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A new strategy for treating nets. Part 1: formulation and dosage

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Summary The conventional dosages of pyrethroid insecticides on mosquito nets assume that nets will be retreated at 6–12 month intervals. However, dosage should be related to washing of nets; if nets are only washed once or twice a year, their dosage requirements will be different to those which are washed fortnightly. A 'low-dose, frequent-wash' retreatment system might be technically more appropriate and more affordable where nets are washed frequently, as they are in Dar es Salaam. Moreover, for use as a domestic insecticide, water-based formulations of pyrethroid are preferable to the more commonly used emulsifiable concentrates (ECs). This paper reports laboratory evaluations of three formulations (ECs, Flowable, CS) of three pyrethroids (deltamethrin, lambdacyhalothrin, permethrin). Insecticidal activity was tested using serial bioassays at a range of dosages using *Anopheles gambiae*. The water-based formulations were no less effective than the ECs, even at the lowest dosages. Nets treated with 3 mg/m^2 and then repeatedly washed and retreated after each wash with either 3 mg/m^2 or 1 mg/m^2 were subjected to gas chromatography analysis. This showed that the amounts of pyrethroid in the nets accumulated rapidly over the first few wash-retreatment cycles and then remained fairly stable over subsequent cycles. These nets gave consistently high bioassay mortalities throughout the experiment, while the mortality declined rapidly after several washes with the nets that were treated at 3 mg/m^2 but not retreated. Experimental huts were used to compare the effectiveness of these 2 net retreatment regimes and nets which were not retreated. All nets caused high mortality rates amongst *Anopheles* females, but had negligible effects on culicines; either in killing them or in preventing feeding. Therefore use of a high 'loading' dose for initial treatment with lower 'maintenance' doses for retreatment may be preferable to ensure that net users promptly perceive the benefits of the insecticide against culicines.

keywords Low dose net treatment, pyrethroid, bednets, bioassays, verandah hut trials, net washing practices

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Introduction

Pyrethroid-treated nets dramatically reduce malaria morbidity and mortality in children in Africa. There remains some doubt as to whether the short-term impact seen in recent field trials will be sustained in the long term (Lines & Armstrong 1992; Marsh & Snow 1995; Trape & Rogier 1996), and it is therefore important to continue asking questions about efficacy. In the meantime, however, there is enough evidence to justify action to make treated nets and net treatment accessible on a large scale. Unlike house-spraying and attacks on breeding sites, treated nets protect the user whether or not others in the community join in. Moreover, untreated nets are already a well-known household item in most places, even where they are rare. For these reasons, and because public health structures in Africa remain generally weak, we should consider the potential role of commercial channels in distributing the insecticide as well as the nets.

In operational treated net projects, retreatment rates have often been disappointing, and in several cases have declined markedly when charges for the insecticide were introduced. This might be because people simply did not perceive or appreciate the benefits of the insecticide, but it may also be related to the fact that in most of these projects, insecticide was made available only for a short period (often just one day) at the start of the transmission season. This was convenient for the supplier, but presumably less so for net owners who were unable to attend the 'dipping day', or who happened to be

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short of cash on that day. A more commercial approach, perhaps, is to arrange for insecticide to be permanently and conveniently available whenever and wherever the consumer is able and willing to buy. This could be achieved by means of a 'dip-your-net' service, but our experience in Dar es Salaam (Tanzania) suggests that this is a labour-intensive and inefficient approach (Lines 1996). A potentially attractive alternative is the 'dip-it-yourself' approach. This shifts the responsibility for the dipping process from the insecticide supplier to the net owner, but does not necessarily mean much more work for the user, since dipping could conveniently be made part of the normal household washing routine. It also has the advantage of giving net users much more autonomy in deciding whether and when their nets are to be treated.

In Dar es Salaam we have been developing a 'dip-it-yourself' kit for treatment of nets at home. Our goal was a product that could be sold in small shops alongside coils and sprays, and that could be used safely and effectively even by inexperienced and untrained people. This paper reports a series of studies evaluating aspects of the problem in technical terms; a parallel series of studies evaluating users' attitudes and responses are reported in a companion paper.

Not all formulations of pyrethroid insecticide are suitable for use in a home treatment kit. Lines (1996) recommended that only water-based formulations ('suspension concentrates') should be used for this purpose, since they are less hazardous than emulsifiable concentrates (ECs), especially in terms of acute oral toxicity. We therefore began by comparing the performance of such formulations in laboratory bioassays.

Another question which must be re-examined in the dip-it-yourself approach is that of dosage. The dosages normally recommended by manufacturers, and conventionally used in public health projects, are those that provide good residual activity for 6–12 months, for example 200–500 mg/m^2 of permethrin and 10–25 mg/m^2 of deltamethrin and lambdacyhalothrin (Miller 1990, 1994; Lindsay *et al*. 1991; Jana-Kara *et al*. 1995). These dosages are appropriate when insecticide distribution is carried out in a coordinated fashion by a centrally organized system (as in China and Vietnam, for example), since the interval between retreatments can be imposed by the supplier. With home treatment, however, the frequency of retreatment is determined by the net owner. This may be an advantage, because in places where net use is common, many people consider a dirty net to be a health hazard as well as a sign of poor domestic hygiene. In such places, the interval between washes is often 1–2 months (1–2 weeks in some households), and it is unlikely that efforts at persuasion could extend this very much. The advantage of the 'dip-it-yourself' approach therefore is that it allows a net to be treated as often as it is washed.

Washing removes most of the insecticide on a net, but not all of it. Miller *et al*. (1991) found that after 3 washes, nets treated with cypermethrin, deltamethrin, lambdacyhalothrin, permethrin and pirimiphos-methyl had lost between 85 and 96% of the active ingredient. Repeating the standard dosage every few weeks after each wash would therefore not only be very expensive, but might also lead to the accumulation of undesirably high dosages of insecticide on the net. This suggests that a 'low-dose, frequent-treatment' system might be technically more appropriate. It might also be more affordable, since poor people often prefer a little-and-often pattern of expenditure to paying larger amounts at longer intervals. So, if retreatment is to be done frequently, what dosage should be used? In the studies described here, we therefore compared the immediate and residual insecticidal activities of water-based and EC formulations at standard and lower doses, and investigated the accumulation of insecticide over successive wash and retreatment cycles.

Materials and methods

Serial bioassays comparing formulations and dosages

Twenty-one pieces of mosquito netting (SiamDutch, 100 denier, polyester) measuring 1 m × 1 m were treated with permethrin EC ('Peripel' 55%, AgrEvo), lambdacyhalothrin EC ('Icon' 2.5%, Zeneca Public Health), lambdacyhalothrin CS ('Icon CS' 10%, Zeneca Public Health) and deltamethrin Flowable ('K-Othrin' 10%, AgrEvo). The required amount of pyrethroid concentrate was added to the amount of water necessary to fully saturate the piece of netting without allowing it to drip. This ensured that all of the measured insecticide was deposited on the net, and that it was as evenly distributed as possible.

The target deposit densities were

(a) Permethrin EC: 400, 200, 100, 50 and 25 mg/m^2

(b) Lambdacyhalothrin EC: 15, 10, 5, 3 and 1 mg/m^2

(c) Lambdacyhalothrin CS: 15, 10, 5, 3 and 1 mg/m^2.

(d) Deltamethrin Flowable: 15, 10, 5, 3 and 1 mg/m^2.

A piece of netting was dipped in water to serve as an untreated control. Bioassays were carried out on the treated netting pieces at 2, 5, 8 and 10 weeks after treatment. The pieces treated with permethrin and lambdacyhalothrin EC were also bioassayed 48 h after treatment. Laboratory-reared, three day-old, glucose-fed, mixed sex *Anopheles gambiae* were used.

Bioassays were performed using the 'ball' system rather than bioassay cones. A ball of netting about 10 cm in diameter was secured with a clothes peg, and 10–15 mosquitoes were introduced through a small slit in the top; this ensured that the mosquitoes could only rest on treated netting. After a 3-minute exposure period, the mosquitoes were removed, placed in a cardboard cup, and offered cotton wool soaked in 10% glucose solution. Three replicate bioassays were carried out on each

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piece on each occasion. Knock-down was recorded after 1 h and mortality after 24 h. Between bioassays the pieces of netting were hung on a clothes line indoors (ambient conditions: temp 26–32 °C; rh 65%). Results were compared between treatments and within timepoints using the χ^2 test.

Analysis of insecticidal build-up in successive wash-retreatment cycles using gas chromatography and mosquito bioassays

Lambdacyhalothrin CS was chosen as it showed the most durable insecticidal activity in the serial bioassays. Nine whole nets (SiamDutch, 100 denier, polyester) measuring 150 × 180 × 70 cm were used, 3 in each of the 3 treatment groups shown in Table 1. Washing was done by hand, by a Tanzanian woman using Komesha, a block soap. She spent about 5 min washing each net and the order in which the nets were presented for washing was changed each time (in case she washed more vigorously at the beginning than at the end).

Samples of netting were taken for analysis of their lambdacyhalothrin content by gas chromatography (GC). After the initial treatment, and before the first wash, 4 pieces of netting measuring 10 cm × 10 cm were cut out of the nets, 2 from the sides and 2 from the ends. Each block of 4 wash-retreatment cycles took a week, with an interval of 24 h between each wash and retreatment. After each block of 4 cycles, 4 more pieces of netting were cut out from each net as before. This continued for 12 cycles. Thus, samples were taken after 0, 4, 8 and 12 wash-retreatment cycles. Note that the samples were taken after retreatment, so that those taken after, for example, 4 cycles had been washed 4 times but treated 5 times. At the same time as the samples were cut from the nets, bioassays were carried out on one net in each treatment group, using the procedure described above.

Analysis of insecticidal build-up in successive wash-retreatment cycles using experimental huts

Verandah-trap experimental huts are built on stilts surrounded by moats to prevent the entry of ants which would eat the mosquito corpses. They have window and verandah

Table 1 Patterns of treatment and retreatment in successive washing cycles

Treatment group	Initial treatment (mg/m²)	Number of washes	Retreatment after each wash (mg/m²)
A	3	12	0
B	3	12	1
C	3	12	3

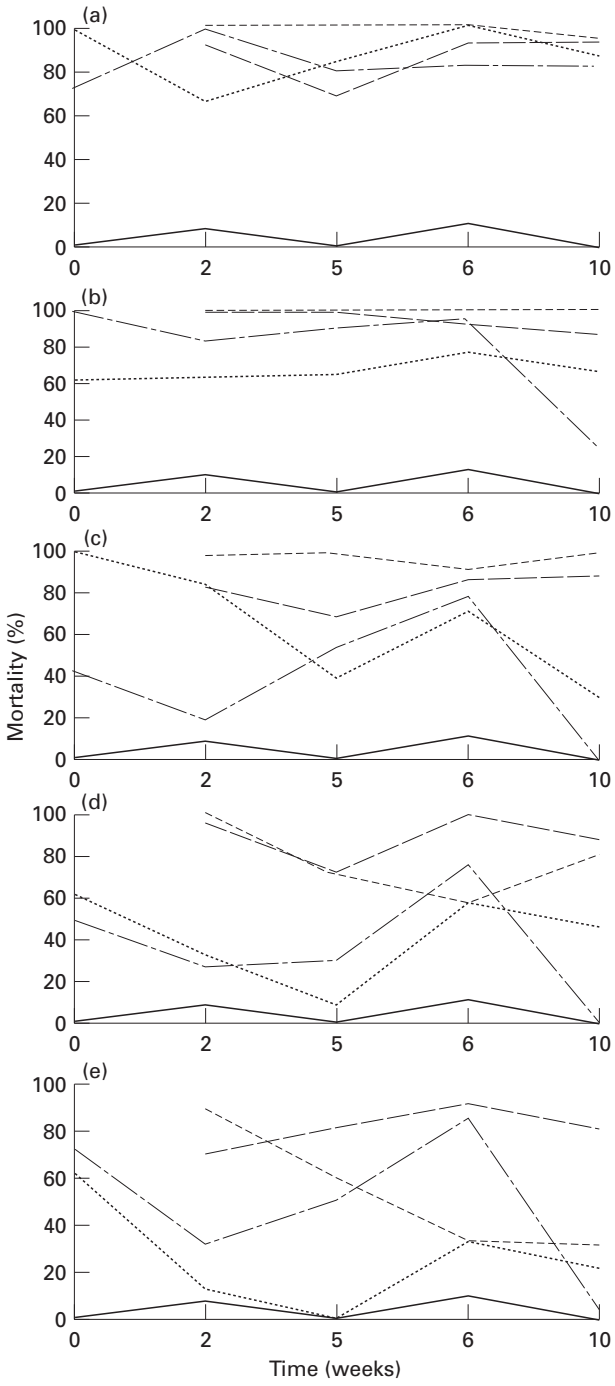


Figure 1 Mortality of *An. gambiae* after 3 min exposure to netting treated with either deltamethrin 'Flow' (—); lambdacyhalothrin microencapsulated suspension (— — —); Lambdacyhalothrin emulsifiable concentrate (·····); permethrin (– · – · – ·) or water (—). Panels (a–e) show netting treated with (a) 400 mg/m² (b) 200 mg/m² (c) 100 mg/m² (d) 50 mg/m² (e) 25 mg/m² permethrin and (a) 15 mg/m² (b) 10 mg/m² (c) 5 mg/m² (d) 3 mg/m² (e) 1 mg/m² all other treatments.

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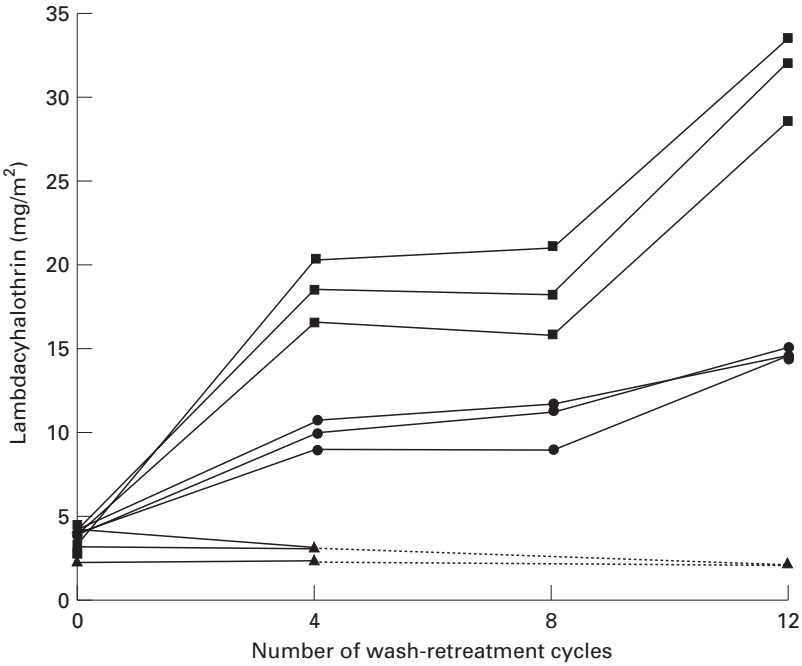


Figure 2 Gas chromatography analysis of lambdacyhalothrin deposit (mg/m^2) on groups of nets after repeated treatment-wash-retreatment cycles, using various dosages of lambdacyhalothrin. ▲ nets treated initially and after every wash with 3 mg/m^2. ■ nets treated initially with 3 mg/m^2, and subsequently after each wash with 1 mg/m^2. ● nets treated with 3 mg/m^2 and then not retreated.

traps on 2 of their 4 sides so that one half of the mosquitoes exiting can be caught. In this way, the number of mosquitoes entering, feeding and dying can be calculated for different net treatments (Lines *et al*. 1987; Miller *et al*. 1991).

Four whole nets (see above) were used. Three of the nets were treated with one of the treatments shown in Table 1; each net was treated 5 times and washed four times. After the final treatment, 4 pieces of netting measuring 10 cm × 10 cm were cut out of each net, 2 from the sides and 2 from the ends to simulate a badly torn net. The fourth net was washed 4 times but not treated and holes were not cut in it.

The 4 nets were tested in the experimental huts in Muheza, Tanzania. The estimated total number of females entering the huts in the presence of the control (untreated) net was arbitrarily designated as 100%, and the numbers observed with each treatment on other nets were expressed as percentages relative to this figure.

Results

Mortality in serial bioassays of formulations and dosages

The results are shown in Figure 1. At the highest dosages tested, consistently high mortality was seen throughout the 10-week study period with all four formulations. In the first week, the lower dosages of lambdacyhalothrin EC and permethrin EC showed significantly lower mortality than the highest dosages ($\chi^2 = 12.9$ and 32.7, $P = 0.0003$ and $P < 0.0001$, respectively), although this was followed by considerable fluctuation over the following weeks (perhaps due to batch variation between the test insects). In the case of deltamethrin flowable, high levels of mortality were seen at first with all dosages; however, this was sustained only with the higher dosages. Of all the formulations tested, lambdacyhalothrin CS was the only one to show consistently high mortality, even at the lowest dosages of 3 mg/m^2 and 1 mg/m^2, throughout the 10 weeks of testing.

Low-dose, frequent-wash and retreatment regime – GC and bioassays

The results of the GC analysis are presented in Figure 2, and those of the bioassays on these nets are shown in Figure 3. The deposits detected after the first treatment, without washing, were close to the target dosages, and the three replicate nets in each treatment group gave very similar results throughout. The bioassay mortalities on the net that was washed but not retreated declined rapidly over successive washing cycles, and after 12 washes were close to control levels; the insecticide deposit detected by GC analysis, by contrast, declined much more slowly, and only about one third of it had been washed out after 12 washes. The amounts of pyrethroid in the nets that were retreated with 1 or 3 mg/m^2 accumulated rapidly over the first 4 wash-retreatment cycles, remained apparently stable over the next four cycles, but increased again between the 8th and 12th cycles. These nets both gave consistently high bioassay mortalities throughout the experiment.

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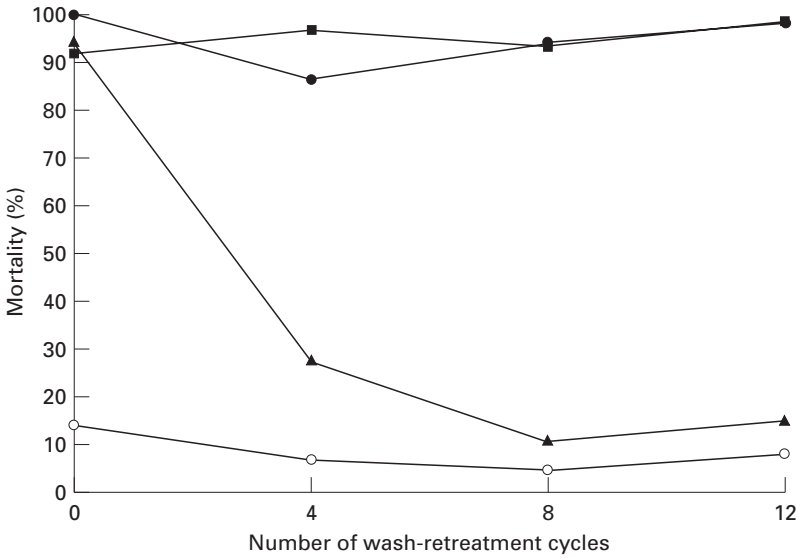


Figure 3 Mortality of *An. gambiae* after 3 min exposure to polyester nets, after repeated treatment-wash-retreatment cycles, using various dosages of lambdacyhalothrin. Symbols as in Figure 2. ○ control nets, dipped only in water.

Low-dose, frequent-wash and retreatment regime – experimental huts

The results of the experimental hut trial are shown in Figure 4, for Anopheles mosquitoes (mainly An. gambiae). An estimated total of 745 female Anopheles entered the huts in the presence of the control (water-treated) net; this figure was arbirarily designated as 100%, and the estimated numbers feeding and dying with each treatment were expressed as percentages relative to this. The observed numbers caught within each category were too low for formal statistical analysis, but some clear patterns can nonetheless be seen. The estimated total numbers entering (represented by the heights of the columns) were not consistently lower with the treated nets than with the control net, suggesting that female mosquitoes were not deterred from entering the hut. All the treated nets killed a large proportion of the Anopheles females that entered the huts, and this proportion was slightly higher in the nets that had been retreated after each wash. The numbers of culicines caught was even smaller (data not shown), but with all the nets (including the untreated control), the observed mortality rates were very low (< 10%), and some 30% to 40% of the females succeeded in feeding.

Discussion

The two water-based formulations used in this study, deltamethrin flowable and lambdacyhalothrin CS, are both very much less dangerous in terms of acute oral toxicity than the equivalent ECs (Lines 1996); for example in the case of deltamethrin the quoted rat oral LD50 is some 40-fold greater for the 2.5% flowable than for the 2.5% EC (WHO 1991). The results of the serial bioassays in this study indicate that water-based formulations are not inferior to the equivalent ECs in terms of residual insecticidal activity on nets. Both the water-based formulations gave high bioassay mortalities immediately after treatment even at the lowest dosages, but

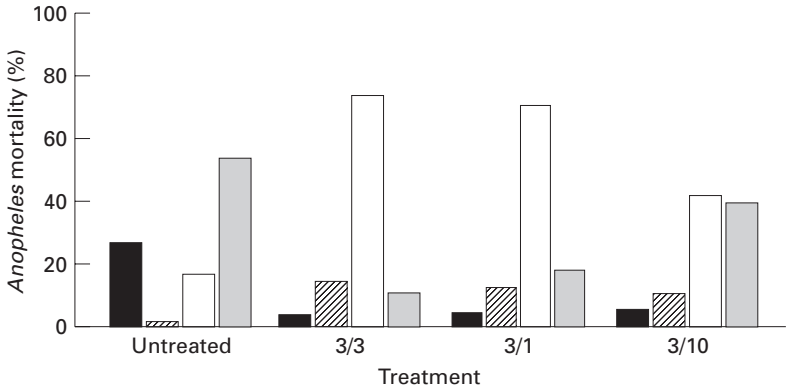


Figure 4 Experimental hut data for *An. gambiae* s.l. using nets that had been washed and retreated 12 times. 3/3, nets treated initially and after every wash with 3 mg/m². 3/1, nets treated initially with 3 mg/m², and subsequently after each wash with 1 mg/m². 3/0, nets treated with 3 mg/m² and then not retreated. ■ Fed, alive; ▒ Fed, dead; ▨ Unfed, alive; □ unfed, dead.

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the insecticidal activity was clearly more persistent over time with low dosages of lambdacyhalothrin CS than with the same dosages of either deltamethrin flowable or lambdacyhalothrin EC.

Whilst water-based formulations of other compounds would probably also be suitable, we selected lambdacyhalothrin CS for further work developing a low-dose frequent-treatment system for home dipping. It should be noted, however, that the insecticidal activity in 3-minute bioassays may not be an entirely reliable guide as to the protection given to the user. For example, in experimental huts, nets treated with lambdacyhalothrin tend to kill a slightly larger proportion of the mosquitoes that come to bite than nets treated with permethrin, but if the net is damaged, permethrin tends to be slightly better at preventing feeding (Curtis *et al*. 1992).

Treated nets can work in either or both of two ways; by preventing mosquitoes feeding on the net user (personal protection) and/or by killing mosquitoes which may or may not have first fed. When treated nets are used widely within a community and many mosquitoes are killed, the so called 'mass killing effect' may occur and reductions may then be found not only in the density of the mosquito population but also in parous and sporozoite rates (Magesa *et al*. 1991).

In the experimental huts, nets which had been treated with low doses all caused high mortality rates amongst malaria vector mosquitoes, whether or not they had been retreated after washing. It should be noted that the treated nets used in this study had had holes cut in them, while the control (water-treated) net was intact. It is therefore not surprising that the degree of protection against feeding was not very different in the treated and control nets.

In any case, the results are entirely consistent with previous evidence that interventions with impressive effects on Anopheles may have no discernable impact on Culex both in laboratory and field populations (Magesa *et al*. 1991; Jana-Kara *et al*. 1995; Curtis *et al*. 1992). This is important because in many places Culex mosquitoes are responsible for much of the nuisance biting – often more than 90% of it, in urban areas. Nuisance biting is the first reason cited by most people for buying protection against mosquitoes. People who fail to perceive an effect on nuisance biting when trying out a new method of personal protection are unlikely to continue buying it. This applies equally to the many people for whom protection against malaria is another important reason for avoiding mosquitoes. If one cannnot tell the difference between a bite from an Anopheles or a bite from a Culex, then it makes sense to avoid them both and to distrust any intervention that fails to reduce mosquitoes in general. Nevertheless, and fortunately, we have evidence that users can at least initially perceive an effect on nuisance biting by Culex with treatment dosages as low as those used here (Miller *et al*. 1999).

Research in Dar es Salaam (Evans 1994) has found that people wash their nets as regularly as once a month, usually because kerosene lamps are used for for light and these create soot, dirtying the nets. Net owners also report frequent washing habits in rural Tanzania (Dr K. Reed, personal communication). One Gambian study found that people do not wash their nets quite as often as they claim when asked, but that nonetheless, about 50% of nets had been washed at least twice over a period of 16 weeks (Miller *et al*. 1995). People in Dar es Salaam express unwillingness to refrain from such frequent washing; there is a strong feeling that a dirty net is unhygienic and people worry about what their visitors and the neighbours will think (Evans 1994; Miller *et al*. 1999).

It is difficult, using existing techniques, to assess exactly how insecticide deposits will vary with different rates of wear-and-tear, of washing, and of retreatment. This is because GC analysis is so expensive, specialized and time-consuming, and only a very limited number of samples can be tested. The results of our preliminary tests of a frequent wash and low-dose retreatment regime are nevertheless encouraging. The pyrethroid deposit builds up rapidly over the first few washes, presumably because more is added than is washed away in each cycle. With a 1-mg/m^2 retreatment after each wash, the amounts found by GC were at the lower end of the range that is conventionally recommended, while with a 3-mg/m^2 retreatment cycle, they were in the upper end of this range or slightly beyond it. We are investigating whether a frequent treatment/wash regime using conventional doses would cause the insecticide to accumulate to unacceptably high levels on the net.

This study needs to be repeated with a wider variety of net fabrics, and with the retreatment being carried out immediately after washing while the net is still damp. We plan to do this when more easily replicable quantitative tests to measure the insecticide deposit become available. Meanwhile, allowing for some additional loss through wear and tear (which was excluded in this study), it appears that a regular postwash retreatment of about 3 mg/m^2 would be suitable in most cases. In a separate paper (Miller *et al*. 1999), we report studies of what users think of nets treated with low doses; these suggest that it may be preferable to use a 'loading dose' (i.e. a high dosage at the initial treatment), with lower 'maintenance doses' used for retreatment, in order to ensure that users promptly perceive the benefits of the insecticide.

Conclusion

We conclude that water-based formulations are no less effective than ECs, and are preferable for safety reasons for use at home. The main advantages of a home-dipping system are that it allows the net user more autonomy and saves work for insecticide suppliers. If net owners are to dip their own nets,

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and are to decide for themselves when this needs to be done, it is preferable and more realistic to adapt the re-dipping regime to fit people's washing habits, rather than vice-versa. The results here suggest that a low-dose retreatment after each wash may be suitable for the purpose of malaria control when washing is frequent. However, in order for such a regime to be accepted and desired by net users, it will also have to protect against nuisance mosquitoes. The results on the use and acceptability of a community-level field trial of such a regime are currently being analysed.

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