THE EFFICACY AND PHYSICAL CONDITION OF OLYSET INSECTICIDE-TREATED NETS AFTER 5 YEARS USE IN RURAL LAO PDR

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Abstract. We assessed the insecticidal efficacy of Olyset nets after 5 years of use in rural villages of Lao PDR and evaluated the relationship between the physical condition of the nets and their insecticidal effect. Our results showed that most of the Olyset nets remained effective after 5 years of use; however, there was no significant relationship between the physical condition of the nets (*ie*, presence of holes, level of stains) and the insecticidal effect. The presence of large holes in polyester nets compared to the Olyset nets suggest the Olyset nets are stronger; however, nearly half of Olyset nets had small holes or had been previously repaired. Interestingly, the insecticide concentration and knockdown (KD) rate for 3 stored nets was low compared to the other nets routinely used in the house. To maintain the effectiveness of Olyset nets in rural villages of Lao PDR and other areas, residents should be advised to repair and store the nets appropriately and avoid exposure to high temperatures and direct sunlight for long periods.

Keywords: malaria, LLINs, Olyset net, efficacy, Lao PDR

INTRODUCTION

It has been reported that insecticide-treated nets (ITNs) considerably reduce morbidity and mortality due to malaria in various settings; however, it is difficult to implement regular insecticide retreatment (Lengeler, 2004). The World Health Organization (WHO) has advocated the use of long-lasting insecticidal nets (LLINs) whereby insecticide treatment will last for the lifetime of the net so that further insecticide treatment would not be required (Teklehaimanot *et al*, 2007). Of the LLIN brands recommended by the WHO, Olyset Net (Sumitomo Chemicals) is the first to be given a full recommendation (Teklehaimanot *et al*, 2007). Olyset nets are made out of wide-meshed, high-density polyethylene in which permethrin, the pyrethroid insecticide, is incorporated into the fiber at a 2% weight/weight (w/w) concentration (corresponding to 1 g/m^2 surface concentration). The insecticide constantly diffuses across the surface of the yarn over time.

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Experimental trials regarding the efficacy of the Olyset net conducted in several countries have produced encouraging results (Doannio *et al*, 1999; Henry *et al*, 1999; N'Guessan *et al*, 2001; Sharma *et al*, 2009); however, a large variability in the net-to-net insecticide content has been reported after 7 years of use (Tami *et al*, 2004). Some bed nets undergo tough usage in the field resulting in poor physical condition (holes and/or stains), which may significantly reduce the insecticidal effect of nets. In this study, we assessed the insecticidal effect of Olyset® nets based on permethrin content and biological performance after 5 years use in rural villages of Lao PDR. We also evaluated the relationship between the physical condition of the nets and their insecticidal effect.

MATERIALS AND METHODS

This study was conducted in conjunction with the ITN distribution project in Khammouane Province, central Lao PDR. Between 1999 and 2000, Olyset nets were distributed to rural villagers in Boualapha District, which is located close to the Lao-Vietnam border. In November 2006, we retrieved the Olyset nets from 3 of the villages (Taphachon, Kouanboun, and Napong) in the district after 5 years of use.

We randomly selected 30 households in the villages (10 of 41 households in Taphachon, 10 of 40 in Kouanboun, and 10 of 75 in Napong) and offered them new Olyset nets in exchange for their used nets. Oral consent was obtained from senior household members before the nets were exchanged. We noted the presence of holes and repairs in the Olyset nets, as well as in other insecticide-untreated polyester bed nets. The color of the new Olyset nets is light blue; however, the nets become stained by dirt during regular use. The color change of the sample nets was evaluated by stain level based on 5 categories (1 = light blue, equivalent to a new Olyset net, 2 = muddy light blue, 3 = greyish blue, 4 = olive blue, and 5 = dark green). We carried out interviews to obtain information about net use (routinely used or stored, place of use, place of storage).

After the household visit, sets of 50 x 50 cm samples from the roof of each Olyset net were individually packaged in plastic bags and maintained at room temperature until shipment. The samples were sent to the manufacturer (Sumitomo Chemical, Tokyo, Japan) for performance of bioassay tests and assessment of permethrin content. The bioassay and chemical tests were performed in accordance with standard procedures outlined in WHO documents (WHO/CDS/CPC/MAL/98.12; WHO/IS/NI/331/2002). For bioassay testing, susceptible laboratory-reared female *Anopheles albimanus* mosquitoes were exposed to net samples in standard WHO cones for 3 minutes. The mosquitoes were then held in plastic cups covered with netting with a honey solution provided and maintained for 24 hours at 30ºC and 80% humidity; the percent mortality of the mosquitoes was recorded. The bioassay tests were conducted 3 times with each sample, and the average score was calculated for each sample. The samples used in the bioassay were also used for the chemical tests. During bioassay and chemical testing, the investigator was blinded to the attributes of each Olyset net sample. The authors analyzed the relationship between the laboratory data and field data independently. Quantitative data were summarized using proportions and means. Statistical analysis was performed using the Student's *t*-test and chi-square test. Significance was determined at the 5% level.

Table 1
Permethrin content and bioassay efficacy of 22 Olyset nets.

Condition	*n*	%	Permethrin content (mg/m^2)		Bioassay efficacy			
					KD60 (%)[a]		Mortality (%)	
			Mean	*p*	Mean	*p*	Mean	*p*
Presence of holes								
Holes/fixed[b]	10	45.5	546.5		93.2		33.1	
No holes	12	54.5	600.8	0.4	99.5	0.2	52.7	0.2
Stain level[c]								
Strong	14	63.6	576.1		95.3		48.3	
Weak	8	36.4	604.4	0.5	98.9	0.5	35.9	0.4
Status of use[d]								
Routinely-used	19	86.4	599.7		99.6		45.6	
Stored	3	13.6	426.7	0.03	78.3	0.002	32.2	0.5

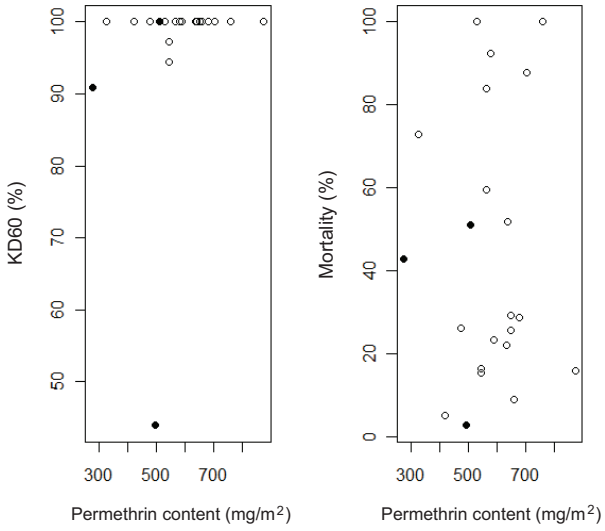
[a]KD60, knockdown rate at 60 minutes; [b]3 nets were previously repaired; [c]weak, muddy light blue (level 2) or greyish blue (level 3); strong, olive blue (level 4); [d] "routinely-used" nets were hung in the house during the day, whereas "stored" nets were stored in the roof lining of the house and sometimes used for sleeping outdoors.

Approval for this study was obtained from the Center for Malariology, Parasitology and Entomology, and the Research Committee of the Ministry of Public Health, Lao PDR.

RESULTS

All 30 households included in the study were in possession of at least 1 Olyset net; the residents agreed to exchange the net and participate in the study. The average permethrin content of 29 samples (1 sample was lost) obtained from the households was 579.0 ± 143.8 mg/m^2 [mean ± standard deviation (SD)], indicating that an average of 58% of the initial insecticide dosage (1,000 mg/m^2) was retained. However, there was great variability in insecticide content (275-875 mg/m^2). Although the mosquito mortality was low (45.3 ± 32.0%), the mean knockdown (KD) rate at 60 minutes (KD60) (97.5 ± 10.5%), met the definition (*ie*, 95% or higher KD rate) for a long-lasting insecticidal effect after 3 years of use (WHO, 2005).

The physical condition of 22 of the 29 nets was inspected during the household visits. Table 1 shows the permethrin content and bioassay efficacy based on the condition of the nets. Of the 22 samples, 12 (54.5%) had neither detectable holes nor were previously repaired or torn, 10 nets had holes or were repaired previously (7 nets had holes and 3 had repaired holes); 14 nets (63.6%) were strongly stained and the original color had changed from light blue to olive green; 19 nets were hung in the house during the day (*ie*, routinely used), whereas 3 were not routinely used and were stored at the roof lining of the houses (*ie*, stored). Owners of the stored nets reported that these nets were some-



○ Routinely-used Olyset nets; ● Stored Olyset nets

Fig 1–Permethrin content and bioassay efficacy by status of use.

times used for sleeping in the forest and/or huts in the field. There was no significant relationship between the physical condition (*ie*, presence of holes and level of stain) and the insecticidal effect of the nets. The KD rate of almost of all the nets was very high regardless of the insecticide concentration; however, we found that the insecticide concentration and the KD rate of the stored nets were significantly lower compared with the routinely-used nets. The insecticide concentration did not seems to affect the mortality rate at all (Table 1, Fig 1).

In the 30 households studied, we also inspected 38 polyester bed nets. None of the polyester nets were treated with insecticide during the previous year and the average duration of use was 2.7 ± 2.3 years (mean ± SD). Of the 13 polyester nets used for 4-6 years (which corresponds to the duration of use of the Olyset nets), more than half (7/13 or 53.9%) the nets had at least 1 large hole (*ie*, a hole the investigators could penetrate with a forefinger). Fewer Olyset nets (4/22 or 18.2%) had large holes compared to the polyester nets ($p = 0.03$).

DISCUSSION

Our results suggest most of the Olyset nets distributed to rural Laotian villages retained their insecticidal effect after 5 years of use. These results are consistent with those of a previous report conducted in Tanzania (Tami *et al*, 2004). We found the insecticide content and bioassay efficacy varied greatly among nets. The Olyset nets stored in the house had lower insecticidal content and less bioassay efficacy. Houses in rural Lao PDR are constructed of woven bamboo for walls and tin or palm frond roofs. Three of the unused Olyset nets were stored in the palm roof of the houses, where daytime temperatures are very high. Since stored Olyset nets were often used by residents who slept outdoors while hunting or cultivating the land, these nets may have had a greater chance of being exposed to high temperatures and direct sunlight while being carried and used under such conditions. Therefore, the permethrin content of the stored Olyset nets may have been rapidly lost since the nets were stored at high temperatures and exposed to ultraviolet light, which can cause degradation of the permethrin (Gimnig *et al*, 2005; Sreehari *et al*, 2009).

Our data suggest Olyset nets are stronger than polyester nets; however, nearly half the Olyset nets had small or repaired holes. The results of a recent study on experimental huts suggested mosquitoes can pass through Olyset nets despite high levels of insecticide if the nets

have holes and/or inappropriately used (Malima *et al*, 2008); hence, it is important for Olyset nets to be repaired.

We expected the Olyset nets in poor physical condition (*ie*, more holes and/or stains) would have a lower insecticidal effect than those in better condition; however, only storage in the roof lining of the house and occasional outdoor use seems to reduce the insecticide efficacy. It has been reported the washing method, frequency and drying conditions can affect insecticidal efficacy of Olyset nets (Sreehari *et al*, 2009). It is not clear whether washing frequency significantly affects the permethrin content under field conditions; however, the Olyset nets in our study area may have been washed less frequently (Olyset net owners reported that they washed their nets by hand in a river 1-3 times a year) as compared to a laboratory study where nets were washed at 7-day intervals up to 20 times (Sreehari *et al*, 2009). Washing frequency of nets may be less important than their place of use and storage conditions. Although the main malaria vector species in Southeast Asia (*An. dirus* and *An. minimus*) were not used for the bioassay test because these species were not maintained, it is highly likely the storage conditions and outdoor use, which caused depletion of the insecticide in the Olyset nets, would also result in reduction of insecticidal efficacy against these species.

Although additional surveys in larger areas with more Olyset net samples are needed to confirm our findings, our study is the first report from Southeast Asia which evaluated the insecticidal effect of Olyset nets over a long term use under field conditions. The human habitat and ecology of malaria vector mosquitoes in Southeast Asia are associated with the maintenance of malarial transmission in rural areas. Although the incidence of malaria sharply decreased in our study area after the distribution of ITN (Shirayama *et al*, 2008), rural residents may still be at risk of malaria because they are often outdoors during food gathering, hunting, crop cultivation, and timber cutting. Such individuals may become infected with malaria as a result of exposure to *An. dirus* s.l., which is known to be the primary vector of malaria in Southeast Asian forests (Erhart *et al*, 2004, 2005; Sanh *et al*, 2008). To maintain the efficiency of Olyset nets in rural villages of Lao PDR and other areas with similar conditions, residents should be instructed to repair and store Olyset nets appropriately to avoid exposure to high temperatures and direct sunlight for long periods.

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