CERTIFICATION

The undersigned certify that he has read and hereby recommend for acceptance by Muhimbili University of Health and Allied Sciences a dissertation titled: ***Use of Insecticide Treated Nets and Temporal Concentration of Insecticide in Olyset® Nets in Dar Es Salaam, Tanzania,*** in fulfillment of the requirements for the degree of Master of Science (Tropical Disease Control) of Muhimbili University of Health and Allied Sciences.

.....

Dr. D.S. Tarimo
(Supervisor)

Date: .....

DECLARATION

AND

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I, **Remigi Cosmas**, declare that this **dissertation** is my own original work and that it has not been presented and will not be presented to any other University for similar or any other degree award.

Signature.....Date.....

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ACKNOWLEDGEMENT

First and foremost, I wish to express my sincere appreciation to my supervisor, Dr. D.S. Tarimo of the Department of Parasitology and Medical Entomology (Muhimbili University of Health and Allied Sciences, MUHAS) for his acceptance, guidance and supervision of this dissertation work to its successful completion.

Exclusive word of thank goes to my lecturers at MUHAS; Prof. K.F. Hirji and Dr. R. Mpembeni for their invaluable assistance in statistical issues during research proposal stage, Dr. C. K. Makwaya for his tireless assistance in data analysis and data presentation which made this dissertation work possible. This dissertation work would not be have been successful without technical inputs of Dr. A.V.F. Ngowi of MUHAS.

I appreciate the technical support I received from the laboratory scientists at the Tropical Pesticides Research Institute (TPRI). More specific, I am indebted to Mr. Joseph Malulu for his tireless effort during chemical analysis of the Olyset net samples regardless of frequent power cuts and water shortage that inevitably over-prolonged the laboratory work.

I am also grateful to my sponsor, the Science and Technology Higher Education Project (STHEP) which is administered by the Dar es Salaam University College of Education (DUCE) for financial support of this research and my M.Sc. studies at large.

Lastly, I am grateful to my colleagues who have voluntarily assisted me at different stages of research proposal writing, field work and report writing as their inputs has greatly contributed to the successful completion of this study. I wish I could mention all who have contributed to this work but those not mentioned are not the least but equally important. I thank them and may the Almighty God bless you all.

ABSTRACT

Background

Long-lasting insecticidal mosquito nets (LLINs) have been recommended by the World Health Organization as major malaria control tool in malaria endemic countries. Despite the large body of literature supporting the effectiveness of these nets for protection against malaria over at least 5 years, it is unclear to what extent the applied insecticide persists throughout the five year use period. Frequent washing under different methods, detergents and drying methods may conceivably lead to decrease in concentration of the insecticide incorporated in the nets.

Objective

This study aimed to determine ITNs ownership and use, and the temporal concentration of permethrin in Olyset® nets currently in use and the potential factors that might lead to decrease in concentration of insecticide.

Methodology

A community-based cross-sectional household survey, using multi-stage random sampling design was carried out in Kijitonyama and Mbezi wards – Dar es Salaam in July and August 2011. ITNs ownership and use were assesed in 601 households followed by random recovery of 50 Olyset® nets from four randomly selected streets. The sampled nets were analyzed for permethrin concentration by Gas Chromatography method. The obtained data were analyzed at a confidence level of 95% to provide descriptive statistics and determine association between variables. A *P*-value < 0.05 was considered indicative of a statistically significant difference.

Results

A very high net ownership (93.2%) and use (83.5%) were observed; most of which were Olyset® brand (50.3%). Chemical analysis indicated that the nets were losing its insecticide concentration at a rate of 101mg/kg per month. Weak negative correlations existed between the concentration of insecticide and the reported age of nets: r^2 = 0.111 (95%CI = - 0.189 - - 0.01) as well as with washing frequency of nets: r^2 = 0.035 (95%CI = - 0.098 - 0.021). Furthermore, no significant associations

were found between the concentration of insecticide in nets and the types of detergents used for washing (x^2 =3.406, p = 0.671) neither with drying regimens of nets (x^2 =1.344, p = 0.649).

Conclusion and Recommendations

The LLINs of Olyset brand lose insecticide at the rate of 101mg/kg per month, and after 22.2 months of use in the field will on the average retain 47.3% of its initial insecticide dose. Only about a third (36%) of the analysed nets were above the minimum concentration limit required for the nets to be efficacious. The use period of the net did not significantly affect the concentration of insecticide in the nets; similarly to other factors like frequency and methods of washing, the detergents used and the drying methods. Further studies are needed to investigate on long-term effectiveness of Olyset nets.

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ABBREVIATIONS

GC-FID	Gas Chromatography with Flame Ionisation Detection
IHI	Ifakara Health Institute
ITNs	Insecticide-treated nets
LLINs	Long-lasting insecticidal mosquito nets
LSHTM	London School of Hygiene & Tropical Medicine
NIMR	National Institute of Medical Research
WHO	World Health Organization
WHOPES	World Health Organization Pesticide Evaluation Scheme
TPRI	Tropical Pesticides Research Institute

1. INTRODUCTION

1.1. Background

All mosquito nets act as a physical barrier, preventing access by vector mosquitoes and thus providing personal protection against malaria to the individual(s) using the nets. The pyrethroid insecticides used to treat nets, have an excito-repellent effect that adds a chemical barrier to the physical one offered by the net fabric, further reducing human–vector contact and increasing the protective efficacy of treated nets. Most commonly, the insecticide kills the malaria vectors that come into contact with the insecticide-treated nets (ITNs). By reducing the vector population in this way, ITNs, when used by a majority of the target population (coverage rates > 80 %) has a mass effect by offering protection for all people in the community, including those who have no nets (Binka, 1998; Hawley, 2003).

ITNs protect users against malaria in several ways; firstly, the fabric provides a physical barrier, so reducing man-mosquito contact. Secondly the impregnated insecticide knocks down and often kills mosquitoes that come into contact with the net. The insecticide dosage required for knockdown is well below the threshold level of mammalian toxicity (WHO, 2006). Thirdly impregnated insecticide is excito-repellent hence will prevent entry of mosquitoes into households with ITNs (Curtis *et al.*, 1998, 2003). The two extra properties make ITNs advantageous over untreated nets, such that a treated net can be effective even if torn. If ITNs are used by the whole community the insecticidal effect results in reduced longevity of female *Anopheles* mosquitoes so that few survive for the time required for development of the malaria parasite to the sporozoite stage in the mosquito thereby reducing malaria transmission.

Pyrethroids were developed after prolonged efforts to improve biological and chemical stability of the natural pyrethrins, which have long been known for their insecticidal effects. As well as causing a knockdown effect, insects encountering pyrethroids show restless behaviour and hyper excitability caused by involvement of the central nervous system. Pyrethroids may also have an anti-feeding effect on *Anopheles* mosquitoes (Becker *et al.*, 2003).

The use of ITNs treated with synthetic pyrethroids is increasing fast in many malarial parts of the world including, sub-Saharan African countries. The efficacy and effectiveness of ITNs in terms of mortality and morbidity reduction in malaria endemic countries have been very well documented (Lengeler, 2004). The WHO currently advocates their universal use by all household members in malaria endemic areas.

Previously, nets had to be re-treated every 6-12 months, more frequently if the nets were washed in between re-treatment. Nets were re-treated by dipping them in a mixture of water and insecticide and allowing them to dry in a shady place. Re-treatment kits (NGAO) were commercially available in retail shops, however not affordable by the needy majority, thus leading to low re-treatment rates (Chavasse *et al.*, 1999; Mubyazi *et al.*, 2002; Kolaczinski *et al.*, 2004; Abdullah *et al.*, 2005). The need for frequent re-treatment was a major barrier to widespread use of ITNs in endemic countries. For ITNs to be fully effective re-treatment rates need to be very high (WHO, 2003). The additional cost of the insecticide kit (NGAO) and the lack of understanding of its importance resulted in low retreatment rates in most African countries.

One of the main programmatic issues affecting the conventional ITNs is the necessity for regular re-treatment. For a number of reasons (such as frequency and washing methods, wear and tear, UV light and others) the pyrethroid insecticide on net fabric wears off over time and has, therefore, to be replaced. There is convincing evidence that after 3 to 5 washes, the insecticide is removed from nets treated by conventional dipping. Under programmatic conditions, unless nets can be systematically be retreated after 3 washes as recommended by WHO (1998) the protective efficacy might be much lower than expected, especially if nets are frequently washed. While it is relatively easy to socially market mosquito nets to the population of endemic countries, the idea of a regular insecticide re-treatment has been found very difficult to implement (Minja, 2001; Armstrong-Schellenberg, 2002).

The barriers associated with the conventional ITNs that require re-treatment have been mitigated by the invention of long-lasting insecticidal mosquito nets (LLINs). Several companies have developed LLINs that maintain effective levels of insecticides for the life of the net fabric estimated to 5 years mitigating the need for conventional re-treatment. LLINs are treated in the factory with insecticide incorporated into or coated around the fibres, which makes them capable to resist multiple washes. 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 (Tami *et al.,* 2004). 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 (WHO, 2003). Currently several insecticide and net manufactures are active in developing long lasting nets technology (WHO, 2011).

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. Currently, Olyset® nets are locally produced in East Africa by the A-Z Textile Industry in Arusha, Tanzania. Olyset® nets have permethrin incorporated into the polyethylene material during the manufacturing process at a concentration of 20g/kg (±3g/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. This migration being a temperature-dependent process, exposure of nets to heat after washing (e.g. few hours to sun) can accelerate it.

Olyset® nets are very strong due to their thick fibres of high density polyethylene which gives them strength of about 180 denier despite the wide mesh of 4mm. Their durability and long-lasting insecticidal properties make them suitable for use in most rural settings in malaria endemic areas in Sub-Saharan Africa (Tami *et al.,* 2004).

1.2. Statement of the Research Problem

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 (2001), a number of important practical issues remain unresolved. It is yet unclear to which extent Olyset® nets can retain the chemical content throughout 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 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 five 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 product (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 (2005) has provided guidelines on standardized procedures for washing and drying of LLINs, however, 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 so as to examine how different washing and drying methods can affect the concentration of insecticide in the Olyset® nets to as to propose alternative washing and drying methods that would ensure continued protection against malaria vectors over the five year physical life of the net. This study therefore gathered data from a community-based net survey that aimed to examine ITNs use, the temporal concentration of insecticide in Olyset® nets and the factors associated with changes in insecticide concentration over time.

1.3. Objectives

1.3.1. Broad Objective

To examine the use of insecticide treated nets and temporal changes in insecticide concentration in Olyset® nets and associated washing and drying practices of the nets in Dar es Salaam.

1.3.2. Specific Objectives

i. To determine the ownership of the bed nets among households in Dar es Salaam.

ii. To determine the proportion of household members who slept under the bed net the previous night in Dar es Salaam.

iii. To determine the rate of loss of insecticide per month of the Olyset® nets used by household members in Dar es Salaam.

iv. To examine the factors associated with loss of insecticide concentration in Olyset® nets among households in Dar es Salaam.

1.4. Significance of the Study

This study will provide important information on household ITNs use practices and the gradual change in concentration of insecticide in Olyset nets over the five year physical life of the net. The study will also provide information on whether washing and drying practices are associated with loss of the insecticide concentration in the net that may conceivably reduce the protective efficacy over time. Findings from this study will also inform manufacturers to develop nets that can withstand the observed household practices so as to have a sustained protection againt malaria vectors over the phjysical life span of the net.

2. LITERATURE REVIEW

2.1. Ownership and use of bed nets

Insecticide-treated nets (ITNs) have become one of the major tools in the global fight against malaria. ITNs reduce the number of clinical malaria episodes by roughly 50% and improve child survival by nearly a fifth (Lengeler, 2000). Large-scale distribution of ITNs is proceeding at an accelerating pace, especially in East Asia and sub-Saharan Africa. Globally, ITNs were shown to substantially reduce morbidity and mortality from malaria in numerous settings and are currently deployed on a large scale in many malaria-endemic countries (Lengeler, 2004).

It is estimated that with a high coverage of ITNs in malaria endemic settings between 6 and 35 lives could be saved each year per 1000 underfives (Schellenberg *et al.,* 2001; Alaii *et al.,* 2003). ITNs also reduce incidence of malaria episodes by about 48% and reduce incidence of severe malaria by up to 45% (WHO, 2003). It was also shown by Curtis *et al.,* (1998) that anaemia associated with malaria in children of all ages was reduced. Furthermore use of ITNs reduces the risk of maternal anaemia and incidence of malaria by 45% in first time pregnancy and by 63% in subsequent pregnancies (D'Alessandro *et al.,* 1995; Garner & Gulmezoglu, 2000; Abdullah *et al.,* 2001; Ekwaru *et al.,* 2004).

Entomological studies show that ITNs reduce the Entomological Inoculation Rate (EIR) i.e the number of infective mosquito bites per year (Magesa *et al.,* (1991); Maxwell *et al.,* (1999); D'Alessandro *et al.,* (2001). This is attributed to the reduction of Anopheles population density and sporozoite rates in mosquitoes due to the killing effect of the insecticide. Untreated bed nets alone do not provide much protection against malaria once they become torn (Mwangi *et al.,* (2003). Studies conducted in Tanzania, Ghana and Kenya showed that people without nets that sleep near to people with insecticide treated nets are partially protected against mosquito biting and/or malaria indicating that ITNs have a mass effect (Binka *et al.,* 1998; Howard *et al.,* 2000).

In Monitoring and Evaluation Household surveys carried out in 2007 by Ifakara Health Institute and London School of Hygiene and Tropical Medicine, it was established that 65% of surveyed households reported owning at least one any net, and 36% reported owning at least one ITN in Tanzania. Also, personal use of any net and ITN by individuals living in a net owning household was 59% and 37.6% respectively (Hanson *et al*., 2009).

2.2 Long-lasting insecticidal mosquito nets (LLINs)

Long-lasting insecticidal mosquito nets (LLIN) is a factory-treated mosquito net made with netting material that has insecticide incorporated within or bound around the fibres. The net must retain its effective biological activity without re-treatment for at least 20 WHO standard washes under laboratory conditions and three or five years of recommended use under field conditions (WHOPES, 2005). Long-lasting insecticidal mosquito nets (LLINs) in which the insecticide treatment is intended to last for the lifetime of the net are advocated by the World Health Organization for protection against malaria (WHO, 2007).

Pyrethroids are the only class of insecticides that are currently recommended for use on mosquito nets. Treated nets should be non-toxic for children and non-irritant when in contact with exposed skin. The insecticides recommended by the WHO Pesticide Evaluation Scheme (WHOPES) are alpha-cypermethrin, cyfluthrin, deltamethrin, etofenprox, lambda-cyhalothrin and permethrin (Zaim *et al.,* 2000; Nagera & Zaim, 2004).

Three LLINs have been fully recommended by the WHO and are now commercially available: Olyset®, PermaNet® 2.0 and Yorkool LLIN (WHO, 2011). An additional six LLINs have an interim recommendation from the WHOPES for the prevention of malaria: DawaPlus® 2.0, PermaNet® 2.5, PermaNet® 3.0, Netprotect®, Duranet® and Interceptor®. Data on the efficacy of the interim recommended nets remains limited (Banek *et al.,* 2010; WHO, 2011).

Olyset nets are made of polyethylene fibres and are manufactured with a 4mm mesh which is considerably larger than the 1.5mm of most bed nets; the larger mesh has the advantage of allowing more ventilation for the sleeper. The pyrethroid insecticide, permethrin is incorporated into the polyethylene fibres during manufacturing and diffuses gradually from the core to the surface. Surface concentrations are regenerated after removal of permethrin by washing or general use (N'Guessan *et al.,* 2001; Gonzales *et al.,* 2002; Graham *et al.,* 2005). Olyset nets has been in use in some Tanzanian villages for several years and according to cone bioassay tests retains insecticidal activity for at least seven years (Tami *et al.,* 2004).

2.3 Effectiveness of Long-lasting insecticidal mosquito nets (LLINs)

LLINs are treated with special binders which are wash durable, hence do not require any further re-treatment throughout their lifetime (WHOPES, 2005; Kulkarni, 2006; WHO, 2007). LLINs require minimum surface concentration of about 0.15g/kg to provide effective protection (WHO, 2006). Several studies have confirmed that LLINs offer longer time protection than conventional ITNs (Lindblade *et al.,* 2005; Smith *et al.,* 2007; Killian *et al.,* 2008).

After up to 5 years of household use, Olyset® nets still retain 50% of the original permethrin content and about 65% of these nets are still effective (cause more than 80% mosquito mortality after 24 hours and/or above 95% Knock Down (KD) after 60 minutes post-exposure) as per WHO definition (WHOPES, 2005). Olyset nets were shown to be much more durable than ordinary polyester nets and their insecticide content and effectiveness to persist for more than five years (Tami *et al.,* 2004).

Experimental hut trials are used to study the impact of insecticide treated mosquito nets on: deterrence (fewer mosquitoes enter the room), excito-repellency (increase in exophilic behaviour), feeding inhibition (those that do enter have a lower probability of feeding), and killing (fraction of blood-seeking females killed, before or after they have succeeded in feeding). The first three of these are indicators of personal protection, and benefit individual users. The fact that some blood-seeking females

are killed is primarily important because community-wide use of treated nets can, in some circumstances, produce a "mass effect", i.e. a reduction in the density of infective mosquitoes in the area and, by the way, protect the whole community, including those not using ITNs (WHOPES, 2001).

In Sudan, Olyset nets were reported to be efficacious after 1.5 years of use with mortality >80% after 24h against *Anopheles arabiensis* (Hassan *et al.,* 2008), and even after 3 years of field usage against *Anopheles gambiae* in Cote d'Ivoire (N'Guessan *et al.,* 2001). Similar observations were made in Tanzania where washing did not have significant effect on knockdown or mortality of mosquitoes (Tami *et al.,* 2004; Malima *et al.,* 2008).

Other studies showed that the pyrethroid insecticides used for net treatment have some persistence on polyester but that regular washing does eventually reduce the insecticide dose below an effective level (Lindsay *et al.,* 1991; Curtis *et al.,* 1996; Jawara *et al.,* 1998; Miller *et al.,* (1999); Adams *et al.,* 2002; Ordonez-Gonzalez *et al.,* 2002) reported that users perceive the effect of reduced insecticide concentration on mosquitoes.

2.4 Factors associated with loss of insecticide in LLINs

Although LLINs offer an alternative solution to regular net re-treatment, their actual wash durability under field conditions is not known. For example the frequency of washing, the washing methods, and drying regimens that are used in the field are some of the factors which are likely to affect their efficacy and durability. The standard WHOPES recommended washing procedure is not applicable under field conditions and might be underestimating the abrasive washing practices that are used in the field and, therefore, overestimating the biological durability of LLINs in many countries (Gimnig *et al.,* 2003).

Morris *et al.,* (2006) reported that LLINs have been promoted for use in reducing human-vector contact for a long time. However, there is limited information on the effect of different washing and drying practices, and how they affect the efficacy of

current brands of LLINs, especially under tropical conditions. It has also been demonstrated that direct sunlight is harmful to pyrethroid-based insecticide because UV rays break down pyrethrin molecules thereby rendering the insecticide ineffective. It has also been documented that heat accelerates the rate of migration of permethrin molecules in the fibers, thereby increasing the bioavailability of insecticide on the net surface, especially if they are polyethylene based (Atieli *et al.*, 2010).

3 METHODOLOGY

3.1 Study Area

This study was carried out in randomly selected four streets in the two wards of Kijitonyama and Mbezi (neighbouring Kimara and Kibamba) in Kinondoni district; one of the administrative districts of Dar es Salaam, others being Ilala and Temeke. According to 2002 Census information, the city has 73 wards (Kinondoni 27, Ilala 22 and Temeke 24). Dar es Salaam city is located at 6°48' South, 39°17' East; is the biggest and economically most important city of Tanzania, with a population of about 5 million inhabitants and an area of 1450 km^2 along the coast of the Indian Ocean.

Dar es Salaam has a hot and humid tropical climate with two rainy seasons – a long one observed during the months of March and April, and a short one occurring in November and December. A theoretical model based on climate conditions (rainfall and temperature) characterizes all the districts of Dar es Salaam as an area with endemic and perennial malaria, with transmission occurring during the whole year (MARA/ARMA, 2002). Combined with the hot and humid climate, this results in excellent breeding opportunities for Anopheles mosquitoes, the main species being *Anopheles gambiae* and *Anopheles funestus* as the main malaria vectors. *Anopheles gambiae* prefers small collections of clean and fresh water, although some subspecies can breed in salty water while *Anopheles funestus* prefer permanent water, especially near inland marshes. Different types of breeding sites are found in urban areas of Dar es Salaam, and they are the result of the physical characteristics of the area (presence of creeks) and of the activities undertaken by the population (such as agriculture). They include '*matuta'* (raised planting beds), salt pans, paddy fields, sand pits, seepage, swamps, mangrove swamps, pits for house construction, roadside puddles, and marshes prevalent in the study area.

3.2 Study Design

Community-based cross-sectional household, multi-stage random sampling design was applied to survey selected households in Dar es Salaam. The survey was a quantitative study that employed structured questionnaires to examine household ownership and use of mosquito nets, followed by random selection of Olyset LLINs

for the analysis of insecticide concentration in the nets. The household survey was conducted during the dry season in July and August followed by chemical analysis starting from September 2011.

3.3 Study Population

Study population included all households and nets in the study area while the study sample included the randomly selected households and nets located in the randomly selected wards and streets. Study participants that were involved in the survey included those households that have stayed in the selected wards and streets for atleast three months. The Olyset® nets samples was randomly selected from the households in the randomly selected wards and streets. The samples that were recovered from the surveyed households were used for chemical analysis to determine the temporal concentration of the insecticide contained in these nets.

3.4 Sample size estimation

Household survey for determination of bed net ownership, type and use

Given that, Dar es Salaam was estimated to have 5 million people; estimated proportion of household owning ITNs was 36; Design effect of 2; maximum allowable error of 5%; then sample size 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. The primary outcomes of interest of this sample were to determine bed net ownership, type and use.

Olyset® nets for Chemical Analysis

The sample size was calculated to detect monthly rate of loss of insecticide of the Olyset nets of 0.125g/kg per month under a Linear Regression Model. Power of the study was set at 90%; Standard deviations for the age of the net (duration of use) and for the initial concentration of the insecticide of the Olyset nets were 6 months and 1.5g/kg respectively. Sample size was calculated by using G*Power software *version* 3.1.2 (Faul *et al.,* 2009) and estimated to be 34 Olyset nets. 15% was added to adjust for non-respondents, the sample size was increased to 39 nets. When 10% was added

for each of pre-specified variables, the total sample size was estimated to be 50 Olyset nets.

3.5 Sampling procedure and data collection techniques

Multi-stage random sampling was applied to select the households for survey. From 2002 Census information, wards located in the randomly selected district was listed alphabetically where two wards were randomly selected using computer-generated random numbers. In each of the two randomly selected wards, two streets were randomly selected using simple random sampling. Prior to data collection in each street, each street was visited and "ten cell unit" leaders were briefed about the study and the assistance that was needed from them as part of prior informed consent. This was helpful to ensure that the local government at the street level is well informed and work could start soon after arrival in the street on the next day.

Finally, within each randomly selected street, 150 households were chosen using a modified EPI-type sampling procedure. This level of sampling was done in the field. After the researcher/interviewer had introduced himself and explained the purpose of the study to the street leader, the researcher/interviewer and the street leader went to centre point of the street for sampling the households. At the centre point of the street, sampling procedures were that; the researcher/interviewer spin/threw the pen/bottle so that it indicated the random direction. The researcher/interviewer walked in the direction to the edge/periphery of the street while drawing a sketch map of the location of each house, and at the same time assigning an identification number for each household (in this context a household means a group of people who cook and eat their meals together). He selected one of the households from this list randomly. This became the starting point for sampling. The researcher/interviewer had to return to the randomly selected house – the starting point. This was the household number 1. To sample more households it followed throwing the pen/bottle again and walking in the random direction it indicated. Each household he passed in that direction was sampled – names of household heads were written down and a rough map of the route was drawn. Each time he came to a road junction he had to throw the pen/bottle again and to change the direction if indicated.

He continued with this exercise until he obtained a sample of 150 households from that street. Each of those households was then surveyed.

A household survey of selected households was done to investigate various aspects of the bed 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 of the net, duration of use of the net, whether the net was treated or not, frequency and methods of washing, and drying method of the nets.

Olyset® nets taken for laboratory analysis were systematically selected from the studied households. A net was collected from every second household found with Olyset® net. For each street, a total of 13 Olyset nets were collected and replaced with new ones of the same brand. If the household had more than one Olyset net, the first net to be investigated was collected. A total of fifty (50) Olyset® nets from selected streets in the two wards were collected for laboratory chemical analysis. Each net was packaged in a separate plastic bag and kept at room temperature for chemical analysis at the TPRI laboratory in Arusha.

3.6 Laboratory testing of Olyset® net samples

Gas chromatography method was used to measure insecticide concentration on and in the net fibres. General method for sampling rectangular and conical nets was applied where each net sample consisted of 5 pieces taken according to the pattern recommended by World Health Organization. Testing was performed following standard procedures on Permethrin-Incorporated Mosquito Net outlined by World Health Organization (WHO, 2006).

In order to measure permethrin content on the surface of the yarn, a 10 × 10 cm piece of net (corresponding to about 600mg) was cut from each Olyset® net sample, accurately weighed and introduced into a 100mL conical flask. A 30mL of acetone, was then added and the flask thoroughly shaken by hand for one minute. The acetone

extract was quantitatively transferred into a 50mL volumetric flask, concentrated using Soxhlet extractor and filled up to 10mL with acetone. A 2mL internal standard solution (Tetramethrin) was added; the flask was filled up to volume with acetone. The extract was put into an injection vial and analysed by capillary gas chromatography with flame ionisation detection (GC-FID) for determination of permethrin using the internal standard calibration.

In order to determine the permethrin content inside the Olyset® yarn after the first acetone wash, the net sample was cut into 2–3mm pieces and homogenised. A sub-sample of 400mg was accurately weighed and introduced into a 100mL conical flask, then 30mL of xylene was added; permethrin was extracted by heating under reflux for 60minutes. The extract was allowed to reach ambient temperature and quantitatively transferred into a 50mL volumetric flask, 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 analysed as above. The tests were repeated to confirm the results of samples that showed no detectable insecticide. All laboratory work was done at Tropical Pesticides Research Institute, Arusha.

3.7 Recruitment and training of research assistants

Four research assistants with basic experience on health-related household surveys were identified and recruited by the Principal Investigator. Each assistant was responsible for interviewing about 10 households each day. To ensure quality data collection, a one day training was offered to research assistants regarding the purpose of the study, household sampling, the consenting process, how to administer questionnaire, and selection and storage of the net samples. Prior to actual data collection, a pre-testing of the research tools was done at Mwenge, Dar es Salaam. For quality control purposes, only experienced interviewers were recruited in the study. The field supervision was done by the Principal Investigator by carrying out repeated 2-4 household interviews independently each day, through accompanied interview and re-interview. The Quality Control check was used to compare the original

interview and re-interview and identified discrepancies were verified with the respondents.

3.8 Ethical considerations

Ethical clearance was obtained from the Research and Publication Committee of the Muhimbili University of Health and Allied Science. Permission to conduct the study was obtained from the Kinondoni Municipal Directorate, the district in which the two surveyed wards were located. The local authorities in the wards and streets were consulted prior to the selection of the households. The purpose of the study was explained to relevant authorities as well as to the study participants. Written informed consent was obtained from each head/representative of the household after the participant had read and understood the informed consent form. Confidentiality was adhered to.

3.9 Data management and analysis

The data collected 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 insecticide level (in g/kg) as the outcome variable and the age of nets (in month) as the independent variable after removing the outliers. The slope was estimated from the model and 95% CI for the slope was calculated. Additionally, a multivariate linear model was fitted using associated factor (washing frequency of the net) as additional independent variable.

4 RESULTS

4.1 Demographic characteristics of the households

A total of 601 households were surveyed in four streets where heads/representatives of households were interviewed on various aspects of bed nets. The mean household's size was 4.5 (±2.4) while the mean number of children under five years old in the household with at least one child was 1.4 (±0.9). The mean number of sleeping places (beds) was 2.3 (±1.3) per households, the lowest number of sleeping places being 1 while the highest being 9. Most of the households, 560/601 (93.2%) owned at least one bednet while the overall use of the nets was found to be 552 (91.8%) of all households surveyed. The descriptive statistics for demographic characteristics of the households is presented in Table 1.

Table 1. Descriptive Statistics for Demographic Characteristics of the Households

Variable	*n*	*Sum*	*Mean*	*Std. Dev.*	*Min.*	*Max.*
The size of the household	601	2701	4.49	2.40	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	2.40	0	14
Use of bednet by underfives in the household	348	434	1.25	0.73	0	6

4.2 Households' ownership of nets.

4.2.1 The overall ownership of nets

The point estimate for household ownership of nets showed that out the 601 households surveyed, 560 (93.2%) of households owns at least one (any) bednet. A total of 1218 nets were owned by all households where each household owned a varying number of nets from 0 to 8 giving a mean of 2.0 (±1.3) nets per household (Fig. 1).

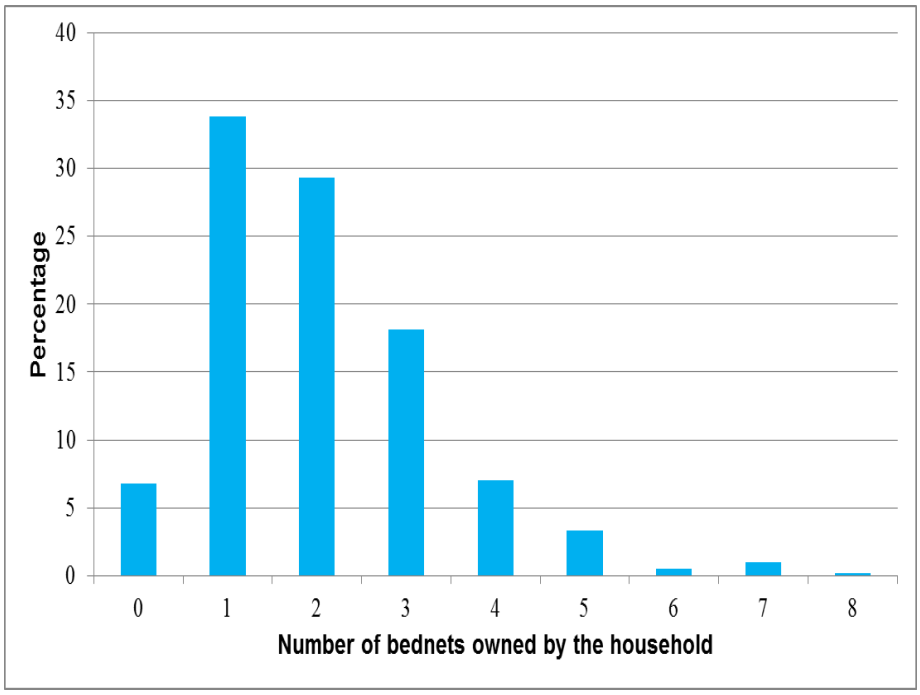


Figure 1. The Percentage Ownership of the Number of Bednets by the Households.

A total of 41 (6.8%) households did not own a net. Reasons for not owning any bed nets included: inability to buy 24/41 (58.5%), dislike/not prefer using the 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%), the use of sprays/no mosquitoes around 4/41 (9.8%) and the net is worn out, torn out or damaged 2/41 (4.9%).

4.2.2 Households' ownership of nets by brand

Out of the investigated bednets, majority 1082/1218 (88.8%) were identified by their brand names of which 544 (50.3%) were Olyset nets (Fig. 2).

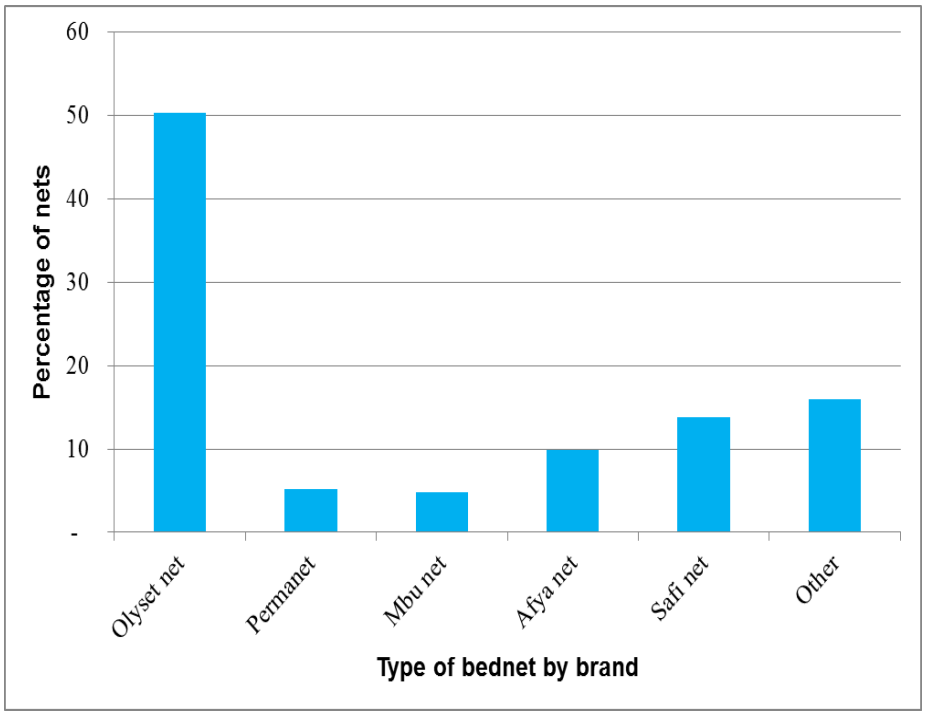


Figure 2. The Percentage of Bednet by Brand in the Households

4.2.3 Households' ownership of nets by insecticide treatment status

Among households' nets, 1021/1218 (83.8%) were identified based on their insecticide treatment status. More than half of the nets, 600/1021 (58.8%) were LLINs (factory pre-treated) while 252/1021 (24.7%) were ITNs (excluding LLINs) and 169/1021 (16.6%) were untreated nets (Fig. 3).

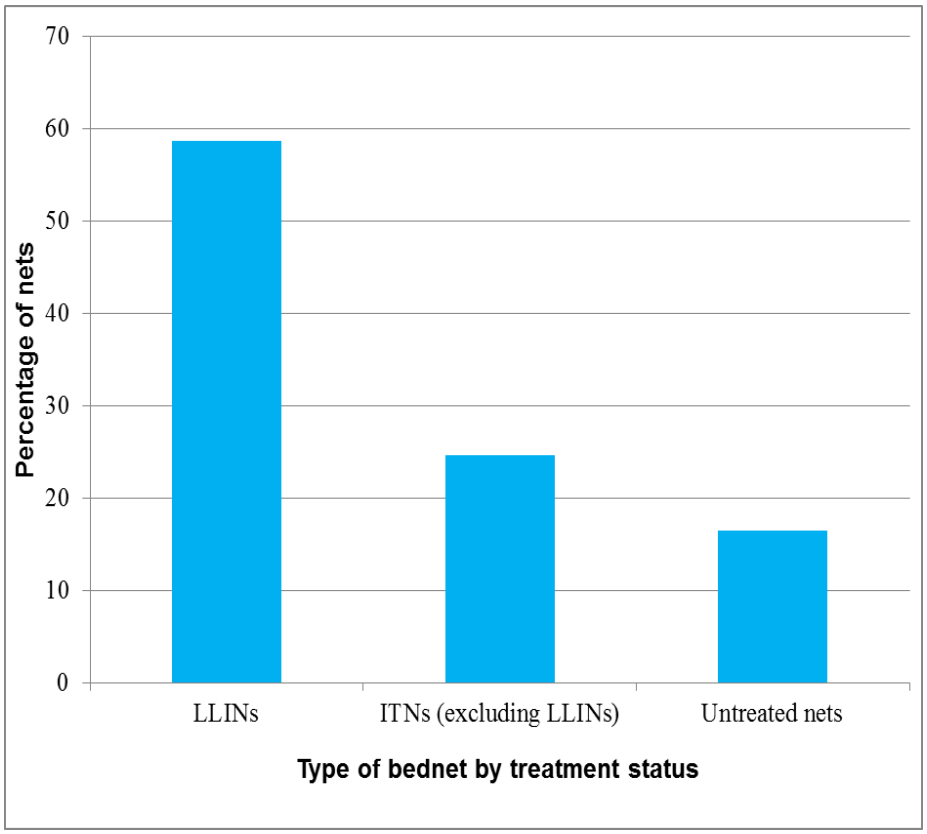


Figure 3. The Percentage of Bednets by insecticide treatment status in the Households

4.3 Use of nets

4.3.1 Use of nets at households' level

Sleeping under a net in the night preceding the survey was an indicator of use of net. Heads/representatives of household were asked to indicate the number of household members that had slept under a net in their household the previous night, giving an indication of net usage among the general population. It was found that a large majority, 552/601 (91.8%) of households surveyed, at least one member slept under a net (Fig. 4).

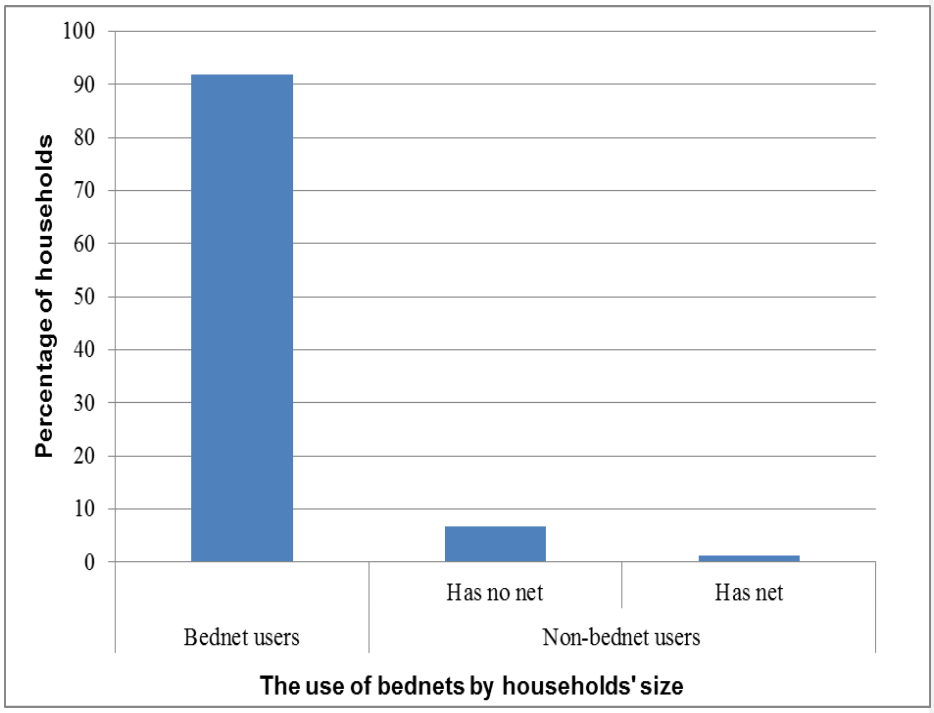


Figure 4. The Percentage Use of bed nets by the households

4.3.2 The overall use of nets for households' members

Point estimates of households' use of nets were derived from the question about sleeping under a net in the night preceding the survey. The overall net use for the total population surveyed was 2256/2701 (83.5%) of household members; for underfives the use was 434/501 (86.6%) (Fig. 5)

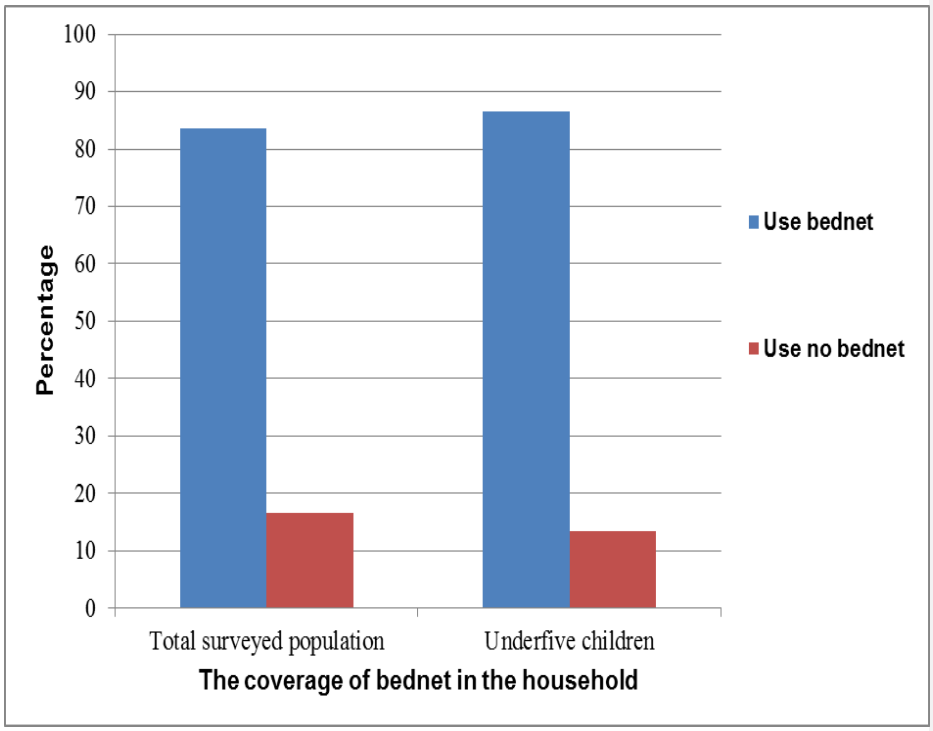


Figure 5. The Percentages Use of Bednets for Members in the Households

4.4 Chemical analysis of insecticide content in Olyset® nets

The reported average age of the sampled Olyset nets was 22.2(±16.9) months. It was found that 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 101mg/kg per month. On average, 47.3% of the initial insecticide dose (20,000 mg/kg) was still present. Only 36% of the analysed nets were above the minimum surface concentration limit required for good efficacy and the diferrence was significant (p = 0.004). The analysis clearly indicated that the insecticide was still very much concentrated inside the fibres: average inside:outside ratio of 22. The correlation between surface concentration and the internal concentration of the net was very weak and not significant: r^2 = 0.002 (95%CI = -0.041 - 0.058). There seemed to be a very weak negative correlation between the

concentration of the insecticide and the reported age of the net: $r^2 = 0.111$ (95%CI = - 0.189 - - 0.01). When multiple regression analysis for the numeric variables (age and washing frequency of the nets) were conducted to determine how they affect the concentration of insecticide of the nets, it was found that variation in age and washing frequency could explain just 9.2% of the variation in concentration of insecticide of the nets (R^2 = 0.0921; 95%CI = -0.19 - 0.015). Figure 6 shows temporal insecticide residues of 50 Olyset® net samples collected in the field and tested by capillary Gas Chromatography with flame ionization detection (GC-FID)

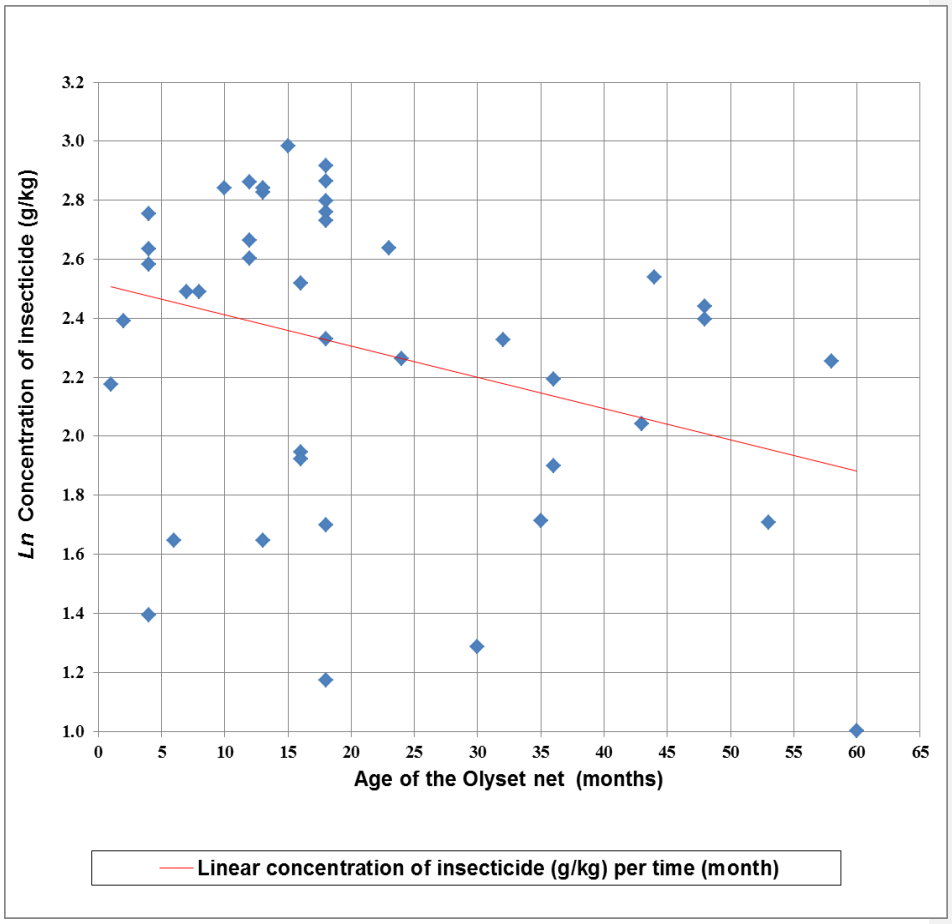


Figure 6. Temporal Insecticide Residues in the Olyset® Net samples

4.5 Assessment of factors associated with loss of insecticide in Olyset® nets

Various factors associated with the loss of insecticide in the nets were investigated. These factors included: the number of washes of the net (washing frequency), the washing methods, the types of detergents used and the drying methods.

It was found that the sampled Olyset® nets were washed at an average of 22 (±17) times per year and 32 (±28) times in the lifetime of the net, the washing frequency ranged from 2 to 50 and 2 to 103 respectively. There was a very weak non-significant correlation between the concentration of insecticide in the nets and the washing frequency: $r^2 = 0.035$ (95%CI = - 0.098 - 0.021).

When respondents were asked on how they washed their nets, they all (100%) reported to use hand washing "smooth" and not machine washing "abrasive". This implies that no association could be made between the washing methods and the concentration of insecticides in the Olyset® nets.

Regarding the type of detergents used for washing the nets, 14.0% reported to use bar soap, 2.0% used liquid soap while 84.0% combined both bar and powder soap. It was found that there were no significant association between the concentration of insecticide in the net with the types of detergents used for washing (x^2 =3.406, p = 0.671).

After washing the nets, 22.0% were reported to be dried under the shade while the remaining 78.0% were dried under the sun. Likewise, no significant association was found between the concentrations of insecticide in the nets and the drying methods (x^2 =1.344, p = 0.649).

5. DISCUSSION

This study examined household ITNs use practices, the temporal concentration of permethrin 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 the pregnant women and underfives launched in 2004 and 2006 respectively as well as free nets distribution for underfives (underfives catch up campaign). These interventions have achieved to considerably increase the ownership of the nets. If delivery of ITNs to young children and pregnant mothers continues to increase it may be possible to reach a point of saturation within a household as nets are shared among household members (an average of two people share a single bed). The present findings show major achievements in comparison to earlier observation by Hanson *et al.,* (2009) whereby in the year 2007 only 65% of surveyed households owned at least any net while 36% of them owned at least one ITN. Ownership of nets might have increased as a result of free universal distribution of nets implemented in late 2011 after the household survey of this study had already taken place.

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.,* (2009) 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 were shown to be popular, durable than ordinary polyester nets, conceivably being one of the best choices for ITN programmes 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 (WHOPES, 2001). Being one of the indicators of the effectiveness of the Olyset net it was important to determine the concentration of the insecticide of 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. These results are consistent with those of a previous studies conducted in Tanzania (Tami *et al.,* 2004) and in India (Tsuzuki *et al.,* 2011) where they found 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 have produced very low surface concentration of the 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 more or less rapid 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 test whether these nets with surface concentration below minimum limit are effective or not. Some authors like Tami *et al.,* (2004) and Malima *et al.,* (2008)

found that Olyset nets maintained their insecticidal activity for at least 7 years of use while Maxwell *et al.,* (2006) reported that Olyset net with 4 years of domestic use had a similar performance as new Olyset nets in terms of mortality of mosquitoes.

The current study found that the concentration of the insecticides in the sample Olyset nets is not significantly associated with washing frequency. Other study by Sharma *et al.,* (2009) on bioefficacy showed that the Olyset nets maintained high bioefficacy (>80%) even after repeated washings against *Anopheles culicifacies* and *Anopheles fluviatilis*, which is in agreement with another study in India by Sharma *et al.,* (2006). Results from a previous study in Africa also showed good insecticide persistence even after 3 year of use, and the Olyset nets were found effective in causing >80% mortality in bioassay tests (N'Guessan *et al.,* 2001). 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 (2006) and Atieli *et al.,* (2010a) found that vigorous washing procedures had effect on loss of insecticidal power and insecticide concentration of the nets respectively. Sreehari *et al.,* (2009) concluded that hand washing is more appropriate to retain the efficacy of Olyset net than the machine washing method.

Regarding detergents, it has been observed that the type of detergents used for washing nets have no significant impact on the concentration of insecticide in the nets. Although theoretically it is argued that washing nets using detergents with bleaching effects tend to fade away the chemical content in the nets, there is limited information on field studies that have systematically tested the impact of various washing detergents on the concentration of insecticide in the net after use.

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.,* (2010a) 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 while being dried under such conditions. 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 the 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.,* (2006) and Atieli *et al.,* (2010a) has found the 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 to maintain their efficacy.

The determination of concentration of insecticide might be useful indicator of effectiveness of the net. It should be kept in mind that bioassays only give us a very rough estimation of the situation in the field. Bioassays can be standardized to a certain extent but variations are likely to occur. Bioassays, however, give an estimate for the insecticide on the surface of the net that can potentially be absorbed by mosquitoes. The dosage that was measured by Gas Chromatography reflects the content of insecticide within and on the netting. A previous study by WHO (1998), found that the mean knockdown time was directly correlated to the insecticide concentration over the surface for fast-acting insecticides such as pyrethroids. In contrast, Tami *et al.,* (2004) found a very weak (non-significant) correlation between the insecticide concentration and the bioassay results. Likewise, Atieli *et al.,* (2010a) observed that, the loss in efficacy corresponded to the reduction in residual insecticide contents for all the nets except Olyset.

Study limitations

This study examined few practices that might affect the concentration of insecticide in the nets after use in the field. 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 were reported to be washed by hand. Since the variation in age and washing frequency could explain just 9.2% of the variation in concentration of insecticide of the nets, other factors that are responsible for the loss of the concentration of insecticide of the nets could not be examined in this study.

This study did not involve bioassay tests which would have tested whether the 64% of nets that were below the minimum surface concentration limit would have any impact on: deterrence (fewer mosquitoes enter the room), excito-repellency (increase in exophilic behaviour), feeding inhibition (those that do enter have a lower probability of feeding), and killing effect. Cost and time constraints were the major limitations to carryout bioassay tests.

This survey was a cross sectional study and as such only measure indicators such as net ownership and use at a point in time and seasonal changes in ownership and use cannot be accounted for. Due to the limitation of cost and time to carryout prospective longitudinal studies, it was inevitable to use reported information from the respondents, e.g. age of the net, treatment status of the net, washing frequency of the net, washing and drying methods of the net to enable to establish some correlations with the concentration of the insecticide of the sample Olyset nets.

6. CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

This study concludes that the ownership and use of the nets in the study sites in Dar es Salaam is high (93.2% and 83.5% respectively) and Olyset nets, being one of three LLINs recommended by WHO are widely (50.3%) used by different households.

This study supports the confidence vested in Olyset nets that this product is treated with long lasting insecticides. The Olyset nets lose its insecticide at the rate of 101mg/kg per month and the nets retain, on average, 47.3% of its initial insecticide dose after 22.2 months of use in the field. Only about a third (36%) of the analysed nets were above the minimum surface concentration limit required for good efficacy. The concentration of the insecticides is not significantly affected by the age of the net and other factors like washing frequency, washing methods, the detergents used in washing and the drying methods of the nets.

5.2 Recommendations

Since it has been found that not all individuals own an LLIN, future efforts by all stakeholders in the fight against malaria should target getting bed nets to the remaining populations that still do not have them, address eventual replacement of conventionally teated nets with LLINs, and encourage proper washing and drying practices of the bed nets.

Although LLINs are recommended for malaria control purposes, their performance should be monitored in the field under various ecological settings to assess their long-term effectiveness for malaria prevention and control. Further studies are needed to investigate on long-term effectiveness of Olyset nets, including: longevity, physical integrity, attrition rate, persistence of bio-efficacy and chemical content under the operational conditions in different settings that may have varying human behaviour and cultural practices of net usage.

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8. APPENDICES

Appendix 1. A household survey questionnaire for investigating various aspects of bed nets in Dar es Salaam, 2011 (*English version*)

A HOUSEHOLD SURVEY FOR INVESTIGATING VARIOUS ASPECTS OF BED NETS IN DAR ES SALAAM

MODULE 1: HOUSEHOLD INFORMATION		
1.	Name of the Ward	_____ ward
2.	Name of the Street	_____ street
3.	Number of Household	|__|__| houseno
4.	Interviewer initials	|__|__| intinitials
5.	Date of interview (dd/mm/yyyy)	|__|__|/|__|__|/|__|__| date
6.	Name of head of the household	_____ hhname
7.	Who is the respondent? 1= Head of the Household 2=Representative	|__| whoresp
8.	Sex of the head of household 1 = Male 2 =Female	|__| hhsex
9.	What is the education level of head of the household? 1 = No formal education 2 = Primary education 3 = Secondary education 4 = College level 5 = Undergraduate 6 = Postgraduate	|__| hhedu
10.	What is your occupation? 1 = Farming/fishing/forestry 2 = Mining 3 = Non-agricultural day-labour 4 = Professional/clerical 5 = Services (eg repair, hair dressing, tailoring) 6 = Petty trade 7 = Business 8 = Domestic worker 9 = Student 10 = Not employed	|__| occup
11.	What is the size of the household?	|__| hsize
12.	How many children under five years of age are in this household?	|__| U5no
13.	How many sleeping places are there in this house? ***(Sleeping place is an area of the house where one or more people sleep together on the same bed or mat that could be covered by a single bednet)***	|__| Sleeppc

MODULE 2: BEDNET OWNERSHIP, USE AND TREATMENT INFORMATION (*Ask to observe nets)*				
14.	Does this household own any net? 1 =Yes 2 = No	|__| hasnet **IF Q14 = 1, SKIP TO Q16** **IF Q14 = 2, GO TO Q15**		
15.	Why doesn't your household own net? 1= unable to buy 2=I don't like/prefer it 3=I don't believe if its protective 4=it makes me uncomfortable on bed 5=Its too hot 6=Child cries 7=No mosquitoes around 8=Net too small 9=Other (specify)	|__| Whynonet **END THE INTERVIEW**		
16.	How many nets do this household own?	|__| netno		
17.	Did anyone sleep under the mosquito net last night? 1 = Yes 2 = No	|__| Netsleep **IF Q17 = 1, SKIP TO Q19** **IF Q17 = 2, GO TO Q18**		
18.	Why did no one sleep under this net last night? (**select 1 response**) 1=Its too hot 2=Its too cold 3=Child cries 4=Net was being washed 5=Net not hung up 6=No mosquitoes around 7=Net worn out/poor condition 8=Net too small 9=Other (specify)	|__| Whynotsleep		
19.	How many people in this household slept under a net last night?	|__| Netsleepno		
20.	How many children under five years of age slept under a net last night?	|__| NetU5sleepno		
		Net # 1	Net # 2	Net # 3
21.	Is the net observed? 1 =Yes 2 = No	|__| netseen1	|__| netseen2	|__| netseen3
22.	Is the net hanged up? 1=Yes 2=No	|__| Nethanged1	|__| Nethanged2	|__| Nethanged3
23.	How many months ago did your household start using this mosquito net? **Don't know/not sure, write 99**	|__|__| Netage 1	|__|__| Netage 2	|__|__| Netage 3
24.	How was the net obtained? (**select one**) 1 = Discount voucher for pregnant women 2 = Discount voucher for infants 3= Equity voucher 4= Upgraded Discount voucher for pregnant women 5= Upgraded Discount voucher for infants 6=free net campaign 7= bought the net **99=don't know**	|__| howobtain1	|__| Howobtain2	|__| Howobtain3
		IF ANY OF Q24 =1,2,3,4,5,7,8, SKIP TO Q26 **IF Q24 = 6, GO TO Q25**		

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25.	What free net campaign did it come from? 1=U5cc in 2010 2=NGO/private project 3=Present 4= Other (Specify)	|__| freenet1	|__| freenet2	|__| freenet3
26.	What is the brand name of the net? (The identity of the Olyset Net, should be verified by the manufacturer's label and/ or the netting material) 1= Olyset® net 2= Permanet 3= Mbu net 4= Afya net 5=Safi net 6= Other (Specify)	|__| Netbrand1	|__| Netbrand2	|__| Netbrand3
27.	What size is this net (**cm**)? **Rectangular** *(Length x Width x Height)* 1= Double: 180x100x150 **(3ftx6ft)** 2= Family: 180x130x150 **(4ftx6ft)** 3=Large family: 180x160x150 **(5ftx6ft)** 4=Extra family: 190x180x150 **(6ftx6ft)** **Conical (***Circumfer. x Height x Roof, d)* 5=Small: 850x220x56 6=Medium: 1050x220x56 7=Large: 1250x250x65	|__| Netsize1	|__| Netsize2	|__| Netsize3
28.	What is the colour of the net? 1=white 2=green 3=blue 4=other (specify)	|__| Netcolour1	|__| Netcolour2	|__| Netcolour3
29.	Has this net ever been treated with an insecticide (**check for pre-treated nets**)? 1=Yes 2=No **If don't know/not sure, write 99**	|__| Evertreat1	|__| Evertreat2 **IF Q29 = 1, GO TO Q30** **IF Q29 = 2,99, SKIP TO Q32**	|__| evertreat3
30.	What type of insecticide was used? 1='Ngao' 2=Better 'Ngao' 3=Pre-treated from factory **If don't know/not sure, write 99**	|__| Insecttype1	|__| Insecttype2 **IF Q30 = 1,2 GO TO Q31** **IF Q30 = 3,99 SKIP TO Q32**	|__| Insecttype3
31.	When was the last time (**in months**) this net re-treated? **If don't know/not sure, write 99**	|__|__| lasttreat1	|__|__| lasttreat2	|__|__| lasttreat3
32.	How frequently (**weeks**) do you wash this net? **If never been washed, write 88** **If don't know/not sure, write 99**	|__|__| Washfreq1	|__|__| Washfreq 2 **IF Q32 = 88, END THE INTERVIEW**	|__|__| Washfreq 3
33.	How long (**weeks)** since the last time this net was washed? **If don't know/not sure, write 99**	|__|__| Whenwashed	|__|__| Whenwashed	|__|__| Whenwashed
34.	How do you wash this net? 1=hand washing 2= washing machine 3= other method (Specify)	|__| Howwash1	|__| Howwash2	|__| Howwash3

35.	After washing, how do you dry up the net? 1= sunlight heating 2= under the shade 3= other (specify)	|__| Howdry1	|__| Howdry2	|__| Howdry3
36.	What is the type of detergent do you use? 1 = soap bar 2 = soap bar 3 = liduid soap 4 = both bar and soap powder 5 = Other (specify)	|__| Detergentyp1	|__| Detergentyp2	|__| Detergentyp3

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Appendix 2. A household survey questionnaire for investigating various aspects of bed nets in Dar es Salaam, 2011 (*Swahili version*)

UTAFITI WA KAYA KUCHUNGUZA VYANDARUA VYENYE DAWA KATIKA JIJI LA DAR ES SALAAM

SEHEMU 1: TAARIFA ZA KAYA		
1.	Kata	_____ ward
2.	Mtaa	_____ street
3.	Namba ya kaya	|__|__| houseno
4.	Vifupisho vya majina ya mhojaji	|__|__| intinitials
5.	Tarehe ya uhojaji (dd/mm/yyyy)	|__|__|/|__|__|/|__|__| date
6.	Jina la mkuu wa kaya	_____ hhname
7.	Mhojiwa ni nani? 1=Mkuu wa kaya 2= Mwakilishi wa mkuu wa kaya	|__| whoresp
8.	Jinsia ya mkuu wa kaya 1 = Mwanaume 2 = Mwanamke	|__| hhsex
9.	Mkuu wa kaya ana kiwango gani cha elimu? 1 = Hana elimu ya darasani 2 = Elimu ya msingi 3 = Elimu ya sekondari 4 = Elimu ya chuo cha kawaida 5 = Shahada ya kwanza 6 = Shahada ya juu	|__| hhedu
10.	Unajihusisha na shughuli gani? 1=Kilimo, misitu, uvuvi; 2=Madini; 3=Kazi ya kutwa isiyo shamba; 4=Mtaalamu/karani; 5=Huduma (ufundi, ususi, ushonaji); 6=Muuzaji (mboga, bidhaa); 7=Biashara; 8=Mfanyakazi wa ndani; 9=Mwanafunzi; 10=Sijaajiriwa	|__| occup
11.	Kaya hii una watu wangapi?	|__| hsize
12.	Kaya hii ina watoto wangapi wenye umri chini ya miaka mitano?	|__| U5no
13.	Je, katika kaya ya hii kuna jumla ya sehemu ngapi za kulala zinatumika na wanakaya hata kama siyo kwenye kitanda? (***sehemu za kulala ni sehemu ambapo mtu mmoja au zaidi wanaweza kulala pamoja kwenye kitanda kimoja au hata kama siyo kwenye kitanda lakini panaweza kufunikwa na***	|__| Sleeppc

	chandarua kimoja)			
SEHEMU 2: TAARIFA ZINAZOHUSU VYANDARUA NA MATUMIZI YAKE (*Omba kuona/kukagua vyandarua)*				
14.	Je, kaya hii ina chandarua/vyandarua? 1 = Ndiyo 2 = Hapana	|__| hasnet **KAMA Q14 = 1, NENDA Q16** **KAMA Q14 = 2, NENDA Q15**		
15.	Kwa nini kaya yako haina chandarua? 1= sina uwezo wa kununua 2=sipendelei 3=siamini kama inazuia mbu 4=sijisikii vizuri nikilala kwenye chandarua 5= Joto sana 6=mtoto analia 7=hakuna mbu 8= Chandarua kidogo 9= Nyingine (taja)	|__| Whynonet **MALIZA MAHOJIANO**		
16.	Kaya hii ina vyandarua vingapi?	|__| netno		
17.	Je, kuna mtu yeyote alilala kwenye chandarua usiku uliopita? 1 = Ndiyo 2 = Hapana	|__| Netsleep **KAMA Q17 = 1, NENDA Q19** **KAMA Q17 = 2, NENDA Q18**		
18.	Kwanini hakuna aliyelala kwenye chandarua hiki usiku uliopita? (**chagua jibu moja**) 1=Joto sana 2=Baridi sana 3=Mtoto analia 4=Chandarua kilikuwa kimefuliwa 5=Hakikutundikwa 6=hakuna mbu 7=kimechanika/kimeharibika 8=Chandarua kidogo 9=Nyingine (taja)	|__| Whynotsleep		
19.	Watu wangapi katika kaya hii walilala kwenye vyandarua usiku wa kuamkia leo?	|__| Netsleepno		
20.	Watoto wangapi wenye umri chini ya miaka mitano walilala kwenye vyandarua usiku wa kuamkia leo?	|__| NetU5sleepno		
		Chandarua #1	Chandarua#1	Chandarua#1
21.	Je, chandarua hiki kilikaguliwa/umekiona? 1 = Ndiyo 2 = Hapana	|__| netseen1	|__| netseen2	|__| seennet3
22.	Je, chandarua kimetundikwa? 1= Ndiyo 2=Hapana	|__| Nethanged1	|__| Nethanged2	|__| Nethanged3
23.	Kaya yako ilianza kutumia chandarua hiki miezi mingapi iliyopita? **Kama hajui/hana hakika, andika 99**	|__|__| Whenobtain1	|__|__| Whenobtain2	|__|__| Whenobtain3
24.	Chandarua hiki kilipatikanaje? (**chagua jibu moja**) 1 = *Hati Punguzo* ya mjamzito 2 = *Hati Punguzo* ya mtoto 3 = *Hati Punguzo* maalum 4 = *Hati Punguzo* mpya ya mjamzito 5 = *Hati Punguzo* mpya ya mtoto 6 = Alipata bure/kampeni 7 = Nilinunua 8 = Sijui	|__| howobtain1	|__| Howobtain2	|__| Howobtain3
		KAMA Q24 =1,2,3,4,5,7,8, NENDA Q26 **KAMA Q24 = 6, NENDA Q25**		

25.	Chandarua hiki cha bure kilipatikana katika kampeni gani? 1= ya vyandarua vya bure kwa watoto chini ya miaka 5 ya 2010 2=shirika lisilo la kiserekali/mradi binafsi 3=Zawadi 4=Nyingine (taja)	|__| freenet1	|__| freenet2	|__| freenet3
26.	Chandarua hiki ni cha aina gani? **1= Olyset** **2= Permanet** 3= Mbu net 4= Afya net 5=Safi net 6= Nyingine (elezea)	|__| Netbrand1	|__| Netbrand2	|__| Netbrand3
27.	Chandarua hiki kina ukubwa gani? **Mstatili** *(Urefu x Upana x Kimo)* 1= Double: 180x100x150 **(3ftx6ft)** 2= Family: 180x130x150 **(4ftx6ft)** 3=Large family: 180x160x150 **(5ftx6ft)** 4=Extra family: 190x180x150 **(6ftx6ft)** **Mviringo (***Chini. x Kimo x Juu-kipenyo)* 5=Kidogo: 850x220x56 6=Wastani: 1050x220x56 7=Kikubwa: 1250x250x65	|__| Netsize1	|__| Netsize2	|__| Netsize3
28.	Chandarua hiki kina rangi gani? 1=nyeupe 2=kijani 3=bluu 4=nyingine (elezea)	|__| Netcolour1	|__| Netcolour2	|__| Netcolour3
29.	Je chandarua hiki kilishawahi kuchovywa kwenye dawa ya chandarua? **(angalia kama chandarua kilichovywa kiwandani)** 1 = Ndiyo 2 = Hapana **Kama hajui/hana hakika andika 99**	|__| Evertreat1	|__| Evertreat2 **KAMA Q29 = 1, NENDA Q30** **KAMA Q29 = 2,99, NENDA Q32**	|__| evertreat3
30.	Ni aina gani ya dawa ya chandarua ilitumika? 1=Ngao 2=Ngao iliyoboreshwa 3= Chandarua kilichovywa kiwandani **Kama hajui/hana hakika andika 99**	|__| Insecttype1	|__| Insecttype2 **KAMA Q30 = 1,2 NENDA Q31** **KAMA Q30 = 3,99 NENDA Q32**	|__| Insecttype3
31.	Chandarua hiki kilichovywa kwende dawa miezi mingapi iliyopita? **Kama hajui/hana hakika, andika 99**	__|__| lasttreat1	|__|__| lasttreat2	|__|__| lasttreat3
32.	Unafua chandarua hiki kila baada ya muda gani? **(andika kwa majuma)** **Kama hakijawahi kufuliwa, andika 88** **Kama hajui/hana hakika, andika 99**	|__|__| Washfreq1	|__|__| Washfreq 2 **KAMA Q32 = 88, MALIZA MAHOJIANO**	|__|__| Washfreq 3
33.	Chandarua hiki kilifuliwa lini kwa mara ya mwisho? **(andika kwa majuma)** **Kama hajui/hana hakika, andika 99**	|__|__|__|__| whenwashed	|__|__|__|__| Whenwashed	|__|__|__|__| Whenwashed
34.	Unafuaje chandarua hiki? 1=kwa mikono 2= kutumia mashine ya kufulia 3= nyingine (elezea)	|__| Howwash1	|__| Howwash2	|__| Howwash3

35.	Baada ya kufua chandarua unakikaushaje? 1= juani 2= Kivulini 3= Nyingine (elezea)	|__| Howdry1	|__| Howdry2	|__| Howdry3
36.	Unatumia sabuni gani kufulia chandarua? 1= sabuni ya kipande 2= sabuni ya unga 3 = sabuni ya maji 4 = mchanganyiko wa kipande na unga 5= nyingine (elezea)	|__| Detergentyp1	|__| Detergentyp2	|__| Detergentyp3

Appendix 3. Informed Consent Form (*English version*)



MUHIMBILI UNIVERSITY OF HEALTH AND ALLIED SCIENCES
DIRECTORATE OF RESEARCH AND PUBLICATIONS
INFORMED CONSENT FORM

HOUSEHOLD NO. ☐☐☐☐

Greetings! My name is Remigi Cosmas and I am doing a research project with the objective to determine the temporal concentration of insecticide of the Olyset® nets and how the potential factors associates with the loss of the insecticide of the nets in Dar es Salaam, Tanzania.

Purpose of the study

The study is intended to collect information about the households' ownership and use of Olyset nets and the concentration of the insecticide in nets as well as associated aspects in selected households in Dar es Salaam. Findings from the study will help the principal investigator to write a dissertation which is a partial fulfillment of his M.Sc. (TDC) studies for academic year 2010/2011.

What Participation Involves

If you agree to participate in the study, you will be requested to be interviewed on various aspects of bed nets.

Confidentiality

All collected information we will be entered into computers with only the participant identification number. Confidentiality will be adhered to, and no unauthorized persons will have access to the data collected.

Risks

We assured you that no harm will happen to you because of your participation in this study.

Rights to Withdraw and Alternatives

Participation in this study is completely voluntary. You can stop participating in this study at any time, even if you have already given your informed consent. Refusal to participate or withdrawal from the study will not involve penalty or loss of any benefits to which you are otherwise entitled.

Benefits

If you agree to take part in this study, where applicable, you may benefit by getting a new Olyset® nets with the exchange of the used one.

Who to contact

If you have any question about this study, you may contact Dr. D. Tarimo – the Supervisor of this study, P. O. Box 65001, Dar es Salaam. If you have any question about your rights as a participant, you may contact Prof. M. Abood, the Chairman of the Senate Research and Publications Committee, P. O. Box 65001, Dar es Salaam, Tel 2120302-6

Participant agrees……. Participant does not agree ……

I _____have read/listened and understood the information contained in this form and I agree to participate in this study.

Signature of participant _____

Signature of Researcher / Research Assistant_____

Date _____

Appendix 4. Informed Consent Form (*Swahili version*)



CHUO KIKUU CHA AFYA NA SAYANSI SHIRIKISHI MUHIMBILI
KURUGENZI YA UTAFITI NA MACHAPISHO
FOMU YA RIDHAA KWA MSHIRIKI

NAMBA YA KAYA ☐☐☐☐

Habari! Jina langu ni Remigi Cosmas, mwanafunzi wa shahada ya uzamili katika chuo kikuu cha afya Muhimbili. Ninafanya utafiti unaohusu matumizi ya vyandarua na kiwango cha dawa ya kuua mbu iliyowekwa kwenye vyandarua vya Olyset® katika jiji la Dar es Salaam.

Dhumuni la utafiti huu ni kuweza kupata taarifa zinazohusu matumizi ya vyandarua na kiwango cha dawa ya kuua mbu iliyowekwa kwenye vyandarua vya Olyset® pamoja na sababu zinazoathiri kiwango cha dawa kwenye vyandarua hivyo katika jiji la Dar es Salaam. Matokeo ya utafiti huu yatamwezesha mtafiti kuandika tasnifu ambayo itafanya sehemu ya masomo yake ya shahada ya uzamili kwa mwaka wa masomo 2010/2011.

Kushiriki katika utafiti huu kunahusisha mshiriki kukubali kuhojiwa baadhi ya maswali hasa yanayohusiana na vyandarua**.**

Taarifa zote zitakazokusanywa katika utafiti huu zitakuwa siri na hakuna mtu yeyote asiyehusika na utafiti huu atakayerusiwa kuzitumia kwa namna yoyote ile**.**

Tunakuhakikishia kuwa hutapata madhara yoyote kwa kushiriki kwako katika utafiti huu.

Ushiriki wako katika utafiti huu ni wa hiari. Katika muda wowote unaweza kuamua kutoendelea kushiriki hata kama utakuwa umeshatoa ridhaa ya kushiriki. Kukataa au

kujiondoa kwako hakutakusababishia adhabu yoyote wala hatakufanya ukose faida ambazo ungezipata kama ungekubali kushiriki.

Kama utakubali kushiriki katika utafiti huu, unaweza kupatiwa chandarua kipya chenye ngao ya muda mrefu (Olyset®) kwa kutoa chandarua chako kilichotumika iwapo utakuwa miongoni mwa washiriki wataokuwa wamechaguliwa kwa mfumo wa kubahatisha.

Iwapo una maswali yoyote kuhusu utafiti huu kama mshiriki unaweza pia kuwasiliana na Dr D. Tarimo ambaye ndiye msimamizi wa utafiti huu. Kama una swali lolote kuhusu haki zako kama mshiriki unaweza kuwasiliana na Prof. M. Abood, mwenyekiti wa seneti ya utafiti na machapisho ya Chuo kikuu cha afya na sayansi shirikishi Muhimbili kwa S.L.P 65001, Dar es Salaam au kwa simu namba 2120302-6.

Mshiriki amekubali……. Mshiriki hajakubali ……

Mimi _____nimesoma/nimesomewa na nimeelewa taarifa zilizopo katika fomu hii ya ridhaa na nimekubali kushiriki katika utafiti huu.

Saini ya mshiriki _____

Saini wa Mtafiti/Mtafiti Msaidizi_____

Tarehe _____

Appendix 5. Approval of Ethical Clearance for the Study