Wash Resistance and Bioefficacy of Olyset Net — A Long-Lasting Insecticide-Treated Mosquito Net Against Malaria Vectors and Nontarget Household Pests

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ABSTRACT During recent years, long-lasting insecticide-treated nets (LLINs) have been developed to overcome the problems of low retreatment rates, washing, and erratic dose of the insecticide resulting in the dilution of efficacy of the conventional insecticide-treated mosquito nets. These nets are treated at factory level with insecticide either incorporated into or coated around fibers. Olyset net, a polyethylene net with 2% permethrin incorporated within fibers, is one type of LLIN. Therefore, these nets were evaluated for their wash resistance and bioefficacy against malaria vectors *Anopheles culicifacies* Giles and *Anopheles fluviatilis* James (Diptera: Culicidae) and other nontarget species. Cone bioassay tests produced 100% mortality in these two vector species with 3-min exposure. Results of the bioassays on washed nets showed 100% mortality in *An. fluviatilis* even after 20 washings, whereas in *An. culicifacies* 100% mortality up to 11 washings and 80% mortality up to 20 washings were observed. Cone bioassay tests also were performed on nontarget mosquito species *Culex quinquefasciatus* Say; house fly, *Musca domestica* L.; American cockroach, *Periplaneta americana* (L.); head louse, *Pediculus humanus capitis* De Geer; and bed bug, *Cimex lectularius* L. with 30-min exposure. Except for bed bugs, 100% mortality was observed in these nontarget species after 24-h recovery period. In bed bugs, only 25% mortality was observed. The density of *An. culicifacies* and *An. fluviatilis* was significantly reduced in houses with Olyset nets compared with those with untreated nets or no nets. Thus, it may be concluded that Olyset nets are highly effective against malaria vectors and moderately against other nontarget household insects.

KEY WORDS *Anopheles culicifacies*, *Anopheles fluviatilis*, bioefficacy, Olyset net, wash resistance

Mosquito nets treated with pyrethroids have proved to be an important tool for the control of malaria and other vector-borne diseases (WHO 1989). Several field efficacy trials in many malaria endemic countries have shown the usefulness of insecticide-treated mosquito nets (ITMNs) in reducing morbidity and mortality from malaria (Lengeler 2004). Insecticide-treated mosquito nets are now a part of operational strategies adopted by the National Vector Borne Diseases Control Programme, Government of India.

One of the main operational problems with ITMN-oriented intervention is the necessity to retreat nets regularly because the effective dose of pyrethroid insecticide only persists for a limited period. Moreover, repeated washings and erratic dose of the insecticide resulted in dilution of efficacy of the nets. Although it is relatively feasible to distribute mosquito nets through social marketing in malaria-endemic countries, regular insecticide retreatment has been found to be difficult to implement, resulting in very low retreatment rates (Schellenberg et al. 2002). During recent years, long-lasting insecticide-treated nets (LLINs) have been developed to overcome these problems (Guillet et al. 2001). These nets are treated at the time of manufacturing with insecticide either incorporated into or coated around fibers and are resistant to multiple washes. The biological activity lasts as long as the net itself (3 to 4 yr for polyester nets and 4 to 5 yr for polyethylene nets). So far, there are two LLINs on the market that are recommended by the WHO Pesticide Evaluation Scheme (WHO 2001). Olyset net manufactured by Sumitomo Chemicals Co. Ltd. is made out of wide-meshed high-density polyethylene in which the insecticide (permethrin) is incorporated directly into the fiber at a 2% (wt:wt) concentration (corresponding to 1 g/m^2 surface concentration). The extensive laboratory and field experience with Olyset nets is reviewed in WHO (2001). Olyset nets have only recently been introduced into India. Therefore, studies on the bioefficacy of Olyset nets against local malaria vectors and other nontarget species were carried out. The results of this study are reported here.

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Materials and Methods

Study Area. The study was conducted in Jharbeda village under Bisra Primary Health Centre in Sundargarh district, Orissa. The village is located at 22° 14′ N and 85° 11′ E. The study village is predominantly inhabited by ethnic tribal communities and is located in a forest area ≈35 km away from Rourkela. The area consists of undulating uplands intersected by forested hills, rocky streams, and river and paddy fields containing mud houses with thatched roofs clustered in hamlets. Three hamlets in the Jharbeda village consisting of 18, 20, and 17 houses were selected on the basis of baseline entomological data and were randomly assigned as treated net, untreated net, and no net hamlets, respectively. These hamlets have a total population of 94, 107, and 86, respectively. *Anopheles culicifacies* Giles and *Anopheles fluviatilis* James are the major malaria vectors in this area, responsible for persistent transmission throughout the year. Malaria is a major cause of morbidity. The nearest health facility for the residents of the study village 10 km distant in Bisra.

Mosquito Nets and Distribution. The Olyset nets were made of white polyester polyfilament of 150 denier strength with 4- by 4-mm mesh size. Untreated nets were of the same specifications but were pink. These nets were used as untreated control. Ten houses in treated and untreated hamlets were randomly selected and provided with treated nets and untreated nets free of charge based on sleeping pattern survey, so that all the occupants of these houses were covered under nets. Mosquito nets were distributed in the first week of January 2004.

Bioassay Test. The persistence of insecticide on nets in regular use was determined by contact bioassays using a standard WHO procedure (WHO 1998). The tests were performed at monthly intervals beginning in January 2004 up to June 2004. Wild-caught blood-fed female *An. culicifacies* and *An. fluviatilis* were used in the bioassays. Ten mosquitoes were released in each cone with the help of a suction tube and exposed to Olyset net for 3 min. The mosquitoes knocked down after the exposure period and mortality after 24 h were recorded. Mosquitoes were provided with cotton swabs soaked in 10% glucose solution during the recovery period. For each species, there were four replicates. The untreated nets served as controls. The percentage of corrected mortality was calculated using Abbott's formula (Abbott 1925).

Bioassay tests on Olyset nets also were performed on nontarget species such as mosquito C*ulex quinquefasciatus* Say; house fly, *Musca domestica* L.; American cockroach, *Periplaneta americana* (L.); head louse, *Pediculus humanus capitis* De Geer; and bed bug, *Cimex lectularius* L. with an exposure time of 30 min for each species, and mortality was recorded after 24 h.

Bioassay Test on Washed Nets. The impact of washing on the efficacy of Olyset nets was tested through washing the net with detergent at an interval of 24 h up to 20 washes. Surf Excel, manufactured by M/S Hindustan Lever Limited, Mumbai, and procured from the local market was used as the detergent in all washings. Five grams (1 tsp) of the detergent powder was dissolved in 5 liters of water, and Olyset net was dipped in the detergent solution for 10 min. Subsequently, the net was washed and thoroughly rinsed with tap water. The washed nets were exposed to the sun light for 30 min, and the cone bioassay on malaria vector species was performed as per the procedure described above. Untreated washed nets were used as controls. The percentage of corrected mortality was calculated using Abbott's formula.

Entomological Evaluation. Indoor-resting adult mosquitoes were collected between 0600 and 0900 hours monthly in 12 houses selected randomly with four houses each for Olyset net, untreated net, and no net. Mosquito collections were made by three insect collectors, one each in houses with Olyset nets, untreated nets, and no nets. The mosquito collections were made for 15 min in each dwelling. The insect collectors and houses were rotated every month to avoid any bias in mosquito collections. The mean monthly density of indoor-resting mosquitoes was calculated as person-hour density. Preintervention mosquito collections were made in December 2003, and postintervention data were collected from January to June 2004. The percentage of reduction in the density of mosquitoes was calculated by the following formula: % reduction = mosquito density in control − density in experimental/density in control × 100.

Results

Results of the bioassay tests on regularly used Olyset nets in the study village showed 100% mortality in *An. culicifacies* and *An. fluviatilis* with 3-min exposure. Results of the bioassay tests on washed Olyset net at an interval of 24 h up to 20 washings are shown in Table 1. The results showed 100% mortality with 3-min exposure in *An. fluviatilis*, even after 20 washes, whereas 100% mortality was observed up to 11 washings for *An. culicifacies;* but in subsequent washing, there was a gradual decline in bioefficacy to 80% mortality after 20 washings of Olyset net. Results of the bioassay tests on new unwashed Olyset net against nontarget species such as C*x. quinquefasciatus*, *M. domestica*, *P. americana*, *P. humanus capitis*, and *C. lectularius* are shown in Table 2. Initially, these species were exposed for 3 min, but there was no knockdown effect or mortality; therefore, the exposure time was increased to 30 min. There was 100% mortality in all these species except bed bugs after 30 min of exposure to the Olyset net. In bed bugs, only 25% mortality was observed even after 30-min exposure. However, mortality was 100% when exposed for 1 h.

The impact of Olyset net on the indoor-resting density of malaria vectors *An. culicifacies* and *An. fluviatilis* is shown in Figs. 1 and 2, respectively. Results showed that average person-hour density of *An. culicifacies* during preintervention was 24.0, 21.5, and 19.0 in houses with the Olyset nets, untreated nets, and no nets, respectively, and no significant difference in mosquito productivity was observed in all the three

Table 1. Impact of washing of Olyset nets on its bioefficacy against malaria vectors after 3 min of exposure and 24-h recovery period

No. of washings	No. of replicates for each species[a]	Mean mortality/replicate ± SD		% mortality	
		An. culicifacies	*An. fluviatilis*	*An. culicifacies*	*An. fluviatilis*
1–11	44	10 ± 0	10 ± 0	100	100
12–13	8	9.5 ± 0.76	10 ± 0	95	100
14–17	16	9.0 ± 0.82	10 ± 0	90	100
18–19	8	8.5 ± 0.76	10 ± 0	85	100
20	4	8.0 ± 0.82	10 ± 0	80	100

[a] Ten mosquitoes were exposed in each replicate.

hamlets ($\chi^2 = 0.58$, df = 2, $P > 0.05$). However, during the intervention period, the density of *An. culicifacies* was reduced drastically in houses with the Olyset nets (Fig. 1). The percentage of reduction in density of *An. culicifacies* in houses with the Olyset nets was 83.5 and 92.0% in comparison with houses with untreated nets and no nets, respectively. There was a significant reduction in the density of *An. culicifacies* in houses with Olyset nets compared with houses with untreated nets ($\chi^2 = 25.41$, df = 1, $P < 0.001$) and no nets ($\chi^2 = 68.51$, df = 1, $P < 0.001$). The density of *An. fluviatilis* during preintervention period in houses with the Olyset net, untreated net, and no net was 29.0, 25.0, and 22.5, respectively, and there was no significant difference in the density of this species in three hamlets ($\chi^2 = 0.84$, df = 2, $P > 0.05$). During the intervention period, the density of *An. fluviatilis* was remarkably low in houses with Olyset nets in comparison with houses with untreated nets and no nets (Fig. 2). There was 91.2 and 94.3% reduction in the density of *An. fluviatilis* in houses with the Olyset nets in comparison with houses with untreated nets and no nets, respectively. A significant reduction in the density of *An. fluviatilis* in houses with Olyset nets was observed in comparison with houses with untreated nets ($\chi^2 = 34.73$, df = 1, $P < 0.001$) and no nets ($\chi^2 = 63.80$, df = 1, $P < 0.001$).

Discussion

The Global Malaria Control strategy emphasizes the need for selective and sustainable preventive measures of vector control for reducing malaria problems (WHO 1993). To achieve this objective, insecticide-treated mosquito nets can contribute because it revolves around the community-based action-oriented program. Untreated mosquito nets were reported to provide some protection against malaria and mosquitoes (Bradley et al. 1986), but they cannot give complete protection against mosquitoes (Lindsay et al. 1989). Therefore, the protective effect of mosquito nets may be enhanced by impregnating the fabric with an insecticide such as pyrethroids (Curtis and Lines 1985). The recently introduced long-lasting insecticide-treated mosquito nets provide a solution to the operational problem of retreating the nets at 6-mo intervals to maintain efficacy of nets against mosquitoes.

Several studies have demonstrated the efficacy of Olyset nets against malaria vectors in different countries, and these nets are being considered as a long-lasting and cost-effective intervention tool in disease vector control (Itoh and Okuno 1996, Nguyen et al. 1996, N'Guessan et al. 2001). The current study showed a high bioefficacy of Olyset nets against primary malaria vectors *An. culicifacies* and *An. fluviatilis.* The study also showed that repeated washings of the nets did not reduce the efficacy. Results from a previous study also showed good insecticide persistence even after 3 yr of use, where Olyset nets were still fully effective in bioassays and provided >80% mortality after 3 min of exposure and were as effective as new Olyset nets when tested in experimental huts (N'Guessan et al. 2001). Recently, an evaluation of Olyset nets distributed 7 yr previously in Tanzania revealed 34% mortality in *An. gambiae* after 3-min exposure, and they found that Olyset nets are

Table 2. Bioefficacy of Olyset nets against nontarget house hold pests after 30 min of exposure and 24-h recovery period

Species	Nos. exposed	Mortality after 24 h ± SD	% mortality
Cx. quinquefasciatus	40	40 ± 0	100.0
M. domestica	40	40 ± 0	100.0
P. americana	10	10 ± 0	100.0
P. humanus	10	10 ± 0	100.0
C. lectularius	8	2 ± 0	25.0[a]

[a] 100% mortality was observed after 1-h exposure.

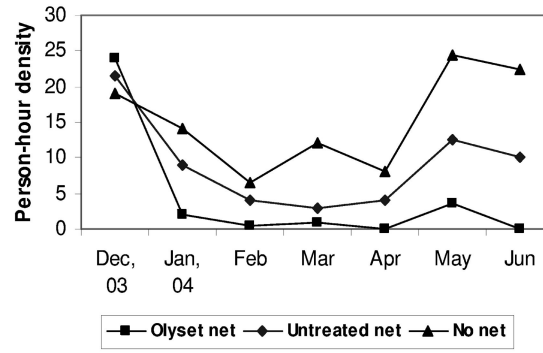

Fig. 1. Person-hour density of malaria vector *An. culicifacies* in houses with the Olyset nets, untreated nets, and no nets. The adult mosquito density was measured in indoor resting hand catch collections in four houses each with the Olyset nets, untreated nets, and no nets once every month during December 2003 (preintervention) and January to June 2004 (postintervention).

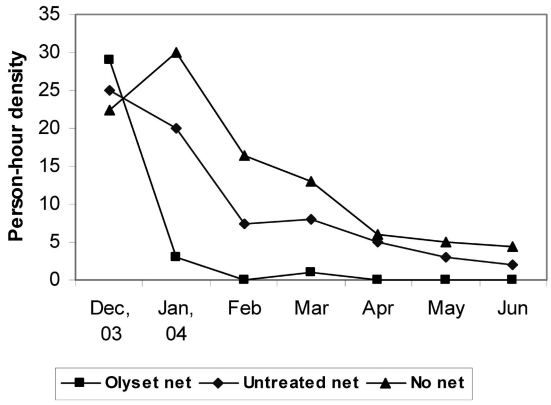

Fig. 2. Person-hour density of malaria vector *An. fluviatilis* in houses with the Olyset nets, untreated nets, and no nets.

popular, durable, and had much longer insecticide persistence than ordinary polyester nets (Tami et al. 2004). The high efficacy of Olyset nets even after repeated washing is due to the reactivation of the insecticide under tropical conditions (30 ± 2°C and 80% RH). The killing effect of Olyset nets get enhanced if dried under sunlight, probably because of the acceleration in migration of permethrin in the fibers, thereby increasing the bioavailability of insecticide on the net surface. The Olyset nets also were found to be moderately effective against nontarget species such as *Cx. quinquefasciatus*, *M. domestica*, *P. americana*, *Pediculus humanus capitis*, and *C. lectularius*, which is an added advantage of using Olyset nets for personal protection. Although it was not a primary objective of the current study to measure a reduction in the density of malaria vectors in houses with the Olyset nets in comparison with those using untreated nets or no nets, but just to corroborate the results of bioefficacy of Olyset nets with direct observation in the field, monitoring of indoor-resting adult mosquitoes in houses with Olyset nets, untreated nets, and no nets was carried out only for 6 mo during intervention period. It was observed that the density of mosquitoes was significantly lower in houses with Olyset nets compared with houses with untreated nets or no nets. The reduction in mosquito density can be due to excito-repellency, deterrence, or mass killing of mosquito vectors, or various combinations (Hossain and Curtis 1989). The excito-repellent action of mosquito nets treated with different pyrethroids also has been reported in India (Ansari and Razdan 2000). None of the users of Olyset nets reported any side effects.

Malaria transmission is perennial in Sundargarh district and malaria attributable morbidity is considerably high in the young age groups among the tribal communities living in the forest ecotype (Sharma et al. 2004b). In Sundargarh district, the peak of malaria incidence coincides with the peak vector density of *An. fluviatilis* during postmonsoon months (October–December). The species is known to have high human blood index and entomological inoculation rate, whereas, *An. culicifacies* plays a complimentary role in the intermediate and low transmission seasons (Sharma et al. 2004a). Therefore, the use of Olyset nets in such areas will provide year-round protection from malaria vectors.

The primary malaria vector *An. culicifacies* is developing multiple resistance to DDT, malathion, and deltamethrin in some parts of India (Sharma et al. 2004c). Alternatively, the biological agents such as biolarvicides, larvivorous fish, and the environmental management of disease vectors have limitations and may not be feasible in highly malaria-endemic forested areas in India. Therefore, use of insecticide-treated mosquito nets provides an effective alternative strategy. The mosquito net approach has the advantage that it provides immediate and direct relief not only from mosquitoes but also from other hematophagous insects (Njunwa et al. 1991), as also observed in the current study. Compared with the cost of indoor residual spraying, distribution of long-lasting insecticide-treated mosquito nets in highly malarious areas seems to be cost-effective, especially because their effectiveness lasts for several years. However, the issues involved with such type of intervention are mainly acceptability and sustainability, which require continuous education and community awareness. This is particularly important in high-risk tribal areas where socioeconomic and cultural factors play important role in maintaining a high degree of malaria transmission (Sharma et al. 2001). Therefore, in such situations a simple and cost-effective intervention tool such as Olyset nets may be more acceptable to the community.

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References Cited

Abbott. 1925. A method for computing the efficacy of insecticide. J. Econ. Entomol. 18: 265–267.

Ansari, M. A., and R. K. Razdan. 2000. Relative efficacy of insecticide treated mosquito nets (Diptera: Culicidae) under field conditions. J. Med. Entomol. 37: 202–204.

Bradley, A. K., B. M. Greenwood, A. M. Greenwood, K. Marsh, P. Byass, S. Tulloch, and R. Hayes. 1986. Bed nets (mosquito nets) and morbidity from malaria. Lancet 2: 204–207.

Curtis, C. F., and J. D. Lines. 1985. Impregnated fabrics against malaria mosquitoes. Parasitol. Today 1: 147.

Guillet, P., D. Alnwick, M. K. Cham, M. Neira, M. Zaim, D. Heymann, and K. Mukelabai. 2001. Long-lasting treated mosquito nets: a break through in malaria prevention. Bull. World Health Organ. 79: 998.

Hossain, M. I., and C. F. Curtis. 1989. Permethrin impregnated bed nets: behavioural and killing effects on mosquitoes. Med. Vet. Entomol. 3: 367–376.

Itoh, T., and T. Okuno. 1996. Evaluation of polyethylene net incorporated with permethrin during manufacturing of thread on efficacy against *Aedes aegypti* (Linnaeus). Med. Entomol. Zool. 47: 171–174.

Lengeler, C. 2004. Insecticide-treated bed nets and curtains for preventing malaria. Cochrane Database Syst. Rev. CD000363.

Lindsay, S. W., F. C. Shenton, R. W. Snow, and B. M. Greenwood. 1989. Responses of *Anopheles gambiae* complex mosquitoes to the use of untreated bed nets in The Gambia. Med. Vet. Entomol. 3: 253–262.

N'Guessan, R., F. Darriet, J. M. Doannio, F. Chandre, and P. Carnevale. 2001. Olyset net efficacy against pyrethroid resistant *Anopheles gambiae* and *Culex quinquefasciatus* after 3 years field use in Cote d'Ivoire. Med. Vet. Entomol. 15: 97–104.

Nguyen, H. T., T. V. Tien, N. C. Tien, T. U. Ninh, and N. T. Hoa. 1996. The effect of Olyset net screen to control the vector of dengue fever in Viet Nam. Dengue Bull. 20: 87–91.

Njunwa, K. J., J. D. Lines, S. M. Magesa, A.E.P. Mnzava, T. J. Wilkes, M. Alilio, K. Kivumbi, and C. F. Curtis. 1991. Trial of pyrethroid impregnated bed nets in an area of Tanzania holoendemic for malaria. Part 1. Operational methods and acceptability. Acta Trop. 49: 87–96.

Schellenberg Armstrong, J.R.M., H. Minja, H. Mponda, N. Kikumbih, A. Mushi, R. Nathan, S. Abdulla, O. Mukasa, T. Marchant, M. Tanner, et al. 2002. Re-treatment of mosquito nets with insecticide. Trans. R. Soc. Trop. Med. Hyg. 96: 368–369.

Sharma, S. K., P. Pradhan, and D. M. Padhi. 2001. Socioeconomic factors associated with malaria in a tribal area of Orissa, India. Indian J. Public Health 45: 93–98.

Sharma, S. K., R. Chattopadhyay, K. Chakrabarti, S. S. Pati, V. K. Srivastava, P. K. Tyagi, S. Mohanty, S. K. Misra, T. Adak, B. S. Das, et al. 2004a. Epidemiology of malaria transmission and development of natural immunity in a malaria-endemic village, San Dulakudar, in Orissa state, India. Am. J. Trop. Med. Hyg. 71: 457–465.

Sharma, S. K., P. K. Tyagi, K. Padhan, T. Adak, and S. K. Subbarao. 2004b. Malarial morbidity in tribal communities living in the forest and plain ecotypes of Orissa, India. Ann. Trop. Med. Parasitol. 98: 459–468.

Sharma, S. K., A. K. Upadhyay, M. A. Haque, O. P. Singh, T. Adak, and S. K. Subbarao. 2004c. Insecticide susceptibility status of malaria vectors in some hyperendemic tribal districts of Orissa. Curr. Sci. 87: 1722–1726.

Tami, A., G. Mubyazi, A. Talbert, H. Mshinda, S. Duchon, and C. Lengeler. 2004. Evaluation of Olyset insecticide-treated nets distributed seven years previously in Tanzania. Malar. J. 3: 1–9.

[WHO] World Health Organization. 1989. The use of impregnated bed nets and other materials for vector-borne disease control. WHO/VBC/89.981, Geneva, Switzerland.

[WHO] World Health Organization. 1993. Global Malaria Control. Bull. World Health Organ. 71: 281–284.

[WHO] World Health Organization. 1998. Test procedures for insecticide resistance monitoring in malaria vectors, bio-efficacy and persistence of insecticides on treated surfaces. WHO/CDS/CPC/MAL/98.12, Geneva, Switzerland.

[WHO] World Health Organization. 2001. Review of Olyset Net; Bifenthrin 10% WP. Report of the Fifth WHOPES Working Group Meeting, WHO/HQ, Geneva, 30–31 October 2001. Document WHO/CDS/WHOPES/2001.4.

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