

Field efficacy and persistence of Long Lasting Insecticide treated mosquito Nets (LLINs) in comparison with conventional Insecticide Treated mosquito Nets (ITN) against malaria vector in Thailand

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Abstract

In Thailand, the conventional Insecticide Treated mosquito Nets or ITN have been used over the years by the villagers. These mosquito nets are treated with permethrin 10%w/w EC manually as under the guidance of the health workers. These treated nets have efficacy for 6 months and need re-treated again. Long Lasting Insecticide treated mosquito Nets or LLINs, which can retain persistence for at least 3 years, are being considered to replace the conventional ITN. This study is intended to monitor the bioefficacy of two products of LLINs under field conditions in Thailand. These nets are PermaNet[®] and OlysetNet[®]

The study was carried out in a malaria endemic area of Kanchanaburi province. PermaNet[®], OlysetNet[®] and conventional ITN were distributed to the households in Baan-Su-Phan hamlet of village no.5, Loom-Soom subdistrict of Sai-Yok district and the households were allowed to use the bed nets. The nets were washed at every 6 months intervals and only conventional ITN were re-treated after washings. WHO standard procedures for cone bioassay tests were conducted with the bed net samples collected from the households that were using the nets and laboratory reared *Anopheles minimus*. The mosquitoes were exposed to the mosquito net samples for 3 minutes and the mortality rates were measured after 24 hours recovery period. Bioassays were carried out every month continuously for 3 years. The community acceptance of LLINs was studied by informal interview of the net users.

Results of the study showed that both LLINs (PermaNet[®] and OlysetNet[®]) offered > 80% mortality on *Anopheles minimus* over the entire 3 years period of field evaluation. The conventional ITN performed similar to LLINs except the fact that ITN were re-treated at 6 months intervals. Interestingly the ITN offered only 15% mortality after 6 months use and were washed without re-treatment.

The qualitative data on community acceptance of LLINs revealed that the majority of the households prefer PermaNet[®] due to its soft nature of net material, easy to wash and pack after use.

Key words : conventional insecticide treated mosquito nets, long lasting insecticide treated mosquito nets, bioefficiency, bioassay test, *Anopheles minimus*

Introduction

Malaria is considered as a main vector-borne diseases worldwide. In Thailand, malaria remains a major public health problem with approximately 30,000 to 40,000 cases being reported annually. Among the vector-borne diseases, the highest rate of fatality is due to malaria with about 100 to 200 deaths reported every year¹.

In Thailand the hilly forest regions are highly endemic for malaria transmitted through the mosquito vectors, *Anopheles minimus* and *Anopheles dirus*. In the recent years *An. minimus* has been considered as the most important vector of malaria because it is widely distributed in malaria endemic area all over the country while *An. dirus* has a restricted distribution with low density.

Indoor residual spraying (IRS) is the main vector control method used in high malaria endemic areas in Thailand, while conventional insecticide treated mosquito nets are used in moderate and low malaria endemic areas. The usage of these products is implemented in the country based on the recommendations of World Health Organisation (WHO)². Deltamethrin 5% w/w WP is used twice in a year for IRS at a target dosage of 20 mg a.i./m² surface. Permethrin 10% w/w EC is used to impregnate mosquito nets once in 6 months at a target dosage of 300 mg a.i./m² net as a general recommendation, while in some parts of the country Deltamethrin 25% w/w WT is used at a target concentration of 25 mg a.i./m² net.

With the innovation of long lasting insecticide treated mosquito nets (LLINs) technology, which do not require insecticide re-treatment have been introduced to replace the conventional ITN which need 6 months interval re-treatment. An LLINs is a factory-treated mosquito net expected to retrain its biological activity for at least 3 years of normal use under field conditions. The purpose of this study was determine the long lasting efficacy of LLINs which have been recommended by WHO to evaluate the field net samples for its persistence nature of treated insecticides against the major malaria vector *An. minimus* under malaria field conditions in Thailand.

Materials and Methods

PermaNet[®] and OlysetNet[®] were the two LLINs selected for the study since they have been recommended by WHO for malaria prevention and control³. PermaNet[®] is a long lasting insecticide treated mosquito net manufactured and supplied by Vestergaard Frandsen S.A, Switzerland. It is treated with Deltamethrin at 55mg a.i./m² mixed in a resin that coats the netting polyester fibres and releases the insecticide progressively in order for the net to retain efficacy after repeated washings³. OlysetNet[®] is a long lasting Permethrin treated mosquito net manufactured and supplied by Sumitomo Chemicals, Japan. It is an insecticide incorporated polyethylene polymer before yarn extrusion and is blended with Permethrin 2%w/w as active ingredient corresponding to 1,000 mg of a.i./m²⁴ Conventional ITN was treated with Permethrin 10%w/w EC at a target dose of 300 mg a.i./m² which requires treatment at every 6 month intervals was also included in the study for comparison.

The bioefficacy of long lasting insecticide treated mosquito nets was carried out in a malaria endemic area of Kanchanaburi province which is located 150 kilometers from Bangkok and is the western part of the country near Thai-Myanmar border. Baan-Su-Phan hamlet in Village No.5, Loom-Soom Sub-District of Sai-Yok District was selected for the study. The hamlet consists of 30 houses located near foot-hill of the forest and most of the villagers were farmers who cultivate cassava and make bamboo mats for their livelihood. A total of 15 houses were selected randomly from the hamlet. The 15 houses were grouped into three and each group was supplied with PermaNet[®], OlysetNet[®] and conventionally treated ITN. Mosquito net samples were distributed to the households at the rate of one net per house. The nets were washed at every 6 months intervals by the users and were washed by hand using detergent available

in the local shop. WHO kit for cone bioassay test was used to evaluate the efficacy of bed net samples. Laboratory reared *Anopheles minimus* from the insectary of the Bureau of Vector-borne disease, Ministry of Public Health was used to conduct bioassay as an indirect method to evaluate the long lasting efficacy and persistence of field net samples for the entire study program. Bioassays were carried out every month continuously for 3 years using the bed net samples collected from the households that were using the nets. All nets samples were washed once in 6 months and only ITN were re-treated after washings. WHO standard procedures for cone bioassay tests⁵ were conducted in the village conditions by using laboratory reared *An. minimus* mosquitoes. Four bioassay cones were used per sample net, each cone being fixed at the center of each side of a field collected net sample. A batch of 15 numbers of sugar fed, 2-5 days old female *Anopheles minimus* mosquitoes were transferred to each cone and exposed to net samples for 3 minutes following which they were removed from the cones to holding tubes with access to sugar solution. About 60 mosquitoes were tested on each net. Mortality was recorded after 24 hours. Mosquitoes exposed to untreated nets were used as control. Abbott's formula⁶ was used to correct mortality rates where control mortality ranged between 5 and 20 percent. The temperature and relative humidity during the trial period were recorded. Bioassay results were pooled and reported as percentage mortality for every month. The community acceptance of LLINs was studied by informal interview of all users who were supplied with net samples.

Results

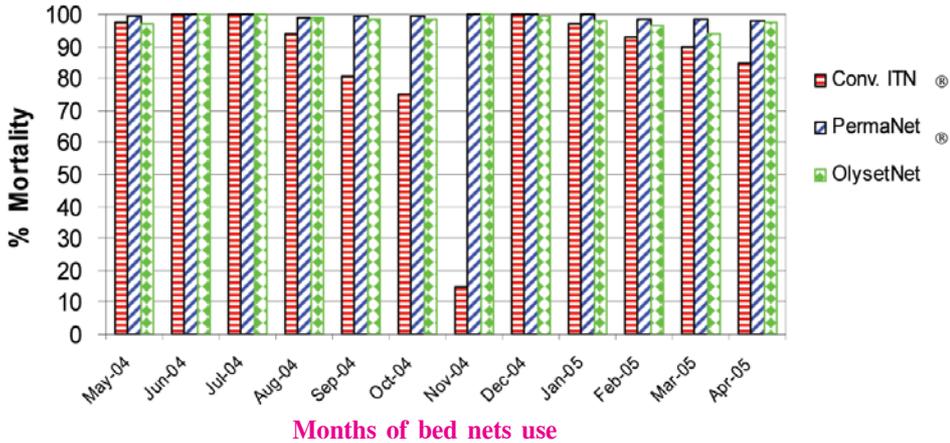
During the 3 years of study, the bioassays were carried out in the morning to avoid high temperatures in the afternoon. The average temperature records were between 28°C – 34°C and relative humidity were between 59 – 79%. After exposure to the net samples, the tested mosquitoes were kept in cooler boxes with some ice and cover with damp towels to maintain low temperature and high humidity entire holding period before reading results. The mortality rates which are shown in table 1-3 have already corrected by Abbott's formula where the control mortality rates were between 5-20%.

The results of the first year study are presented in table 1 and figure 1. PermaNet[®] offered 98% mortality on *An. minimus* samples over the first year while OlysetNet[®] offered 93.7% mortality. The conventional ITN showed good results up to 5 months and mortality declined considerably to 75% after 6 months. All net samples were washed in October after performing bioassay tests. In November, bioassay tests were carried out before re-treatment of conventional ITN. The mortality dropped to 15%. This indicated that the conventional ITN's loose bioefficacy after repeated washing, In contrast, both LLINs remained very high mortality after washing (100% mortality in November). After re-treatment, the conventional ITN gave 100% mortality again in December and declined to 84.7% in April (after 5 months use).

Table 1 First year results of bioassay tests showed mortality rates (mean ± SD) of *An. minimus*

Nets	May-04			Jun-04			Jul-04			Aug-04			Sep-04			Oct-04		
ITN	97.7	±	4.3	100.0	±	0.0	100.0	±	0.0	94.0	±	3.5	80.7	±	5.6	75.0	±	8.4
PermaNet [®]	99.7	±	0.7	100.0	±	0.0	100.0	±	0.0	99.0	±	2.2	99.7	±	0.7	99.7	±	0.7
OlysetNet [®]	97.0	±	3.0	100.0	±	0.0	100.0	±	0.0	99.0	±	2.2	98.3	±	2.4	98.3	±	3.7
Nets	Nov-04			Dec-04			Jan-05			Feb-05			Mar-05			Apr-05		
ITN	15.0	±	2.4	100.0	±	0.0	96.7	±	4.7	93.0	±	4.5	89.7	±	3.2	84.7	±	3.6
PermaNet [®]	100.0	±	0.0	100.0	±	0.0	100.0	±	0.0	98.3	±	1.7	98.3	±	2.4	98.0	±	1.8
OlysetNet [®]	100.0	±	0.0	99.7	±	0.8	98.0	±	2.7	96.3	±	3.8	93.7	±	6.7	97.7	±	1.9

Figure 1 First year results of bioassay test showed mortality rates (mean \pm SD) of *An. minimus*

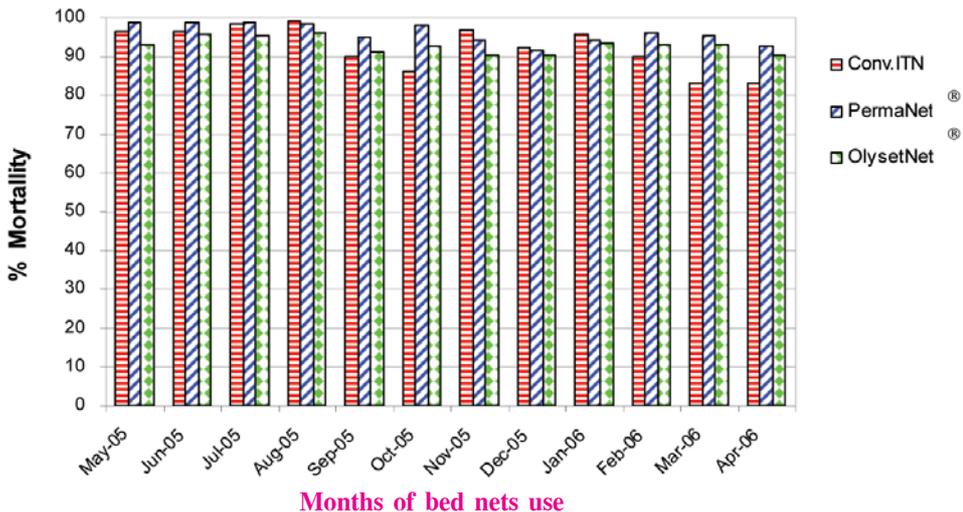


The results of the second year study are presented in table 2 and figure 2. PermaNet[®] yielded 91.7-99% mortality rates, slightly declined compared to the first year results, same was with OlysetNet[®]. The mortality rates of *An.minimus* samples caused by OlysetNet[®] were between 90.3-96.0%. The conventional ITN exhibited 86.3% and 83.3% mortality on October and April (6 months after re-treatment). All net samples were washed after bioassays in October and April. Re-treatment of conventional ITN was carried next day after washing. Results of bioassays in November and May obtained after re-treatment of conventional ITN.

Table 2 Second year results of bioassay tests showed mortality rates (mean \pm SD) of *An. minimus*

Nets	May-05		Jun-05		Jul-05		Aug-05		Sep-05		Oct-05	
ITN	96.7	\pm 3.9	96.7	\pm 2.6	98.3	\pm 2.0	99.3	\pm 1.5	90.0	\pm 9.1	86.3	\pm 7.2
PermaNet [®]	98.7	\pm 1.4	99.0	\pm 1.5	99.0	\pm 1.5	98.3	\pm 2.0	95.0	\pm 4.9	98.0	\pm 2.7
OlysetNet [®]	93.0	\pm 5.2	95.7	\pm 4.5	95.3	\pm 5.1	96.0	\pm 5.6	91.3	\pm 0.7	92.7	\pm 7.2
Nets	Nov-05		Dec-05		Jan-06		Feb-06		Mar-06		Apr-06	
ITN	97.0	\pm 1.4	92.3	\pm 5.5	95.7	\pm 1.5	90.0	\pm 4.6	83.3	\pm 1.7	83.3	\pm 2.0
PermaNet [®]	94.4	\pm 1.0	91.7	\pm 1.7	94.4	\pm 1.0	96.1	\pm 1.9	95.6	\pm 2.5	92.8	\pm 1.9
OlysetNet [®]	90.4	\pm 5.2	90.4	\pm 2.1	93.7	\pm 1.4	93.3	\pm 1.7	93.3	\pm 1.7	90.3	\pm 2.7

Figure 2 Second year results of bioassay test showed mortality rates (mean \pm SD) of *An. minimus*



The results of the third year study are presented in table 3 and figure 3. PermaNet[®] produced 80.6-91.7% mortality against mosquito test samples, while OlysetNet[®] produced 80.3-87.7% mortality. The conventional ITN's showed 83.3-86.3% mortality after 6 months use before re-treatment.

Table 3 Third year results of bioassay tests showed mortality rates (mean ± SD) of *An. minimus*

Nets	May-06			Jun-06			Jul-06			Aug-06			Sep-06			Oct-06		
ITN	97.7	±	2.8	96.7	±	2.0	90.3	±	1.4	83.7	±	1.8	83.3	±	4.7	86.3	±	7.2
PermaNet [®]	91.7	±	7.3	90.0	±	1.7	88.3	±	4.4	90.6	±	2.5	86.7	±	1.7	85.0	±	1.7
OlysetNet [®]	88.0	±	3.0	88.3	±	1.2	87.7	±	1.9	87.3	±	1.9	87.0	±	2.7	83.0	±	5.2
Nets	Nov-06			Dec-06			Jan-07			Feb-07			Mar-07			Apr-07		
ITN	95.0	±	1.7	97.7	±	1.5	90.3	±	2.2	86.3	±	4.1	83.3	±	5.1	83.3	±	2.0
PermaNet [®]	83.3	±	4.4	83.9	±	2.5	81.1	±	1.0	82.2	±	3.8	82.2	±	1.0	80.6	±	1.0
OlysetNet [®]	81.3	±	5.9	83.0	±	3.6	82.0	±	3.0	83.0	±	1.4	80.3	±	1.8	80.3	±	0.7

Figure 3 Third year results of bioassay test showed mortality rates (mean ± SD) of *An. minimus*

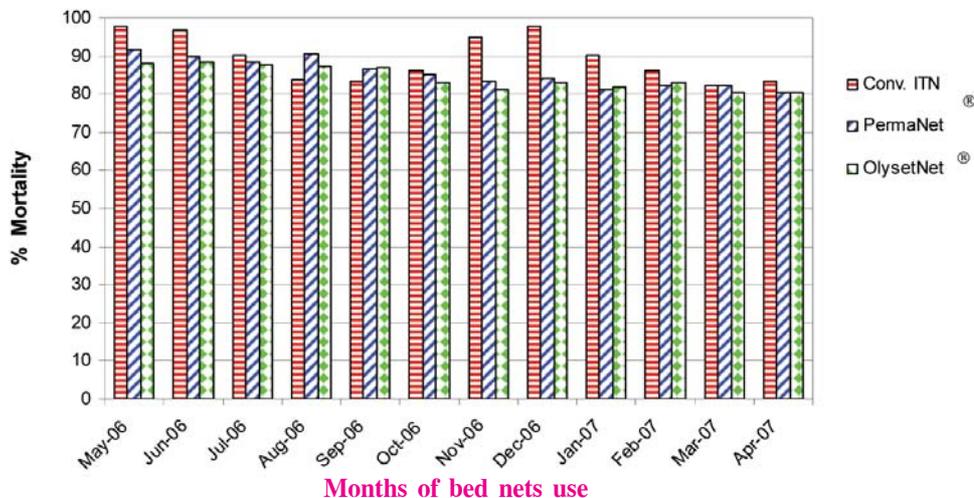
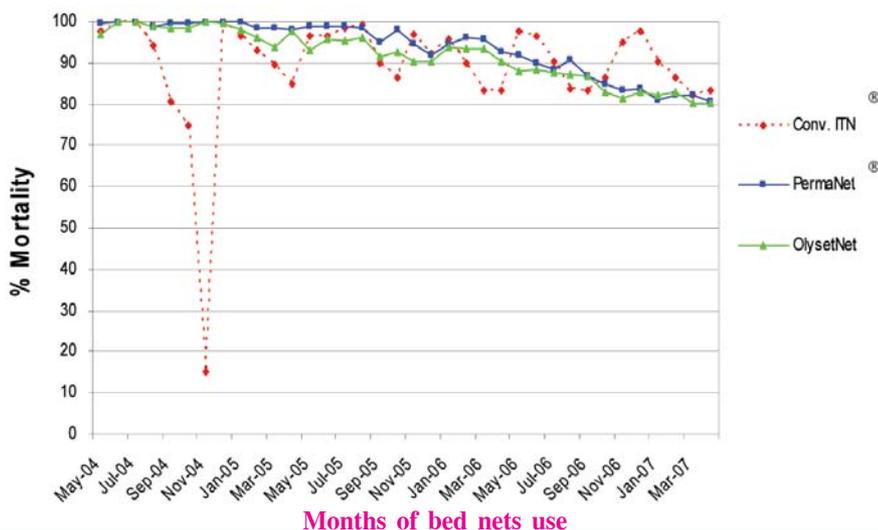


Figure 4 Comparative efficiency during May 04 to April 07 (3 years)



Conclusion and Discussion

PermaNet[®] and OlysetNet[®] which were normal regularly used by households offered >80% mortality on *Anopheles minimus* laboratory strain over the entire 3 years period of field evaluation (figure 4). In the present study both the LLINs exhibited long lasting efficacy against the most important malaria vector in Thailand all along despite repeated washed at 6 month intervals. From enquiry the local people in the study area, mosquitoes nets washing frequency were usually every 3-6 months as normal practice. For the conventional ITN program, household were advised by local health workers to wash their nets just before re-treatment.

Overall the conventionally treated ITN performed similar to LLINs except the fact that ITN were retreated at 6 months intervals. Interestingly the ITN offered only 15% mortality against malaria vector when repeat treatment did not take place immediately after the 6 months use and were washed. This indicated the failure of ITN when re-treatment was not properly scheduled exactly at 6 monthly intervals. This is the main reason for WHO emphasizing the need for LLINs like PermaNet[®] and OlysetNet[®] in malaria prevention for endemic areas. Pyrethroid impregnated bed nets are one of the preventive methods of the WHO Global Strategy for malaria control, and their large scale use in the next decade is one of the goals of the recently developed programme Roll Back Malaria. However, the successful implementation of ITN at community level has several technical, operational, economical and social factors such as the low re-treatment rates usually observed, which limit the sustainability of ITN programmes and, therefore, their efficacy in reducing malaria transmission and morbidity on a long term basis as envisaged by WHO². The study further revealed that if re-treatment of ITN fails due to some reasons, there is likely a possibility of control failure of vectors transmitting malaria. In order to solve the issue of re-treatment of ITN, WHO has encouraged introduction of “long lasting” and “wash resistant” mosquito nets (LLINs). LLINs are supposed to last longer than “hand treated” nets (3-5 years instead of 6-8 months) and to keep their efficacy even after several (20 or more) washes^{7,8}.

The qualitative data on community acceptance of LLINs from the household users revealed that PermaNet[®] was considered preferably due to its soft nature of the polyester fabric than OlysetNet[®] which was made up of polyethylene nets. The polyethylene nets by nature are rough in texture. The other advantages of PermaNet[®] over OlysetNet[®] was, easy to wash and pack after use in view of the soft polyester fabric, while OlysetNet[®] were considered difficult to wash and pack after use due to polyethylene fabric.

The recent years LLINs have been used all over the world for effective malaria control, these nets do not require repeated treatment with insecticides and can be used for a minimum period of 3 years continuously or to a minimum of 20 standard washes whichever is earlier. LLINs are treated with insecticides in the factory through a specialized process for a long lasting biological activity under field conditions. LLINs are recommended by World Health Organization as an effective tool in malaria control all over the world. However conventionally treated mosquito nets requiring repeat insecticide treatments (once in 6 months) failing of which has resulted in resurgence of malaria in many parts of the world, WHO emphasizes the need for use of LLINs to avoid operational failures of repeated treatment of conventionally treated mosquito nets. LLINs are preferred to re-treatment of mosquito nets with insecticides. Even though the initial costs of LLINs are higher than mosquito nets that are retreated regularly, in the long run LLINs are more cost effective than ITN⁹.

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