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Evaluation of long-lasting insecticidal nets after 2 years of household use

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Summary Development of long-lasting insecticidal nets (LLINs) may eliminate the need for insecticide retreatment of ITNs. While two LLINs (Olyset®, Sumitomo Chemical Co., Japan; and PermaNet® 1.0, Vestergaard-Frandsen, Denmark) have received recommendations from the World Health Organization Pesticide Evaluation Scheme, field-testing under normal use has been limited. We used a survival analysis approach to compare time to net failure of conventional polyester bednets treated only with deltamethrin to two LLINs and two candidate LLINs (Olyset®; PermaNet®; Insector, Athanor, France; and Dawa®, Siamdutch Mosquito Netting Co., Thailand). Additionally, we evaluated nets treated with a process designed to increase the wash-durability of permethrin-treated nets through the addition of cyclodextrin (a starch) in the treatment process. Houses in western Kenya were randomly assigned to one of the six net types and nets were distributed to cover all sleeping spaces. Households were visited monthly to assess reported side effects in inhabitants and washing frequency. Nets were evaluated for insecticidal activity by periodic WHO cone bioassays with mortality assessed at 24 h. Nets with bioassay mortality <70% were assayed monthly until failure, defined as the first of two consecutive bioassay mortality rates <50%. Time to failure was analyzed using an extended Cox Proportional Hazards model controlling for the cumulative number of washes. We distributed 314 nets to 177 households in June–July 2002; 22 nets (7.0%) were lost to follow-up and 196 (62.4%) failed during the first 2 years of the evaluation. Controlling for cumulative number of washes, PermaNet® 1.0 [Hazard Ratio (HR) 0.14, 95% Confidence Interval (CI) 0.06–0.31] had a significantly lower risk of failure than conventional nets while Insector had a significantly higher risk of failure (HR 2.57, 95% CI 1.06–4.15). The risks of failure of the remaining nets (Olyset®: HR 1.29, 95% CI 0.79–2.10; Dawa®: HR 0.58, 95% CI 0.32–1.18; cyclodextrin: HR 0.65, 95% CI 0.40–1.1) were not significantly different from that of a conventional net. PermaNet® 1.0 performed significantly better than conventional nets and should be recommended to malaria control programs.

keywords malaria prevention, long-lasting insecticide treated bed nets

Introduction

Several community-randomized controlled trials have shown that insecticide-treated bednets (ITNs) substantially reduce pediatric malaria morbidity and all-cause mortality in sub-Saharan Africa (Phillips-Howard *et al.* 2003; Lengeler 2004). Consequently, as a key strategy of the Roll Back Malaria initiative, ITNs are being implemented as part of national malaria control programs around the world (WHO 2002, 2003).

ITNs reduce malaria transmission through the combination of a physical barrier (which prevents mosquitoes from feeding on occupants) and an insecticide (which both kills and repels malaria vectors). Comparisons of treated and untreated nets have highlighted the importance of the insecticide in realizing significant public health benefits (Lengeler 2004). The presence of the insecticide likely augments a community-level effect: through mass killing of malaria vectors, individuals without ITNs but living near a household with ITNs receive some protection against malaria (Hawley *et al.* 2003). Additionally, recent data have highlighted the importance of timely retreatments of ITNs with insecticide in reducing malaria transmission and child mortality. In western Kenya, when retreatment was delayed more than 6 months there was a 79% loss of efficacy in reducing indoor-resting densities of *Anopheles* mosquitoes (Gimnig *et al.* 2003) and a 35% loss of efficacy in reducing child mortality (Phillips-Howard *et al.* 2003).

Despite the important role of the insecticide in reducing malaria transmission, retreatment rates in communities generally lag far behind net use (Armstrong-Schellenberg *et al.* 2001). Low retreatment rates have been attributed to the extra expense and effort required by net owners to access retreatment as well as the perception that it is the physical barrier of the net, and not the insecticide, that provides protection (Armstrong-Schellenberg *et al.* 1999; Snow *et al.* 1999).

In response to the difficulty of promoting net retreatment, technical solutions that reduce or eliminate the need for retreatment of bednets have been sought. Several manufacturers have developed processes to incorporate insecticide into polyethylene fibers, which are then used to make netting material, or to bind insecticide onto polyester fibers during the manufacturing process using a resin. Processes to make the insecticide on ITNs already in use more wash-resistant are also under development. Under laboratory or controlled conditions, long-lasting insecticidal nets (LLINs) have been shown to retain lethal doses of insecticide after frequent washing (Ordonez Gonzalez *et al.* 2002). However, field-testing of LLINs under normal use conditions has been limited.

We evaluated 2 LLINs (PermaNet® 1.0, Vestergaard-Frandsen, Denmark; and Olyset®, Sumitomo Chemical Co., Japan) and two candidate LLINs (Dawa®, Siam Dutch, Thailand; Insector, Athanor, France) developed by commercial net manufacturers. Additionally, we evaluated nets that were treated with a combination of permethrin and cyclodextrin (a starch) to render the nets more wash-resistant (Division of Parasitic Diseases, Centers for Disease Control and Prevention). All nets were used by villagers in western Kenya under normal household conditions and were compared to conventional nets treated with deltamethrin (K-O Tab®).

Methods

Long-lasting insecticide-treated nets

Characteristics of the LLINs and candidate LLINs (hereafter, all test nets are simply referred to as 'LLINs') evaluated in this study are described in Table 1. All nets were treated with either permethrin or deltamethrin. Polyethylene fibre Olyset® nets came with special instructions to wash the net every 6 months and then place the net in the sun in a clear plastic bag provided by the manufacturer to regenerate the insecticide. Olyset® was the only coloured net we evaluated.

In addition, we tested white polyester bednets treated with permethrin (1 g/m^2) and cyclodextrin at a molar concentration 1.5 times that of the pesticide (4.6 g/m^2) to render the insecticide more wash-resistant (henceforth referred to as the 'cyclodextrin' net). All nets were compared to a conventional white polyester net treated by our study staff with deltamethrin (available locally in a sachet as K-O Tab®) at a concentration of 25 mg/m^2 (henceforth referred to as the 'conventional' net). In all, six different types of nets were tested, with three types treated with permethrin and three treated with deltamethrin.

Study area

The study was conducted in western Kenya near the Centre for Vector Biology and Control Research of the Kenya Medical Research Institute (KEMRI), approximately 10 km from the city of Kisumu. Most residents of the area are of the Luo ethnic group and live in scattered family compounds consisting of one or more houses and surrounding agricultural fields. An average of 5–6 people live in each house. Malaria transmission in this area is intense and occurs year-round with peaks after the two annual rainy seasons (March–June and October–November) (Beier *et al.* 1994). The primary vectors in this region are *Anopheles gambiae*, *A. arabiensis* and *A. funestus*. A global positioning system was used to map all of the houses in the study area in August 2002; details of the number of occupants and sleeping spaces in each house were also recorded.

Study design

We employed a cohort design to compare time to failure of the four LLINs and the cyclodextrin net to the

Table 1 Characteristics of nets included in this evaluation

Brand name and manufacturer	Type of material	Insecticide	Concentration of insecticide (mg/m^2)	Colour
PermaNet® 1.0	Polyester	Deltamethrin	50	White
Dawa®	Polyester	Deltamethrin	50	White
Insector	Polyester	Permethrin	1500	White
Olyset®*	Polyethylene	Permethrin	1000†	Blue
Cyclodextrin net	Polyester	Permethrin	1000	White
Conventional net	Polyester	Deltamethrin	25	White

* Manufacturer's instructions: wash net every 6 months; place net in plastic bag and leave in the sun after washing.
† Manufacturer gives the active ingredient as 2% permethrin w/w, which is approximately 1000 mg/m^2.

conventional net. Heads of households were provided with the manufacturer's instructions for net washing and use, if any; no additional instructions were provided by the study team. Nets were bioassayed regularly to determine whether the insecticide remained effective in killing insecticide-sensitive anophelines from a laboratory colony. Washing frequency and any retreatment with insecticide were measured monthly throughout the study. If households reported washing their Olyset® nets, we asked whether they had followed the manufacturer's instructions and placed the net in a plastic bag in the sun. Potential side effects in adults (upper respiratory symptoms, headache or skin rashes) were measured for the first 3 months of the study only.

Sample size calculations

We calculated that with 44 nets of each type we would have an 80% probability of detecting a difference in time to net failure between an LLIN and conventional net, assuming a median time to failure of 1 year for conventional nets and 2 years for LLINs and a two-sided alpha level of 0.05.

Distribution of nets

Nets were distributed to households in June and July 2002 and were followed for 2 years. Because there were three deltamethrin and three permethrin net types comprising the six arms of the study, and to ensure that neighbouring houses contained nets with the same insecticide, only compounds with exactly three occupied houses were eligible for participation. Additionally, to avoid a situation whereby a few houses with large numbers of sleeping spaces might unduly influence results, only compounds where each house had three or fewer sleeping spaces were eligible. Eligible compounds were paired according to geographic proximity and pairs were selected according to a randomly assigned order until minimum sample size requirements for all arms of the study were met. One compound of each pair was randomly assigned to deltamethrin nets and the other to permethrin nets. Within each compound, each house was randomly assigned one of three net types within the insecticide group. The number of nets allocated to each house was determined from the number of sleeping spaces. Each net was permanently marked with an identifier. The number and type of net distributed to each house were recorded.

Determination of net failure

Standard WHO cone bioassays (WHO 1975) were conducted on 4–10 nets of each type at baseline and on all nets at approximately 6-month intervals. Colony-reared, 2–3-day-old unfed female *A. gambiae* of the Centers for Disease Control and Prevention (CDC) pinkeye strain (susceptible to pyrethroids) were transported to and from the field in cool boxes in cardboard cups covered by mosquito netting and draped with a damp cloth. Cotton wool soaked in a sugar solution was put on the top of the cup to reduce starvation-associated mortality. A plastic WHO cone and a circular piece of cardboard were clipped together on either side of the net in three different locations (two sides and one top). The net was left in its resting position. Approximately 10 mosquitoes were transferred into each cone. Mosquitoes remained in the cone, exposed to the net, for 3 min before being transferred back to cardboard cups. Mortality was recorded at 24 h. Each day, four cups with control mosquitoes were carried to the field and exposed for 3 min to an untreated polyester net. If control mortality was found to be greater than 20%, all assays for that day were repeated. No attempt was made to blind the either the study participants or the technicians doing the bioassays to the identity of the net types.

We chose a bioassay mortality rate <50% to indicate a failed net, based upon results from a community-randomized trial of ITNs conducted near the study area. That study indicated that the efficacy of ITNs in reducing infant mortality decreased significantly when retreatment of ITNs was delayed more than 6 months (Phillips-Howard *et al.* 2003). In the same study, the average WHO cone bioassay mortality rate of a sample of 15 nets that had been retreated more than 6 months earlier was 55.4% (Gimnig *et al.* 2003). Initially, nets were defined to have failed when they had a single bioassay mortality rate of <50%. Any net with a bioassay mortality rate <70% was followed monthly until failure or a bioassay mortality rate ≥70%, when it returned to the regular schedule. Due to the unexpected high failure rate during the first round of bioassays, a second bioassay was performed approximately 1 month later on nets with initial mortality rates <70%. Failure during the first round was defined as an average bioassay rate of <50% of two consecutive bioassays. From the second round onwards, a more stringent definition of failure was employed requiring two consecutive bioassay mortality rates of <50%. All failed nets were replaced with conventional nets treated with deltamethrin.

Regeneration of Olyset® nets

The first ten Olyset® nets that failed were brought to the laboratory for further testing. The nets were bioassayed again in the laboratory then washed, placed in plastic bags provided by the manufacturer and exposed to the sun for 1–2 days before conducting another set of bioassays. All bioassays on Olyset® nets collected from the field were conducted with three WHO cone bioassays, one on the top of the net and one on each of two sides. Ten female *A. gambiae* were introduced into each cone bioassay and exposed for 3 min. Mortality was recorded 24 h after exposure.

Evaluation of house entering/exiting behaviour, feeding success

In August 2002, after nets were distributed, we randomly selected 10 pairs of compounds, one with deltamethrin nets and the other with permethrin nets, to evaluate numbers of anophelines entering, exiting and feeding according to net type. Half of the exterior of all houses in each compound was wrapped with a Colombian curtain which allowed for collection of mosquitoes exiting via windows and eaves (Service 1993). The eaves on the side not under the curtain were open. At dawn, mosquitoes that had exited the house were collected from the inside surface of the curtain or the exterior house walls with manual aspirators. In the morning, indoor-resting mosquitoes were collected by pyrethrum knock-down spray catches (PSC) (Service 1993). Approximately 1 year later, PSC was conducted in all houses where only originally assigned study nets were present.

Chemical analyses

Gas chromatography was used to measure insecticide concentration in the net fibres on a single swatch from one side of the net at baseline and after net failure. Samples from permethrin-treated nets (cut to 15 × 30 cm) were prepared for gas–liquid chromatographic analysis by adding 100 μl of 100 mg/ml didecyl phtahalte in chloroform, followed by the addition of 20 ml chloroform. Samples from deltamethrin-treated nets (cut to 15 × 30 cm) were prepared for gas–liquid analysis by adding 100 μl of 10 mg/ml alphacypermethrin in acetone, followed by the addition of 20 ml acetone. The samples were subjected to sonication for 30 min and then left standing overnight. One microliter of extract was injected into a gas–liquid chromatography system using a megabore DB-17 (J & W Scientific, Folsom, CA) capillary column. A flame ionization detector and an electron capture detector were employed for the analysis of permethrin and deltamethrin, respectively. For quantification, relative responses were compared to that of a standard solution (Richards *et al.* 1994).

Data analysis

The date of net failure was considered to be date of the first bioassay with mortality <50% that was followed by a second consecutive bioassay with mortality <50%. Nets that did not fail were censored as of the date of the last bioassay. An extended Cox Proportional Hazards model (PROC PHREG, SAS release 8.02, SAS Institute, Cary, NC) with time-varying covariates was used to model the time to net failure. A counting process procedure was used to allow the movement of nets into and out of the risk set as several nets travelled out of the study area with household members.

To control for the different washing rates between net types, the cumulative number of washes was included in the model. Interactions between the individual net types and cumulative washing frequency were evaluated to determine whether washing differentially altered the survival of different net types. The cumulative number of months that the net was hanging was evaluated as a predictor of failure to determine whether time or exposure of the net to household dust or handling reduced its effectiveness. The robust sandwich estimate was used when calculating the standard errors to account for the clustering of nets within a household.

The proportion of households reporting any side effects during the first 3 months after nets were distributed was compared among net types using Fisher's exact test. Baseline bioassay mortality rates were transformed using the arcsine of the square root and compared between net types using ANOVA procedures after testing for normality. Differences in the numbers of indoor-resting mosquitoes caught using PSC were compared between net types using a non-parametric Kruskal–Wallis test. We compared bioassay mortality rates in failed Olyset® nets before and after our study team washed and regenerated the nets using a paired *t*-test, after transforming the proportion killed with the arcsine of the square root and testing for normality of the difference between pre- and post-washing bioassay mortality rates.

Ethical review

The protocol for this study was reviewed by the institutional review boards of both CDC (Atlanta, GA, USA) and KEMRI (Nairobi, Kenya). Written, informed consent was received from all participating households.

Results

We distributed 314 nets to 177 houses in 60 compounds in June and July 2002. Between 47 and 60 nets of each type were distributed; numbers of nets distributed differed between net types due to variation in the number of sleeping spaces in houses randomly assigned to the different arms of the study (Table 2). We observed nets for 3741

Table 2 Number of nets distributed, lost and failed, months of observation and washing frequency by type

Net type	Distributed (*n*)	Lost *n* (%)	Failed* *n* (%)	Months of observation	Number of months between washes
PermaNet® 1.0	54	9 (16.7)	8 (17.8)	910.4	3.7
Dawa®	52	5 (9.6)	27 (57.4)	720.1	2.9
Insector	52	2 (3.8)	50 (100.0)	381.0	2.6
Olyset®	49	0 (0.0)	39 (79.6)	556.7	8.3
Cyclodextrin net	47	3 (6.4)	28 (63.6)	669.5	3.3
Conventional net	60	3 (3.3)	44 (77.2)	751.2	3.4

* Percentage of nets that failed is calculated as a proportion of the number distributed minus the number lost.

net-months and recorded 1134 net-washing episodes. There were 22 (7.0%) nets lost to follow-up and 196 (62.4%) net failures. No households reported retreating any nets with insecticide.

Detergent was used every time nets were washed; bar soap was used in 898 (79.2%) and powder soap in 236 (20.8%) washes. The frequency of washing differed by net type (Table 2). Olyset® was washed at a rate of once every 8.3 months whereas Insector was washed once every 2.6 months. The washing frequency likely reflects the colour, durability and dirt resistance of the products.

Only 19 households (10.8%) reported any possible side effects (rash, runny nose, sneezing, cough) during the first 3 months. There was no statistically significant difference by net type ($P = 0.34$).

Bioassay mortality

Baseline bioassay mortality rates did not differ by net type ($P = 0.22$). We conducted 1269 bioassays in four rounds. We had originally planned to assay approximately 14 nets per day in order to assay all nets in 1 month. The daily requirement for a minimum of 420 unfed 2–3-day-old female *A. gambiae* proved to be unfeasible so we conducted four rounds of bioassays (October–January 2003; March–May 2003; July–January 2004; and March–July 2004), inclusive of follow-up assays, in 2 years.

The average mortality of control mosquitoes was 6.5%. Control mortality was >20% on one day when three nets were bioassayed but was not noted until data were analyzed; the corrected mortality rate indicates that one of these nets (a cyclodextrin net) likely would have been categorized as failed if the background mortality had been lower.

Mortality rates corrected for control mortality using Abbott's formula were highest for PermaNet® 1.0 (>70% in all surveys) and lowest for Insector (<50% in all but the baseline survey) (Figure 1). Each round of bioassays included only nets that had survived from the previous round. There was some indication that the nets surviving into the fourth round of bioassays had higher bioassay mortality rates on average than in previous rounds.

Survival analysis

After 2 years of normal household use, the number and percentage of the different net types remaining effective in killing susceptible anophelines was as follows: PermaNet® 1.0, 37 (82.2% of nets not lost to follow-up); Dawa®, 20 (40.8%); Insector, 0 (0%); Olyset®, 10 (20.4%);

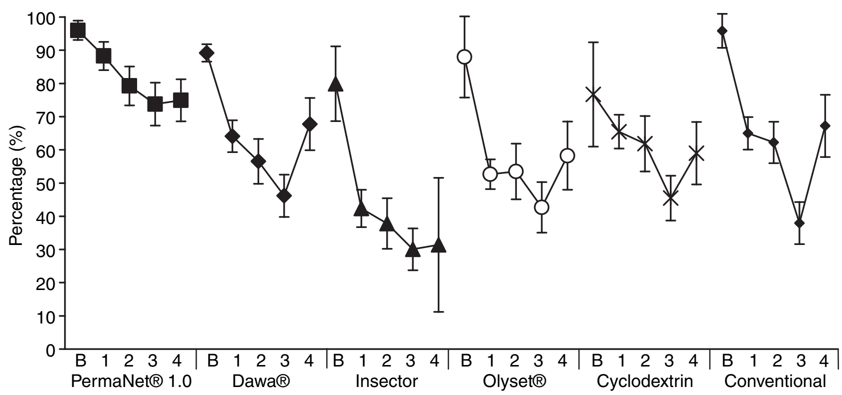

Figure 1 Mean bioassay mortality rates (corrected) and 95% confidence intervals by net type at baseline (B) and each round (numbers in parentheses indicate the number of nets tested in each round; failed nets were included in the round in which they failed but removed from subsequent rounds).

cyclodextrin, 16 (36.4%); and conventional, 13 (14.0%). Although the washing rate differed by net type, the cumulative number of washes was not significantly associated with failure [hazard ratio (HR) 1.06, 95% confidence interval (CI) 0.99–1.14]. Despite the lack of statistical association, we included cumulative number of washes in the final model to control for potential confounding. None of the 2-way interaction terms for cumulative number of washes and net type was significant, indicating that there was no effect modification of the association between net type and failure rate by total number of washes. The number of months the net was hanging was not significantly associated with failure (HR 1.02, 95% CI 0.97–1.08).

There were significant differences in time to net failure for two of the LLINs compared to the conventional net (Figure 2). The risk of failure of PermaNet® 1.0 was 86% less than a conventional net (HR 0.14, 95% CI 0.06–0.31). The risk of failure of the Insector net was more than twice that of a conventional net (HR 2.57, 95% CI 1.06–4.15). The risks of failure of the remaining nets (Dawa®: HR 0.58, 95% CI 0.32–1.08; Olyset®: HR 1.29, 95% CI 0.79–2.10; cyclodextrin: HR 0.65, 95% CI 0.40–1.1) were not significantly different from that of a conventional net.

House entering/exiting and feeding success

A total of 59 houses in 10 paired compounds was sampled for entering and exiting mosquitoes in August 2002 immediately after nets were distributed. A total of 17 anophelines (14 *A. gambiae* s.l. and 3 *A. funestus*) was found resting indoors but there was no difference by net type ($P = 0.12$). No anophelines were found to have exited during the night. Only 1 anopheline (in a house with Olyset® nets) was found to have successfully blood fed.

Of 109 houses eligible for evaluation of indoor-resting mosquitoes in June 2003, 99 (90.8%) were available for sampling. We collected 120 *A. gambiae* s.l. and 6 *A. funestus*. Houses containing conventional nets had the lowest indoor-resting density of anophelines [0.57 mosquitoes/house (m/h)] while houses with Dawa® nets had the highest (2.05 m/h). However, there was no statistically significant difference by net type ($P = 0.5$).

Regeneration of Olyset® nets

Ten Olyset® nets that had failed and been removed from the field after approximately 3 months of use yielded an average bioassay mortality of 44.1% (95% CL:

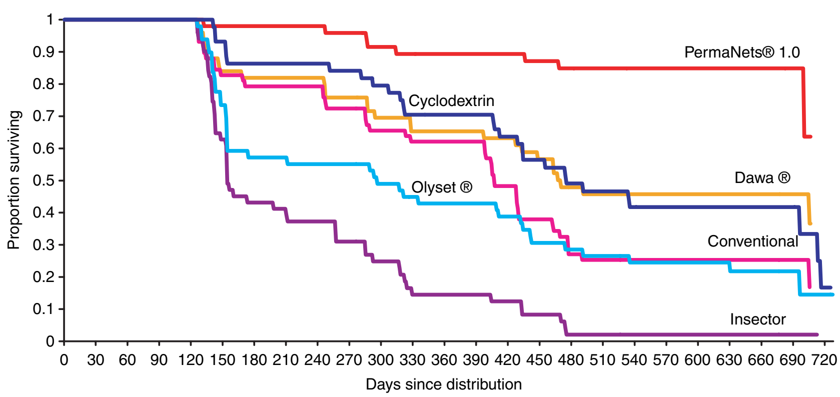

Figure 2 Kaplan–Meier survival estimates of the proportion of nets remaining effective.

Table 3 Concentration of insecticide on failed nets

Net type	n	Baseline mean concentration of insecticide (mg/m^2) (95% CI)*	n	Failure mean concentration of insecticide (mg/m^2) (95% CI)*
PermaNet® 1.0	3	44.9 (43.3–46.5)	1	11.1†
Dawa®	3	236.5 (131.8–341.2)	10	37.5 (13.3–61.7)
Insector	3	922.7 (762.9–1082.4)	30	590.2 (467.5–712.9)
Olyset®	3	936.7 (931.8–941.6)	12	1118.7 (1001.8–1235.5)
Cyclodextrin net	3	759.0 (592.7–925.3)	6	278.3 (173.1–383.5)
Conventional net	3	14.0 (8.2–19.8)	20	5.2 (3.5–6.9)

* 95% confidence interval of mean concentration.
† 95% confidence interval could not be calculated because only one net was tested.

35.3–53.0). After washing and regeneration, the nets yielded an average bioassay mortality of 86.4% (95% CL: 80.1–92.7). The average difference in bioassay mortality before and after washing plus regeneration was 42.2% (95% CL: 32.0–52.5). In a paired *t*-test, the differences were statistically significant ($P < 0.001$).

Chemical analyses

Baseline chemical analyses on three nets of each type found that none of the nets analyzed reached their target dose of insecticide, except for Dawa® nets which averaged nearly five times the target dose. Insecticide concentration was measured on 79 nets after failure. All failed nets except Olyset® showed insecticide concentrations much less than the baseline concentrations and the target dose (Tables 1 and 3). Only 3 of the 12 failed Olyset® nets tested showed concentrations less than the target of 1000 mg/m^2, indicating that the failure of these nets was due not to a lack of sufficient insecticide but rather to some problem with bio-availablity of the insecticide on the surface of the net fibre.

Discussion

LLINs have the potential to increase effective coverage of ITNs in sub-Saharan Africa if the efficacy of the insecticide bound to or incorporated into the net fibres lasts significantly longer than conventionally treated nets. We compared two LLINs, two candidate LLINs, and one process designed to render nets already in use more wash-resistant, with conventional polyester nets treated with deltamethrin. Nets were distributed to villagers in western Kenya who were advised to follow the manufacturer's recommendations, if any, but otherwise were left to use and wash the nets as they chose. Nets were compared based upon the time to failure with net failure defined as a confirmed 24-hour mortality of susceptible *A. gambiae* exposed to the nets for 3 min of <50%. We found that one of the commercial LLINs (Vestergaard-Frandsen's PermaNet® 1.0) performed significantly better than conventional nets. Approximately 2 years after nets were distributed, 82.2% of the PermaNet® 1.0 in the field remained effective in killing susceptible anophelines compared to only 22.8% of the conventional nets; the risk of failure of PermaNet® 1.0 was significantly less than conventional nets. The Insector net by Athanor performed significantly worse than conventional nets, with no nets remaining effective after 2 years and more than a 2.5-fold increase in the risk of failure. The other commercial LLINs, Olyset® and Dawa®, and the cyclodextrin net performed no better than conventional nets.

PermaNet® 1.0 has been evaluated in only a few published studies. Early versions of PermaNet® 1.0 manufactured before August 2002 showed rapid loss of insecticidal activity with washing (Müller *et al.* 2002). After the manufacturers improved their quality control procedures, the new generation of PermaNet® 1.0 performed significantly better with 79% bioassay mortality after 3 years of use, although only four nets were tested (Kroeger *et al.* 2004). After repeated testing on almost 50 PermaNet® 1.0 for a period of 2 years, our data support the conclusion that PermaNets® 1.0 retain their insecticidal activity for significantly longer than conventional nets. PermaNet® 1.0 has now received provisional WHOPES recommendation (WHOPES 2004) and a new generation, PermaNet® 2.0, which reportedly can withstand a greater number of washes, was introduced in April 2003.

Despite high mortality in baseline bioassays and high concentrations of permethrin within the net fibres, the Olyset® nets in our study lost their biological activity rapidly. The original manufacturer's instructions recommended heating the nets after washing to promote regeneration of the insecticide within the net fibres. Although participants were requested to follow these instructions, failed Olyset® nets that we removed from the field were easily regenerated from average bioassay rates below 50% to an average of 85%. A previous study indicated that Olyset® nets regenerate fully within two weeks at a constant 30°C and 80% relative humidity (WHOPES 2001). Maximum temperatures in the study area averaged 28.7°C during the 2 years of the study. However laboratory studies we conducted indicated that heating Olyset® nets to 60°C, but not 30 or 35°C, restored the biological activity of these nets after washing (Gimnig *et al.* 2005). These results suggest that regular heat-assisted regeneration of the Olyset® net may be necessary to maximize its efficacy for the prevention of malaria.

The poor performance of Olyset®, a WHOPES recommended net (WHOPES 2001), contradicts previously published studies evaluating the insecticidal activity of these nets. Doannio *et al.* (1999) assayed three nets after 3 months of normal use and found them to kill 55.3% of exposed susceptible *Anopheles*. One net used for 15 months had a bioassay mortality rate of 98% and another net used for 26 months had a bioassay mortality of 100%. N'Guessan *et al.* assayed three nets with susceptible *Anopheles* after 3 years of normal use and found mortality rates of 55–83% (N'Guessan *et al.* 2001). There are several possible reasons for the observed performance of the Olyset® net in the current study. First, the strain of mosquito used may have had some resistance to permethrin or the WHO cone tests themselves may have been biased against permethrin as it has strong irritant properties that may limit the time mosquitoes spend on the netting, either by resting on the cone or, because the Olyset® net has a wide-mesh, by resting on the cardboard backing rather than on the test netting. We do not believe either seriously influenced the results as unused and regenerated Olyset® nets had adequate mortality. Furthermore, the cyclodextrin net, which was also treated with permethrin, performed better than the conventional deltamethrin treated net in this study. Second, the Olyset® nets used in this study were ~6 years old at the time of distribution and it was suspected that spontaneous migration of permethrin to the surface of the fibres was slower in these older nets. However, laboratory studies indicated that spontaneous regeneration at 30°C or 35°C did not occur in either 8 year old or 6 month old Olyset® nets (Gimnig *et al.* 2005). Third, the differences may simply be in interpretation. Tami *et al.* (2004) recommended Olyset® nets as an LLIN when average bioassay mortality in 7 year old nets was 34%; by our definition, these nets would have been considered 'failed'.

We defined net failure as a confirmed 24-hour bioassay mortality rate of <50%, a choice that was based on epidemiological data. However, there has been no real epidemiological confirmation of the relevance of this cutoff. Using this definition, the median time to failure for conventional nets treated with K-O Tab® was 400 days (1.1 years) in this study. As nets treated with deltamethrin are recommended for retreatment every 6 months, it is likely, therefore, that defining net failure as a bioassay mortality <50% actually overestimates the effectiveness of nets before failure. In contrast, several authors have noted the repellent effects of pyrethroid insecticides, particularly permethrin, and have suggested this may be the method by which ITNs provide personal protection, but not additional community benefits (Miller *et al.* 1991). We did not employ tests to measure the repellent effects and therefore may have underestimated the longevity of some of the LLINs tested with regard to personal protection. More work is required to understand the relationship between the killing/repellent action of ITNs and LLINs with regard to mosquitoes exposed in bioassays and the public health benefits, particularly from the community-effect, that are expected from ITN and LLIN programs.

Although commercial LLINs are an important way to make LLINs available to large numbers of households, a treatment process to lengthen the effective life of ITNs that can be applied easily in the field would be of substantial benefit. There are several million nets currently in use in sub-Saharan Africa, and most of these are untreated. A long-lasting field treatment would significantly increase the efficacy of these nets in protecting against malaria transmission and disease. In addition, a simple and inexpensive long-lasting treatment process that could be applied after nets are manufactured would allow for local manufacturing of nets that could be converted into long-lasting ITNs, without burdensome investment in expensive equipment. This would result in an increased supply of nets and competition among local net manufacturers, making LLINs more affordable in Africa. The cyclodextrin treatment process extended the duration of insecticidal activity of permethrin on polyester nets, although the difference in time to failure was not statistically different from a conventional net. Logistical constraints, however, may limit the use of this process under field conditions. Cyclodextrin is difficult to dissolve in water and must be mixed thoroughly with permethrin before applying to nets; we have achieved the best results by mixing the cyclodextrin and permethrin in water and stirring for 30 min or more. We will continue to develop this process further in hopes of improving efficacy and removing some of the constraints.

New products, especially new LLINs, need to be evaluated quickly to ensure the shortest possible time between development and use by people at risk from malaria. WHOPES evaluation and testing of insecticides calls for Phase I laboratory testing, Phase II small-scale field evaluations and Phase III medium or large-scale field trials (WHOPES 1996). While large-scale, community-randomized field trials have been the gold standard for determining the impact of ITNs on morbidity, mortality and entomologic inoculation rates, the time, logistics and cost of these major studies prohibit this approach as a practical strategy for future trials. Our use of survival analysis to compare LLINs is a novel approach to comparing the efficacy of several ITNs at one time and is particularly effective at determining whether LLINs are a significant improvement on conventionally treated nets. Sample size calculations indicate that a minimum hazard ratio of 0.4 could be detected with 80% power if 55 LLINs were followed monthly for 6 months and compared with conventional ITNs treated with K-O Tab®. Bioassays of 110 nets per month (five nets per working day) would be feasible with a small insectary.

LLINs hold great promise for controlling malaria transmission in sub-Saharan Africa. PermaNet® 1.0 demonstrates significantly better retention and bio-availability of insecticide than a conventional net and should be recommended to malaria control programs. The performance of the Olyset® net was disappointing, in contrast to previous studies of this LLIN. However, this study may not have been appropriately designed to measure the repellent effects of permethrin nets, such as Olyset®, which may be important in providing personal protection from malaria.

Further studies are needed to better understand the relationship between laboratory tests of the biological activity of nets and the protective efficacy of ITNs and LLINs for malaria prevention.

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