Malaria Control & Elimination

ISSN: 2470-6965

Tarimo and Cosmas, Malaria Contr Elimination 2016, 5:2
DOI: 10.4172/2470-6965/1000149

Research Open Access

Use of Olyset® Insecticides Treated Nets and Changes in Insecticide Concentration Over Time in Kinondoni district, Dar es Salaam, Tanzania

Donath S Tarimo[1*] **and Remigi Cosmas**[2]

[1]*Department of Parasitology and Medical Entomology, Muhimbili University of Health and Allied Sciences (MUHAS), Tanzania*

[2]*Department of Biological Sciences, Dar es Salaam University College of Education (DUCE), Tanzania*

[*]**Corresponding author:** Donath S Tarimo, Department of Parasitology and Medical Entomology, Muhimbili University of Health & Allied Sciences (MUHAS), Tanzania; E-mail: mailto:dontarimo@gmail.com

Received date: May 25, 2016; **Accepted date:** June 28, 2016; **Published date:** June 30, 2016

Abstract

Background: Olyset® nets are effective for malaria control; approved as best choice for rural areas. It is unclear to what extent insecticide persists in the use period. Washing frequency under different methods, soap and drying methods may conceivably lead to a change over time in insecticide concentration. This study examined net ownership, types, handling; and changes in permethrin concentration in Olyset® nets over time.

Methods: A quantitative cross-sectional survey was carried out in Kinondoni district, Tanzania July to September 2011. Nets ownership and use were assessed in 601 households using structured questionnaires with observed (net brand, colour, shape and size) and reported aspects (purchase date, duration of use, whether treated or not, frequency and methods of washing and drying, and soap used). Fifty Olyset® nets were randomly recovered from four randomly selected streets for analysis of permethrin concentration.

Results: Very high net ownership (93.2%) and use (83.5%) were observed; half (50.3%) being Olyset®. Chemical analysis showed Olyset®nets were losing permethrin concentration at a rate of 101mg/kg per month; after 22.2 months of use, will on the average retain 47.3% of initial concentration. Only 36.0% of nets were above the minimum concentration limit required for being effective. Weak negative correlations existed between permethrin concentration with age of nets: r^2 = 0.111 (95%CI = -0.189-0.01), and washing frequency: r^2 = 0.035 (95%CI = -0.098-0.021). No association was found between permethrin concentration with the soap used for washing (x^2 =3.406, p = 0.671) and drying methods (x^2 = 1.344, p = 0.649).

Conclusion: Net coverage was very high, half being Olyset®; loosing insecticide concentration at the rate of 101mg/kg per month and after 22.2 months will on the average retain 47.3% of initial insecticide concentration required for being effective.

Keywords: Malaria; LLINs; Olyset net; Ownership, Washing and drying; Changes in insecticide concentration

Introduction

The barriers associated with the conventional insecticide treated nets (ITNs) that require re-treatment have been mitigated by the invention of long-lasting insecticide treated nets (LLINs). LLINs are designed to survive for several years in the field without further maintenance, and to withstand for at least 20 washes without losing insecticidal activity. Application of LLINs is currently growing in many malaria endemic areas and these nets retain their insecticidal properties for their physical life in domestic use despite many washes [1]. The net fabric can either be polyesters with a physical life of 3-4 years or polyethylene with a physical life of 4-5 years [2].

Several brands of nets are available in the country including Olyset, Permanet, Mbu net, Afya net, Safi net and others. Olyset nets have been found to be popular and durable than ordinary polyester nets. Olyset® nets have permethrin incorporated into the polyethylene material during the manufacturing process at a concentration of 20 g/kg (± 3 g/kg), 2% w/w (1.7-2.3% limits). The manufacturer has reported that, over time, insecticide migrates to the surface of the yarn, replacing the one that has been removed by washing; insecticide migration being temperature-dependent is accelerated by exposure of nets to sun heat after washing.

Despite the large body of literature documenting the effectiveness of Olyset® nets in providing personal protection against malaria vectors for at least 5 years and their use recommended by WHOPES [3], a number of important practical issues remain unresolved. It is yet unclear to which extent Olyset® nets can retain the insecticide in the 5 year physical life period especially when subjected to frequent and different washing methods, different detergents and drying methods. Exposing Olyset® net to sun heat for few hours after washing would accelerate permethrin migration thus increasing potency, but on the other hand over exposure may degrade the insecticide conceivably shortening the expected duration of potency.

While it is inevitable to wash the nets over the 5 years of physical life, this must be done cautiously to ensure that there is no excessive release of the active ingredient after washing. Since the active ingredient is incorporated into filaments, rapid total loss of active ingredient is not likely to occur during washing but, if redistribution to the surface is too slow, the Olyset® net may be ineffective for an

unacceptable period after washing. Alternatively, if the redistribution is too rapid, the surface concentration could become higher than expected, leading to higher losses of active ingredient during washing and possibly increased user exposure to the active ingredient.

Although WHOPES [4] has provided guidelines on standardized procedures for washing and drying of LLINs, it may not be possible to standardize washing methods in every region or country due to local economics and general practices. It is, therefore, necessary to undertake studies to examine how different washing and drying methods can affect the concentration of permethrin in Olyset® nets so as to propose alternative washing and drying methods that would ensure persistence of insecticide concentration required for the nets to be efficacious over the 5 year physical life. This study examined LLINs ownership and use, permethrin concentration in Olyset® nets over 2-5 years of use and the factors associated with changes in insecticide concentration over time.

Materials and Methods

A quantitative cross-sectional household survey was carried out from July to September 2011 in four streets selected by a multi-stage random sampling from two wards of Kijitonyama and Mbezi in Kinondoni district, Dar es Salaam, Tanzania; an area with perennial malaria transmission [5].

(H2)Sample size estimation

The primary outcome was to determine net ownership, type and use; while the secondary outcome was changes over time of insecticide concentration in Olyset® nets; thus two study samples were required: households for the survey of LLINs ownership and use; and Olyset® netsto be recovered from the surveyed households for chemical analysis to determine the changes over time of permethrin concentration in the nets.

Sample size of households for the survey on net ownership and use

Dar es Salaam was estimated to have 5 million people; the estimated proportion of household owning LLINs was 36%,with a design effect of 2 and a maximum allowable error of 5%; the minimum sample was estimated to be 354 households (Calculated using Epi Info version 6.04D). After adjusting for non- participation rate of 15% and 10% for each pre-specified variables, the sample size was estimated to be 600 households.

(H2)Sample size of Olyset® nets for permethrin chemical analysis

The sample size was calculated to detect monthly rate of loss of insecticide inOlyset® nets at the rate of 0.125 g/kg per month under a Linear Regression Model. Setting the power of the study at 90%; standard deviation for the age of the net (duration of use) 6 months and standard deviation for the initial concentration of permethrin in Olyset® nets at 1.5 g/kg; by the sample size was calculated by G*Power software version 3.1.2 [6] to an estimated sample of 34 Olyset® nets. Adding 15% to adjust for non-response; a sample of 39 nets was obtained. When 10% was added for each of pre-specified variables, the total sample size was estimated to be 50 Olyset® nets.

Sampling procedure and data collection procedures

Household survey for net ownership and use: Multi-stage random sampling technique was used to select households to be included in the survey. From 2002 Census information, wards located in the randomly selected district were listed alphabetically then two wards was randomly selected using computer-generated random numbers. In each of the two randomly selected wards, two streets were randomly selected using simple random sampling.

Within each selected street, 150 households were chosen using a modified EPI-type sampling procedure; this level of sampling was done in the field. A centre point of the street for sampling households was identified, then a pen was span so as to indicate a random direction. Researchers walked in the direction to the edge of the street while drawing a sketch of the location of each house, and at the same time assigning an identification number for each household; from this list one household was randomly selected as household number one. The procedure was repeated to sample more households until a sample of 150 households from that street was obtained. The male or female head of household was interviewed to gather data on the various aspects of mosquito nets using structured questionnaires with closed- ended questions. The key features of the questionnaire consisted of a mixture of observed and reported aspects. Observed aspects of the net included: brand, colour, shape and size.

Reported aspects of the net included: date of purchase, duration of use, whether treated or not, frequency and methods of washing, type of soap used for washing and drying methods.

Recovery of Olyset® nets and chemical analysis: Olyset® nets were systematically selected from the selected households. A net was collected from every second household with an Olyset® net. For each street, a total of 13 nets were collected and replaced with new ones, same brand. If the household had more than one Olyset net, the first net to be investigated was collected. A total of 50 Olyset® nets were collected for laboratory chemical analysis of permethrin concentarion. Each net was packaged in a separate plastic bag and kept at room temperature for chemical analysis at the Tanzania Pesticides Research Institute (TPRI) laboratory in Arusha, Tanzania.

The gas chromatography method was used to measure permethrin content on and in the net fibers. A general method applied to take rectangular and conical net samples where 5 pieces were taken from each net according to the pattern recommended and testing was performed following standard procedures on permethrin-incorporated mosquito nets as outlined by the World Health Organization [2].

To measure permethrin content on the surface of the yarn, a 10 × 10 cm piece of net (corresponding to about 600 mg) was cut from each Olyset® net sample, accurately weighed and put into a 100 mL conical flask. A 30 mL of acetone, was then added and the flask thoroughly shaken by hand for one minute. The acetone extract was quantitatively transferred into a 50 mL volumetric flask, concentrated using Soxhlet extractor and filled up to 10 mL with acetone. A 2 mL internal standard solution (Tetramethrin) was added; the flask was filled up to volume with acetone. The extract was put into an injection vial and analyzed by capillary gas chromatography with flame ionisation detection (GC-FID) for determination of permethrin content using the internal standard calibration. To measure permethrin content inside the net yarn after the first acetone wash, the net sample was cut into 2–3 mm pieces and homogenized. A sub-sample of 400 mg was accurately weighed and put into a 100 mL conical flask, then 30 mL of xylene was added; permethrin was extracted by heating under reflux for 60 minutes. The extract was allowed to reach ambient temperature and quantitatively transferred into a 50 mL volumetric flask, then concentrated using Soxhlet extractor and filled up to 10mL with

xylene. A 2mL internal standard solution (Tetramethrin) was added; the flask was filled up to volume with xylene. The extract was put into an injection vial and analyzed as above. The tests were repeated to confirm the results of samples that showed no detectable insecticide.

Ethical considerations: The study was ethical approved by the Muhimbili University of Health and Allied Science IRB; permission to conduct the study was obtained from the Kinondoni Municipal Directorate, while informed verbal consent for participation in the study was obtained from each participant.

Data management and analysis: Data were entered, cleaned and analyzed by using SPSS software version 16.0. Descriptive statistics was used to summarize characteristics of the households surveyed and study sample. Primary analysis was done by fitting a Linear Regression Model with the permethrin insecticide level (g/kg) as the outcome variable and the age of nets (months) as the independent variable after removing the outliers. The slope was estimated from the model and 95% CI for the slope calculated. Additionally, a multivariate linear model was fitted using associated factors such as (frequency of washing the net) as additional independent variable.

Results

Demographic characteristics of the households

A total of 601 households were surveyed in four streets. The mean household size was 4.5 (± 2.4) persons; in households with at least one underfive, the mean number of underfives was 1.4 (± 0.9). The mean number of sleeping places (beds) was 2.3 (± 1.3) per household, the lowest number of sleeping places being 1 and the highest being 9. Most households, 560/601 (93.2%) owned at least one net while the overall use of the nets was found to be 552/601 (91.8%) of all households. The descriptive statistics for demographic characteristics of the households is presented in Table 1.

Variable	n	Sum	Mean	Std. Dev.	Min.	Max.
The size of the household	601	2701	4.49	2.4	1	15
Children under five years in the household	350	501	1.43	0.87	1	10
Sleeping places (beds) in the household	601	1382	2.3	1.33	1	9
Nets owned by the household	601	1218	2.03	1.33	0	8
Use of bednet in the household	601	2256	3.75	2.4	0	14
Use of bednet by underfives in the household	348	434	1.25	0.73	0	6

Table 1: Descriptive statistics for demographic characteristics of the households.

Households' ownership of nets

The point estimate for household nets ownership showed that 560/601 (93.2%) households owned at least one net. A total of 1218 nets were owned by all households where each household owned a varying number of nets ranging from 0 to 8 with a mean of 2.0 (± 1.3) nets per household (Figure 1).

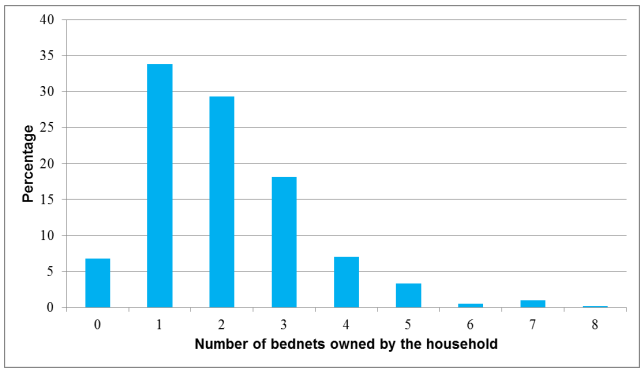

Figure 1: Percentage ownership of nets by households.

A total of 41 (6.8%) households did not own a net; reasons being inability to buy 24/41 (58.5%), dislike or not preferring use of net 6/41 (14.6%), net makes the user uncomfortable on bed 1/41 (2.4%), the weather is too hot 4/41 (9.8%), prefer use of sprays to keep mosquitoes away 4/41 (9.8%) and the net was worn out, torn out or damaged 2/41 (4.9%).

According to brand, of the investigated nets, the large majority 1082/1218 (88.8%) were identified by their brand, of which 544 (50.3%) were Olyset nets (Figure 2).

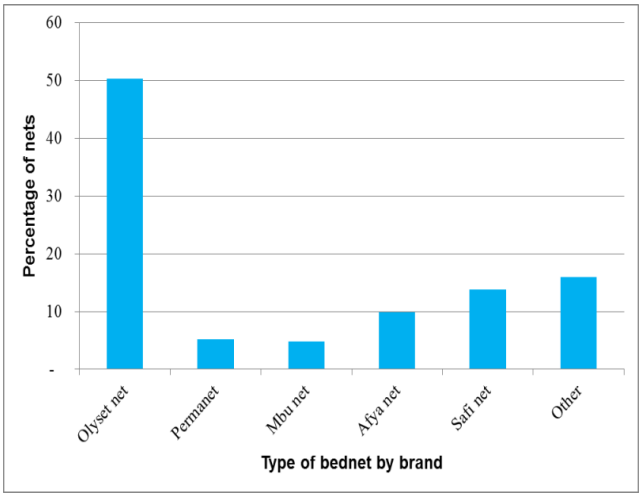

Figure 2: Types of nets by their brand.

Regarding insecticide treatment status, 1021/1218 (83.8%) nets could be identified on the basis of their insecticide treatment status; of which 600 out of 1021 (58.8%) were LLINs (factory pre-treated) while 252/1021 (24.7%) were ITNs that required re-treatment with insecticide while 169/1021 (16.6%) were untreated nets (Figure 3).

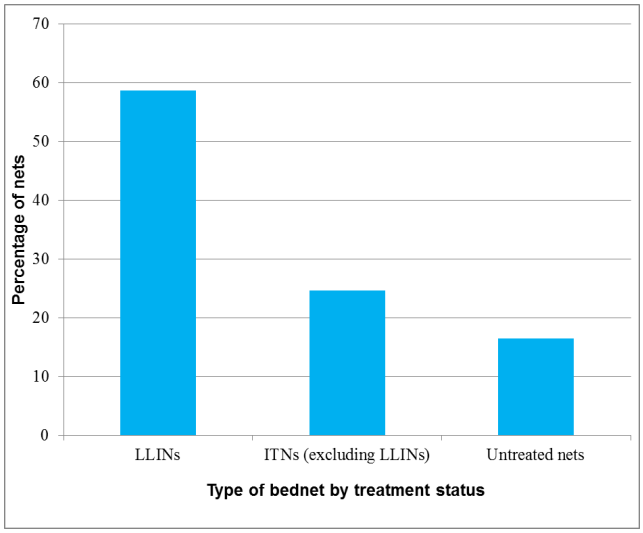

Figure 3: Distribution of nets according to insecticide treatment and type of insecticide.

Use of nets: Sleeping under a net in the night preceding the survey was an indicator of use of net. Male or female heads of households were asked to indicate the number of household members who slept under a net the previous night as an indicator of net use in the general population. It was found that in a large majority, 552/601 (91.8%) of households, at least one member slept under a net (Figure 4).

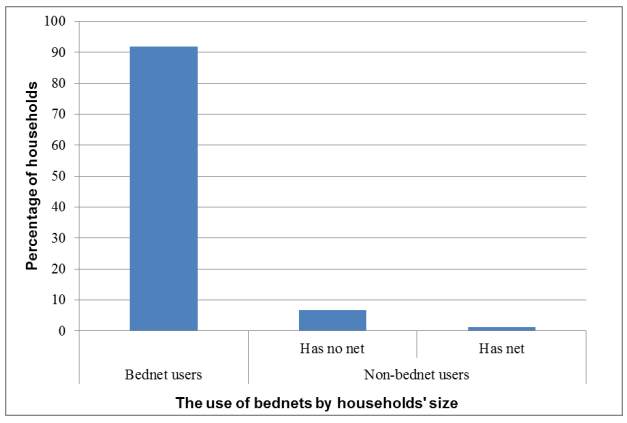

Figure 4: Percentage use of nets by households.

The point estimates fornet use showed that, of all household members 2256/2701 (83.5%) and 434/501 (86.6%) underfives used nets (Figure 5).

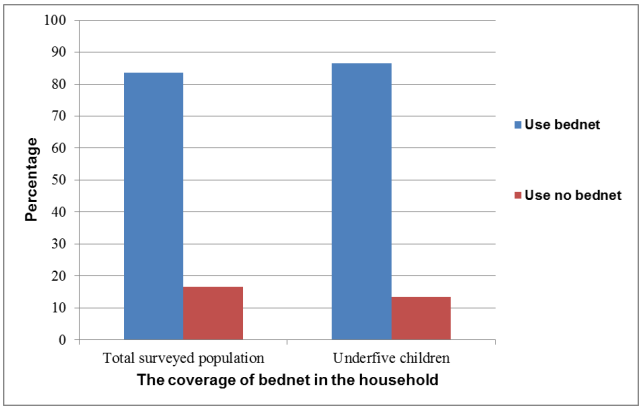

Figure 5: Point estimates for netuse among household members and underfives.

Chemical analysis of permethrin insecticide concentrationin Olyset® nets

The reported average age of the sampled Olyset nets was 22.2 (±16.9) months. It was found that the mean concentration of permethrin in the Olyset nets was 9439 (±5796) mg/kg. The minimum and maximum concentration of permethrin in Olyset nets were 12 and 19762mg/kg respectively. It was found that the nets were losing the insecticide at the rate of 101 mg/kg per month. On average, 47.3% of the initial insecticide dose (20,000 mg/kg) was still present. Only about a third (36%) of the analyzed nets were above the minimum surface concentration limit required for good efficacy and the difference was statistically significant ($p = 0.004$). The analysis clearly indicated that the insecticide was still very much concentrated inside the fibers with an average inside to outside ratio of 22. The correlation between surface concentration and the internal concentration of permethrin in the nets was very weak and non-significant: $r^2 = 0.002$ (95% CI = -0.041-0.058). There seemed to be a very weak negative correlation between the concentration of permethrin in the nets withthe reported age: $r^2 = 0.111$ (95% CI = -0.189-0.01). When multiple regression analysis for the numeric variables (age and washing frequency) were conducted to determine how they affect the concentration of permethrin in the nets, it was found that variation in age with washing frequency could explain just 9.2% of the variation in permethrin concentration in nets ($r^2 = 0.0921$; 95% CI = -0.19 - 0.015).

The changes over time in concentration of permethrin in the 50 Olyset® nets sample collected in the field are shown in Figure 6.

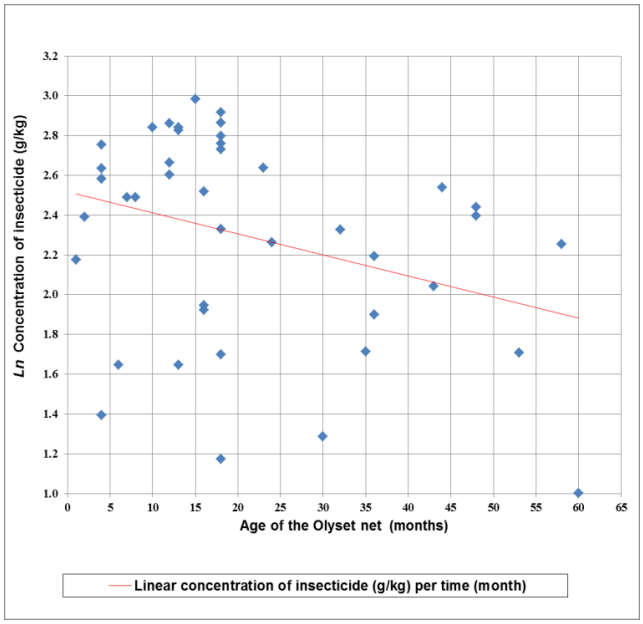

Figure 6: Changes over time in permethrin concentration in Olyset®nets.

Factors associated with loss of permethrin content in Olyset® nets

A number of factors associated with loss of permethrin in Olyset® netswere investigated including: washing frequency, washing methods, type of soap used for washing and the drying methods. It was found that the nets were washed at an average rate of 22 (±17) times per year and 32 (±28) times over the lifetime of the net, the washing frequency ranged from 2 to 50 times per year and 2 to 103 times over the lifetime of the net. There was a very weak and non-significant correlation between the concentration of permethrin in the nets with the frequency of washing: $r^2 = 0.035$ (95%CI = - 0.098 - 0.021).

All (100%) of the respondents reported to use hand (smooth) washing method for washing the nets; none reported using machine (abrasive) washing. This implies that no association could be made between washing methods and permethrin concentration in the nets. The reported type of soap used for washing the nets included bar and liquid soaps with 84.0% reporting using both types. It was found that there were no significant association between the concentration of permethrin in the net with the typeof soap used for washing (x^2 = 3.406, p = 0.671). After washing the nets, 22.0% of the nets were reported to be dried under shade while the remaining 78.0% were dried under the sun. Likewise, no significant association was found between the concentrations of permethrin in the nets with the methods used for drying the nets (x^2 =1.344, p = 0.649).

Discussion

This study examined household net coverage and permethrin concentration in Olyset® nets and the factors associated with changes in insecticide concentration over time. As expected, household ownership of nets was very high (93.2%); however, only 83.4% of the nets were ITNs. Usage was also very high with 83.5% of all household members and 86.6% of underfives reported to use ITNs. The high ownership and use has been partly attributed by ITN interventions that had been implemented in the country. These interventions includes Discount Voucher "Hati Punguzo" scheme for pregnant women and underfives launched in 2004 and 2006 respectively as well as free nets distribution for underfives (underfives catch up campaign). The present findings show major achievements in comparison to earlier observation by Hanson et al. [7] whereby in the year 2007 only 65% of surveyed households owned at least any net while 36% of them owned at least one ITN.

In this study, net usage was also examined focusing on personal use of nets amongst individuals in households with nets. These estimates were not reported as the universal use estimates since they are not representative of the population level. However they do provide an interesting insight into the nature of the gap between ownership and use in Dar es Salaam for this survey. Findings from this study show that in households which owned at least one net, 89.7% of members were reported to have slept under a net in the night before the survey representing a 30.7% increase in use compared to the observation by Hanson et al. [7] WHO reported use of 59% in the year 2007. This finding underlines the importance of household ownership of net as a step in the causal chain leading to effective protection by a net. However this would depend largely on the longevity of the intact net and persistence of insecticide in the ITNs.

Olyset nets have been shown to be popular, durable than ordinary polyester nets, conceivably being one of the best choices for ITN programs in rural malaria-endemic areas. This would be true if the efficacy of the insecticide incorporated into the net fibres lasts significantly longer than conventionally treated nets. This survey was carried out after more than 10 years of Olyset nets use following recommendation by World Health Organisation [3]. Being one of the indicators of the effectiveness of Olyset nets it was important to determine the concentration of insecticide in the nets as it is affected by several factors. Findings from this study showed that there was a considerable varying degree of concentration of insecticide in the nets. Several factors like washing frequency, methods of washing and drying may account for the varied concentrations of insecticide in the nets. Findings of this study are consistent with findings of previous studies conducted in Tanzania [1] and in Cambodia [8] which showed that the insecticide content and bioassay efficacy varied greatly among nets. The variability in insecticide content may conceivably explain the observation that four Olyset nets had no detectable surface concentration of insecticide. Furthermore, some Olyset nets with very high internal concentration produced very low surface concentration of insecticide. So far there have been no reported manufacturing problems which could explain this variability. It is possible that more in-depth studies on the use of individual nets would elucidate the specific factors which are responsible for the rapid or slow depletion in insecticide.

It was observed that, on average, for a period of 22.2 months of use of the nets, more than half (52.7%) of the initial insecticide dose in the nets had been degraded. Likewise, about two third (64%) of the analysed nets were below the minimum surface concentration limit required for good efficacy. The application of bioassay could have tested whether these nets with surface concentration below minimum limit are effective or not; this was not possible in this study. Studies have shown that Olyset nets can remain effective even after 4-7 years of use as demonstrated by Tami et al. [1] and Malima et al. [9] who found

that the nets maintained their insecticidal activity for at least 7 years of use, while Maxwell et al. [10]found that the nets with 4 years of domestic use were as effective as new Olyset nets.

This study found that the concentration of permethrin in Olyset nets is not significantly associated with washing frequency. The study by Sharma et al. [11] showed that Olyset nets, even after repeated washings maintained high bio-efficacy (>80%) against *Anopheles culicifacies* and *Anopheles fluviatilis*, which is in agreement with the findings by Sharma et al. [11,12]. A previous study on Olyset nets in Africa also showed good insecticide persistence even after 3 year of use, and the nets were found to be effective in causing >80% mortality in bioassay tests[13]. All these previous studies indicate that the bioavailability of insecticide in Olyset nets remains effective for a longer period even after multiple washings.

Although in this study it was not possible to examine the association between washing methods with concentration of insecticides due to the fact that all nets (100%) were reported to be washed by hand, Magoma [14] and Atieli et al. [15] found that vigorous washing procedures reduced insecticidal activity as well as insecticide concentration in the nets. Sreehari et al. [16] concluded that for Olyset nets to retain insecticidal power and insecticide concentration hand washing would be preferable tomachine washing.

The type of soap used for washing nets has been demonstrated to have no significant impact on the concentration of insecticide in the nets [16]. Although theoretically it can be argued that washing nets using soaps with bleaching effects may cause loss of insecticide content in the nets, there is limited information on the impact of various types of soap used for washing nets on the insecticide concentration in the nets.

In this study the concentration of the insecticides in the sample Olyset nets was not significantly associated with the method of drying nets after washing. This is in contrast with findings from Atieli et al. [15] who reported that the amount of insecticide retained was higher in the nets air-dried under shade (80.7%) than nets dried in direct sunlight (<70.8%), a difference conceivably attributed to differences in regeneration time. Olyset nets that were dried under the sun might have greater chance of being exposed to high temperatures and direct sunlight. Therefore the permethrin content of such Olyset nets might have been rapidly lost since the nets were dried at high temperatures and exposed to ultraviolet light which can cause degradation of permethrin compared to nets that were air-dried under shade. In most areas, it is a normal practice for washed nets to be dried outdoors for an extended period, sometimes for the whole day. Although this study has not demonstrated that different drying practices affect significantly the loss of the insecticide in the net, studies by Maxwell et al. [11] and Atieli et al. [15] have found an association between loss of the insecticide in the nets and drying the nets in direct sunlight. This means that nets used in the field might be losing insecticide at a faster rate if dried under direct sunlight. These findings suggest that it is important to counsel users to dry nets under shade so as to avoid degradation of insecticide content.

The concentration of insecticide in nets might be a useful indicator of effectiveness of the net; thus a previous study by WHO [17] found that the mean knockdown time was directly correlated with the insecticide concentration on the surface for fast-acting insecticides such as pyrethroids. In contrast, Tami et al. [1] found a very weak (non-significant) correlation between the insecticide concentration and the bioassay results. However, Atieli et al. [15] demonstrated that reduction in residual insecticide content in Olyset nets was not correlated with loss of effectiveness.

This study examined few practices that might affect the concentration of insecticide in nets after use in the field; hand washing was the universal method of washing nets, thus other factors are likely to be responsible for the loss of insecticide concentration as age and washing frequency could explain only 9.2% of the variation in insecticide concentration. Since bio-efficacy was not part of this study, it was not possible to establish whetherthe nets (64.0%) that had below minimum insecticide concentration were still effective against mosquitoesor not.

Conclusion and Recomendations

There is a very high coverage of LLINs in the study area, half of which are Olyset®; these were losing insecticide at the rate of 101mg/kg per month, and after 22.2 months of use will on the average retain 47.3% of initial insecticide. Only about a third (36%) of the Olyset® nets had the minimum insecticide concentration required for the nets to be efficacious. The observation that all household members wash their nets using hands should be encouraged. Further studies on larger samples of Olyset nets are needed to establish the factors that are associated with a so fast loss of insecticide concentration and effectiveness of the nets under such conditions.

Acknowledgements

This study received financial support from the Science and Technology Higher Education Project (STHEP) administered by the Dar es Salaam University College of Education (DUCE). Thanks are due to the household members for devoting their time during the survey. Special thanks go to Mr. Joseph Malulu of the Tropical Pesticides Research Institute (TPRI) for his technical support in chemical analysis of the samples of Olyset nets.

References

1. Tami A, Mubyazi G, Talbert A, Mshinda H, Duchon S, et al. (2004) Evaluation of Olyset insecticide-treated nets distributed seven years previously in Tanzania. Malar J 3: 19.
2. (2006) WHO World Health Organization Specification and Evaluationsfor Public Health Pesticides: Permethrin, Long Lasting (incorporated itno filements) Insecticidal Net. 1–17.
3. (2001)WHO Report of the Fifth WHOPES Working Group meeting.
4. (2005) WHO Guidelines for Laboratory and field testing of Long Lasting Insecticidal Mosquito Nets.
5. MARA/ARMA. MARA LITe for Africa. South African Medical Research Council, Malaria Research Programme.
6. Faul F, Erdfelder A, Buchner A, Lang AG (2009) Statistical power analyses using G*Power 3.1: tests for correlation and regression analyses.Behav Res Methods 41: 1149–60.
7. Hanson K, Marchant T, Nathan R, Mponda H, Jones C, et al. (2009) Household ownership and use of insecticide treated nets among target groups after implementation of a national voucher programme in the United Republic of Tanzania: plausibility study using three annual cross sectional household surveys. BMJ 339: b2434
8. Tsuzuki A, Khamlome B, Kawada H, Eto H, Phompida S, et al. (2011) The efficacy and physical condition of olyset insecticide-treated nets after 5 years use in rural Lao PDR. Southeast Asian J Trop Med Public Health 42: 268–73.

9. Malima RC, Magesa SM, Tungu PK, Mwingira V, Magogo FS, et al. (2008) An experimental hut evaluation of Olyset nets against anopheline mosquitoes after seven years use in Tanzanian villages. Malar J 7: 38.
10. Maxwell CA, Myamba J, Magoma J, Rwegoshora RT, Magesa SM, et al. (2006) Tests of Olyset nets by bioassay and in experimental huts. J Vector Borne Dis 43: 1–6.
11. Sharma SK, Upadhyay AK, Haque MA, Tyagi PK, Mohanty SS, et al. (2009) Field Evaluation of Olyset Nets: A Long-Lasting Insecticidal Net Against Malaria Vectors Anopheles culicifacies and Anopheles fluviatilis in a Hyperendemic Tribal Area of Orissa , India. J Med Entomol 46: 342–350.
12. Sharma SK, UpadhyayAK, Haque MA, Padhan K, Tyagi PK, et al. (2006) Wash Resistance and Bioefficacy of Olyset Net - A Long- Lasting Insecticide-Treated Mosquito Net Against Malaria Vectors and Non-target Household Pests. J Med Entomol 43: 884-888.
13. Guessan R, Darriet F, Doannio J, Chandre F, Carnevale P (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.
14. Magoma J (2006) Bioassays on Olyset long lasting insecticidal nets.MSc Thesis,University of London.
15. Atieli FK, Munga SO, Ofulla AV, Vulule JM (2010) Wash durability and optimal drying regimen of four brands of long-lasting insecticide-treated nets after repeated washing under tropical conditions. Malar J 9: 248
16. Sreehari U, Raghavendra K, Rizvi MM, Dash AP (2009) Wash resistance and efficacy of three long-lasting insecticidal nets assessed from bioassays on Anopheles culicifacies and Anopheles stephensi. Trop Med. Int Health 14: 597–602.
17. (1998) WHO Test procedures for insecticidal resistance monitoring in malaria vectors, bio-efficacy and persistence of insecticides on treated surfaces 1-45.