

Field issues related to effectiveness of insecticide-treated nets in Tanzania

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Abstract. Insecticide-treated nets (ITNs) impregnated with pyrethroid insecticides have become one of the most promising interventions to prevent malaria in highly endemic areas. Despite the large body of experience documenting their health impact and the best way to distribute them, some key practical issues remain unresolved. For example, the duration of effective life of a net under field conditions is unknown. The most important factor affecting net effectiveness is the issue of regular re-treatment with insecticide. Washing is also an important determinant of insecticide longevity in the field. Trials were undertaken to provide some essential field information on ITNs within the site of an extended ITN programme in the Morogoro region of Tanzania. It was found that 45% of all nets were in bad condition (defined as more than seven large holes). It is concluded that an effective 'life' for polyester nets is 2–3 years. Further, two-thirds of the 20% of nets that were reported as having been re-treated within the last 12 months had less than 5 mg/m² of insecticide. According to the World Health Organization this is insufficient to be effective. People reported that they washed their nets four to seven times per year, usually with soap. Observations showed that such washing does not harm the nets and that the wash-water was unlikely to have an impact on the environment. Finally, bioassays were carried out with *Anopheles gambiae* on polyester netting with 0.5, 2, 5, 10 and 30 mg/m² of deltamethrin, alphacypermethrin and lambdacyhalothrin to assess the effectiveness of pyrethroids. The results confirmed that even with low insecticide concentrations, nets can still provide partial protection.

Key words. *Anopheles*, insecticide, insecticide re-treatment, insecticide-treated nets, malaria, malaria prevention, pyrethroid, wash resistance, Tanzania.

Introduction

Insecticide-treated nets (ITNs) have become one of the major tools in the global fight against the burden of malaria. ITNs reduce the number of clinical malaria episodes by roughly 50% and improve child survival by nearly a fifth (Lengeler, 2000). Large-scale implementation of ITNs is proceeding at an accelerating pace, especially in East Asia and sub-Saharan Africa.

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Recently, social marketing was found to be a good way to distribute ITNs with a mixture of public and private sector channels. In southern Tanzania, social marketing has led to a marked increase in ITN coverage in a remote rural area within the Kilombero Net Programme – KINET (Armstrong-Schellenberg *et al.*, 2001). During this programme, ITNs were associated with a 27% increase in survival in young children and an effective protection of over 60% against anaemia and parasitaemia (Abdulla *et al.*, 2001; Armstrong-Schellenberg *et al.*, 2001).

Despite the large body of literature documenting ITNs' health impact, a number of important practical issues remain unresolved. The most important factors affecting

long-term net effectiveness are (i) their effective life under field conditions and (ii) their regular re-treatment with insecticide. Regarding effective life (defined as the period from purchase until the time when the nets are no longer in a physical state to offer protection against mosquitoes), not enough is known. A survey conducted in rural Tanzania demonstrated that nets still were effective 3–4 years after they had been installed (Maxwell *et al.*, 2002). The ITN-programme in that area was based on free provision and re-treatment of nets. Conventionally, a life of 3–5 years has been assumed (Feilden, 1996) but hardly any systematic study on ITNs has been conducted to date.

With regard to net re-treatment more data are available. The basic problem is that without insecticide treatment, nets have only half or less of their full potential entomological and epidemiological impact (Maxwell *et al.*, 1999; Lengeler, 2000). Hence, adding the insecticide doubles the ITN's effectiveness at very little additional cost. Unfortunately, most ITN programmes to date have reported poor re-treatment rates when insecticide is marketed by public health services, in comparison to free-of-charge distribution (mainly in China and Vietnam), or in the course of scientific trials (Maxwell *et al.*, 2003a).

It has been reported that people in ITN-programme areas were reluctant to buy re-treatment kits, especially if the insecticide had been given out free of charge beforehand (Winch *et al.*, 1997; Snow *et al.*, 1999). In the KINET area, over 80% of households owned at least one net in 1999 and more than half the households had re-treated their nets at least once over a 2-year period; unfortunately, few had made a regular habit of it (Armstrong-Schellenberg *et al.*, 2002). There are a number of reasons for this. First, in some areas in Africa *Culex quinquefasciatus* Say are resistant to pyrethroids (Chandre *et al.*, 1998), which might suggest re-impregnation is useless. Minja (2001) showed that some users noticed that mosquitoes did not touch the net within the first 2 weeks of re-treatment but did soon after. This could be a source of disappointment and a reason for abandoning re-treatment. It was also found that mothers of young children feared that their child might be poisoned. Occasional unfounded rumours, for example, of an adverse effect on male fertility, further reduced compliance despite health education (Minja, 2001). On the other hand, money did not seem to be a major impediment. First, nets were affordable by the majority of the population and were sold at over 10 times the price of a re-treatment kit. Second, a limited promotional scheme of discount vouchers for net treatment was carried out with the assistance from a large local employer, but less than 1% of these vouchers were used (Armstrong-Schellenberg *et al.*, 2002). Nevertheless, even under operational conditions, when insecticide re-treatment is organized and free of charge, ITNs are an attractive health investment (Hanson *et al.*, 2003).

Another factor affecting insecticide dosage on nets is washing. Clean nets are very important to some net users. As the insecticidal effect is usually not well appreciated, most users will prefer a clean net at the risk of losing entomological impact (Feilden, 1996). It was reported that

in some regions of South America people liked to wash their net at least every 2 weeks (Kroeger *et al.*, 1997). A recent series of surveys conducted by the Netmark project in Nigeria, Zambia, Uganda, Senegal and Mozambique reported that 50–77% of nets were washed at least once per month, with little difference between urban and rural areas, except in Senegal (66 vs. 43%) and Nigeria (71 vs. 83%) (Netmark, 2003). Unfortunately, net washing data have not yet been validated properly and it is possible that householders exaggerate the reported frequency of net washing.

A number of studies showed that the pyrethroid insecticides used for net treatment have some persistence on polyester but that regular washing does eventually reduce the insecticide dose below an effective level (Lindsay *et al.*, 1991; Curtis *et al.*, 1996; Jawara *et al.*, 1998; Adams *et al.*, 2002; Ordonez-Gonzalez *et al.*, 2002). Miller *et al.* (1999) reported that users did perceive the reduced effect on the mosquitoes when the insecticide dose was low. Recent work on ITNs and pregnant women suggested a better impact on parasitaemia and anaemia among those who had treated or re-treated their nets less than 6 months ago, compared to those who had not, or had untreated nets (Marchant *et al.*, 2001).

Here a number of key parameters affecting the effectiveness of insecticide-treated nets in the KINET programme are described, with a focus on the usable life of nets, washing habits, insecticide treatment status and the entomological effect of low doses of insecticide.

Materials and methods

Study area and social marketing

The present study was conducted in the Kilombero and Ulanga Districts in the Morogoro region in south-western Tanzania. The area has a rainy season from November to May. The population is about 480 000 with an average density of 11 people/km². There are 109 villages in the two districts. Average household size is 5.5 people. Malaria is the foremost health problem as reported through health services and as perceived by local people, for both adults and children (Tanner *et al.*, 1991). Malaria transmission due to *Plasmodium falciparum* is intense and perennial, despite marked seasonality in mosquito densities when rains peak (Smith *et al.*, 1993).

The main motivation for net use was mosquito nuisance rather than malaria control, with use being widely reported as seasonal (Fraser-Hurt & Lyimo, 1998). KINET was initiated in 1997 (Armstrong-Schellenberg *et al.*, 1999). It supplied wholesalers with nets, which were then distributed further by commercial outlets. Advertisement and promotion were carried out by local health workers. Both ITNs and insecticide re-treatment kits were subsidized. KINET nets (brand name: 'Zuia Mbu') were manufactured initially by Siamdutch Ltd. (Thailand) and later by A to Z (Arusha,

Tanzania) and TMTL (Dar es Salaam, Tanzania). The fibre of all nets was 75 or 100 denier polyester. All nets were pre-treated with pyrethroid insecticides such as lambdacyhalothrin, deltamethrin or alphacypermethrin. Recently, the project settled on 75 denier and deltamethrin for pre-treatment (K-Othrin TM) and re-treatment (KO-Tab TM). In parallel, a Demographic Surveillance System (DSS) was established in 25 villages where ITNs were first launched in May 1997 (Armstrong-Schellenberg *et al.*, 1999) and this provided the sampling frame for the present work.

Cross-sectional net survey

To investigate indicators for describing the condition of ITNs, a questionnaire in Kiswahili with 23 questions was designed and piloted. The key features of the questionnaire consisted of a mixture of reported and observed parameters (Erlanger, 2002). Reported parameters included date of purchase of the net, brand, whether the net was treated or not, and when it was last treated. Unfortunately there were no markings or serial numbers allowing the brands and purchase dates to be validated, thus the respondent's replies could not be cross-checked. Treatment and re-treatment dates were also impossible to evaluate as this was done entirely at the owner's discretion. Observed parameters included colour, size and shape, fabric, fibre strength and number of holes larger than 2 cm.

In a two-stage random cluster sampling in six villages, 144 household representatives were interviewed between May and July 2002 and their nets (218) were observed. Only households with at least one net were considered for sampling from the DSS database.

Laboratory testing of net samples

During interviews, 50 nets that were bought or re-treated during the past 12 months were exchanged for new ones, and insecticide residues were measured with high performance liquid chromatography (HPLC) according to Enayati *et al.* (2001).

For each net four sides were chosen and 10×10 cm net samples were collected from each side, wrapped in aluminium foil in separate plastic bags, and kept at 4°C until testing. Later in the laboratory, these pieces were soaked in 5 mL acetone in a glass vial. The tubes were shaken for a few minutes and the pieces of bednet were washed with 2 mL excess acetone in the same vial. The extract was allowed to evaporate at room temperature with a continuous air current from a ventilator to speed up the process. The residues from the four samples were combined by washing each vial sequentially with 3 mL of acetone. The solution was placed into two 1.5 mL Eppendorf tubes and centrifuged for 30 min at 16 000 g. The supernatant was divided into four aliquots, three of which were placed into separate glass tubes. The last aliquot was placed in a clean Eppendorf tube. The extracts in glass tubes were used in a

new colorimetric assay (see below), whereas the extract in the Eppendorf tube was dried, dissolved in 50 µL acetonitrile, centrifuged at 16 000 g for 10 min and used for HPLC analysis. Appropriate control replicates were prepared by extracting materials from untreated bednets with the same colour and size.

HPLC measurements were performed using a Beckman System Gold with a 166 UV/Visible detector and a 126 solvent module pump, equipped with an ODS ultrasphere reverse phase C18 column (Beckman System Gold, High Wycombe, U.K.). The column was equilibrated with methanol:acetonitrile:distilled water (72.5:12.5:15) at ambient temperature with a flow rate of 0.7 mL/min and the peaks were detected at 210 nm by the UV detector. The extracted samples were re-dissolved in 50 µL acetonitrile and were injected into the system manually. Insecticides were identified based on the shape and the retention time of the peaks on the chromatogram and quantified against standard curves of analytical grade insecticide.

Investigating washing patterns

A second questionnaire was designed to describe the washing habits of people. In total, 66 people were questioned about how many times they washed their nets per year, what kind of soap they applied, whether washboard or similar hard surfaces were used for washing and what happened to the rinse-water afterwards.

In addition, 17 people were observed while they were washing their nets. Items of interest were: pH of the washing and rinsing solution, duration of the washing process, how they carried it out and where they poured the washing and rinsing water afterwards.

Bio-assays of nets with different concentrations of insecticide

Nets were treated with target doses of 0.5, 2, 5, 10 and 30 mg/m² of deltamethrin, alphacypermethrin or lambdacyhalothrin and were tested with bioassays. *Anopheles gambiae* (Giles) *sensu stricto* from a strain reared at the Ifakara Health Research and Development Centre (IHRDC) were used. Impregnated pieces of netting were wrapped around a ball-shaped cage of 10 cm diameter, made of iron wire, and batches of 11 mosquitoes per bioassay were exposed for 3 min to the netting. After exposure they were removed carefully with a mouth aspirator and put into a paper cup with access to sugar water. The knockdown rate was observed after 15 and 60 min, and the mortality rate after 24 h. For each insecticide and concentration four repeats (total 44 mosquitoes) were carried out. Controls were tested with untreated netting and results of a series were discarded if the 24-h mortality exceeded 10%. Subsequently, the insecticide dosage was determined by HPLC (as described above). All data were processed by EpiInfo 6.02b (CDC, Atlanta, GA, U.S.A.). Confidence intervals (CI) are given at the 95% level.

Results

Observations on nets in the field

In the study area 107 out of 218 (49%; CI: 42–55%) of all nets were from the KINET programme. Most nets were made of polyester, with only six nets made of cotton and one made of polyethylene (a net from the Philippines from a previous ITN project). All project nets except nine were green medium-sized nets ($1.5 \times 1.8 \times 2.1$ m). Thirty-one per cent of all nets were white, 17% blue and 3% square green non-programme nets. Eighteen per cent (CI: 13–24%) of all 218 nets were round-shaped, mostly blue and white.

Overall, 6% (CI: 4–12%) of nets were 40 denier, 61% (CI: 54–68%) were 75 denier and 32% (CI: 25–37%) were 100 denier. The denier rating of the fibre of two nets could not be determined with certainty.

Table 1 shows the age of nets reported by interviewees. Roughly half the nets were reported to be aged 2 years or less. Forty-five per cent of the observed nets appeared to be in bad condition (defined as more than seven large holes). The median number of holes larger than 2 cm in diameter was 7, the mean number of holes was 12.0 (SD: 13.6). Only 14% (CI: 10–20%) of all nets were undamaged. Many holes are likely to be caused either by rough wooden bed frames, by rats that gnaw through nets to eat kapok mattresses or by fire from different sources (oil lamps, candles, sparks from cooking).

There was no difference in the number of holes in nets with 75 denier (mean: 12.3 holes) and 100 denier (mean: 9.7 holes), but a significant difference was found between 45 denier (mean: 20.5 holes) and 100 denier nets ($P < 0.05$). A significant difference ($P < 0.01$) was also found between the number of holes in nets purchased during the past 2 years (mean: 8.5 holes) and nets older than 2 years (mean: 16.0 holes).

Twenty-two per cent (CI: 17–28%) of the respondents reported having re-treated their nets at least once since purchase and 20% (CI: 15–26%) reported re-treating their nets at least once in the past 12 months. The similarity of these results suggests that recall might be a problem beyond a period of 6–12 months.

Table 1. Age group of nets (as reported by respondents) in proportions and cumulative proportions. Total number of nets = 218; 95% confidence intervals are indicated in brackets

Age group (years)	Proportion (%)	Cumulative proportion (only nets of known age: $n = 207$)	
		Age group (years)	Proportion (%)
Unknown	5 (2–8)		
≤ 0.5	12 (8–18)	≤ 0.5	11 (7–16)
$> 0.5 \leq 1$	23 (29–44)	≤ 1	37 (31–44)
$> 1 \leq 2$	13 (9–19)	≤ 2	52 (45–59)
$> 2 \leq 3$	18 (13–24)	≤ 3	71 (64–77)
$> 3 \leq 4$	11 (8–16)	≤ 4	83 (77–88)
$> 4 \leq 5$	5 (2–8)	≤ 5	87 (82–92)
> 5	13 (9–19)	≤ 6	93 (88–96)

Insecticide doses on field nets

Table 2 shows insecticide residues of 50 nets collected in the field and assessed by HPLC. All of these nets were reported to be purchased (pre-treated) or re-treated within the last 12 months. Overall, only 32% of these nets had an insecticide dose ≥ 5 mg/m², which is considered as the lower limit of effectiveness (WHOPES, 2003). More than one-third (34%) had no detectable insecticide. Nets not older than 12 months had a mean insecticide dose of 10.5 mg/m² (SD: 17.1). On average, nets between 1 and 2 years of age had 7.9 mg/m² (SD: 14.5), nets between 2 and 3 years 8.7 mg/m² (SD: 17.8) and nets older than 3 years 7.7 mg/m² (SD: 13.2).

Twenty (80%) out of 25 nets whose owners claimed to have re-treated them during the last 12 months had detectable insecticide on them and six (24%) had more than 10 mg/m² of insecticide. Of the 19 KINET project nets 12 (63%) had detectable insecticide and six (32%) had more than 10 mg/m² of insecticide.

Washing of nets

All 83 respondents to the questionnaire on net washing habits reported using a detergent but none reported rubbing the net against a washboard, a piece of stone or wood. It can therefore be assumed that the netting was handled gently. 83% (CI: 73–90%) of the interviewees reported that they used soap bars and 17% (CI: 10–27%) used commercially available soap powder (such as 'OMO Blue' TM).

While observing washing habits in 17 cases we measured the pH of the soapy liquid remaining in the buckets after the procedure was finished. We found a relatively high average pH of 9.3 (SD: 0.47), with a range of 8.5–9.9. An experiment with local soap dissolved in water from local wells and rivers (pH range: 6.5–7.4) revealed that small amounts of soap (≥ 1 g/L) made the solution alkaline (range: 7.7–10.7). The pH never went higher than 10.7, even with a large amount of soap (> 30 g/L).

Table 2. Insecticide residues in mg/m² of 50 nets collected in the field and assessed by HPLC. 95% confidence intervals are indicated in brackets

Concentration (mg/m ²)	Proportion (%)	Cumulative proportion	
		Concentration (mg/m ²)	Proportion (%)
0	34 (21–49)		
$> 0 \leq 2$	26 (15–40)	> 0	66 (51–79)
$> 2 \leq 5$	8 (2–19)	> 2	40 (26–55)
$> 5 \leq 10$	8 (2–19)	> 5	32 (20–47)
$> 10 \leq 15$	0 (0–7)	> 10	24 (13–38)
$> 15 \leq 20$	4 (0–14)	> 15	24 (13–38)
$> 20 \leq 25$	0 (0–7)	> 20	20 (10–34)
$> 25 \leq 30$	6 (1–17)	> 25	20 (10–34)
> 30	14 (6–27)	> 30	14 (6–27)

The interviewees were also asked where they disposed of the washing water. Eighty-nine per cent (CI: 79–96%) answered that they poured the water on the ground or in a latrine close to the house. All others poured the solution into the river where they had washed the net.

Answers to the question on frequency of washing gave a wide range of answers. Forty-five per cent (CI: 33–58%) reported washing their nets between zero and five times per year and 32% (CI: 21–44%) more than five times. All other respondents could not remember. The mean number of washes was 8.6 times per year (SD: 10.1) and the median four. The interviewees stated that it took them 14 min (SD: 13.5 min) to wash a net. However, the 17 observed cases revealed that it actually took less time for this task (mean 6 min; SD: 2.2 min).

In our sample the number of washes of coloured nets ($n=59$; mean: 9.3) exceeded the number of washes of white nets ($n=24$; mean: 7.1). This does not support the decision of ITN programmes to prefer coloured nets because white nets are believed to appear dirty after a short time and are therefore washed more often.

Bio-assay on net samples with known insecticide concentration

Although the target doses were 0, 0.5, 2, 5, 10 and 30 mg/m² the effective insecticide doses as measured by HPLC were found to be: 0, 0.7, 2.5, 4, 6.4 and 29.3 mg/m² for deltamethrin, 0, 0.5, 2.2, 5.4, 11.2 and 31.1 mg/m² for alphacypermethrin and 0, 0.6, 2.9, 7.6, 17.3 and 42.7 mg/m² for lambdacyhalothrin.

Figures 1–3 show knockdown rates after 15 min (KD 15), 60 min (KD 60) and mortality rates after 24 h (mort 24 h). These results show that for deltamethrin a dose of 4 mg/m² still gave a KD 60 rate over 80%. For alphacypermethrin the KD 60 was good at 5.4 mg/m² (80%) but the 24-h mortality rate was lower: 38%. With this insecticide only the highest dose gave a 100% KD 60 and mortality. Similar

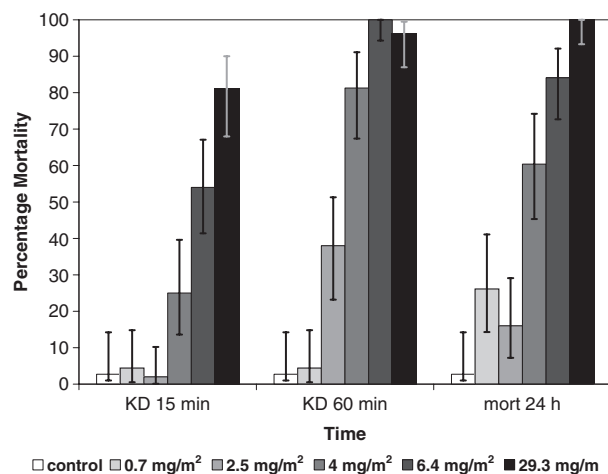


Fig. 1. Knockdown rates after 15 min (KD 15 min), 60 min (KD 60 min) and mortality rates after 24 h (mort 24 h) on nets treated with deltamethrin. Vertical bars indicate 95% confidence intervals.

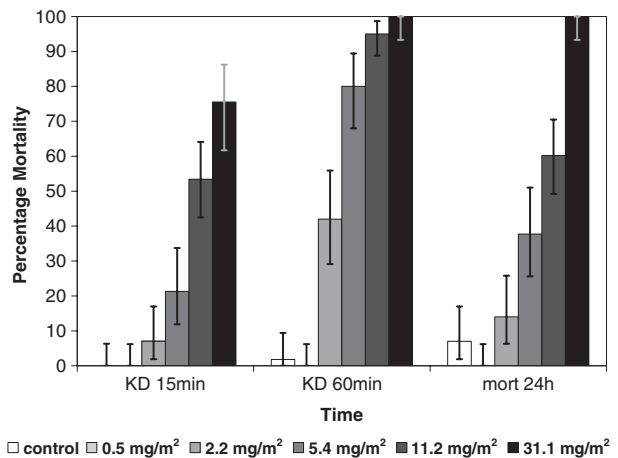


Fig. 2. Knockdown rates after 15 min (KD 15 min), 60 min (KD 60 min) and mortality rates after 24 h (mort 24 h) on nets treated with alphacypermethrin. Vertical bars indicate 95% confidence intervals.

but slightly better results were obtained with lambdacyhalothrin. For more details see Erlanger (2002).

Discussion

The results from a field survey carried out as part of a large-scale ITN social marketing programme 4 years after implementation are presented here. The nets found in the field were mostly made of polyester. They were both an indicator of people's preferences and also an indication of stock sold by the local shops. It was surprising that non-programme nets were so numerous in an area with an active ITN-programme. This indicated that the commercial sector had been stimulated by KINET, a fact confirmed by the social marketing manager (H. Mponda, personal communication).

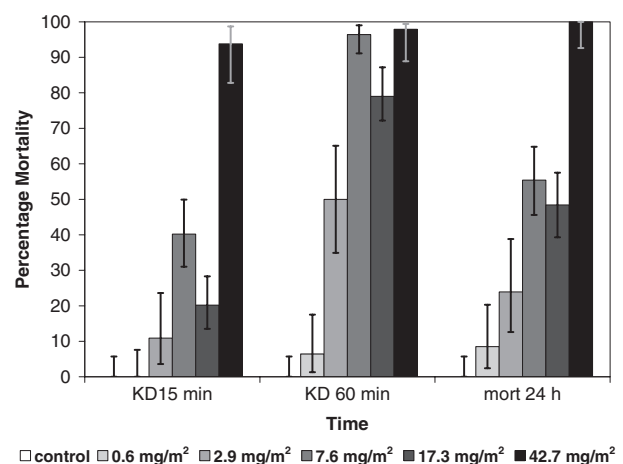


Fig. 3. Knockdown rates after 15 min (KD 15 min), 60 min (KD 60 min) and mortality rates after 24 h (mort 24 h) on nets treated with lambdacyhalothrin. Vertical bars indicate 95% confidence intervals.

Information about the useful life of polyester nets is scarce in the literature. Although previous sources often cited 5 years (Feilden, 1996), others found that nets are still effective 3–4 years after their implementation (Maxwell *et al.*, 2002). As the ITN-programme in that area (Tanga Region, Tanzania) was based on free provision and re-treatment of ITNs, net coverage was high and more than 90% were re-treated. Thus, due to mass killing of vectors, the overall health benefit of these ITNs was still very high, even if they were torn. Here the effective usable life of rarely or non-re-treated nets was found to be closer to 2–3 years. After that time nets were dirty, hardly with any insecticide and full of holes. This estimate seems to concur with that of Kroeger *et al.* (1997) in different South and Central American countries (estimated duration of effective life: 2.3–4 years). Nevertheless, it seems that nets still provide some protection even if torn, dirty and without insecticide (Clarke *et al.*, 2001).

Unfortunately, the KINET programme did not establish a system to record sales and track individual nets. To obtain reliable data for calculating the duration of the effective life of nets in a certain area they have to be labelled and tracked for at least 5 years. Another approach could be a follow-up examination of nets within a scientific trial in which all nets are distributed at a given point in time. In any case, the findings reported here suggest that ITN-programmes should seriously consider distributing nets with a higher fibre weight than 100 denier.

Compliance with insecticide re-treatment appeared to be very low, despite substantial efforts in promoting and subsidizing re-treatment (Armstrong-Schellenberg *et al.*, 2002). The survey reported here confirmed that most of the nets in the field are never re-treated. Even for those that are re-treated, according to their owners, less than one-third had enough insecticide ($\geq 5 \text{ mg/m}^2$) to guarantee a sufficient entomological impact. Hence, it is likely that responses given regarding treatment were not reliable. Unfortunately, this could not be further validated. At least there is the hope that this problem will lose its importance in the future with the development and commercialization of nets treated with a long-lasting insecticide (Guillet *et al.*, 2001).

Project managers should expect that nets will be washed regularly with soap. A previous study showed that a low pH leads to significantly higher insecticidal uptake (Lindsay *et al.*, 1991). To date, no investigation has tested systematically the impact of the pH of washing solutions on the fading of insecticide on netting. Such an effect is to be expected, especially if the pH exceeds 10.

It is likely that householders exaggerate the frequency of net washing. Research in a rural setting in The Gambia found that it is more likely that nets are washed only between two and three times per year (Miller *et al.*, 1995). This was also confirmed by Maxwell *et al.* (2003b), who reported mortalities close to 100% even after about three washes and 15 months of domestic use. On the other hand, work in an urban setting in Tanzania (where one would expect washing to be more frequent because of different social norms) showed that nets were indeed washed frequently (Miller *et al.*, 1999).

The fact that most people poured the used wash-water into latrines or on to the ground confirms that the impact of pyrethroids from ITNs to the environment is expected to be minimal because pyrethroids degrade quickly in the soil (Hirsch *et al.*, 2002).

The mortality rates recorded here in the bioassays with *An. gambiae* exposed to deltamethrin were similar to those of Curtis *et al.* (1996) and Lindsay *et al.* (1991), who both found 100% mortality with 25 mg/m^2 . Adams *et al.* (2002) carried out large numbers of bioassays with laboratory-reared *An. gambiae*, as well as chemical assays on samples from nets that had been in domestic use in Malawi. He found a very high mortality (97.7%) on nets that had been treated with deltamethrin at only 3.2 mg/m^2 . For alphacypermethrin, bioassays also carried out by Adams *et al.* (2002) with 6.5 mg/m^2 and 27.5 mg/m^2 resulted in 93.5% and 96.4% mortality, respectively. For the lower dose this was substantially higher than our mortality of 40% with 5.4 mg/m^2 . For nets impregnated with lambdacyhalothrin, our observed mortality rates were also different from the ones reported in the literature. For 10 mg/m^2 , Curtis *et al.* (1996) found a 100% mortality in *An. gambiae*, whereas Jawara *et al.* (1998) found 25%. WHOPES (2003) recommends 5 mg/m^2 deltamethrin or lambdacyhalothrin as the lower limit of effectiveness. Nevertheless, Curtis *et al.* (1996) found a high mortality in experimental huts with 3 mg/m^2 of deltamethrin or lambdacyhalothrin.

Several factors may be responsible for these inconsistencies, mainly relating to the testing itself. The target doses used here were confirmed by reliable HPLC assessment and hence should not be the problem. However, bioassays done in different settings under various environmental conditions may lead to different results. The *Anopheles* strain and its susceptibility to insecticide is certainly of importance. Some investigators used wild-caught insects, which are likely to be older and blood-fed. It is also relevant whether *Anopheles* mosquitoes were blood- or glucose-fed before testing. Most importantly, it should be kept in mind that bioassays only give us a very rough estimation of the situation in the field. Bioassays can be standardized to a certain extent but variations are likely to occur. The dosage that is measured by HPLC reflects the content of insecticide within and on the netting. Bioassays, however, give an estimate for the insecticide on the surface of the net that can potentially be absorbed by mosquitoes. A more vexing problem is the fact that the relationship between bioassay results and epidemiological impact is not known. Hence, it is unclear at what level of insect killing a full epidemiological protection is still afforded. This is of major importance given the poor re-treatment record in all ITN programmes to-date, and should be investigated as a matter of priority.

The present work has attempted to provide some essential field data on ITNs as used in a real-life programme. Many basic parameters remain yet to be measured in order to plan future large-scale programmes more effectively. Opportunities for such studies should arise increasingly as more ITN programmes are starting. This, plus the constant development of new products (more resistant netting, longer-lasting

insecticide treatment), and their increased availability and affordability, will ensure that the major health impact that ITNs can potentially deliver will be realized.

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