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SUMMARY

A Phase III household randomized trial of PermaNet® 3.0 LLINs in comparison with PermaNet® 2.0 was carried out in Ghana evaluate the insecticidal activity and fabric integrity, as well as community acceptability and usage practices that impact of the effectiveness of the LLINs in the field. A total of 1755 of both set of PermaNet® 3.0 and PermaNet 2.0 Nets were randomly to households as per the protocol in five communities in the district with a total population of 1,895. Out of these total, 460 (230 PermaNet® 3.0 and PermaNet® 2.0) were subjected to Cone bioassay and tunnel test at baseline, 6, 12, 24, 30 and 36 months. The average 24 hr mortality for PermaNet® 3.0 decreased from 88.3% at 12 months to 82.4.0% at 18 months and a further decrease by 8.2% in 24 months. Compared to the baseline, the pass rate at 36 months for PermaNet® 3.0 and PermaNet® 2.0 decreased by 20% and 48% respectively. At 36 months, the pass rate for PermaNet® 3.0 was 80% compared to 58% for PermaNet® 2.0. During the 12 months, a total of 330 nets (172 for PermaNet® 3.0 and 158 for PermaNet 2.0) were surveyed. Out of this, 47.1% of PermaNet® 3.0 (81) and 50.0% (79) of PermaNet® 2.0 had been washed at least once. At 24- and 36-months post-distribution, over 54% of both PermaNet® 3.0 and PermaNet® 2.0 has washed their nets at least once. At 6 months, a total of 371 respondents (186 for PermaNet® 3.0 and 185 for PermaNet® 2.0) were interviewed. Out of this number of respondents for both sets of nets, over 38% had secondary education, 26% primary education with 18% having no formal education with only 5% of the respondents had higher education. However, 36 months, the proportion of households using PermaNet® 3.0 and PermaNet® 2.0 Nets with secondary education was 52% and 48% respectively. Comparing with baseline and 36 months, net ownership decreased from 98% to 92% for PermaNet® 3.0 and 93% to 86% for PermaNet® 2.0 Nets. The net usage pattern was similar to what was observed during the 30-month survey. The PermaNet® 3.0 survivorship rates ranged from 80% at 6 months to 51% after 3 years of distribution. Comparatively, the rates for PermaNet® 2.0 Nets ranged from 71.4% at 6 months to 42% after 3 years. The survivorship rate after 3 years for both nets was relatively low. An evaluation of the deltamethrin content on the side and roof of PermaNet® 3.0 indicated that after 1 year, 2 years and 3 years, the mean deltamethrin content was 3.77 g/kg, 3.6 g/kg and 3.24 g/kg, corresponding to a loss of 19%, 23%

and 30% of the original dose. There was minimal loss of deltamethrin insecticide on the roof compared to the side of the PermaNet® 3.0. Comparatively, after 1 year, 2 years and 3 years of household use, the mean deltamethrin content in PermaNet® 2.0 was 0.97 g/kg, 0.88 g/kg and 0.62 g/kg, corresponding to a loss of 24%, 31% and 51% of the original dose respectively. However, after 3 years of household use, the mean piperonyl butoxide content was 9.99 g/kg, corresponding to a loss of 61% of the original dose respectively.

Based on the results of the evaluation, it can be concluded that although the physical integrity of both set of nets was good, PermaNet® 2.0 failed to meet the criteria for efficacy whilst PermaNet® 3.0 complied with the WHO recommended criteria after 3-years post-distribution and usage.

1.0 INTRODUCTION

The development of the technology of insecticide-treated materials (ITMs) has been one of the major innovations in the field of malariology during the past two decades. The importance of ITMs on malaria transmission in experimental trials in sub-Saharan Africa has been proven as shown by reduction of various entomologic indices (Mbogo, 1996; Bogh *et al*, 2001;) and more importantly, by reduction of morbidity and mortality due to malaria, especially in children (D'Alessandro *et al*, 1996; Binka *et al*, 1996; Lengeler *et al*, 1996). The National Malaria Control Programme (NMCP) recognizes the use of Insecticide Treated Nets and Materials (ITM) as one of the multiple prevention tools in the control of the malaria vectors. The Demographic Health Survey in 2003 revealed that an average of 18% of households owned at least one mosquito net whether treated or untreated. Only 3% of households owned at least one recently treated net (ITN). It also showed a great variation of ownership across the 10 regions. Only 3.5% of children under five years and 2.7% of pregnant women slept under an ITN the previous night. However, recent data from the Demographic Health Surveillance system of the NMCP showed that net usage across all the category of the population has increased over the years (DHS, 2018).

Unfortunately, coverage estimates of under-five children sleeping under ITNs remain well below the target levels specified in the Millennium Development Goals (WHO 2005). This low coverage with ITNs has been ascribed to cost of nets, cultural practices, poor access to markets for ITNs and low re-treatment rates among others (Nuwaha 2001; Okrah *et al* 2002; Simon *et al* 2002). Apart

from low re-treatment rate of nets, which has been a major challenge in the field, another important factor is the rapid loss of insecticidal efficacy of ITNs due to washing by users. Removing the need for re-treatment of nets can eliminate a major operational problem faced by net owners and ITN projects. Currently, some manufacturers in collaboration with WHO have developed long-lasting insecticidal nets (LLINs) that in principle retain effective concentrations of insecticide after long-term use and repeated washings. By their nature, LLINs have less bio-available insecticide on their surface compared to conventionally treated nets and are expected to have less adverse effects than ITNs, which require frequent re-treatment (at least once a year or more as required). The ultimate goal set for the use of LLINs is for them to remain efficacious for their expected lifespan of a minimum of 3 years, with repeated washing. For new LLINs, this requires evaluation in the field in particular for persistence in their insecticidal efficacy, fabric integrity and community perceptions on their use. This is because implementation of ITNs at community level is hampered by several technical, operational, economic and social factors. The rapid loss of bio-efficacy of ITNs due to washing, and low re-treatment rates, particularly in difficult-to-reach areas, limit the operational effectiveness of ITNs programme. Although it is relatively feasible to distribute mosquito nets through social marketing in malaria endemic countries, however, regular insecticide re-treatment has been found to be very difficult to implement resulting in very low re-treatment rates (Schellenberg *et al.* 2002).

To overcome these operational problems, WHO has been collaborating with industry to develop long-lasting insecticidal mosquito nets (LLINs), in which the insecticide is either incorporated into the fibers at the time of extrusion or coated on the fibers of the net at the factory level. It is recommended that long lasting nets should maintain their insecticidal bio-efficacy and after a minimum of three years of usage and withstand 20 washes (Kilian *et al*, 2008).

New brands of LLINs require field evaluation before they are recommended for use in malaria vector control, in collaboration with WHO and the LLIN-industry. Several LLINs are recommended and prequalified by WHO for malaria control[1].

The PermaNet® 3.0 was given WHOPES interim recommendation in 2009, which was subsequently converted to prequalification in 2018. PermaNet® 2.0 LN was given full recommendation by WHOPES in 2009 following interim recommendation awarded in 2004; it

[1] https://www.who.int/pq-vector-control/prequalified-lists/en/

was also converted to WHO prequalification. As a further WHO testing requirement, PermaNet® 3.0 was tested in a 3-year long Phase III trial in some 5 communities in Asutsuare, in the Greater Accra Region of Ghana according to the WHO guidelines (WHO, 2013).

Test products

PermaNet® 3.0 is a mosaic net combining different LN technologies. The roofing of the net utilizes deltamethrin and a synergist, piperonyl butoxide (PBO) incorporated into monofilament polyethylene yarn of 100 denier (warp-knitted fabric, with weight of 40 ± 15% g/m2) at the target dosages of 4.0 g AI/kg and 25 g AI/kg of netting material respectively. The side panels of are made of multi-filament polyester fibres, treated with deltamethrin in a resin coating (75 ± 5% denier, warp knitted fabric, atlas construction). The side netting has two parts: a strengthened lower part, so called border (70 cm) by using 75 ± 5% denier (weight 40 ± 10% g/m2) and a side panel made of 75 ± 5% denier (weight 30 ± 10% g/m2) The target dosage of deltamethrin in the side panels is 2.8 g AI/kg of netting material, i.e 115 mg AI/m2 of the border and 85 mg AI/m2 of the remaining of the side panels.

PermaNet® 2.0 is a deltamethrin-coated LN manufactured by Vestergaard SA (Switzerland). PermaNet 2.0 received WHOPES full recommendations in 2009. The net is made of knitted poly-filament polyester fibres and is treated with deltamethrin to a target concentration of 55 mg/m^2 (= 1.4 g/kg for a 100-denier net; 1.8 g/kg for a 75-denier net).

2.0 OBJECTIVES

The proposed study was to undertake a Phase III household randomized trial of PermaNet® 3.0, LNs according to standard WHO guidelines and procedures (WHO, 2013). The WHOPES Phase III studies are large-scale trials to determine efficacy, longevity and fabric integrity in real-life situations, as well as community acceptance of LNs. Accordingly, household randomized trial is being undertaken in Dangme West District of Ghana with the following objectives:

- To evaluate the insecticidal activity and fabric integrity of PermaNet® 3.0 using PermaNet® 2.0 as a positive control LN over a period of three years of continuous use by households under field conditions;

- To assess community washing methods and washing habits for PermaNet® 3.0 and PermaNet® 2.0 LNs, and

- To assess and compare the community acceptability of PermaNet® and PermaNet® 2.0 LNs over three years of use by households under field conditions

3.0 STUDY AREA

3.1.1 Osudoku District

The study was carried out in 5 communities located in the south-eastern part of Ghana and lies between latitude 5˚ 45' South and 6˚ 05' North and longitude 0˚ 05' East and 0˚ 20' West (Figure 1). It is about 40.8 km away from the national capital Accra. The district is rural but is gradually catching up with the rapid urbanization of the peripheral areas surrounding the city of Accra. It has the largest land surface area [about 1,700 square km] in the region and is bounded on the North by the Akuapim Ranges; to the South by Tema; to the East by River Volta and the West by Ga District. The land is flat and at sea level with isolated hills. Baseline information gathered for the setting up of a demographic surveillance system in the district indicated that the population of the district is 98, 681. Most of population live in scattered small communities of less than 2000 people. The communities are mostly rural with subsistence farming, fishing and petty trading being the most predominant occupation. The main produce of the farmers is rice with maize and vegetables as minor crops. Average yields are around 38 bags per acre of cultivable land. Of the net irrigable area of 1,000 hectares at Asutsuare, approximately 800 hectares have been developed by 1991 and currently being worked by 580 (registered) farmers. Malaria transmission in the district is perennial with an estimated 20 infectious bites per person/year. However, the prevalence of malaria shows considerable seasonal variation, peaking

during and immediately after the rains. *P. falciparum* constitutes 98% of all infections (Afari et al, 1995). There are 4 health centres, one in each of the 4 sub districts. In total, there were six community clinics, two in Dodowa, one in Prampram, two in Osudoku and one in Ningo, which also serves as a Community Health and Planning Services (CHPS).

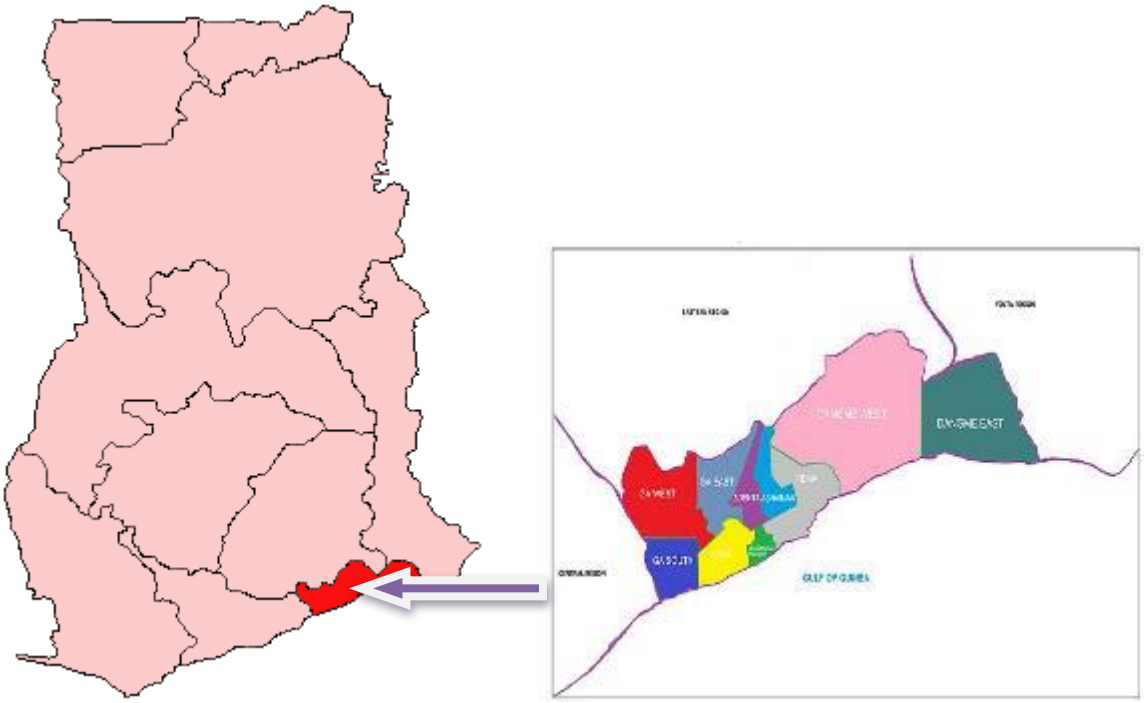

Fig. 1. Map of Ghana showing the locations of Osudoku District where the study was conducted.

4.0 METHODS

4.1 Study design

The efficacy of candidate LN PermaNet® 3.0 was compared with PermaNet® 2.0 Net, for which full recommendation by WHOPES is available, to determine if their efficacy is as good as that of the latter. This was a prospective household single blinded randomized trial with nets as unit of observation. The efficacy of LLINs was monitored for three years of continuous use under the field conditions.

4.2 Selection of villages

It was proposed to include about 1500 households in the study in up to eight villages in the Osudoku District in consultation with the State/District Health Authorities. Each household in the eight villages was allocated at least with one coded net and followed up for 3 years. It was envisaged that over 1440 coded nets in the households will be sampled by the end of the study. Selection of the study villages was made considering malaria endemicity, reach, net use and multiple ethnicities. The two brands of LLIN were distributed to equal number of households by random allocation. All sleeping places in a given household received the allocated LLIN to ensure universal coverage of the selected community/village.

In the selected villages, written informed consent was obtained from headperson of every household to participate in the study by door-to-door visit before distribution of LLIN. The participants were informed in detail about the purpose of the study, consequent sampling procedure and replacement of sampled net with new ones. Help of local health workers and community leaders was solicited to inform the inhabitants about the objectives of the trial and its components.

4.3 Baseline household survey and census

A baseline household survey was carried out in all the selected villages using a structured questionnaire. Information was collected on size of the family, educational status, occupation, average family income, type of house, number of sleeping places in a house, availability of nets, their usage pattern, washing practices etc. Enumeration of houses was done and detailed census with the name, age, sex and level of education of every family member was recorded in registers.

4.4 Distribution of nets

LLINs were randomly distributed free of charge in the selected households by door-to-door visit in the study villages. Randomization took place at household level.

A net master list was created to include the following information:

- All nets to be distributed had in addition to the factory label a blank label (about 3x4 cm) stitched to the net seams for writing twice the same ID number (6 digits) of the net: once with wash-resistant ink, and once with a water soluble ink as a quality control for the assessment of washing;
- A random list of households required for the study according to the sample size was produced for allocation to PermaNet® 3.0 or PermaNet® 2.0 Net. After random allocation of the households to the two groups of nets, and corresponding nets were distributed to the households to assure that all sleeping places have a bed net;
- The net master list inter alia included the following information: ID number (highest number in the household), the full code of the factory, batch number, type of net, household number, name of the householder, position of the household and dates of net distribution, sampling and replacement, if any.
- Only the Principal Investigator and field investigators had the full master list to ensure a correct follow up in the field.
- People were asked not to remove the ID labels from the nets and the nets were given free of charge to all households.

4.5 Community entry

Community level meetings were organised to educate all the people in the selected communities on the objectives of the trial, benefits as well as adverse consequences of malaria, the benefits of using treated/long-lasting nets, correct handling and use of nets in line with WHO recommendation[2] and the need for reporting any adverse events.

The information that the nets have been marked with water-soluble ink as well as the purpose of such marking was provided to the participants in the interest of transparency. People were asked not to remove the ID labels from the nets. Informed consent was obtained from all heads of households enrolled in the study at the time of census survey when all potential households were visited by the investigators. To obtain informed consent of illiterate people, the informed consent form was read and explained by a member of the investigating team in local language in the presence of two community witnesses. Upon their consent, such people were asked to mark a thumb impression on the form and the two witnesses will be asked to sign. Those who refuse to participate in the study had the option to accept or refuse LLINs.

If at any point in time during the study a participant decided not to participate any further, he/she was allowed to do so. All such participants withdrawing from the study were allowed to retain their net. Record of all such participants was kept separately and their data was eliminated in the final analysis of study outcome. At the time of distribution of an LLIN, every head person of the household was informed about the need for reporting any adverse effect of the nets, as well as appropriate use and maintenance of their nets.

4.6 Sampling and testing of LLINs

The sampling of nets was carried out at the baseline, every 6-months for bioassay and durability assessment (physical integrity, survivorship and community practices) i.e. at months 6, 12, 24, 30 and 36. Sampling for chemical content analysis was carried out at the baseline, 12, 24, and 36 months.

4.6.1 Selection of net pieces for bioassay and chemical content analysis

Thirty (30) of each type of net were sampled at baseline, and at 6, 12, 24 and 30 months. Fifty (50) nets were sampled at 36 months. For baseline sampling, five pieces of 30 cm x 30 cm size were cut from positions 1 to 5 as per WHO LLIN guidelines. These five sub-samples were then assembled as one sample for chemical analysis in a WHO Collaborating Centre. In subsequent samplings, only four sub-samples from positions 2 to 5 were cut and assembled for chemical analysis. The sub-samples were rolled up and placed in labelled, new, clean aluminium foil for storage prior to the assay. Netting pieces cut from both the LLINs were sent to the WHO

collaborative centre, Gembloux (Belgium). The sampling scheme was according to the LLIN durability guidelines (WHO, 2013)[3]

4.6.2 Biological assays

4.6.3 Cone bioassays: The cone bioassays were done once at the start of the study, and every 6 months thereafter up to 3 years. 30 nets of each brand were sampled in each survey, except for 50 nets in the survey after 36 months.

Cone bioassays were performed as recommended by WHO, 2013 guidelines). In each sampling, bioassays were carried out on net pieces cut from the side of position 1 to 5 (adjacent to chemical assay samples). On each netting sample, standard WHO cone was placed and held in place using a plastic manifold. Five laboratory-bred susceptible Kisumu *Anopheles gambiae* s.s. mosquitoes (sugar fed, 2-5 day old) were introduced into each cone and exposed for 3 minutes. For each net piece (sub-sample), 5 mosquitoes were exposed in the cone and the test was replicated giving a total of 10 mosquitoes per net piece. The total number of mosquitoes exposed per net was 50 (5 net pieces x 5 mosquitoes per test x 2 replicates). After the exposure, the mosquitoes were removed gently from the cones and kept separately in plastic cups provided with cotton-wool moistened with 10% glucose solution. Knockdown was recorded after 60 minutes and mortality after 24 hours. Mosquitoes exposed to untreated nets were used as controls. The bioassays were carried out at 27±2 °C and 80±10% RH. LLINs which caused knockdown of <95% and a bioassay mortality of <80% were subjected to a tunnel test.

4.6.4 Tunnel test

The tunnel test was carried out in the laboratory to assess blood feeding inhibition by comparing the proportion of blood-fed females (alive or dead) in treated and control tunnels. Overall mortality was measured by pooling the mortalities of mosquitoes from the two sections of the tunnel. From each LLIN that did not meet WHOPES efficacy criteria in cone test, only one out of four net pieces was selected for the tunnel test. This is the piece that gave mortality closest to the average mortality

in the cone test on four pieces. The selected piece will be fixed in the tunnel for test. One tunnel with untreated netting was used as a negative control.

Non-blood fed female Anopheline mosquitoes, aged 5–8 days were released in a tunnel (square section 25 cm x 25 cm) made of glass, 60 cm length (WHO, 2005b). At each end of the tunnel, a 25-cm square mosquito cage covered with polyester netting was fitted. At one third of the length, a disposable cardboard frame was placed, and a 20 cm x 20 cm piece of netting sample was fixed between two pieces of cardboard and set next to the rabbit. The surface of netting "available" to mosquitoes was 400 cm^2 (20 cm x 20 cm), with nine holes each 1 cm in diameter: one hole was located at the centre of the square; the other eight were equidistant and located at 5 cm from the border. In the shorter section of the tunnel, a small rabbit used as a bait was placed, which was unable to move but available for biting. In the cage at the end of the longer section of the tunnel, 100 female mosquitoes Kisumu susceptible *An. gambiae* s.l. were introduced at 18:00. The following morning at 09:00, the mosquitoes were removed by using a suction glass tube and counted separately from each section of the tunnel and mortality and blood feeding rates was recorded. During the test, tunnel was placed in a place maintained at 27 °C ± 2 °C and 80% ± 10% relative humidity under subdued light.

Interpretation of study results: Results of the cone and tunnel tests were considered together to judge on LLIN performance. A candidate net was deemed to meet the requirements of a LLIN if at the end of the study period of 3 years, at least 80% of sampled nets retained bio-efficacy based on WHO cone bioassay and/or the tunnel test as detailed in the WHO guidelines. If mortality in mosquitoes exposed to such 3year old LLINs in the WHO cone bioassays falls below 80% and knockdown falls below 95%, nets were tested using the tunnel test.

As blood-feeding in controls has a considerable impact on mortality in the presence of treated samples (i.e. the host-seeking behaviour increases the chance of contact with treated fabric), a minimum cut-off value of blood-feeding rate in controls was established for tunnel tests.

4.7 Net survivorship

The surveys (at the end of months 6, 12, 24 and 36) was conducted by visiting houses door-to-door to recorded physical presence/absence and fabric integrity of the nets, where applicable, to estimate the annual attrition rate, besides information on people's perceptions and practices as mentioned above using structured standard questionnaire.

For the net survivorship survey, 250 LLINs codes were randomly selected from the Net Master list for each LLIN product. Through household visits, all 250 nets were surveyed to record physical presence/absence of the nets. When a net is found to be no longer present in a household, the house holders was questioned to determine the reason for its loss or absence.

4.8 Fabric integrity

At 6, 12, 24 and 36-month post-distribution of nets, a randomly selected sample of 150 LLINs of each brand were inspected for physical integrity (the size and distribution of holes) by draping the nets over a frame and counting the number of holes of different sizes according to location on the net (top, upper side, lower side). A questionnaire was completed to record net durability.
For each of the selected nets, number of holes (including tears in the netting and split seams) by their location on the net and their size was assessed. Holes were classified into the following categories:

- smaller than a thumb (0.5–2 cm),
- larger than a thumb but smaller than a fist (2–10 cm),
- larger than a fist but smaller than a head (10–25 cm) and
- larger than a head (> 25 cm).

Holes less than 0.5 cm were ignored. Evidence of repairs to the net fabric and the type of repair was also recorded on the form. Holes count was made by removing each net and arranging it over a frame and returning the nets after measuring physical integrity in the field. Hole index was calculated based on standard WHO criteria.

4.9 Community perceptions and practices

At the end of months 6, 12, 24 and 36, an adult participant in 30 each of selected households (and 50 households at the end of 36 months) was interviewed by door-to-door visit to assess net utilization patterns (including early morning observations), method and number of washes, and type of soap used as per the standard questionnaire. Interview assessment of washing frequency

may not always be reliable therefore another net in the household, or a net in a neighbouring household, was marked with a water-soluble marker and revisited one month later to obtain a more accurate picture of washing frequency in the community.

An assessment of the adverse effects was made using a standard questionnaire. The people were advised on such possibilities as part of information given in the Informed Consent form. The Principal Investigator selected required ID numbers from each LLIN arm from their respective master lists using a random selection procedure. The list of selected nets was re-sorted in ascending order and given to the field team who visited the study area one week and one month after distributing the nets to record perception of the participant users and to record any adverse effects. The Principal Investigator informed the Medical Officer of the area about possible reporting of adverse effects of use of nets by the participants so as to provide medical care as necessary.

4.10 Efficacy criteria for Phase III

Using data obtained through questionnaire, SPSS software was used to assess the community acceptance of LLINs (use rate, perceived benefits in malaria control, any adverse effects, washing and upkeep practices) and attrition rate.

A candidate LLIN was considered to meet the efficacy criteria for phase III studies if, after 3 years, at least 80% of nets meet the criteria for WHO cone tests (≥95% knockdown and/or ≥80% mortality) and the tunnel test (≥80% mortality or ≥90% blood-feeding inhibition).

4.11 Insecticide susceptibility tests

Insecticide susceptibility tests at diagnostic concentrations of the candidate insecticide deltamethrin) were conducted every year during the course of the study. Larval surveys were done to collect *Anopheles* vector larvae from breeding sites in localities. The larvae were reared to adults and used in insecticide susceptibility tests. Laboratory reared 2 to 5 days-old, non-blood fed F1 females obtained were used for the insecticide susceptibility test at diagnostic concentrations of permethrin to determine susceptibility level using the standard WHO methodology (WHO, 2018) for the candidate insecticide. Susceptibility status of the *An. gambiae* colony to be used for bioassays were monitored each year.

4.12 Ethical clearance

The study received ethical clearance from the Noguchi Memorial Institute for Medical Research Intuitional Review Board (NMIMR-IRB) in 2014. All the questionnaires, data tools and informed consent forms were subjected to review and given approval by the NMIMR-IRB.

5.0. RESULTS

5.1 Distribution of LLINs to the population in the field

A total of 1755 of both set of PermaNet® 3.0 and PermaNet 2.0 Nets were randomly to households as per the protocol in five communities in the district with a total population of 1,895 (Table 1). A total of 236 coded PermaNet® 3.0 and 229 PermaNet® 2.0 Nets were distributed to 87 and 98 households for the cohort study translating to an average of 2.7 nets per household for PermaNet® 3.0 and 2.3 for PermaNet® 2.0 (Table 1).

Table 1 The population and number of households in the PermaNet® 3.0 and PermaNet® 2.0 Nets study

					PermaNet® 3.0 for cohort study		**PermaNet 2.0 for cohort study**	
Village	**Population**	**Houses**	**Net required**	**Net distributed**	No. of nets	No. of HH	No. of nets	No. of HH
Atrobinya	415	140	450	500	80	30	80	29
Avakpo	400	98	400	350	32	20	30	22
Natriku	365	90	280	300	20	10	24	12
Kadzanya	350	60	265	265	50	12	50	20
Kuwem	365	103	325	340	54	15	45	15

5.2 Characteristics of respondents

At 6 months, a total of 371 respondents (186 for PermaNet® 3.0 and 185 for PermaNet® 2.0) were interviewed. Out of this number of respondents for both sets of nets, over 38% had secondary education, 26% primary education with 18% having no formal education. Only 5% of the respondents had higher education.

During the 12 and 18-months surveys, a total of 327 (170 PermaNet® 3.0 and 157 PermaNet® 2.0) and 353 (173 PermaNet® 3.0 and 180 PermaNet® 2.0) heads of households were interviewed respectively. Over (38.4%) of heads of households interviewed during the 12 and 18-months usage of both PermaNet® 3.0 and PermaNet® 2.0 had at least secondary education, followed by primary education (20.7%) with less than 18.1% not having any formal education during both surveys.

At 24 and 30 months, 48% and 58% of the proportion of households interviewed who were using PermaNet® 3.0 had secondary education, followed by primary education (32%) respectively. A proportion (18%) of the respondents had no education at all. The demography of households interviewed using both nets was similar in all the communities surveyed.

At 36 months, the proportion of households using PermaNet® 3.0 and PermaNet® 2.0 Nets with secondary education was 52% and 48% respectively.

5.3 Net ownership, utilization and community acceptance by households

The results of the study showed that most, 98% and over 93% of households owned at least one PermaNet® 3.0 and PermaNet® 2.0 LLINs respectively during the 6 months post distribution interview. Ownership of both nets was high in all villages and percentage of household members who slept under LLIN the previous night was 97.1% and 95.9% respectively for PermaNet® 3.0 and PermaNet® 2.0 during the 6 months survey. This indicates that the net acceptance rate among the inhabitants was good.

At 12 and 18 months, the proportion (%) of households that owned at least one PermaNet® 3.0 and PermaNet® 2.0 was 91.5% (170) and 94% (157) respectively with an average of 4 nets owned per household. However, usage of PermaNet 3.0 nets at 12 and 18 months was 54.1%

(170) and 61.1% (157) respectively whereas usage for PermaNet 2.0 during the same periods was the same, 55.2%. Comparing the 18 months post-distribution evaluations of both set of nets to 6 months survey, usage of PermaNet® 3.0 and PermaNet® 2.0 has decreased by 37.1% and 42.5% respectively.

Comparing the 24-month post-distribution evaluations of both set of nets to 6-month survey, usage of PermaNet® 3.0 and PermaNet® has decreased by 13.4% (84%) and 20.3% (76.4%) respectively. At 30 months, a high proportion, 92% and 96% of inhabitants still owned both PermaNet® 3.0 and PermaNet® 2.0 Nets respectively. However, only 48% and 56% of PermaNet® 3.0 and PermaNet® 2.0 Nets had been used for sleeping the previous night.

Comparing with baseline and 36 months, net ownership decreased from 98% to 92% for PermaNet® 3.0 and 93% to 86% for PermaNet® 2.0 Nets. The net usage pattern was similar to what was observed during the 30-month survey.

5.4 Insecticidal activity of PermaNet® 3.0 and PermaNet® 2.0 Nets under field operation

A total of 460 (230 PermaNet® 3.0 and PermaNet® 2.0) were subjected to Cone bioassay and tunnel test at baseline, 6, 12, 24, 30 and 36 months. The susceptible Kisumu strain of *An. gambiae* was used for the test. At 24 months, the overall pass rate (%) for PermaNet® 3.0 and PermaNet® 2.0 was 96.7% and 100% respectively (Table 2).

Compared to the baseline where all LLINs passed, the pass rate at 36 months for PermaNet® 3.0 and PermaNet® 2.0 decreased by 20% and 48% respectively. The decrease in the insecticidal activity was mainly in the nets tested from the side panel. The pass rate of nets at 36 months from the top panel, 84% was significantly ($P<0.01$) higher compared to 58% of samples from the side panels. The same pattern was observed during all the survey months. However, the overall pass rate for PermaNet® 3.0 and PermaNet® 2.0 at 36 months was 80% and 58% respectively.

The average 24 hr mortality for PermaNet® 3.0 decreased from 88.3% at 12 months to 82.4.0% at 18 months and a further decrease by 8.2% in 24 months. At 36 months, whilst PermaNet® 3.0 met the criteria for efficacy, PermaNet® 2.0 with an estimated average mortality of 70.3% did not meet the criteria (i.e. KD ≥95% and/or 24 hr mortality ≥ 80%). After tunnel test

evaluations, 4 of the nets passed the test for efficacy (mortality ≥80% and/or blood feeding inhibition (BFI) ≥90%). The average 24 hr mortality for PermaNet® 2.0 Nets decreased drastically over the 30 to 36 months period (Table 2).

Table 2. Results of cone bioassays of nets on PermaNet® 3.0 and PermaNet® 2.0 Net against Kisumu susceptible *An. gambiae* at baseline, 6, 18, 24, 30 and 36 months.

	WHO cone tests				**Tunnel tests**					
Survey (month)	Average No of nets tested	Mean % KD	Mean % mortality	No meeting WHO criteria for cone Test	No of nets	Mean % mortality	% Blood-feeding inhibition	Number meeting WHO criteria for tunnel test	Number passing cone or tunnel	Overall pass rate (%)
PermaNe®t 3.0										
Baseline	30	100	100	30	-	-	-	-	30	100
6	30	100	99.7	30	-	-	-	-	30	100
12	30	99.9	100	30	-	-	-	-	30	100
18	30	99.5	99.0	29	1	80.0	84.0	0	30	100
24	30	96.0	95.8	29	1	56.0	78.0	0	29	96.7
30	30	90.6	88.1	24	6	69.3	75.3	4	28	93.3
36	50	86.7	73.9	16	34	53.8	81.3	24	40	80.0
PermaNet® 2.0										
Baseline	30	100	99.7	30	-	-	-	-	30	100
6	30	99.7	98.8	30	-	-	-	-	30	100
12	30	99.7	100	30	-	-	-	-	30	100
18	30	99.7	99.7	28	2	77.3	76.2	1	29	96.7
24	30	97.5	97.3	30	-	-	-	-	30	100
30	30	81.6	78.2	21	9	61.3	79.3	6	27	90.0
36	50	68.4	70.3	23	27	60.3	70.5	6	29	58.0

5.5 Washing practices

A total of 337 respondents (167 for PermaNet® 3.0 and 170 for PermaNet® 2.0) who had their nets available were interviewed on the washing practices 6 months after distribution. Out of this, 38.2% and 35.3% of the respondents who used PermaNet® 3.0 and PermaNet® 2.0 LNs respectively said they have washed the nets at least once during the period (Table 2). Over 85.7% of respondents had used a local bar soap to wash both PermaNet 3.0 and PermaNet® 2.0 during the 6-month survey periods.

During the 12 months, a total of 330 nets (172 for PermaNet® 3.0 and 158 for PermaNet 2.0) were surveyed. Out of this, 47.1% of PermaNet® 3.0 (81) and 50.0% (79) of PermaNet® 2.0 had been washed at least once. At 24- and 36-months post-distribution, over 54% of both PermaNet® 3.0 and PermaNet® 2.0 has washed their nets at least once (Table 3).

Table 3 Household washing practices at 6,12,18 and 24. 30 and 36months evaluation of PermaNet® 3.0 and PermaNet® 2.0 nets

Period	**PermaNet® 3.0**		**PermaNet® 2.0**	
	Total nets surveyed	% No of nets washed at least once	Total nets surveyed	% No of nets washed at least once
6 months	167	38.2%	170	35.3%
12 months	172	47.1%	158	50.0%
24 months	180	47.8%	203	64.0%
36 months	150	54.8%	180	88.8%

5.6 Adverse events on net users

The adverse events questionnaire was administered on 100 net users two weeks after the distribution of both PermaNet® 3.0 and PermaNet® 2.0 nets. Most PermaNet® 3.0 net users (15%) reported itching of the skin/paraesthesthesia with 5% reporting irritation and headache (4%). For PermaNet 2.0 users, only 6% reported itching with majority reporting sneezing (13%).

At 1month evaluation of both nets, out of 103 respondents interviewed, 3.6% reported itching/paraesthesthesia during usage of PermaNet® 3.0. No adverse advent was recorded for PermaNet® 2.0 during the period.

5.7 Net survivorship rates

Net survivorship rates estimated at 6, 12, 24 and 36 months is shown in Table 4. The PermaNet® 3.0 survivorship rates ranged from 80% at 6 months to 51% after 3 years of distribution. Comparatively, the rates for PermaNet® 2.0 Nets ranged from 71.4% at 6 months to 42% after 3 years. The survivorship rate after 3 years for both nets was relatively low.

Table 4 Survivalship rates of PermaNet® 3.0 and PermaNet 2.0 LLINs from 6 to 36-months post-distribution

Period	**PermaNet® 3.0**		**PermaNet® 2.0**	
	Total nets	% Survivorship	Total nets	% Survivorship
Baseline	236	100	229	100
6 months	189	80.4	170	74.2
12 months	185	78.7	170	74.2
24 months	162	69.0	203	88.6
36 months	120	51.0	100	42.0

5.8 Inspection of fabric integrity of LLINs under field operation

The number of nets with at least one hole and the proportionate hole index (pHI) is shown in table 5. The proportions of PermaNet® 3.0 and PermaNet 2.0 with at least one hole were 3.0% and 5.8% respectively during the 6 months survey and this increased to 66.7% and 69.4 after 3 years (Table 5).

The increase in the number of holes over the period may be the results of continues handling of the nets during usage. However, the total number of holes on both nets decreased after 3 years as a result of continues education on the proper use of the nets. The size 4 holes observed in both set of nets were caused by mostly candle burns and the rest were caused by the sharp edges of the wooden bed where the beds were being hanged. Higher number of holes of all categories occurred on the lower part than the upper part of both nets.

The average hole index (pHI estimated at 6, 12, 24 and 36 months is shown in Table 5. The pHI for PermaNet® 3.0 ranged from 16.4 at 6 months to 120 at 36-month post-distribution. The pHI for PermaNet® 2.0 ranged from 12.4 to 98.1 during the same period. A high percentage (over 90%) of both PermaNet® 3.0 and PermaNet® 2.0 Nets were in good condition after 36-month post-distribution and many were in serviceable condition and are usable (Table 6).

Table 5 Physical integrity of PermaNet® 3.0 and PermaNet® 2.0 Nets expressed as proportion (%) of nets with at least 1 hole and median proportionate Hole Index (pHI).

Period (months)	PermaNet® 3.0		PermaNet® 2.0	
	Proportion (%) with at least 1 hole	Median pHI (if any hole)	Proportion (%) with at least 1 hole	Median pHI (if any hole)
6	3.0	16.4	5.8	12.4
12	3.2	47.0	8.2	50.3
24	26.9	89.0	30.6	70.0
36	66.7	120.0	69.4	98.1

Table 6 Percentage of PermaNet® 3.0 and PermaNet® 2.0 Nets that are good, damaged, serviceable and torn

	PermaNet® 3.0 Nets				PermaNet® 2.0 Nets			
Period (months)	[N] % Good	% Damaged	% Serviceable	% Too torn	[N] % Good	% Damaged	% Serviceable	% Too torn
6	[167] 99.3	0.4	97.8.	0.03	[170] 98.0	1.6	99.6	0.4
12	[185] 90.2	8.9	99.1	0.9	[170] 97.3	1.5	98.8	2.2
24	[364] 80.3	12.2	92.5	7.5	[360] 89.2	8.9	98.1	1.9
36	[150] 80.0	13.2	93.2	6.8	[180] 80	10	90	10

5.9 Insecticide susceptibility test

Insecticide susceptibility assay was carried yearly in the five communities to assess the susceptibility of *An. gambiae* s.l. to selected insecticides including permethrin. Mosquito larvae were collected from the five communities, pooled together and reared to F1 generation for the test. Results of the test is shown in Table 8. The yearly insecticide susceptibility levels to deltamethrin was low ranging from 15.0% to 26.0% whilst that for permethrin and cyfluthrin ranged from 6.0 – 30.0%.

Table 8 Percentage 24hr mortality of *An. gambiae* s.l. from the study areas exposed to different pyrethroid insecticides

Period	No of mosquitoes exposed/test	Insecticide and % mortality		
		Permethrin	Deltamethrin	Cyfluthrin
Baseline	100	6.0%	25.0%	30.0%
12 months	100	15.4%	18.0%	28.0%
24 months	100	20.0%	26.0%	18.0%
36 months	100	15.0%	15.0%	23.0%

5.10 Chemical analysis of LLINs used under field conditions

For PermaNet 3.0, sides

The mean deltamethrin content in sides of PermaNet 3.0 at baseline is 1.93 g/kg, corresponding to 87.3 mg/mÇ. All the samples of sides of baseline PermaNet 3.0 among the 30 samples analyzed comply with the target dose of 2.1 g/kg ± 25% [1.6 – 2.6 g/kg] (information extracted from test report RE / 14 / U10 / 23807 / 2 - third interim report). The between net variation, expressed as the relative standard deviation (RSD) of the deltamethrin content found on 30 different nets, is 7.6% showing an acceptable homogeneity of the active ingredient distribution between the nets. After 1 year, 2 years and 3 years of household use, the mean deltamethrin content is 1.49 g/kg, 1.2 g/kg and 0.96 g/kg, corresponding to a loss of 23%, 38% and 50% of the original dose respectively (Table 9, Figure 3).

For PermaNet 3.0, roof

The mean deltamethrin content in roof of PermaNet® 3.0 at baseline is 4.65 g/kg, corresponding to 181.5 mg/mÇ. All the samples of roof of baseline PermaNet® 3.0 among the 30 samples analyzed complied with the target dose of 4.0 g/kg ± 25% [3.0 - 5.0 g/kg] (information extracted from test report RE / 14 / U10 / 23807 / 2 - third interim report). The between net variation, expressed as the relative standard deviation (RSD) of the deltamethrin content found on 30 different nets, is 2.9% showing an acceptable homogeneity of the active ingredient distribution between the nets. After 1 year, 2 years and 3 years of household use, the mean deltamethrin content is 3.77 g/kg, 3.6 g/kg and 3.24 g/kg, corresponding to a loss of 19%, 23% and 30% of the original dose respectively (Table 9, Figure 4).

The mean piperonyl butoxide content in roof of PermaNet® 3.0 at baseline is 25.62 g/kg, corresponding to 999.6 mg/mÇ. All the samples of roof of baseline PermaNet 3.0 among the 30 samples analyzed comply with the target dose of 25 g/kg ± 25% [18.75 - 31.25 g/kg] (information extracted from test report RE / 14 / U10 / 23807/ 2 - third interim report). The between net variation, expressed as the relative standard deviation (RSD) of the piperonyl butoxide content found on 30 different nets, is 2.2% showing an acceptable homogeneity of the active ingredient distribution between the nets. After 1 year, 2 years and 3 years of household use, the mean piperonyl butoxide content is 15.64 g/kg, 12.4 g/kg and 9.99 g/kg, corresponding to a loss of 39%, 59% and 61% of the original dose respectively (Table 10, Figure 2).

PermaNet 2.0 (positive control)

The mean deltamethrin content in sides of PermaNet® 2.0 at baseline is 1.27 g/kg, corresponding to 58.5 mg/mÇ. All the samples of baseline PermaNet® 2.0 among the 30 samples analyzed comply with the target dose of 1.4 g/kg ± 25% for 100 denier yarn [1.05 - 1.75 g/kg] (information extracted from test report RE / 14 / U10 /23807 / 2 - third interim report). The between net variation, expressed as the relative standard deviation (RSD) of the deltamethrin content found on 30 different nets, is 5.6% showing an acceptable homogeneity of the active ingredient distribution between the nets. After 1 year, 2 years and 3 years of household use, the mean deltamethrin content is 0.97 g/kg, 0.88 g/kg and 0.62 g/kg, corresponding to a loss of 24%, 31% and 51% of the original dose respectively (Table 9, Figure 5).

Table 9 Deltamethrin average content of PermaNet® and 3.0 and PermaNet® 2.0 Nets at baseline, 12, 24, 36-month post-distribution

	PermaNet® 3.0			**PermaNet® 2.0**	
	Roof	Side	Complied with WHO specifications	Deltamethrin content AI content (g/kg)	Complied with WHO specifications
Period	Deltamethrin content AI content (g/kg)	Deltamethrin content AI content (g/kg)			
Baseline	4.65	1.93	Yes	1.27	Yes
12 months	3.77	1.49		0.97	
24 months	3.60	1.20		0.88	
36 months	3.24	0.96		0.62	

Table 10 Piperonyl butoxide content, within-net variation (RSD) of PermaNet® 3.0 at roof of Net at baseline, 12, 24 and 36 months

Period	PBO content AI content (g/kg)	Complied with WHO specifications	Percentage PBO loss
Baseline	25.62	Yes	0
12 months	15.64		39%
24 months	12.4		52%
36 months	9.99		61%

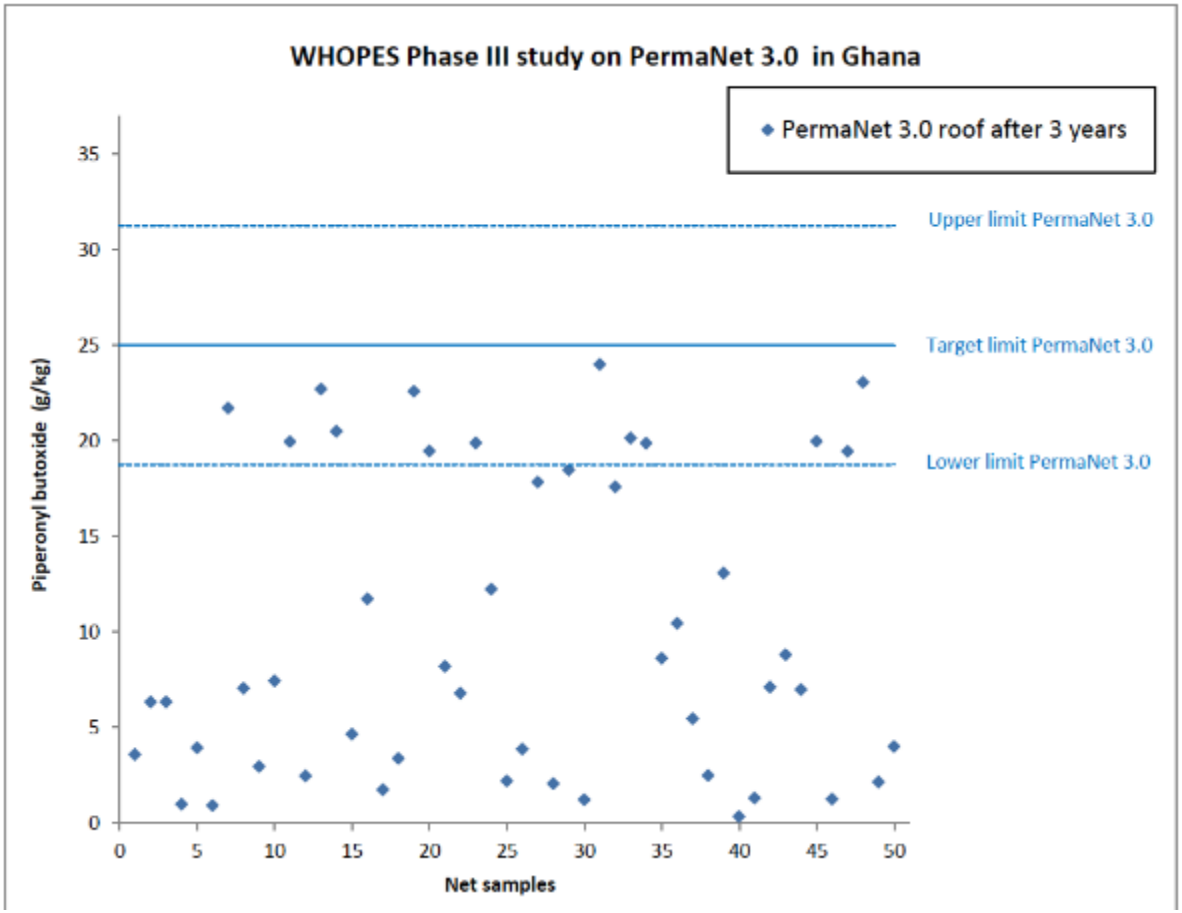

Figure 2 The mean PBO content in the roof of PermaNet® 3.0 after 3 years post-distribution

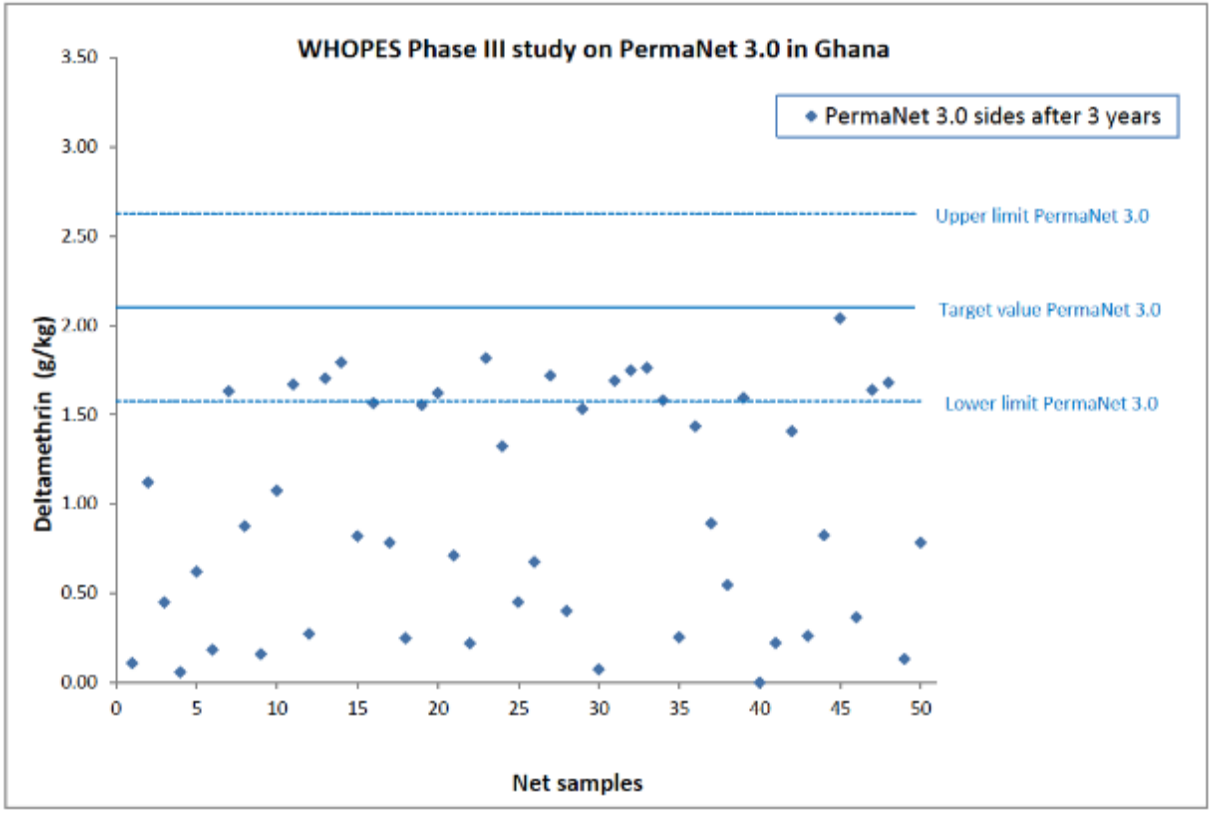

Figure 3 The mean deltamethrin content in the sides of PermaNet® 3.0 after 3 years post-distribution

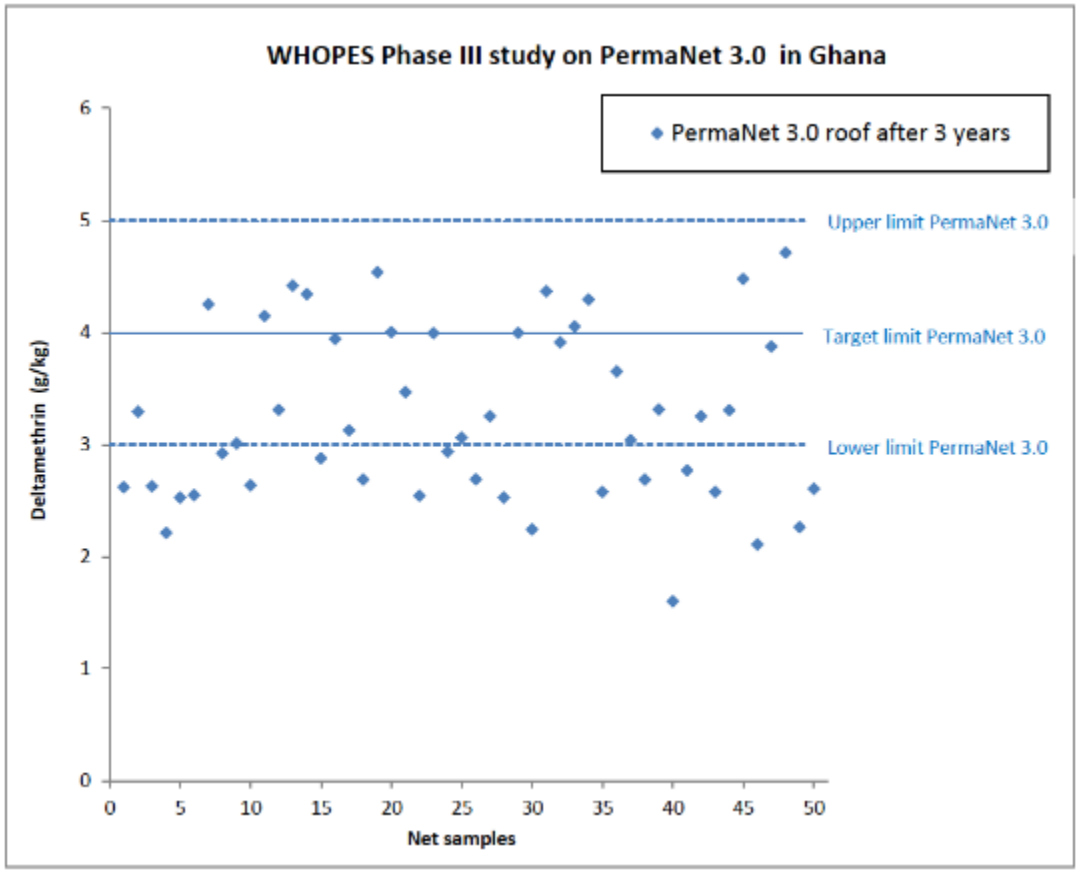

Figure 4 The mean deltamethrin content in the roof of PermaNet® 3.0 after 3 years post-distribution

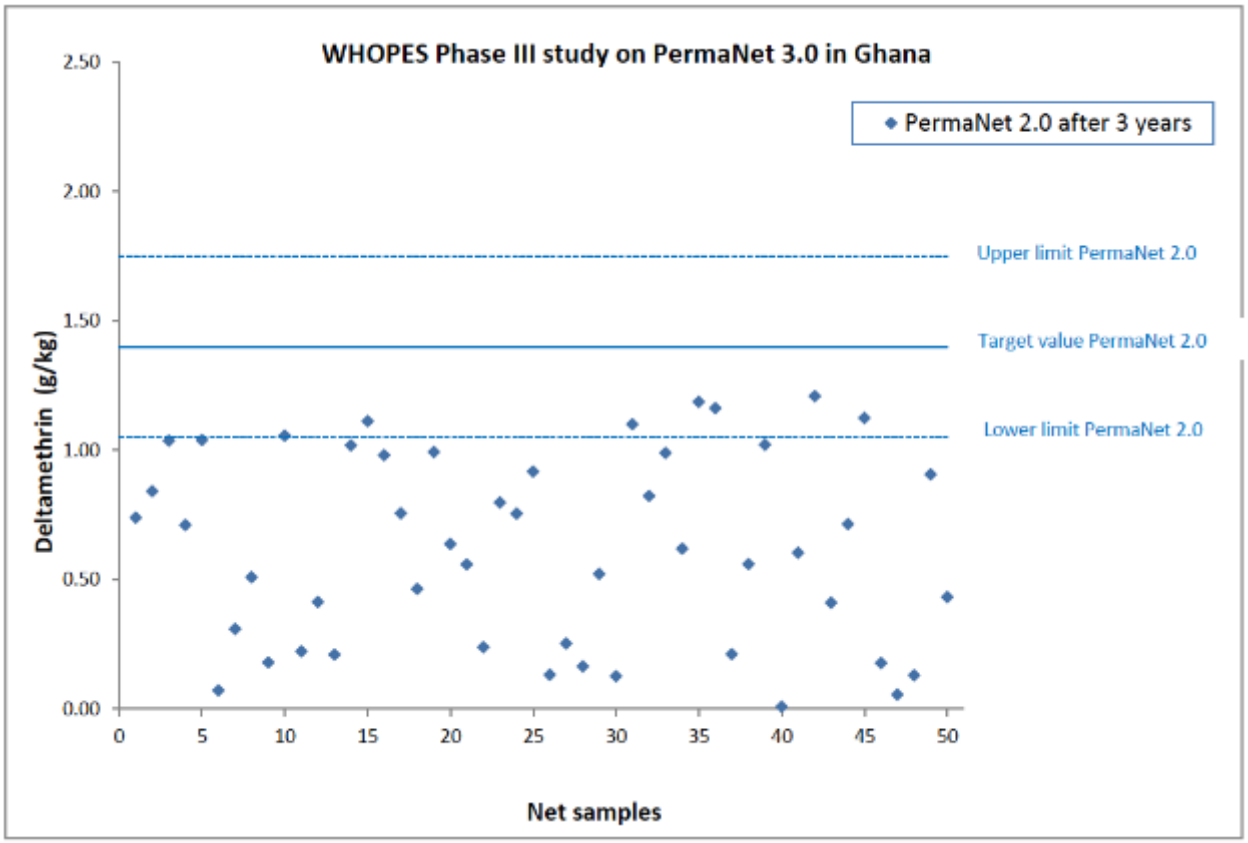

Figure 5 The mean deltamethrin content in PermaNet®2.0 Nets after 3 years post-distribution

Table 11 The mean concentration of deltamethrin and Piperonyl butoxide content and the corresponding % loss in content 0, 1, 2, and 3 years of PermaNet® 3.0 and PermaNet® 2.0 after distribution

Deltamethrin content

		Year after distribution			
		0	1	2	3
PermaNet 3.0 (sides)	N	30	30	30	50
	Mean	1.93 (1.88-1.98)	1.49 (1.30-1.68)	1.2 (1.0-1.4)	0.96 (0.78-1.14)
	% lost	-	**23%**	**38%**	**50%**
PermaNet 2.0	N	30	30	30	50
	Mean	1.27 (1.24-1.30)	0.97 (0.87-1.07)	0.88 (0.78-0.98)	0.62 (0.52-0.72)
	% lost	-	**24%**	**31%**	**51%**

		Year after distribution			
		0	1	2	3
PermaNet 3.0 (roof)	N	30	30	30	50
	Mean	4.65 (4.60-4.70)	3.77 (3.46-4.08)	3.6 (3.3-3.9)	3.24 (3.02-3.46)
	% lost	-	**19%**	**23%**	**30%**

Piperonyl butoxide content

		Year after distribution			
		0	1	2	3
PermaNet 3.0 (roof)	N	30	30	30	50
	Mean	25.62 (25.41-25.83)	15.64 (13.01-18.27)	12.4 (9.4-15.4)	9.99 (7.71-12.27)
	% lost	-	**39%**	**52%**	**61%**

6.0 DISCUSSION

The study was carried out in Osudoku District of Ghana with the objective of evaluating and comparing the efficacy, fabric integrity, community acceptance and washing methods as well as adverse events of the two set of PermaNet® 3.0 and PermaNet® 2.0 LLINs under field conditions.

The results of the evaluation showed that after 36-month post-distribution of both PermaNet® 3.0 Nets and PermaNet® 2.0 nets were all in relatively in good condition. The physical integrity of the nets estimated as hole index was relatively low. However, some of the nets after 36 months of distribution could still not be traced as shown in the relatively low survivorship rates estimated. Many of the nets (over 65%) were lost due to migration of inhabitants in the areas. The inhabitants were mostly fisherman and farmers who tend to migrate to other fertile and productive areas to carry out their activities and therefore will carry the nets with them. The rest were lost due to wear and tear.

The results indicated that those nets that were physically present in the rooms have been used the previous night. This was shown by the relatively high usage rate, over 86% within the households. Most of the holes were of size 1 and were mostly located at the lower end area of the net which was frequently handled when pushing the seam end of the net under the bed.

Although the fabric integrity and acceptance were relatively good for both PermaNet® 3.0 and PermaNet® 2.0, the efficacy as measured by cone assays as we as tunnel test indicated that whilst PermaNet® 3.0 met the efficacy after 36 months usage, PermaNet® 2.0 did not comply with the WHO recommended criteria of efficacy at 3 years of usage. The KD60 and mortality rates for PermaNet® 2.0 after tunnel test were far below the criteria for effectiveness after 3 years. In a multi-country study evaluated by WHOPES, after three years of usage of PermaNet® 2.0, overall the proportion of the nets meeting the criteria based on the cone and tunnel tests was 80%. However, PermaNet® 2.0 only fulfilled these criteria in only three of the five countries, and Ghana was among the two countries where nets collected did not meet the WHOPES criteria (WHOPES 12th report, 2009).

An evaluation of the deltamethrin content on the side and roof of PermaNet® 3.0 indicated that after 1 year, 2 years and 3 years, the mean deltamethrin content was 3.77 g/kg, 3.6 g/kg and 3.24 g/kg, corresponding to a loss of 19%, 23% and 30% of the original dose. There was minimal loss of deltamethrin insecticide on the roof compared to the side of the PermaNet®

3.0. There was 50% loss in deltamethrin content on the side of PermaNet® 3.0 after 3 years. This may be as a result of frequent handling of the sides during usage compared to the roof of the nets. Comparatively, the mean deltamethrin content in PermaNet® 2.0 Nets after 1 year, 2 years and 3 years of household use was 0.97 g/kg, 0.88 g/kg and 0.62 g/kg, corresponding to a loss of 24%, 31% and 51% of the original dose The reason for the variation has still not been established but we postulate that the loss may be driven by household usage of the nets.

A recent study carried out in Ethiopia (Solomon *et al*, 2018) showed low survivalship as well as optimal bio-efficacy for up to 24 months for PermaNet® 2.0 in a semi-arid area. This means that several factors including the ecology and socio-cultural practices can affect the efficacy of LLINs in the field.

It was observed that most people wash both PermaNet® 3.0 and PermaNet 2.0 Nets at least once in six months translating to 2 washes in the years. This indicated that on the average nets were washed on average 6 times for the entire duration of the evaluation. However, after 3 years of usage of both nets, a relatively high percentage of the inhabitants had washed PermaNet 2.0 nets at least once compared to PermaNet® 3.0. It was observed that many inhabitants tend to keep and use both nets inside their rooms, and this may have reduced the accumulation of dirt on the nets and the number of washes. It is known that the frequency of washing of nets may be directly corrected with the pattern of usage and the efficacy of nets as has been shown in other studies (Norris & Norris 2011). The washing frequency of both nets during the study was relatively low and so it is not clear what may have been responsible for the relative loss of both deltamethrin and PBO in PermaNet® 3.0 and PermaNet 2.0. However, it has been shown some nets that have never been washed have lost up to 30% of residual insecticide from the top of the net and 90% from the bottom (Norris & Norris 2011). Although, there was a loss of PBO on PermaNet® 3.0, its impact on mosquito mortality could not be measured in this study. Initial variation in nets have also been shown to be responsible for some variation observed in the field. Most of the inhabitants wash both sets of nets with commercial bar soap using pipe-borne water or water from the lake that is in the area.

The most common adverse effects that net users reported for PermaNet® 3.0 nets two weeks after the distribution included itching and some form of facial burning although the number constituted less than 8% of the users. The complainants were referred to the hospital for examination and it was found that other non-users had similar symptoms and therefore the symptoms could not have been directly as a result of the use of the nets. The inhabitants were

also educated as to how to process new nets before usage. However, no net user complained of any advent event one month after the distribution of both PermaNet® 3.0 and PermaNet® 2.0.

7.0 CONCLUSIONS AND RECOMMENDATIONS

The physical integrity and community acceptance of both nets are relatively good after 36 months of usage. PermaNet® 3.0 met the criteria for efficacy after 3 years of usage. However, PermaNet® 2.0 failed to meet the efficacy criteria probably as a result of the high percentage loss in deltamethin content over years of usage. This was not surprising because in a multi-country study carried out by WHOPES, PermaNet® 2.0 only fulfilled these criteria in only three of the five countries, and Ghana was among the two countries where nets collected did not meet the WHOPES criteria. The deltamethrin content on the roof of PermaNet® 3.0 was relatively high compared to the side after 3 years of usage.

Although, usage practices such as washing and other factors may also have affected the efficacy of the nets over the years, continues education of inhabitants on the proper use of nets also improved efficacy and integrity of both set of nets. Therefore, continues education of net users is very important to improve the effectiveness and protect the integrity of LLINs.

Based on the results of the evaluation, it can be concluded that although the physical integrity of both set of nets was good, PermaNet® 2.0 failed to meet the criteria for efficacy whilst PermaNet® 3.0 complied with the WHO recommended criteria after 3-years post-distribution and usage.

8.0 REFERENCE

Afari EA, Appawu M, Dunyo S, Baffoe-Wilmot A, Nkrumah FK (1995). Malaria infection, morbidity and transmission in two ecological zones in southern Ghana. *Afr J Health Sci*. 2:312–316

Binka, F., Kubaje, A., Adjuik, M., Williams, L., Lengeler, C., Maude, G., Armah, G., Kajihara, B., Adiamah, J. and Smith, P. (1996). Impact of permethrin-impregnated bednets on child mortality in Kassena-Nankana District, Ghana : a randomized controlled trial. *Tropical Medcine and International Health*, **1** :147-154.

Bingham, G., R.V. Gunning, K. Gorman, L.M. Field, G.D. Moores (2007). Temporal synergism by microencapsulation of piperonyl butaoxide and alpha-cypermethrin overcomes insecticide resistance in crop pests. *Pest Manag Sci*., 63: 276-281

Bøgh, C., Clarke, S.E., Pinder, M. (2001). Effect of passive zooprophylaxis on malaria transmission in the Gambia. *Journal of Medical Entomology*, **38 (6):** 822 – 828.

D'Alessandro, U., Langerock, P., Benett, S., Francis, N., Cham, K. and Greenwood, B. (1996). The impact of National Impregnated Bed Net Programme on the outcome of pregnancy in primigravidae in the Gambia. *Transactions of the Royal Society of Tropical Medicine and Hygiene,* **90(5):** 487-492**.**

Lengeler, C., de Savigny, D., and Cattani, J. (1996) From research to implementation. In: Lengeler, C., Cattani, J., & de Savigny, D. (eds.): *Net gain: a new method for preventing malaria deaths*. Geneva, World Health Organization/Ottawa, International Development

Killian A, Byamukama W, Pigeon O, Atieli F, Duchon S, Phan C. Long-term field performance of a polyester-based long-lasting insecticidal mosquito net in rural Uganda. Malar J. 2008;7:49.

Mbogo, C. N. M., Baya, N. M., Ofulla, A. V. O. Githure, J. I. and Snow, Snow, R. W. (1996). The impact of permethrin-impregnated bednets on malaria vectors of the Kenyan coast. *Medical and Veterinary Entomology,* **10**:251-259.

Nuwaha,F. (2001). Factors influencing the use of bednets in Mbara municipality of Uganda. *Americam Journal of Tropical Medicine and Hygiene*, **65:** *877-82.*

Norris, L. C., & Norris, D. E. (2011). Efficacy of long-lasting insecticidal nets in use in Macha , Zambia , against the local *Anopheles arabiensis* population, 1–11

Okrah, J., Traore, C., Pule, A., Sommerfeld, J. and Muller, O. (2002). Community factors associated with malaria prevention by mosquito nets: an exploratory study in rural Burkina Faso. *Tropical Medicine and International health*, **7:** 240-248.

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

Tarekegn Solomon, Eskindir Loha, Wakgari Deressa, Meshesha Balkew, Taye Gari, Hans J. Overgaard and Bernt Lindtjørn Bed nets used to protect against malaria do not last long in a semi-arid area of Ethiopia: a cohort study, Malar J (2018) 17:239

Simon, S.L., Larson, B.A., Zusman, A. and Sydney, R. (2002). How will the reduction of tarrifs and taxes on insecticidetreated bednets affect household purchase? *Bulletin of the World Health Organization*, **80(11):** 892-899.

World Health Organisation. (2018). Test procedures for insecticide resistance monitoring in malaria vectors, bio-efficacy and persistence of insecticides on treated surfaces. WHO unpublished document.

WHO (2009) WHO recommended long-lasting insecticidal mosquito nets. http://www.who int/whopes/long_lasting_insecticidal_nets-Aug09.pdf.

WHOPES 2009, Report of 12th WHOPES working group meeting, Geneva, Switzerland

WHO (2013). Guidelines for laboratory and field-testing of long-lasting insecticidal nets. WHO, Geneva (available at: http://www.who.int/iris/bitstream/10665/80270/1/9789241505277_eng.pdf).

Zaim, M., Aitio, A., Nakashima, N., 2000. Safety of pyrethroid-treated mosquito nets. *Med. Vet. Entomol*. 14, 1-5.