns	3.3ns	1.0ns	31.4	1.9ns	2.4ns	5.3*
8	25	DEsc	est	0	24	5.9				64.9			
9	5	LAec	est	0	12	10.4		11.0***	1.3ns	66.6			
10	10	LAec	est	0	12	3.3	5.8ns	0.2ns	3.1ns	47.0	1.2ns	1.6ns	4.9*
11	15	LAec	est	0	12	2.3				53.8			
12	10	LAcs	est	0	48	5.2	–	6.0*	0.2ns	56.0	–	4.1*	6.7**
13	30	CYew	est	0	12	3.3	1.4ns	1.8ns	9.9**	36.7	0.4ns	0.02ns	1.9ns
14	50	CYew	est	0	12	1.7				46.4			
15	200	ETec	est	0	12	11.5	–	12.8***	1.2ns	64.6	–	2.7ns	5.4*
16	Untreated		est	0	24	7.0	–	7.9**	43.6***	22.8	–	2.2ns	4.0*
17	200	PE40	eth	0	7	2.5	0.6ns	0.5ns	–	41.9	0.1ns	0.7ns	–
18	200	PE40	eth	4	5	0.8				63.6			
19	(2%)	PE40	Oly	0	12	1.5	–	0.8ns	11.4***	76.1	–	7.7**	3.6ns
20	200	PE40	4m	0	12	10.5	1.1ns	12.7***	3.8*	56.5	2.2ns	7.5**	4.6*
21	25	DEsc	4m	0	12	6.5				89.8			
22	Untreated		4m	0	11	19.2	–	15.0***	12.8***	34.2	–	0.9ns	4.3*
23	200	PE40	pro	0	46	14.5	0.1ns	32.5***	–	73.8	1.9ns	11.9***	–
24	200	PE40	pro	4	13	10.9				46.9			
25	200	PE40	ecu	0	12	13.6				37.3			
26	25	DEsc	ecu	0	12	24.1	0.83ns	29.4***	1.3ns	33.0	0.18ns	1.2ns	11.8***
27	10	LAcs	ecu	0	12	21.7				44.6			
28	400	BEwp	ecu	0	12	16.8	–	7.8**	1.3ns	38.1	–	0.01ns	6.4**
29	400	BElv	ecu	0	8	8.8	1.9ns	4.5*	4.0*	19.3	0.73ns	1.1ns	1.4ns
30	400	BElv	ecu	4	4	40.1		7.4**	0.6ns	4.6		3.2ns	0.3ns
31	Untreated		ecu	0	23	28.2	–	32.3***	19.2***	7.8	–	15.7***	1.2ns
32	25	DEsc	wcu	0	60	64.3	2.5ns	73.4***	0.04ns	72.2	1.4ns	19.1***	70.5***
33	No net/curtain				165	69.1	–	116***	–	11.3	–	29.4***	–

Table 2. Data for *Culex quinquefasciatus* in relation to intact nets and curtains: notes and abbreviations as for Table 1.

						Culex (% fed)				*Culex* (% mortality)			
							Kruskal-Wallis *H*				Kruskal-Wallis *H*		
Treatment	Target concentration	Insecticide formulation	Fabric	Prior use	Replicates	Geom. mean	Hetero-geneity	*v.* 200 PE40 est (unused)	*v.* respective untreated	Geom. mean	Hetero-geneity	*v.* 200 PE40 est (unused)	*v*. respective untreated
1	200	PE40	est	0	59	2.8	–	–	2.9ns	2.1	–	–	4.1*
2	500	PE40	est	0	11	8.3	–	3.7ns	0.3ns	4.4	–	1.3ns	5.6*
3	200	PE25	est	0	11	1.2	–	1.4ns	3.7ns	5.2	–	1.6ns	5.9*
4	200	PE40	est	4	4	1.3				3.5			
5	500	PE40	est	4	6	0.6	0.2ns	2.8ns	5.9*	4.2	0.2ns	1.7ns	6.0*
6	200	PE25	est	4	6	0.7				6.0			
7	15	DEsc	est	0	12	3.0	0.7ns	1.9ns	0.6ns	1.4	2.0ns	1.8ns	7.4**
8	25	DEsc	est	0	24	5.9				5.3			
9	5	LAec	est	0	12	3.7		0.01ns	2.1ns	3.2			
10	10	LAec	est	0	12	1.8	9.1**			1.1	1.9ns	0.1ns	4.5*
11	15	LAec	est	0	12	0		8.1**	11.3***	3.9			
12	10	LAcs	est	0	48	2.2	–	0.5ns	4.1*	2.1	–	0.1ns	3.8*
13	30	CYew	est	0	12	0.6	1.7ns	1.8ns	5.8*	0.8	1.2ns	1.2ns	0.1ns
14	50	CYew	est	0	12	2.1				0.2			
15	200	ETec	est	0	12	7.6	–	3.3ns	0.1ns	2.0	–	0.1ns	2.1ns
16	Untreated		est	0	24	5.8	–	2.9ns	44.4***	0.6	–	4.1*	1.2ns
17	200	PE40	eth	0	12	4.7	–	0.6ns	0.2ns	17.1	–	14.6***	–
19	(2%)	PE40	Oly	0	12	2.8	–	0ns	11.2***	2.9	–	0.4ns	1.0ns
20	200	PE40	4m	0	12	10.9	6.6**	6.2*	5.1*	1.6	0.5ns	0.1ns	0.2ns
21	25	DEsc	4m	0	12	1.5		0.8ns	14.7***	1.7			
22	Untreated		4m	0	11	32.8	–	16.9***	9.5**	1.4	–	0.5ns	0.1ns
23	200	PE40	pro	0	46	3.7	–	0.6ns	–	8.3	–	13.1***	–
25	200	PE40	ecu	0	12	12.3				0.2			
26	25	DEsc	ecu	0	12	8.4	0.6ns	10.1***	3.3ns	1.4	1.7ns	3.4ns	0.5ns
27	10	LAcs	ecu	0	12	10.7				1.3			
28	400	BEwp	ecu	0	12	8.9	–	3.7ns	3.0ns	12.7	–	7.5**	12.3***
29	400	BElv	ecu	0	8	11.0	4.9*	4.3*	3.5ns	14.9	1.4ns	7.7**	14.9***
30	400	BElv	ecu	4	4	51.5		10.1**	0.5ns	4.5		0.6ns	5.9*
31	Untreated		ecu	0	23	27.4	–	22.5***	8.4**	0.5	–	5.4*	2.1ns
32	25	DEsc	wcu	0	60	28.9	2.5ns	33.9***	11.7***	1.1	3.5ns	1.9ns	0.1ns
33	No net/ curtain			–	154	63.7	–	99.1***	–	1.1	–	3.7ns	–

Table 3. Data on *An.gambiae s.s.* and *An.funestus* in relation to polyester nets with six holes 4 × 4 cm. r = rectangular net; c = conical net; other abbreviations as in Table 1.

								Anopheles (% fed)				*Anopheles* (% dead)			
									Kruskal-Wallis *H*				Kruskal-Wallis *H*		
Treatment	Target concentration	Insecticide formulation	Net shape	Prior use	Times washed	Times re-tr.	Replicates	Geom. mean	Hetero-geneity	*v.* 200 PE40	vs respective untreated	Geom. mean	Hetero-geneity	*v.* 200 PE40	*v.* respective untreated
34	200	PE40	r	0	0	0	12	19.2				64.6			
35	200	PE40	r	9	0	0	24	6.7	4.0ns	–	16.6***	28.5	5.0ns	–	9.2**
36	200	PE40	r	6	1	0	11	9.4				45.0			
37	200	PE40	c	6	1	0	11	4.9				89.4			
38	500	PE40	r	0	0	0	12	21.0	3.0ns	0.12ns	8.9**	77.9	5.6*	0.9ns	12.0***
39	500	PE40	r	6	1	0	12	8.2				37.5		2.1ns	2.2ns
40	25	DEsc	r	0	0	0	12	45.0	12.9***	15.6***	0.3ns	87.5	1.1ns	0.9ns	13.6**
41	25	DEsc	r	9	0	0	12	2.2		3.2ns	12.5***	36.4			
42	3	DEsc	r	0	0	0	8	23.3				46.3			
43	3	DEsc	r	1	1	1	8	20.0	0.7ns	4.1*	6.4*	43.5	0.3ns	0.9ns	7.5**
44	3	DEsc	r	2	2	2	8	20.7				48.9			
45	5	LAec	r	9	0	0	12	5.3	–	0.4ns	12.1***	37.5	–	0.1ns	5.1*
46	3	LAcs	r	0	0	0	8	24.9	–	2.1ns	0.7ns	40.9	–	0.4ns	3.0ns
47	3	LAcs	r	1	1	0	4	49.6		7.1**	1.5ns	73.8		0.8ns	9.0**
48	3	LAcs	r	2	2	0	8	23.9	1.3ns			73.1	0.1ns		
49	3	LAcs	r	1	1	1	8	29.6		3.0ns	5.4*	64.0		0.1ns	10.6**
50	3	LAcs	r	2	2	2	8	8.7				61.2			
51	10	LAcs	r	9	0	0	24	8.0				31.7			
52	10	LAcs	r	15	0	0	12	25.5	2.9ns	1.4ns	10.4**	73.5	5.9ns	2.2ns	7.0**
53	10	LAcs	c	6	1	0	13	15.1				33.8			
54	50	CYew	r	9	0	0	12	9.3	0.7ns	0.3ns	7.9**	25.3	0.5ns	0.4ns	5.4*
55	50	CYew	r	15	0	0	12	21.3				48.4			
56	200	ETec	r	11	1	0	13	11.8	–	0.3ns	8.0**	55.9	–	0.1ns	6.7**
57	–		Untreated net				12	61.7	–	16.6***	0.3ns	14.6	–	9.2**	1.3ns
58	–		No net				72	63.3	–	60.0***	–	7.2	–	37.6***	–

Table 4. Data on *Cx quinquefasciatus* in relation to polyester nets with six holes 4 × 4 cm. Abbreviations as in Tables 1 and 3.

								Culex (% fed)				*Culex* (% mortality)			
									Kruskal-Wallis *H*				Kruskal-Wallis *H*		
Treatment	Target concentration	Insecticide formulation	Net shape	Prior use	Times washed	Times re-tr.	Replicates	Geom. mean	Hetero-geneity	*v.* 200 PE40	*v.* respective untreated	Geom. mean	Hetero-geneity	*v.* 200 PE40	*v.* respective untreated
34	200	PE40	r	0	0	0	12	13.0	5.1ns	–	14.5***	3.0	6.1ns	–	7.6**
35	200	PE40	r	9	0	0	24	3.6				1.2			
36	200	PE40	r	6	1	0	11	17.4				4.0			
37	200	PE40	c	6	1	0	11	8.5				6.2			
38	500	PE40	r	0	0	0	12	4.6	0.9ns	0.01ns	11.7***	2.0	0.5ns	0.01ns	6.6**
39	500	PE40	r	6	1	0	12	13.0				3.5			
40	25	DEsc	r	0	0	0	12	10.2	2.7ns	0.7ns	11.0***	6.7	4.3*	3.6ns	11.5***
41	25	DEsc	r	9	0	0	12	2.0				0.9		2.3ns	0.5ns
42	3	DEsc	r	0	0	0	8	28.2	3.6ns	1.6ns	4.8*	0.7	5.7ns	0.7ns	4.7*
43	3	DEsc	r	1	1	1	8	5.4				6.0			
44	3	DEsc	r	2	2	2	8	14.7				0.9			
45	5	LAec	r	9	0	0	12	6.2	–	0.1ns	4.1*	4.8	–	0.5ns	4.1*
46	3	LAcs	r	0	0	0	8	16.9	–	1.2ns	3.4ns	1.1	–	1.3ns	2.7ns
47	3	LAcs	r	1	1	0	4	17.3	5.0*	0.4ns	6.9**	1.6	1.3ns	2.3ns	1.4ns
48	3	LAcs	r	2	2	0	8	10.9				0.7			
49	3	LAcs	r	1	1	1	8	3.1		4.9*	20.1***	3.5		0.1ns	5.8*
50	3	LAcs	r	2	2	2	8	2.9				1.4			
51	10	LAcs	r	9	0	0	24	7.9	15.7***	0.01ns	10.2**	3.5	1.1ns	0.01ns	8.2**
52	10	LAcs	r	15	0	0	12	0.9		7.5**	15.8***	1.8			
53	10	LAcs	c	6	1	0	13	28.0		7.1**	2.2ns	2.2			
54	50	CYew	r	9	0	0	12	11.5	0.2ns	0.2ns	10.7**	0.8	1.4ns	1.6ns	3.7ns
55	50	CYew	r	15	0	0	12	12.5				2.3			
56	200	ETec	r	11	1	0	13	26.8	–	6.7**	1.4ns	4.3	–	0.7ns	8.3**
57			Untreated net				12	53.3	–	14.5***	4.2*	0.1	–	7.6**	1.7ns
58			No net				72	70.3	–	67.6***	–	0.7	–	10.9***	–

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Accepted 16 August 1995