Comparison of Efficacy of Five Types of Long-Lasting Insecticidal Nets Against *Anopheles fluviatilis*, the Primary Malaria Vector in East-Central India

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ABSTRACT Five types of long-lasting insecticidal nets (LNs), namely, Olyset, Netprotect, PermaNet, DuraNet, and Interceptor, were tested after 20 washes for efficacy in terms of mortality, deterrence effect, blood-feeding inhibition, and induced exophily of the malaria vector *Anopheles fluviatilis* in experimental huts in Malkangiri district of Odisha State, India. Efficacy of the three synthetic pyrethroids (SPs) used in the LNs was also analyzed. Use of LNs reduced the entry of *An. fluviatilis* into the huts by 73.3–83.2%, and the five LNs were comparable in terms of deterrence. The exit rate of *An. fluviatilis* from the huts with untreated net was 56.3%, and relative to this, Olyset followed by DuraNet induced significantly a higher exophily. In contrast, the exit rate was significantly lower with Interceptor. Among the three SPs, permethrin induced significantly greater exophily relative to the untreated control, and as a result of this, permethrin-treated Olyset produced a lower mortality. Blood-feeding rate of *An. fluviatilis* was significantly lower with all the five LNs than the control. Similarly, all the three SPs significantly inhibited blood feeding compared with the control. Interceptor and DuraNet, both alphacypermethrin-treated LNs, caused relatively a higher mortality of *An. fluviatilis* than the other LNs. The five brands of LNs and three SPs tested in the current study were equally effective in terms of deterrence and blood-feeding inhibition; only exiting and killing effect differed among them. Permethrin-treated LNs induced greater exophily, while, overall, alphacypermethrin-treated LNs killed more *An. fluviatilis* that entered the huts. Advantage of deterrence, excito-repellent, and killing effects of LNs and appropriate selection of SP for net treatment are discussed in this paper.

KEY WORDS DuraNet, Interceptor, Olyset, Netprotect, PermaNet

Vector control remains an essential component of the control of major endemic and epidemic vector-borne diseases (Mosqueira et al. 2013), and it is a fundamental element of the existing global strategy to fight malaria. Indoor residual spraying (IRS) and use of bed-nets treated with insecticide, including long-lasting insecticidal nets (LNs), are the two core and broadly applicable malaria vector control measures in most of the malaria endemic countries, including India (WHO 2013a). Considering the difficulties of maintaining high coverage and quality of IRS, it is likely that insecticide-treated nets, especially LNs, will replace IRS in most areas in India, although IRS will still be the choice to combat epidemics and to protect people who cannot easily be encouraged to use insecticidal nets (NVBDCP 2009a). Bed-nets impregnated with pyrethroid insecticides (ITNs) were well received and shown to give protection against malaria in many countries (Zaim et al. 2000; Jambulingam et al. 2008). However, one of the operational challenges faced by large-scale implementation of ITNs was their retrieval from the households and retreatment at regular intervals. In response to low retreatment rates of ITNs, LNs, which are treated at the manufacturing point (industry) with insecticide either coated with wash-resistant resin on polyester fibers or incorporated into matrix of polyethylene fibers and thereby requiring no further insecticide treatment even after repeated washes throughout their expected life span of about 3 yr or more, have been developed and promoted, as they are operationally more convenient and preferred over the conventional ones (Guillet et al. 2001; WHOPES 2001, 2004, 2007; Müller et al. 2002; Gonzalez et al. 2002; Lengeler 2004; Banek et al. 2010). Furthermore, in India, it was realized that LNs could be the most appropriate vector control option for the control of malaria, as considerable amount of practical difficulty was experienced in organizing repeated

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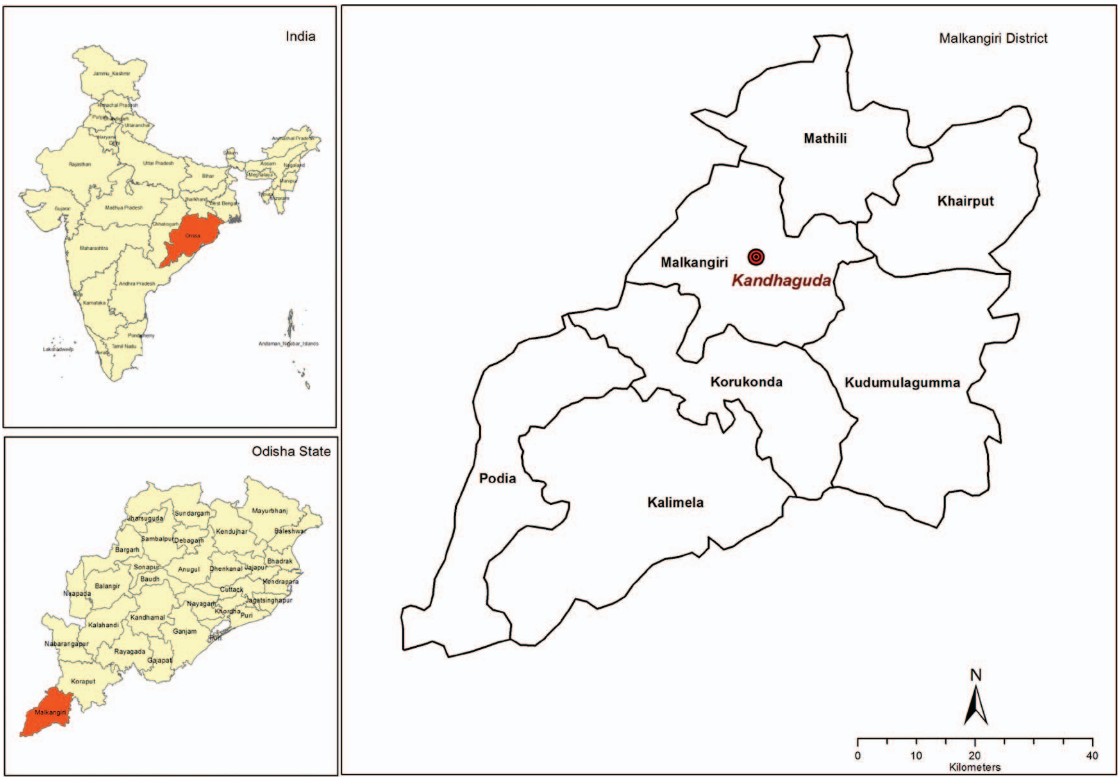

Fig. 1. Map showing the study village, Kandhaguda in Malkangiri Block in Malkangiri district of Odisha State, India. (Online figure in color.)

treatment of nets (NVBDCP 2009b). The National Vector Borne Disease Control Programme (NVBDCP) in India initiated distribution of LNs to high-risk areas in 2009 and endorsed scaling-up of LNs as one of the primary strategies for malaria control with the main objective of achieving complete coverage and maximal utilization of LNs by all populations living in high malaria endemic areas with annual parasite incidence per 1000 populations (API) $\geq$2, with priority to areas with API $\geq$5 to bring down the morbidity and mortality due to malaria significantly (NVBDCP 2009a). Various products of LNs have been recommended for use by the World Health Organization (WHO) on the basis of independent assessment of their performance and safety through the WHO Pesticide Evaluation Scheme (WHOPES 2008, 2009). Olyset, PermaNet, and Interceptor and Netprotect and DuraNet are the LNs given full and interim recommendation, respectively, by the WHO. These five brands of LNs were made available in India for assessing their comparative efficacy in experimental huts, following the Phase-II protocol, as such head-to-head comparison is rarely done. The efficacy of the five types of LNs, in terms of mortality, deterrence, blood-feeding inhibition, and induced exophily, was evaluated and compared after 20 washes against a wild susceptible population of *Anopheles fluviatilis* sensu lato, the major malaria vector in east-central India. The results of this comparative evaluation will help guide LN policy and procurement decisions in India.

Materials and Methods

Study Site. The evaluation in experimental huts was done in Kandhaguda village in Malkangiri District of Odisha state (east-central India; Fig. 1). The terrain of the district is hilly with forest and streams. The climate is characterized by summer (March–June), rainy (July–October), and cold (November–February) seasons. The area has been endemic for *Plasmodium falciparum* malaria transmitted primarily by *An. fluviatilis*, the abundance of which was higher during September to February (Sahu et al. 2008). The vector species, which was endophilic, endophagic, and anthropophagic, preferred to breed in streams and terraced paddy fields (Gunasekaran et al. 1994, Sahu et al. 2008). The vector was susceptible to dichlorodiphenyltrichloroethane, malathion, and synthetic pyrethroids (SPs, Sahu et al. 2013).

Experiment Arms. The study was a blinded one; the nets were coded by a scientist who was not involved in the study. Washing and bioassay before and after washing was done at the Vector Control Research Centre (VCRC), Puducherry, to ensure that the persons who conducted the evaluation in the experimental huts were not aware of the details of the nets. The evaluation included six arms; five treatment arms (the five brands of LNs) and one control arm (untreated nets). The nets of all the arms (size of nets: width 160 cm by length 180 cm by height 150 cm) were evaluated after 20 washes, in six experimental huts constructed in the study village, over a period of 12 wk from November 2009 to January 2010.

Experimental Hut. Six identical experimental huts were built in the Kandhaguda village for the comparative evaluation of the five brands of LNs. The huts were specially designed for recording the entering and exiting mosquitoes and for measuring their response to insecticides/treated nets, including mortality.

The experimental hut consisted of a single room with four windows; size of each window was 0.45 by 0.45 m, grilled with wooden planks fixed horizontally in tilted position one above the other, leaving a gap of 1 cm between two planks through which mosquitoes could enter into the hut but could not exit. There were two windows on the front door side and one on each of the sides and a screened (using nylon mesh) verandah at the backside (verandah/exit trap to collect the exiting mosquitoes). The dimensions of the huts resembled those of the village huts (length 3 m, width 3 m, and height 2.5 m), having brick walls with cement plastering and thatched roof, above which there was tin-sheeted roofing for protecting the thatched roof. There was no space between the thatched ceiling and tin-roof. The huts were constructed 1 foot above the ground level on a platform made up of brick and cement. The platform had a water-filled gutter (moat; 6-inch depth by 6-inch breadth) all around to deter entry of scavenging ants. The gutter was made at 2 feet away from the hut walls, except on the back side of the hut, where it was at 1.5 feet away from the base of the verandah trap. At the center of the hut, the roof was at a height of 2.5 m and near the wall, the height was 2 m; this difference in height was to maintain a slope of the roof. The eave on the backside (facing toward east) had a gap of 1–2 cm and through this gap mosquitoes could exit, but those mosquitoes were collected in the verandah trap. On the other sides, there were no gaps in the eave. In the hut, the only entry points for mosquitoes were the windows. There was one wooden door of 0.75 by 1.5 m facing toward the west.

Suitability of the huts for carrying out the evaluation was ascertained by measuring adult vector density resting indoors, recovery rate of the released mosquitoes inside the huts, and scavenging by ants/insects on mosquito carcasses. The five brands of LNs and the untreated nets were coded, indicating the six arms (five brands of LNs or untreated net) and the six replicates and the two additional nets in each arm. Six replicate nets were used per each arm and each net was tested one night per week. The two additional nets in each arm were used for cone bioassays and chemical analysis before any wash and after washing 20 times.

Washing, Bioassays, and Chemical Analysis. The LNs and the untreated nets (seven nets from each of the six arms) were washed 20 times according to the WHO washing protocol (WHO 2005) during August–October 2009. Bioassays using WHO-prescribed cones were performed on the nets exposing laboratory-reared, blood-fed *Anopheles stephensi* before any wash (to ensure the efficacy of the insecticide treatment of the nets) and after 20 washes (to assess the wash resistance of the treated nets). The target vector species, *An. fluviatilis*, could not be used for bioassays, as rearing facility of this species was not available at the VCRC laboratory, Puducherry, where washing was done. On each net, 5 by 2 cone tests (on each section of the net: roof and four sides) were performed. Five female mosquitoes were exposed per cone test. Exposure to net lasted for 3 min, after which mosquitoes were held for 24 h with access to sugar solution. Knock down was measured 60 min after exposure time and mortality after 24 h. Results were pooled for the 50 mosquitoes tested per net.

Before any wash, 16 pieces of 30- by 30-cm nettings were taken from the designated net of each of the six arms. Similarly, net samples were obtained after 20 washes. The samples were labeled and packed in aluminum foil and subjected to chemical analysis. After washing the nets 20 times and conducting bioassays on the washed nets, the nets were shifted to the field site for evaluation in experimental huts.

Before initiation of the evaluation in experimental huts, bioassays were done on the nets exposing wild-caught blood-fed *An. fluviatilis.* Subsequently, six holes, two each on long sides and one each on front and hind ends, were made (size of each hole was 4 by 4 cm) in all the treated and untreated nets that were used in the experimental huts (six per arm) to simulate the conditions of a torn net and to put emphasis on testing whether the insecticide treatment, rather than the net, effectively prevented mosquito biting on sleepers. After the completion of the hut trial, bioassays were performed on one randomly selected net of each arm used in the huts exposing field-collected blood-fed *An. fluviatilis.*

Selection of Volunteers. Twelve volunteers, two per hut, were selected from the village to sleep in the experimental huts under the mosquito net that was provided to them (two volunteers under one net) from dusk to dawn, with a small break for dinner. The volunteers (sleepers) were informed that they should be available for the entire period of the trial and should maintain the same behavioral patterns (such as time of entering the hut, dressing, nonsmoking, using bedding material, sleeping under net, etc.) throughout, as these could constitute an important source of variation that must be controlled for. Informed consent was obtained from all the volunteers recruited to participate in the study and each one was remunerated. Clearance from the Institutional Ethical Committee was obtained to involve human volunteers for the study.

Evaluation. The nets were evaluated from first week of November 2009 in the six experimental huts; in each hut, one net was used. Six replicate nets were used per arm and each net was tested one night per week. Thus, in 1 wk, the six replicate nets of each arm were tested in one experimental hut in six successive nights. Similarly, nets of the other five arms were tested in the other five experimental huts. Sleepers were organized in six teams, each with two persons. The teams formed in the beginning were not changed.

Rotation of Arms and Volunteers. The nets and the sleepers were rotated between the huts using the Latin Square Rotation scheme. In practice, sleepers were rotated daily, whereas the arms weekly. Six nets (replicates) were used per arm, and each net was tested one night during a week. In the morning, the nets were removed and stored in separate bags labeled arm- and replicate-wise. At the end of each week, mosquito nets together with bedding materials and the white cloth spread on the floor of the room and verandah were removed from the huts. The huts were then cleaned and ventilated to remove any contamination from the nets previously used. The mat and the beds (labeled according to treatment) were rotated with the treatments since they came in close contact with the treated net.

Mosquito Collection. In the previous evening to each day of evaluation, before the volunteers entered the hut for sleeping, clean white cloths were spread on the floor of the hut (room and verandah), and the gutter all around the hut was filled with water. The volunteers entered the experimental huts at 1900 hours in the evening and remained inside until 0530 hours in the morning. They slept under the nets during that period. In the morning, mosquito collections were made separately under the net, followed by from verandah and room. Resting and dead mosquitoes were collected separately from verandah, room (walls, roof, and floor), and inside bed-net using an aspirator. Mosquitoes were stored separately by hut and by collection place (verandah trap, room, and under net) for further processing. The collected mosquitoes were scored as dead or alive, identified morphologically, and graded according to their gonotrophic status (blood-fed/unfed/semigravid + gravid). Results were entered on a daily record sheet, using one sheet per arm, per species, and per week (one sheet each for *An. fluviatilis*, *Anopheles culicifacies*, other anophelines, and culicines). Alive mosquitoes were kept in wax-coated paper cups (each 250 ml capacity) with access to sugar solution for 24 h. After 24 h, mortality was recorded. Mosquito collections were done up to 12 wk post distribution of nets.

The volunteers were interviewed to record any side effects perceived by them after sleeping under the nets in the experimental huts.

Chemical Analysis. At the end of the hut evaluation, 16 pieces of 30- by 30-cm nettings were cut and taken from one net of each of the six arms, which were used by the volunteers for sleeping in the huts. The samples were packed in aluminum foil, labeled, and subjected to chemical analysis.

Determination of Active Ingredient Content in Net Samples. Chemical analysis of the net samples of three batches (before any wash, after 20 washes, and at the end of hut evaluation) of the five LNs and one untreated control net was done at the Walloon Agricultural Research Centre, CRA-W, Gembloux, Belgium. Active ingredient content of deltamethrin in Netprotect and PermaNet, of alphacypermethrin in DuraNet and Interceptor, and of permethrin in Olyset was determined. The 16 pieces of 30 by 30 cm of the same net were combined together to obtain a composite net sample, and in total, 18 such composite net samples (three batches of six nets) were obtained. From each composite net sample, two pieces of 10 by 10 cm were cut with scissors and used for determination of the density, and the remaining were cut with scissors into small pieces of 5–10 mm^2, homogenized, and two analytical portions were weighed for determination of deltamethrin, alphacypermethrin, or permethrin.

Statistical Analysis. The data were analyzed to show the effect of the five brands of LNs in terms of deterrence (the number of mosquitoes caught in a LN hut, as a percentage of the number in the control hut with untreated net), induced exophily (increasing of exit rate [exit rate is the number of mosquitoes caught in the verandah trap, as a percentage of the total number caught in the hut including under bed-net and verandah] in the LN arm compared with the untreated arm), blood-feeding inhibition (the proportional reduction in blood feeding in huts with LNs relative to control with untreated nets), and total mortality (the number of mosquitoes found dead either at dawn [immediate mortality] or 24 h later [delayed mortality] as a percentage of the total numbers entered the hut). Logistic regression analysis was done to estimate the proportional outcomes of the LN effects (mortality, blood-feeding inhibition, and induced exophily), and negative binomial regression was used to analyze counts of mosquitoes entering the experimental huts (Version 10 of the Stata software package). A P value of 0.05% was considered indicative of a statistically significant difference.

Results

Suitability of the Huts. Before hut evaluation, 17 indoor resting collections were done simultaneously in the six experimental huts and in six randomly selected village huts during dawn hours, spending 10 min in each hut. The average per man-hour density (PMD) ± SE of *An. fluviatilis* in the experimental huts (12.8 ± 4.6) was comparable with that in the village huts (13.5 ± 4.4). Field-collected *An. fluviatilis* or *An. culicifacies* females were released into the experimental huts on 10 occasions (40–250 on each occasion) over a period of 4 months. On eight occasions, the recovery rate was around 80% and on two, it was between 70 and 80%. Scavenging was tested on 11 occasions, keeping 30–50 dead *Anopheles* mosquitoes on the floor (in four corners) of the huts in the evening and recording the number present in the following morning. Although the huts were ensured ant-proof by filling the gutter around the hut with water and regular cleaning of the huts and placing ant-traps inside the huts, house crickets, *Gryllodes sigillatus*, were found scavenging on dead mosquitoes. This problem was overcome by cleaning the rooms, verandah, and surroundings of the huts every day.

Table 1. Details of the five brands of LNs evaluated in experimental huts

No.	Brand of LNs	SP used for treatment	Dosage
1	Olyset	Permethrin (PM)	1,000 mg/m^2
2	Netprotect	Deltamethrin (DM)	63 mg/m^2
3	PermaNet 2.0	Deltamethrin (DM)	55 mg/m^2
4	DuraNet	Alphacypermethrin (ACM)	261 mg/m^2
5	Interceptor	Alphacypermethrin (ACM)	200 mg/m^2

LN Treatment. The five brands of LNs and the SPs used for their treatment, with dosages, are presented in Table 1.

Species Composition. In total, 72 collections were done in each of the six experimental arms. Among the total mosquitoes collected (1169), *An. fluviatilis* was the predominant species (88.1%). The proportion of *An. culicifacies,* the secondary vector of malaria, was only 1.4%; other anophelines and culicines were 3.5 and 7.0%, respectively. As *An. culicifacies,* other anophelines, and culicines were collected in very small numbers, further analysis with reference to efficacy of the LNs was restricted only to *An. fluviatilis.*

Entry. Numbers of *An. fluviatilis* females caught in experimental huts with the LNs and with the untreated net over 72 collections conducted during the 12-wk period are presented in Table 2. Hut entry of *An. fluviatilis* differed significantly between the six experiment arms ($\chi^2 = 70.28$, df = 5, $P < 0.0001$). Compared with the control, the entry was greatly reduced with the LNs ($P < 0.05$). Among the five brands of LNs, entry of *An. fluviatilis* was relatively lower with Netprotect (DM), followed by DuraNet (ACM); however, 95% confidence interval (CI) of the incidence rate ratios for the five LNs showed no significant difference between them (Table 3).

The five brands of LNs were grouped by the SPs used for net treatment and analyzed for difference, if any, in their efficacy against the vector, using the same statistical methods applied for the LN brands. Thus, there were three SPs, permethrin (Olyset), deltamethrin (Netprotect and PermaNet), and alphacypermethrin (DuraNet and Interceptor), tested in the current study. The comparative efficacy of the three SPs in terms of deterrence, induced exophily, blood-feeding inhibition, and mortality of *An. fluviatilis* is given in Table 4 and 5.

Exit. Overall, there was a significant difference in exit rate between the six arms ($\chi^2 = 27.2$, df = 5, $P < 0.0001$). Relative to the control, increased exophily was observed with Olyset (PM), PermaNet (DM), and DuraNet (ACM). However, the level of increase was significant only with Olyset (PM) and DuraNet (ACM; $P < 0.05$), and not with PermaNet (DM; $P > 0.05$). In contrast, there was lower exiting with Netprotect (DM) and Interceptor (ACM) than the control; however, the difference was significant only with Interceptor (ACM; Table 2). Among the five LNs, although Olyset (PM) was found inducing higher exophily, it was not significant from the other LNs, except Interceptor (ACM), with which the exit rate was significantly lower than the other LNs, except Netprotect (DM; Table 3).

Blood Feeding. The six experimental arms recorded significantly different blood-feeding rates ($\chi^2 = 330.61$, df = 5, $P < 0.0001$). Relative to the untreated control, all the five LNs greatly inhibited blood feeding of *An. fluviatilis* ($P < 0.05$); however, no significant difference between the five LNs in terms of blood-feeding inhibition was seen, as indicated by the 95% CI of the odds ratios of the LNs (Table 3).

Mortality. The total mortality rate (immediate mortality + delayed mortality) of *An. fluviatilis* in the experimental arms is given in Table 2. Significantly different levels of total mortality were recorded with the six arms ($\chi^2 = 601.28$, df = 5, $P < 0.0001$). All the five LNs caused a greater (total) mortality than the untreated control ($P < 0.05$; Table 2). The difference in total mortality between the five LNs was not significant, although DuraNet and Interceptor, both alphacypermethrin-treated LNs, caused relatively a higher total mortality (Table 3). Among the three SPs, the odds ratios indicated that the total mortality associated with alphacypermethrin was many fold higher than the other two SPs, but this observed difference was not significant (Table 5).

Bioassays. Before washing and after 20 washes, all the five brands of LNs caused 100% mortality of *An. stephensi,* whereas the mortality with the untreated nets was only 0–2%. Similarly, before distribution of the nets to the volunteers, all the five LNs caused a 100% mortality of *An. fluviatilis* in the bioassays and the mortality was only 2% with the untreated net. After the hut trial, in the bioassays, Netprotect (DM), PermaNet (DM), and DuraNet (ACM) produced

Table 2. Comparison of the performance of the five brands of LNs against wild *An. fluviatilis* in experimental huts

Arms	Untreated net	Olyset (PM)	Netprotect (DM)	PermaNet (DM)	DuraNet (ACM)	Interceptor (ACM)
No. of collections	72	72	72	72	72	72
No. of females entered/caught	496	106	83	109	94	133
No. of females caught per night	6.9a	1.5b	1.2b	1.5b	1.3b	1.9b
Deterrence (%)		78.6a	83.3a	78.0a	81.0a	73.2a
Exit rate (%)	56.3a	72.6bd	55.4adc	66.1ad	70.2bd	45.9c
Blood feeding (%)	58.9a	9.4b	4.8b	11.9b	7.4b	6.0b
Blood-feeding inhibition (%)	–	84.0	91.9	79.8	87.4	89.8
Total mortality (%)	1.6a	62.3b	62.7b	62.4b	74.5b	75.9b

Numbers in the same row sharing a letter do not differ significantly ($P > 0.05$). Total mortality, immediate mortality + delayed mortality.

Table 3. Results of statistical analysis of the performance of the five brands of LNs against wild *An. fluviatilis* in experimental huts

Variables	Olyset (PM)	Netprotect (DM)	PermaNet (DM)	DuraNet (ACM)	Interceptor (ACM)
Entry (deterrence)					
IRR (95% CI)	0.213 (0.127–0.359)	0.167 (0.098–0.283)	0.219 (0.131–0.368)	0.189 (0.112–0.319)	0.268 (0.161–0.447)
Z	−5.83	−6.63	−5.74	−6.23	−5.04
P	<0.001	<0.001	<0.001	<0.001	<0.001
Exit (induced exophily)					
Odds ratio (95% CI)	2.065 (1.301–3.279)	0.966 (0.605–1.543)	1.513 (0.980–2.336)	1.833 (1.138–2.951)	0.658 (0.448–0.967)
Z	3.07	−0.14	1.87	2.49	−2.13
P	0.002	0.888	0.061	0.013	0.033
Blood-feeding (inhibition)					
Odds ratio (95% CI)	0.072 (0.037–0.142)	0.035 (0.012–0.098)	0.094 (0.051–0.173)	0.056 (0.025–0.123)	0.044 (0.021–0.093)
Z	−7.60	−6.42	−7.62	−7.14	−8.27
P	<0.001	<0.001	<0.001	<0.001	<0.001
Total mortality					
Odds ratio (95% CI)	100.6 (45.1–224.3)	102.3 (44.6–234.2)	101.1 (45.5–224.9)	177.9 (76.9–411.4)	192.5 (86.1–430.1)
Z	11.28	10.95	11.33	12.11	12.83
P	<0.001	<0.001	<0.001	<0.001	<0.001

Untreated net was the reference category for all variables. IRR, incidence rate ratio.

100% mortality, and Olyset (PM) and Interceptor net (ACM) caused 98.0% mortality; against the untreated net, the mortality was only 4% (Table 6).

Side Effects. The volunteers who slept under the LNs in the experimental huts for a period of 12 wk perceived no side effects. They observed that they did not get any odor from the nets. The perceived benefit by the volunteers was the reduced mosquito bites in the huts throughout the study period.

Chemical Analysis. Results of active ingredient content (g/kg) of SPs and their overall retention index after 20 washes and average a.i. retention index are given in Table 7. The average deltamethrin content in the unwashed Netprotect and PermaNet 2.0 (100 denier) complied with the target dose of 1.8 g/kg (±25%) and 1.4 g/kg (±25%), respectively. The deltamethrin 1R-isomer (non relevant impurity) content in the unwashed Netprotect and PermaNet 2.0 represented 11.7 and 3.4%, respectively, of the deltamethrin content, and this amount did not significantly increase after 20 washes and at the end of the study. After 20 washes, the overall deltamethrin retention index in Netprotect (78.6%) was higher than that in PermaNet 2.0 (35.3%), which corresponds to an average deltamethrin retention index per wash of 98.8% and 94.9%, respectively. The average alphacypermethrin content in the unwashed DuraNet complied with the target dose of 5.8 g/kg (±25%), whereas the average content of the same insecticide in the unwashed Interceptor (100 denier) was a little bit higher (6.38 g/kg) than the target dose of 5.0 g/kg (±25%). The alphacypermethrin *cis I* isomer (nonrelevant impurity) content in the unwashed DuraNet and Interceptor LN represented 11.1 and 1.7%, respectively, of the alphacypermethrin (*cis II*) content, and this amount did not significantly increase after 20 washes and at the end of this study. After 20 washes, the overall alphacypermethrin retention index in DuraNet (74.1%) was higher than in Interceptor (52.3%), which corresponds to an average alphacypermethrin retention index per wash of 98.5 and 96.8%, respectively. The average permethrin content in the unwashed Olyset was equivalent to the target dose of 20 g/kg (±3 g/kg). After 20 washes, the overall permethrin retention index in Olyset was 90.1%, which corresponds to an average permethrin retention index per wash of 99.5%. No active ingredient was found in the control net samples above the limit of quantification (LOQ) level of 0.01 g/kg for deltamethrin, 0.05 g/kg for alphacypermethrin, and 1 g/kg for permethrin.

Discussion

SPs are the recently introduced insecticide in India for IRS and treatment of mosquito/bed-nets (NVBDCP 2009a). Based on the encouraging results from a number

Table 4. Comparison of the performance of the three SPs used for treatment of the five brands of LNs against *An. fluviatilis* in experimental huts

Variables	Untreated control	Permethrin	Deltamethrin	Alphacypermethrin
Number of collections	72	72	72	72
Number of females entered/caught	496	106	96[a]	114[a]
Number of females caught per night	6.9a	1.5b	1.3b	1.6b
Deterrence (%)	–	78.6a	80.7a	77.1a
Exit rate (%)	56.3a	72.6bc	61.5ac	55.9ac
Blood feeding (%)	58.9a	9.4b	8.9b	6.6b
Blood-feeding inhibition (%)	–	84.0	84.9	88.8
Total mortality (%)	1.6a	62.3b	62.5b	75.3b

Numbers in the same row sharing a letter do not differ significantly ($P > 0.05$). Total mortality, immediate mortality + delayed mortality.
[a] Average of two LN brands treated with the same insecticide.

Table 5. Results of statistical analysis of the performance of the three SPs used for treatment of the five brands of LNs against *An. fluviatilis* in experimental huts

Variables	Permethrin	Deltamethrin	Alphacypermethrin
Entry (deterrence)			
IRR (95% CI)	0.213 (0.126–0.359)	0.193 (0.123–0.302)	0.228 (0.147–0.356)
Z	−5.80	−7.23	−6.54
P	<0.001	<0.001	<0.001
Exit (induced exophily)			
Odds ratio (95% CI)	2.065 (1.301–3.279)	1.240 (0.882–1.743)	0.987 (0.719–1.355)
Z	3.07	1.24	−0.08
P	0.002	0.215	0.939
Blood-feeding (inhibition)			
Odds ratio (95% CI)	0.072 (0.037–0.142)	0.067 (0.039–0.115)	0.049 (0.028–0.085)
Z	−7.60	−9.97	−10.65
P	<0.001	<0.001	<0.001
Total mortality			
Odds ratio (95% CI)	100.650 (45.160–224.321)	101.666 (47.677–216.793)	186.267 (87.024–398.689)
Z	11.28	11.96	13.46
P	<0.001	<0.001	<0.001

Untreated net was the reference category for all variables. IRR, incidence rate ratio.

of studies, the Government of India introduced ITNs for malaria control in 100 highly endemic districts under the Enhanced Malaria Control Programme (Jambulingam et al. 2008). However, to overcome the problem of poor retreatment coverage of ITNs, use of LNs has been promoted and scaled-up in high-risk areas (NVBDCP 2012). Further, the malaria control program in India is now gradually shifting toward reducing areas under IRS and increasing coverage with LNs; currently, a deltamethrin-treated LN is being distributed to the people by the program. In this context, the outcome of the current study assumes importance, as it compared efficacy of the five brands of LNs that have been awarded with either full or interim recommendation by the WHOPES. Such comparison is first of its kind in India, and the results would have direct relevance to the program in planning to include more brands of LNs for distribution.

In Malkangiri district, where the current study was conducted, the major malaria vector was *An. fluviatilis*, which was susceptible to SPs (Sahu et al. 2008, 2013) and predominantly endophilic, with peak biting rates at midnight in many parts of India (Gunasekaran et al. 1994; Sahu et al. 2003, 2008).

Among the five brands of LNs evaluated in experimental huts in the current study, Olyset was treated with permethrin, Netprotect and PermaNet with deltamethrin, and DuraNet and Interceptor with alphacypermethrin. Relative to the untreated control net, hut entry of *An. fluviatilis* was significantly lower with all the five LNs, after 20 washes; the five LNs did not differ by deterrent effect. When the hut entry of *An. fluviatilis* was analyzed by SPs, there was a greater reduction in the entry with all the three SPs than with the control; however, the deterrent effect did not vary between the SPs. The levels of deterrence observed in the current study (77–81%) were on a higher side compared with that reported for the same SPs against *Anopheles arabiensis* (25–35.3%; Mosha et al. 2008). However, Corbel et al. (2004) observed a higher deterrence (83–89%) against *Anopheles gambiae* with nets treated with 50, 100, 250, and 500 mg permethrin/m^2. But, against the same vector species, there was no clear evidence of deterred effect of alphacypermethrin-treated (Interceptor) and deltamethrin-treated (PermaNet) LNs (Malima et al. 2013, Badolo et al. 2012). In an experimental hut trial of bed-nets impregnated with SPs or organophosphate (OP) insecticide for mosquito control in The Gambia, it was concluded that permethrin tends mainly to deter mosquitoes from house entry, enhancing personal protection, whereas the OP insecticide kills higher proportions of the endophilic mosquitoes, which would give better community protection against malaria transmission (Miller et al. 1991). The current study, however, recorded a high deterrent effect of all the five brands of LNs and all the three SPs, even after 20 washes, and this could be partly due to the high sen-

Table 6. Results of bioassays

No.	Arm	Before any wash		After 20 washes		Before hut evaluation		After hut evaluation	
		NE	CM (%)	NE	CM (%)	NE	CM (%)	NE	CM (%)
1	Olyset (PM)	50	100	50	100	50	100	50	98
2	Netprotect (DM)	50	100	50	100	50	100	50	100
3	PermaNet (DM)	50	100	50	100	50	100	50	100
4	DuraNet (ACM)	50	100	50	100	50	100	50	100
5	Interceptor (ACM)	50	100	50	100	50	100	50	98
6	Untreated net	50	0	50	2	50	2	50	4

NE, no. exposed; CM, corrected mortality (treated mortality corrected to the control mortality).

Table 7. Results of chemical analysis of LNs

LN (target dose)	Batch	a.i. content (g/kg)	a.i. retention after 20 washes (% of wash 0)	Average a.i. retention[a] (% at each wash)
Olyset Net (PM) (20 ± 3 g/kg)	0 wash	20.6	90.1	99.5
	20 wash	18.6		
	End of study	17.4		
Netprotect (DM) (1.8 g/kg ± 25%)	0 wash	1.88	78.6	98.8
	20 wash	1.47		
	End of study	1.61		
PermaNet (DM) (1.4 g/kg ± 25%)	0 wash	1.45	35.3	94.9
	20 wash	0.51		
	End of study	0.53		
DuraNet (ACM) (5.8 g/kg ± 25%)	0 wash	4.60	74.1	98.5
	20 wash	3.41		
	End of study	4.06		
Interceptor net (ACM) (5.0 g/kg ± 25%)	0 wash	6.38	52.3	96.8
	20 wash	3.33		
	End of study	2.88		

Olyset (permethrin incorporated into polyethylene LN). Netprotect (deltamethrin incorporated into polyethylene LN). PermaNet 2.0 (deltamethrin coated onto polyester LN). DuraNet (alphacypermethrin incorporated into polyethylene LN). Interceptor (alphacypermethrin coated onto polyester LN).

Average a.i. retention index, $n\sqrt{(t_n/t_0)}$; where t_n, active ingredient total content after wash n, g/kg; t_0, active ingredient total content at wash 0 (pre-washing), g/kg; n, no. of washes (=20).

[a] Free migration stage behavior.

sitivity of the targeted vector species to the SPs. Another important biological activity of the vectors is their blood-feeding behavior. All the five LNs and three SPs significantly inhibited blood feeding of *An. fluviatilis* compared with the untreated control. The levels of blood-feeding inhibition obtained with the five LNs or the three SPs were comparable. A Tanzanian study compared the efficacy of the same three SPs against *An. arabiensis* and reported that the highest blood-feeding inhibition was by the nets treated with permethrin (40.6%); deltamethrin and alphacypermethrin inhibited only a lower proportion (22.5 and 27.5%, respectively; Mosha et al. 2008). Relatively higher blood-feeding inhibition of alphacypermethrin-treated LN, washed 20 times, was reported against *Anopheles funestus* (49.8%) and *An. gambiae* (65.8%; Malima et al. 2013). However, compared with these results, the current study reported a very high blood-feeding inhibition. It is apparent from these observations (deterrence and blood-feeding inhibition) that when the LNs and SPs were used in the huts, *An. fluviatilis* was greatly deterred from entering the huts and those mosquitoes that entered the huts were prevented from feeding on the host, despite the presence of holes on the nets, indicating a significant and comparable personal protection offered by the LNs and SPs.

Different results were obtained for vectors' exiting in response to the SPs and LNs when used in experimental huts; some reported induced exophily (Badolo et al. 2012, Malima et al. 2013) and others observed insignificant difference relative to the control (Mosha et al. 2008). Such difference depends also on the innate characteristic of the targeted vector species. The exiting behavior of *An. fluviatilis,* in the current study, differed among the LNs. Relative to the control, Olyset (PM) induced significantly a higher exophily, followed by DuraNet. In contrast, the exit rate was significantly lower with Interceptor (ACM) LN. Among the three SPs, induced exophily was significantly greater with permethrin relative to the control. Such pyrethroids that induce more exiting would reduce the chances of leading to a mass killing (Hawley et al. 2003) of the vector.

Mosquito mortality recorded in experimental huts is an indicator of the potential mass killing effect of the LNs (WHO 2013b). In the current study, the total mortality was greater, although not statistically significant, with Interceptor and DuraNet, both alphacypermethrin-treated LNs. In failing to induce many mosquitoes to exit, nets treated with alphacypermethrin would favor mosquitoes contacting the insecticide on the nets adequately to get killed, as also observed by Mosha et al. 2008. Thus, alphacypermethrin-treated LNs with relatively lower excito-repellent effect and probably with more toxicity killed significantly a greater number of *An. fluviatilis* that entered the huts. In contrast, the low mosquito mortality associated with permethrin (Olyset) was a result of this compound inducing higher exiting of mosquitoes (Mosha et al. 2008). Relatively low permethrin-induced mortalities, compared with other pyrethroids, were observed in several previous studies (Miller et al. 1991, Curtis et al. 1992, N'Guessan et al. 2001, Corbel et al. 2004) also.

The average a.i. retention index (% at each wash) was almost same among all the five types of LNs. Even though PermaNet 2.0 and Interceptor showed relatively lower a.i. retention after 20 washes, the bioavailability of the insecticide was adequate in these two nets, as their efficacy after 20 washes was comparable with the other three types of LNs in terms of deterrence and blood-feeding inhibition. As the average a.i. retention index was almost same in all the five types of LNs; the chemical analysis results would not affect the comparison of the efficacy of the five LNs against the vector. Further, the chemical analysis report indicated that the a.i. content of the insecticide was not higher than the target dose in the LNs, except in Interceptor net, in which the content was above specifications. Such higher content in the unwashed net was a concern, as it raised a question whether an Interceptor that was within the specifications would have performed as well. The volunteers, however, perceived no side effects after sleeping under any of the five LNs in the experimental huts.

The five brands of LNs and three SPs tested in the current study were equally effective in terms of deterrence and blood-feeding inhibition, but they differed by induced exophily and killing effect. Permethrin-treated LN induced greater exophily, whereas, overall, alphacypermethrin-treated LNs killed greater number of mosquitoes that entered the huts. The deterrence effect of SPs in LNs would benefit the households having badly damaged bed-nets or with insufficient number of nets for all the occupants to use. In malaria control programs, however, where coverage with insecticidal nets is often much lesser than 100%, the reduction of the vector population remains an important objective. In such settings, the insecticide used to treat nets needs to kill rather than repel mosquitoes (Mosha et al. 2008), as a mass killing effect is desirable to reduce transmission (Asidi et al. 2005). The strategic plan 2012–2017 in India for malaria control has endorsed scaling-up use of LNs in high-risk areas to protect at least 80% of those people at high risk of malaria. Where complete coverage is not possible, LNs treated with alphacypermethrin could be the preferred option considering their more killing effect. At the same time, it is also important to monitor the potential of selection pressure for development of resistance when LNs are used in the program, as it is not clear whether the less excito-repellent pyrethroids (deltamethrin or alphacypermethrin) would exert a higher selection pressure for resistance than the more repellent permethrin (Gimnig et al. 2003).

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