Large-scale (Phase III) evaluation of efficacy, fabric integrity and community acceptability of PermaNet® 3.0 long-lasting insecticidal nets compared with PermaNet® 2.0 in India

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EXECUTIVE SUMMARY

Long-lasting insecticidal nets (LNs) have proved to be effective in vector control in malaria control programmes throughout the malaria endemic regions all over the world. Keeping in view the over-going demand of LNs, many new innovative products are being developed and tested in various eco-epidemiological settings. Apart from nets manufactured from polyester or high-density polyethylene (HDPE), PermaNet 3.0 and PermaNet 2.0 nets with a polyethylene roof and polyester sides and a polyester net, respectively have been developed by M/s. Vestergaard, Frandsen SA. In the PermaNet 3.0 product, technical deltamethrin complying with requirements of the WHO specification is impregnated on to 75 and 100 denier polyester at the target dose of 2.8 g/kg (range 2.1 g/kg-3.5 g/kg) in side parts and incorporated into 100 denier polyethylene at the target dose of 4 g/kg (range 3.0 g/kg-5 g/kg) along with Piperonyl butoxide (PBO) at the target dose of 25 g/kg (range 18.75-31.25 g/kg) in the roof. Target dose of deltamethrin in PermaNet 2.0 is 1.4 g/kg for 100 denier polyester yarn (range1.05 g/kg-1.75 g/kg). Phase III trial of PermaNet 3.0 as a candidate net along with PermaNet 2.0 as a positive control, was carried out in Gujarat, India from September 2014 to December 2017. The trial design involved a comparison of durability of PermaNet 3.0 with a WHOPES fully-recommended LN (PermaNet 2.0) as a positive control.

Four villages of district Anand, Gujarat, India, namely Rail (Old), Rail (New), Kaneval and Valii were selected for this study. Population census had been done before the distribution of both types of nets. Baseline cone-bioassays were carried on 30 nets samples each of PermaNet 3.0 and PermaNet 2.0 nets. Both types of nets were coded with unique codes before distribution. All houses were given unique codes. A net master list was prepared and accordingly all nets were distributed in these four villages. All households were covered. One net was given to each unit family (2.5 people). House hold consent forms were filled during net distribution. A questionnaire based survey was carried out in 100 house holders of each arm for adverse effects of net use in 15 days and one month interval. However, no major adverse effect was recorded during these surveys. Two lists from master list were prepared for withdrawal and cohort surveys. Net withdrawals were carried out at 6, 12, 18, 24, 30 and 36 months of net use. Thirty nets of each arm were withdrawn at 6, 12, 18, 24, 30 months while at 36 months, 50 nets were withdrawn from each arm. Cone bioassay tests were performed on nets against laboratory-reared glucose-fed *An. stephensi/ An. culicifacies* (2-5 days old) after every withdrawal. One set of nets from both arms were sent for chemical analysis that were withdrawn in baseline, 12 month, 24 months and 36 month post distribution as per WHOPES guidelines.

Another list of 350 nets of each arm was prepared for cohort survey. Four cohort surveys were carried out after 6, 12, 24 and 36 months of net use for the determination of net survivorship, usage pattern and physical integrity. The surveys revealed the presence of 98.9% to 85.5% nets survived in 6 months to 36 months in PermaNet 3.0, whereas it was 99.1% to 92.9% in

PermaNet 2.0 arm. Most net users used the nets tucking under the bed (39.8-83.5%), hanging over the sleeping places (30.7-39.9%). Most nets were used either by draping the wooden bed frame (Stick) (54.1-80.5%) followed by wooden bed frame (7.6-17.8%). Usage pattern increased from 78.2% to 94.2% in both arms. More than 74% nets were being used throughout the year. Washing of nets increased from 25.2% to 100% from 6 to 36 months surveys. Generally, detergent powder and local bar soaps were used to wash the nets. During initial survey at 6 month period, the frequency of net drying out side in the sun was very high >80% which was decreased in subsequent surveys up to 9.2% after IEC activities were carried out with householders.

During cohort surveys, holes on nets increased from 9.3 to 88.1 in PermaNet 3.0 and 3.0 to 70.5% in PermaNet 2.0 nets from 6 month to 36 month surveys. Maximum numbers of holes were found in seams followed upper and lower parts of PermaNet 3.0 nets and lower and upper parts of PermaNet 2.0 nets. Some of holes were found repaired either by stitches or knots. Some missing parts of nets were patched by other cloths. Most of the nets were found clean (range 49.2-84.2%) in all cohort surveys.

Thirty net samples withdrawn from each arm at six to 24 months period and 50 nets at 36 month withdrawal were sent for chemical analysis to Walloon Agriculture Research Centre (CRA-W), Belgium. Analysis of deltamethrin content in side parts of PermaNet 3.0 nets and all parts of PermaNet 2.0 nets was done by Gas Chromatography (GC) technique and deltamethrin and Piperonyl butoxide contents in roof of PermaNet 3.0 nets by High Performance Liquid Chromatography (HPLC) technique. The mean deltamethrin in PermaNet 2.0 at baseline was 1.32 g/kg. All the samples of baseline PermaNet 2.0 among 30 samples analysed complied with target dose of 1.4 g/kg±25% for 100 denier yarn (1.05-1.75) except one sample (1.01 g/kg). Relative Standard Deviation (RSD) of the deltamethrin content found in 30 different nets was 10.7% showing an acceptable homogeneity in the active ingredient distribution between the nets. After 1, 2 and 3 year use, the mean deltamethrin content was 0.99 g/kg, 0.60 g/kg and 0.45 g/kg corresponding to a loss of 25%, 55% and 66% of the original dose, respectively.

The mean deltamethrin content in PermaNet 3.0 lower side at baseline was 2.86 g/kg which comply with target dose of 2.8 g/kg±25%. The relative standard deviation was 9.2% showing an acceptable homogeneity of the active ingredient distribution among the nets. After 1, 2 and 3 years of household use, the mean deltamethrin content in nets was 1.79 g/kg 1.17g/kg and 0.88 g/kg corresponding to loss of 37%, 59% and 69% of the original dose, respectively.

The mean deltamethrin content in PermaNet 3.0 upper side at baseline was 2.69 g/kg complying with the target dose of 2.8 g/kg±25%. The relative standard deviation between 30 nets was 5.8% showing an acceptable homogeneity of the active ingredient distribution. After 1, 2 and 3 years of household use, the mean deltamethrin content was 1.88 g/kg, 1.09 g/kg and

0.79% g/kg corresponding to loss of 30%, 59% and 71% of the original dose, respectively.

The mean deltamethrin content in PermaNet 3.0 roof at baseline was 4.32 g/kg complying with target dose of 4.0 g/kg±25%. RSD value 3.1% showing an acceptable homogeneity of the active ingredient distribution among the nets. After 1 and 2 years of household use the mean deltamethrin content was 3.34/kg and 2.8 kg corresponding to loss of 23% and 35% of the original dose, respectively. The mean Piperonyl butoxide in roof at baseline was 25.54 g/kg complying with the target dose of 25 g/kg±25% except one sample whose content was lower than the lowest limit (17.4 g/kg) and the RSD value of Piperonyl butoxide was 6.7% showing an acceptable homogeneity of the ingredient distribution among the nets. After 1, 2 and 3 years of house hold use the contents of Piperonyl butoxide content was 11.46 g/kg, 6.8 g/kg and 5.1 g/kg corresponding to a loss of 55%, 73% and 80% of the original dose, respectively.

Cone-bioassays carried out at baseline on net samples drawn from 30 samples of PermaNet 3.0 and PermaNet 2.0 met with WHOPES criteria (≥80% mortality). In subsequent withdrawal of nets (6, 12, 18, 24, 30 and 36 months) both nets met WHOPES criteria of ≥80% mortality. Tunnel tests were conducted on nets that did not meet WHOPES efficacy criteria in cone tests at 24 and 30 months. Only four samples of PermaNet 3.0 nets failed in tunnel tests. 50 nets from each arm were withdrawn after 36 months use. Results of cone bioassays and tunnel tests combined revealed that all parts of PermaNet 3.0 nets withdrawn at 0, 6, 12, 18, 24,30 and 36 months met the WHOPES criteria of ≥80% mortality. However, it ranged from 93.3 to 100% in PermaNet 2.0 nets.

Washing of cohort nets from both arms increased from 25.2% to 100% from 6 to 36 months surveys. Generally, detergent powder and local bar soaps were used to wash the nets. Most of the nets were washed directly without soaking in water. Most of the nets were not scrubbed hard during washing (72.3-93.9%). During initial survey at 6 month period, the frequency of net drying out side in the sun was very high (>80%) which was decreased in subsequent surveys up to 9.2% after IEC activities were carried.

1. Background

In India, field trials have shown high effectiveness of conventionally treated nets in malaria control (Yadav et al, 2001, Sampath et al, 1998, Mishra, 1999, Sharma et al, 2005, 2006). Currently, the National Vector Borne Disease Control Programme (NVBDCP) in India has been scaling up use of conventionally treated mosquito nets (ITN) and long-lasting insecticidal nets (LNs) in the malaria endemic and high-risk areas.

Long-lasting insecticidal nets that obviate the need for treating nets at home are now widely used for malaria prevention and control throughout the tropical countries including India. New brands of LNs require field evaluation before they are recommended for use in malaria vector control. Thirteen LNs are

now recommended by WHOPES for malaria control: four LNs with full recommendation and nine with time-limited interim recommendation[1].

The WHO Pesticide Evaluation Scheme (WHOPES) introduced in 2006 a system of time-limited (4 years) interim recommendations. After such a recommendation, the manufacturer is required to submit the candidate LN for a large-scale 3-year long testing failing which interim recommendation can be withdrawn. To maintain a range of LNs there is an urgent need to organize 3-year long prospective studies according to WHOPES guidelines[2].

PermaNet® 3.0 manufactured by M/s. Vestergaard Frandsen SA, Switzerland has received interim recommendation by WHOPES (WHO, 2009) and there is a need to carry out a large-scale Phase III evaluation to generate data for its full recommendation for use in the prevention and control of malaria.

The aims of the present project are to assess the efficacy, fabric integrity and community acceptability of PermaNet 3.0. A comparison was made with PermaNet® 2.0 LNs which has received full WHOPES recommendation (WHO, 2009). The study was carried out in Gujarat as part of the WHOPES multi-centre trial of PermaNet 3.0 in an area where the main malaria vector *Anopheles culicifacies,* has shown some degree of resistance to pyrethroids.

2. Description of nets

2.1. Description of PermaNet 3.0 LN

PermaNet 3.0 is a mosaic net combining different LN technologies. The roofing of PermaNet 3.0 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/m^2) at the target dosages of 4.0 g AI/kg and 25 g AI/kg of netting material respectively. The side panels of PermaNet 3.0 are made of multi-filament polyester fibers, 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/m^2) and a side panel made of 75 ± 5% denier (weight 30 ± 10% g/m^2) The target dosage of deltamethrin in the side panels is 2.8 g AI/kg of netting material, i.e 115 mg AI/m^2 of the border and 85 mg AI/m^2 of the remaining of the side panels.

2.2. Description of PermaNet 2.0 LN

PermaNet 2.0 is a deltamethrin-coated LN manufactured by M/s. Vestergaard Frandsen SA (Switzerland). PermaNet 2.0 received full WHOPES recommendation in 2009[3]. The net is made of knitted poly-filament polyester fibers and is treated with deltamethrin at 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). The insecticide

[1] http://www.who.int/whopes/en/
[2] http://www.who.int/whopes/guidelines/en/
[3] http://whqlibdoc.who.int/hq/2009/WHO_HTM_NTD_WHOPES_2009_1_eng.pdf

is bound in a resin coating that reduces the amount of insecticide lost during routine washing. WHO specifications for its quality control and international trade were published in 2006[4]. PermaNet 2.0 has been widely distributed and used in many countries for the prevention and control of malaria.

Details of nets received for the trial are as follows**:**

Parameters	PermaNet 3.0	PermaNet 2.0
Size	190x180x150 cm	190x180x150 cm
Colour	Roof: Blue Side: White	Blue
Batch Number	1088 14	1239 13
Manufacturing Date	20.4.2014	2.7.2013
Quantity Received	1500	1500
Yarn	Roof: Polyethylene Side: Polyester	Polyester
Denier	Roof: 100 Side: 75	100

3. Objectives

It was proposed to undertake a Phase III (household randomized) evaluation 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 acceptability of LNs. Accordingly, household randomized trial would be undertaken in an area of central Gujarat, India with the following objectives:

- to determine and compare the insecticidal activity and fabric integrity of PermaNet 3.0 LNs with PermaNet 2.0 over three years of use by households under field conditions

- to assess washing mode and washing habits of LNs by the householders, and

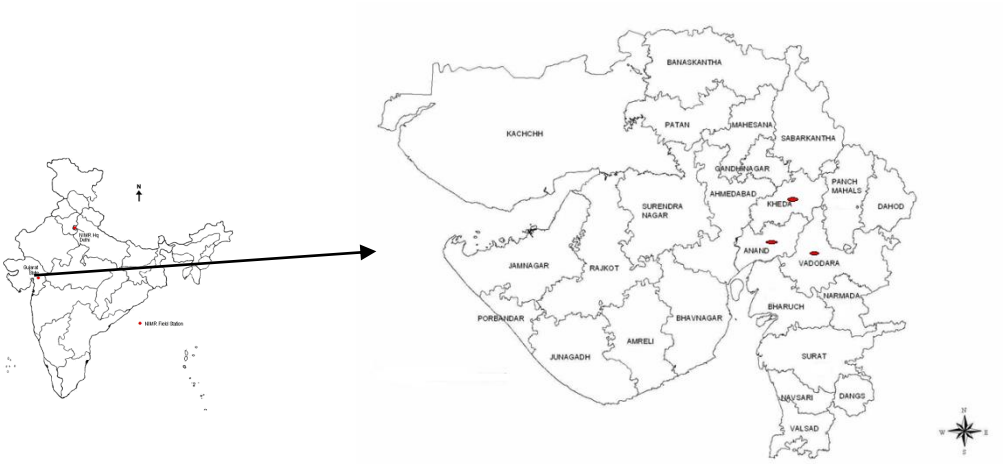
- to assess the community acceptability of LNs over three years of use by households under field conditions.

[4]http://www.who.int/whopes/guidelines/en/

4. Methods

4.1 Study area

In Gujarat, the study was carried out in a rural area of central Gujarat including districts of Kheda (22º45'-22º 75'N and 72º 41'-72º-68'E) having an area of 3943 sq. km and Anand (22º 34'-22º 57' N and 72º 56-72º 93' E) having an area of 4690 sq. km. The villages are well developed and connected by all-weather roads. Most villages in the area have a school and the literacy rate is about 70%. Agriculture is the major occupation of the people and main crops grown in the area are paddy, wheat, groundnut, cotton, millet, banana, tobacco and potato. Almost every village has a milk co-operative society and all villages have respective '*Panchayat*' (village council). *Panchayats* are a part of the local-self government.



4.1.1 District level health services

The District Malaria Officer, who is a vector control person, heads the malaria control programme at the district level. He/she reports to the Chief District Health Officer in the district and to the Joint Director (Malaria) at the state level. The health infrastructure comprises of Sub-centres, Primary Health Centres (PHC), Community Health Centres (CHC) and a district level referral hospital. Malaria is a major public health problem in Gujarat. Under the primary health care there is a system of fortnightly visits by multi-purpose health workers (MPHWs) to each family. Additionally Accredited Social Health Activists (ASHAs) run Fever Treatment Depots where slides are obtained and presumptive treatment given.

4.2 Study design

The efficacy of candidate LN (PermaNet 3.0) was compared with PermaNet 2.0 for which full recommendation by WHOPES is available. This was a prospective, household randomized, equivalence trial with nets as unit of observation. In this study, PermaNet 3.0® LNs was compared with PermaNet 2.0 to determine, if their efficacy is as good as that of the latter.

The efficacy of candidate LNs distributed in the villages was monitored up to three years of continuous use under the field conditions.

4.2.1 Selection of villages

The communities/villages for the study were selected in consultation with the state/district health authorities in an area where communities are conversant with use of nets. No indoor residual spraying was undertaken in the study villages. Four villages, namely Rail (Old), Rail (New), Kaneval and Valli of Rohini PHC of Tarapur taluka of District Anand, were selected for this trial. 869 PermaNet 3.0 and 765 PermaNet 2.0 nets were distributed in these villages. All families were covered with net distribution.

4.2.2 Community counseling

Following activities were scheduled as part of the community entry activities:

- to obtain permission to use the community as a study site;
- to inform the community members of the study objectives and methodologies and;
- to seek community acceptance for use;
- to seek their support in successful conduct of the study.

Community level meetings were organized to educate all the people in the selected villages on the adverse consequences of malaria, the benefits of using long-lasting insecticidal nets, proper handling and use of nets in line with WHO recommendation (WHO, 2002) 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 were 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 to be enrolled in the study at the time of census survey when all potential households were visited by a team of 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 a witness was asked to sign. Those who refuse to participate in the study had the option to accept or refuse LNs.

If at any point in time during the study a participant decides 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 and their data were excluded from the study. The initial sample size was adequate enough for the study.

At the time of distribution of an LN, every headperson 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.

In each study village, a general consent of the village head was obtained and a village meeting was conducted to give details about the purpose of the study. Then, informed consent was obtained from headperson of participating households to participate in the study by door-to-door visits before distribution of LNs. An informed consent form is attached vide Annex 3. 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, head of village council and community leaders was taken to inform about the objectives of the trial and its components.

4.2.3 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 the 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 had been done and detailed census with the name, age, sex and level of education of every family member was recorded in registers.

4.2.4 Distribution of nets

The two brands of LNs were distributed free of cost to households by random allocation by door-to-door visit in the study villages. Randomization had been done at household level. All sleeping places in a given household would receive the allocated LN to ensure universal coverage of the selected community/village.

A net master list was created. 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 to allocate to PermaNet 2.0 or PermaNet 3.0. After random allocation of the households to the two groups of nets (PermaNet 3.0 and PermaNet 2.0), 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, the full code of the factory, batch number, and type of net, household code, net code and dates of net distribution, sampling and replacement, if any. Only the Principal and Co-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.

4.2.5 Sampling of LNs

Sampling of nets for insecticidal activity as well as net survivorship and fabric integrity were done as detailed below:

Month	**[1] Number of LNs of each brand withdrawn destructively for chemical assay & bioassay. Fabric integrity of all these nets is also checked in laboratory**				**[2] COHORT STUDY Sample size for inspection of LNs in situ (for survivorship & fabric integrity)**	
	PermaNet 3.0		PermaNet 2.0		PermaNet 3.0	PermaNet 2.0
	Chemical assays + bioassays	Bio-assays alone	Chemical assays + bioassays	Bio-assays alone	n = 350 LNs*	n = 350 LNs*
0	30		30		-	-
6		30		30	350	350
12	30		30		350	350
18		30		30	-	-
24	30		30		350	350
30		30		30	-	-
36	50		50		350	350

*All these nets or those remaining at HH were inspected

4.2.6 Durability assessment

Durability assessment included the following:

a) Net survivorship and fabric integrity using a cohort design in which 350 LNs of each brand were identified at the beginning and these nets were inspected after 6, 12, 24 and 36 months.

b) Insecticidal activity (bioassays and chemical assays) was monitored at baseline and then every 6 months until the end of the study. A cross-sectional survey of the remaining nets (i.e. excluding the cohort nets) was done in each survey. Detailed procedures are described below:

4.2.6.1 Insecticidal activity

This included tests for bio-efficacy by WHO cone test and, when necessary, tunnel test, as these are direct measures of the amount of insecticide available to contact and kill mosquitoes. Following sampling used for this purpose:

i) Baseline sampling: For quality control purpose, 30 each of the two types of LNs were picked up randomly for baseline chemical assay. From each of these 30 nets, five pieces of 30 cm x 30 cm size for chemical assay and five pieces of 25 cm x 25 cm from adjacent positions for bioassays were cut from positions 1 to 5 as per WHO guidelines (see Figure below) using sharp scissors according to the WHO durability guidelines (WHO, 2011). These two sets of netting pieces were used for chemical assay and bioassays, respectively as described below:

ii) Follow up sampling: For follow up sampling, 30 nets of each brand were withdrawn from randomly selected households at the end of 6 months, year 1 and 2, while 50 nets were withdrawn at the end of year 3. This is called destructively sampling of LNs. These nets were replaced with un-coded LNs of the same brand at the same time. This required selecting 30 each of ID numbers from both LN groups. After destructive sampling and replacement of new nets, such households were excluded from the study. LNs selected randomly and withdrawn from a household for bioassays and or chemical analysis as described above had been replaced with a new net of the same brand.

4.2.6.2 Chemical assays

Selection of net pieces (PermaNet 2.0)

The 5 netting samples (25 x 25 cm size) from 30 nets of PermaNet 2.0 brand were rolled up and placed in labelled, new clean aluminium foil for storage at +4°C temperature prior to dispatch for chemical analysis. The five sub-samples were then assembled as one sample for chemical analysis at WHO Collaborating Centre, Gembloux, Belgium. In subsequent samplings (year 1, 2 and 3), only four sub-samples from positions 2 to 5 were cut and assembled for chemical analysis. Sample from position 1 was not taken since netting fabric at this position is subjected to excessive abrasion in routine use (this portion of net is frequently manipulated while tucking the nets under the bed/mattress).

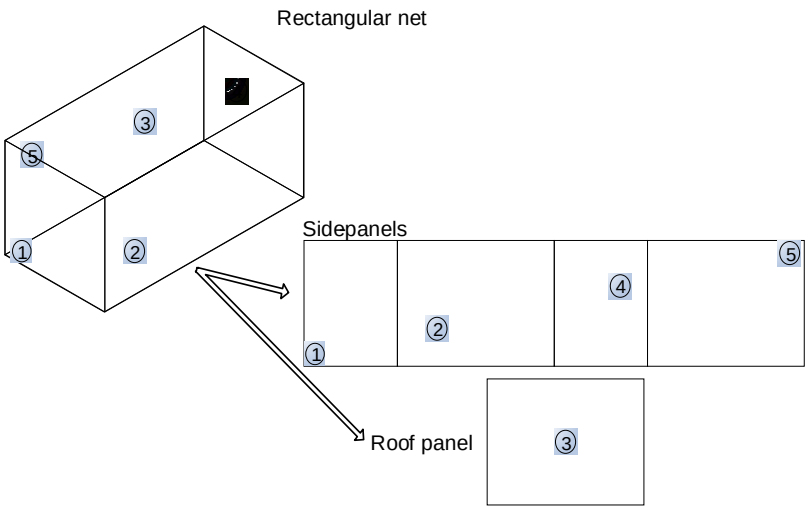


Figure 2. Sampling pattern of PermaNet 2.0

Selection of net pieces (PermaNet 3.0)

From each of the 30 PermaNet 3.0 sampled at baseline, 8 pieces had been cut from the side panels by a diagonal sampling i.e., position number 1 to 4 on the lower part of the side panel (strengthened border), and positions 5 to 8 on the upper part of the side panels (Fig. 3). Two positions each on the lower and upper parts were close to the seams. These 8 pieces were combined in order to provide average deltamethrin content for the side panels[5]. However, at the end of years 1, 2 and 3, samples from position 1 were not taken and the average content of deltamethrin was determined by combining only 7 samples (positions 2 to 8).

Three samples are also proposed to be taken along the longer diagonal of the roof panel. These 3 pieces were combined in order to give an average deltamethrin and PBO content for the roof of the net.

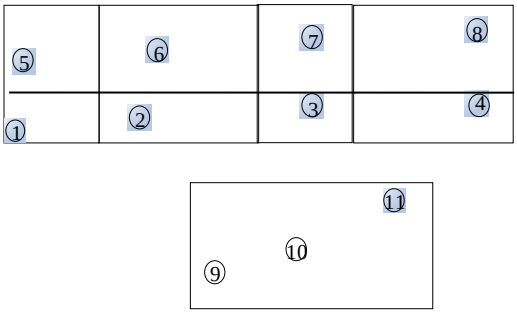


Figure 3. Sampling pattern of PermaNet 3.0

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 PermaNet 3.0 and PermaNet 2.0 were sent for chemical analysis to WHO Collaborating Centre, Gembloux, Belgium under intimation to WHOPES, Geneva. The four sub-samples from each LN brand were combined to provide the average target concentration of the insecticide in each LN. The sampling scheme for PermaNet 2.0 was according to WHO (2013).

Biological assays

Cone bioassays: The cone bioassays were done once at the start (baseline) of the study, and every 6 months thereafter up to 3 years. As mentioned in Table 1, 30 nets of each brand were sampled in each survey; except for 50

[5]Note: The side panels with kitted multifilament polyester (75 denier) fibres are coated with deltamethrin at the target dose of 2.8 g/kg although the fabric of the lower part of the side panels is more densely knitted (40± 10% g/m²) than the upper part (30± 10% g/m²).

nets in the survey after 36 months. The sampling scheme for bioassays is mentioned below.

PermaNet 2.0: Cone bioassays were performed as recommended by WHO (2013). In each sampling, bioassay on net pieces cut from the side of positions 1 to 5 were done (adjacent to chemical assay samples).

PermaNet 3.0 : In each sampling, bioassays were carried out on net pieces cut on the side of positions 2, 3, 7 and 8 (to include different sides and closeness to middle position or to the seam), as well as number 10 from the roof panel. Thus, only 5 net pieces had been cut for bioassays.

On each netting sample, standard WHO cone was placed and held in place using a plastic manifold. Five laboratories reared susceptible *An. culicifacies/An. stephensi* 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 and total 50 mosquitoes were exposed per net (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. Data were recorded in a structured form for further analysis. Nets selected randomly and withdrawn from a household for destructive sampling had been replaced with a new net of the same brand and the households were excluded from the study for future sampling and study.

Withdrawn LNs which failed WHO criteria (knockdown: <95% or a bioassay mortality: <80%), were subjected to a tunnel test. The tunnel tests were performed as described in the WHOPES guidelines with a small rabbit as the bait animal as detailed below:

Tunnel test: The tunnel tests were carried out in the laboratory. The purpose was 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. Data were recorded in structured forms for further analysis.

From each LN that did not meet WHOPES efficacy criteria in cone test, only one out of four net pieces was selected for the tunnel test in PermaNet 2.0 nets. However, in PermaNet 3.0 nets, one piece of each side (lower, upper and roof) was selected for tunnel test. The selected piece was 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, 2013). 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 is 400 cm^2 (20 cm x 20 cm), with nine holes each 1 cm in diameter: one hole is located at the centre of the square; the other eight are equidistant and located at 5 cm from the border. In the shorter section of the tunnel, a small rabbit was placed as bait, 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 were introduced at 18:00 hrs. The following morning at 09:00 hrs, 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 were recorded. During the tests, Tunnels were 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-bioassay and tunnel tests were considered together to judge on LN performance. A candidate net is deemed to meet the requirements of a LN if at the end of the study period of 3 years, at least 80% of sampled nets retain bio-efficacy based on WHO cone bioassay and/or the tunnel test as detailed in the WHO guidelines[6].Whenever the mortality in mosquitoes exposed to such 3 year old LNs in the WHO cone bioassays fell below 80% or knockdown fell 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 <50% of blood-feeding rate in controls had been established for tunnel tests.

4.2.6.3 Net survivorship and fabric integrity

i) Net survivorship rate (cohort survey)

The measure net survivorship, a cohort of study design had been used. From the net master list, all 350 IDs for each of PermaNet 3.0 and PermaNet 2.0 LNs were selected and these were served as a cohort for subsequent follow up after 6, 12, 24 and 36 months. Questionnaire surveys had been used for net survivorship, along with fabric integrity assessment.

To measure survivorship, households provided with 700 nets (350 x 2 brands) were surveyed. Physical presence of the LNs having selected ID was recorded. If the net was still present in the household, the investigator had recorded whether the net was used for its intended purpose. Nets that had

[6]Bio-efficacy criteria:
Cone bioassay: criteria for acceptance- mortality in mosquitoes >80% or knockdown >95%.
Tunnel Test: mortality in mosquitoes >80% or blood feeding inhibition >90%.

never been used were also recorded but excluded from the analysis. Such surveys had provided data on survivorship rate (or conversely attrition rate) of each LN brand.

When a net was found to be no longer present in a household, the householders were questioned to determine the reason for its loss or absence. By this method, it would be possible to monitor the gradual deterioration of individual nets as each net was inspected four times during the 3-year study. However, it was not possible to pinpoint the exact time when a net was discarded or no longer used due to wear and tear because this was the first and only visit to the household.

As explained in Table 1, all 350 LN codes of the cohort were selected from the net master list for each LN product. Through household visits, all 350 identified nets of each LN brand were surveyed to record physical presence/absence of the nets. When a net was found to be no longer present in a household, the house holders were questioned to determine the reason for its loss or absence. An issue to consider with prospective studies was the 'Hawthorne effect', the possibility that the regular visits made people more inclined to retain their nets and less likely to give them away or to dispose of them when damaged as they know that the investigators will return to inspect the nets. As a check on this, the results of the cohort study were compared with the results of the physical integrity assessment of those nets that were destructively sampled for bioassays. While it was possible that nets were less likely to be discarded in the cohort study, the cohort approach did offer a better opportunity to compare the gradual deterioration of individual nets between the 2 study arms and therefore the true fabric integrity of the two types of nets in the study area.

ii) Fabric integrity

Changes in fabric integrity of nets were assessed using the following sampling procedures:

i) Fabric Integrity of all nets that were collected for bioassays were checked in the laboratory at every 6-month sampling period. The data were analysed separately and compared with cohort study as described below.

ii) All 250 nets per brand visited in cohort survey after 6, 12, 24 and 36 months post-distribution (or whatever number surviving and being used by the households) were inspected at the household for physical integrity. For physical integrity assessment, the head person was interviewed by the project team using the questionnaire given in Annex 8 at each survey point (6, 12, 24 and 36 months after net distribution).

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 were assessed (Annex 8, section 4). 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 were 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.

Fabric integrity was calculated as per WHO guidelines. Broadly, following table was used to measure hole index:

Calculation of hole Index (HI)

Hole size categories	Diameter of the hole in cm (mid-point of the hole size category)	R (Radius = Diameter/2)	R^2	Pi (π) value	Area of circle/hole (= Pi * Radius^2)	Divider	Weight
0.5-2.0	1.25	0.625	0.390625	3.142	1.23	1.23	1
2-10	6	3	9	3.142	28.28	1.23	23
10-25	17.5	8.75	76.5625	3.142	240.56	1.23	196
>25	30	15	225	3.142	706.95	1.23	576

Weights for each size category were estimated assuming that the average diameter for holes in the different size categories was the midpoint of each category (i.e. 1.25 cm, 6 cm, 17.5 cm) except for the largest hole size where the average diameter was assumed to be 30 cm. The approximate area of an average sized hole from each category was then estimated by assuming each hole, as approximately circular such that the average area of a hole in each size category could be estimated by the formula: πr^2, where π (pi) = 3.142 and r = diameter divided by 2. Final weights for each size category were then estimated by dividing the area encompassed by a hole in the smallest size category (i.e. 1.23).

4.2.7 Adverse effects

An assessment of the adverse effects was made using a questionnaire. Questionnaire was administered to 100 HH in each study arm after 15 days to 1 month post-net distribution. The people were advised on such possibilities as part of information given in the Informed Consent form. The Principal Investigator had selected required ID numbers from each LN arm from their respective master lists using a random selection procedure. The list of selected nets were re-sorted in ascending order and given to the field team who had visited at the study area once within one month after distributing the nets to record perception of the participant users and to record any adverse effects.

4.2.8 Insecticide susceptibility tests

Insecticide susceptibility tests at diagnostic concentrations of the candidate insecticide (deltamethrin) were conducted every year during the course of the study.

Blood-fed and gravid *An. culicifacies* females were collected from houses using aspirators and maintained in the laboratory to lay eggs that were used for rearing F_1 progeny for susceptibility tests. If necessary, surveys were made to collect *Anopheles* vector larvae from breeding sites in localities where adult collections were made. The larvae were also reared to adults for use in insecticide susceptibility tests. Laboratory reared 2 to 5 days-old, non-blood fed females obtained as described above were used for the insecticide susceptibility test at diagnostic concentrations of deltamethrin to determine susceptibility level using the standard WHO methodology (WHO, 1998) for the candidate insecticide.

Susceptibility status of the *An. culicifacies* colony used for bioassays was monitored each year.

4.2.9 Efficacy criteria for Phase III

Using data obtained through questionnaire, community acceptance of LNs (use rate, perceived benefits in malaria control, any adverse effects, washing and upkeep practices) and attrition rate was assessed.

PermaNet 3.0 as a candidate LN 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 or >80% mortality) and the tunnel test (>80% mortality or >90% blood-feeding inhibition).

5. Ethical and National Clearance

5.1. Ethical clearance: The study involved the ethical issue of protecting people's rights, possible inconveniences caused to them and protecting infringement of privacy of women during the study and more specifically during census and sociological surveys. The survey teams included a sociologist and a woman health worker to ensure that no infringement on human right occurred during the survey.

Animal ethics clearance for this project was obtained for use of rabbits in tunnel tests

Considering these issues, necessary ethical clearance was obtained from the Institutional Ethics Committee of the National Institute of Malaria Research prior to initiating the study as indicated in the activity schedule.

5.2. National clearance: A clearance of the Screening Committee, Ministry of Health & Family Welfare, and Govt. of India was obtained to undertake this study.

6. Results:

6.1. Household survey

Four villages, namely Rail (New), Rail (Old), Kaneval and Valli of Rohini PHC of Tarapur taluka, district Anand, Gujarat, India were selected for the study. Population census was done in the months of December 2013 to January 2014 (Plate 1)**.** A total of 332 population (Male: Female = 51.5: 48.5) living in 805 houses was covered under LNs distribution. Results of population census and other relevant information of villages are presented in Table 1. About 38% houses were pucca followed by Kuchcha (33.66%) and Semi-pucca (28.85%); and 61.45 % population was literate. The main occupation of the villagers is agricultural and animal farming. All villages are electrified and main source of drinking water is pipe line supply. Every family had their own plain nets. During the house hold survey one question was asked for preference of nets and 57.14% prefer any type of nets followed by polyester (33.29%) and nylon (9.69%).

6.2. Distribution of nets

Distribution of LNs was carried in August-September 2014. All nets of both types and houses were coded before net distribution (Plate 2 &3). A total of 1454 nets (689 PermaNet 3.0 and 765 PermaNet 2.0) were distributed in 805 houses covering the population of 3432 (1.8 nets/HH). Each unit family (2.4 people) received single net for protection. Both types of nets (PermaNet 3.0 and PermaNet 2.0) were distributed randomly, i.e. each HH received a particular type of net as per pre-determined distribution plan. Village-wise distribution of nets is presented in Table 2.

6.3. Assessment of adverse effects

Two hundred houses from each arm (PermaNet 3.0 and PermaNet 2.0) were selected for the assessment of adverse effects of net use after 15 days and 30 days use. In all, 209 net users from PermaNet 3.0 and 242 from PermaNet 3.0 net users were interviewed through questionnaire based survey (Table3). No adverse effect was reported by any users after 15 days and 30 days period of net use. However, among PermaNet 3.0 users, itching was reported by 10%, facial burning by 30%, sneezing by 7% and discharge from nose by 25.5% up to 2 to 3 days of net use. Similarly, 7% of PermaNet 2.0 users reported itching, 98.5% facial burning, 3% sneezing and discharge from nose by 1.5% of users up to 2-3 days.

6.4. Net survivorship and usage pattern

6.4.1. Cohort Nets

Initially, 350 nets of each arm were surveyed using questionnaire based survey after six month use (Plate 4 and 5). In all, 291 PermaNet 3.0 were actually surveyed (83.1%), 55 nets were either locked or shifted to other working places by net users and only 4 nets were found lost (1.1% net loss). In 12, 24 and 36 month surveys, the per cent surveyed nets were 89, 71.5 and 85.4% respectively in PermaNet 3.0 arm. The per cent loss of nets varied from 1.1 to 7.17 in 6 months to 36 months surveys. Similarly, in PermaNet 2.0 arm, the per cent nets actual surveyed in 6, 12, 24 and 36 months were 85.1, 91.9, 77.1 and 77.9%, respectively. Net loss varied from 0.9% to 3.0% (Table 4).

Questionnaire based surveys of 350 cohort nets of each arm revealed the presence of 98.9% nets of PermaNet 3.0 and 99.1 % nets of PermaNet 2.0 after 6 month use (Table 5). Net survivorship declined in each study arm from 98.9 to 85.1 % in PermaNet 3.0 and 99.1 to 92.9 % in PermaNet 2.0 over three year period. Sleeping habits of net users gradually increased from 86.6% to 94.8% in PermaNet 3.0 arm from 6 month cohort survey to 36 month cohort survey. Similarly, it was 78.2 to 91.2% in PermaNet 2.0 arm. Net users were asked about net used last night for sleep, the affirmative response varied from 86.6 to 93.6% in different time periods in PermaNet 3.0 arm. In PermaNet 2.0 arm, it varied from 78.2 87.7% in different cohort surveys. Question regarding net use in past week, every night net users varied 62.9 to 83.8% followed by most nights (7.6-27.4%), some nights (2.4-10.8%) and not used/don’t know (5.6-13%) in PermaNet 3.0 arm. Similarly, it ranged from 61.2-73.2% for every night followed by 4.4-17.2% (most nights), 2.2-6.7% (some nights) and 6.5-19.8% (not used/don’t know) in PermaNet 2.0 arm. When asked about duration of period of net use in calendar year, >76% responded for use throughout the year (range 76.2-88.1%) followed by only rainy season (6.5-13.7%) and dry season (2.8-6.9%) in PermaNet 3.0 arm, whereas it was 74.9-81.9% whole year followed by 8.5-13.8% rainy season and 2.3-6.4% in dry season in PermaNet 2.0 arm. Most suitable sleeping places were found to be wooden bed frame (stick) (65.4-82.9%) in both arms. Tucking of PermaNet 3.0 nets varied from 39.8-83.5% in different surveys, whereas it varied from 42.2-60.7% in PermaNet 2.0 arm.

Results of storage of nets of both arms and sleeping places were also checked during every cohort survey. Most of the PermaNet 3.0 nets were found hanging loose over sleeping places (range 33.0-39.9%) followed by hanging tied in knot (range 11.6-23%) stored away (14.8-27.1%) and visible but not hung up (2.4-12.2%). Similarly, in PermaNet 2.0 arm, 32.7-37.9% nets were found hanging loose over sleeping places, 11.1-18.7% hanging tied in knot, 10.7-20.5% visible but not hung up and 19.9-39.3% stored away. During every cohort survey, sleeping places were also checked where nets were found in hanging position. Most preferable places were wooden bed frame (stick) ranging from 64.1 to 0.5% followed by wooden bed frame (7.6-17.8%) in PermaNet 3.0 arm. Similar observations were also made in PermaNet 2.0 net users; it was wooden bed frame (stick) 56.6-75.5% and wooden bed frame (10.4-13.5%).

6.4.3 Withdrawan nets

Thirty nets from each arm were withdrawn after 6, 12, 18, 24, 30 months and 50 nets from each arm were withdrawn after a 36 month period. During the withdrawal of nets, questionnaire based surveys were also carried out in these houses similar to cohort surveys. Table 6 presents the data of pattern of usage and storage practices of withdrawal nets. These nets were found in the house hold during the surveys. It revealed that when HHs were asked the question regarding the use of nets, whether the net had ever been used by them for sleeping, >80% responded positively (range (8100%) in both the arm. Similarly, > 80% respondents answered that they have used these nets last night**.** Question regarding net use in past week, every night net users varied 68 to 93.3% followed by most nights (3.3-20.0.4%), some nights (3.3-10.0%) and not used (0.10.0%) in PermaNet 3.0 arm. Similarly, it ranged 56.6-83.3% for every night followed by 4.4-17.2% (most nights), 2.2-6.7% (some nights) and 6.5-19.8% (not used) in PermaNet 2.0 arm. When asked about duration of period of net use in a calendar year, > 66.7 responded to the all throughout the year (range 66.7-967.1%) followed by only rainy season (0-16.7%) and dry season (3.3-16.7%) in PermaNet 3.0 arm, whereas it was 73.3-94.09% for the whole year followed by 0-6.7% rainy season and 0-10% in dry season in the PermaNet 2.0 arm. Most suitable sleeping places were found to be wooden bed frame (stick) (10-100%) followed by metal bed frame (range 0-80%) in both arms. Tucking of nets varied from 26.7-86.7% in different surveys of both arms.

During withdrawal of nets in different time intervals from both arms, storage of nets of both arms and sleeping places were also checked. Most of the PermaNet 3.0 nets were found hanging loose over sleeping places (range 30.0-63.3%) followed by hanging folded (range 13.3-30%), hanging ties in knots (6.7-20.0%). stored away (6-26.7%%) and visible but not hung up (2.4-12.2%). Similarly, in PermaNet 2.0 arm, 30-40.9% nets were found hanging loose over sleeping places, 10-20% hanging tied in knot, 10.0-33.3.3% stored away and 0-9.1% visible but not hung up and. During every withdrawal, sleeping places were checked where nets were found in the hanging position. Most preferable places were wooden bed frame (stick) ranging from 0-90% followed by wooden bed frame (range 0-20%), and metal bed frame (0-16.7%) in the PermaNet 3.0 arm. Similar observations were also made in the PermaNet 2.0 net users; it was wooden bed frame (stick) 0-90% followed by metal bed frame (0-73.3%) and wooden bed frame (3.3-13.6%).

6.5. Washing behaviour of nets (Community practices)

6.5.1 Cohort nets

Results of washing patters, usage of soaps and drying of nets are presented in Table 7. During 6th month survey, 40% of net were washed which increased gradually from 80% to 97% in successive surveys in the PermaNet 3.0 arm. Similar pattern was also observed in PermaNet 2.0 arm ranging from 25.2-100% in successive period of surveys. When the net users were asked about the time of washing, most of the HHs responded that 3-6 month period (range 13.5-30.5% in PermaNet 3.0) and 13.8-46.7% in the PermaNet 2.0 arm, followed by 1-3 month ago and 1 week to 1 month ago in both arms. Most of the net users prefer detergent powder (range 38.5%-55.3%) followed by local bar soap (range 25.8-38.9%) for washing in both the arms. When asked the question about soaking of nets before washing, most of the respondents did not soak the net (range 79.8-100%). It was also observed that most of the nets users did not scrub hard or beaten the nets during washing (range 72.3-93.9%) in both arms. Interestingly, during 6 month cohort survey, when interviewers were asked about drying place of net after washing, it was found that most of the nets of both arms were dried out side in the sun (84.7% PermaNet 3.0 and 96% PermaNet 2.0), however, the drying pattern had changed from 55.9% to 8% in both arms in one to 3 years cohort surveys after IEC activities were carried out for drying of nets in the study area.

6.5.2 Withdrawn nets

Information of net washing pattern, soap usage and drying patterns and other relevant information were also collected during each withdrawal period having six months gap (Table 8). HHs were asked whether they have washed the nets , the positive answers ranged from 40.0 to 96.7% in different time intervals in the PermaNet 3.0 arm, however, it was 30 to 92% in the PermaNet 2.0 arm. Further, 6.7-77.8% HHs responded that they have washed the nets 3-6 months ago followed by HHs who reported that they had washed after 1-3 months ago (range (8.32 -62.5%) in both arms.

6.6 Physical integrity of nets

6.6.1 Cohort Nets

Cohort nets of each arm (PermaNet 3.0 and PermaNet 2.0) were examined for fabric integrity after 6, 12, 24 and 36 months post distribution. In the first survey after six months, 9.3% nets had developed holes in PermaNet 3.0 arm and 3% of nets were having holes in PermaNet 2.0 arm. Gradually, the percentage of nets having holes had increased from 39.4 to 88.1% in subsequent cohort survey with the PermaNet 3.0. Similarly, the nets having holes in PermaNet 2.0 arm were 37%, 74.1% and 70.5% after 12, 24 and 36 month cohort surveys, respectively (Table 9). Some of the holes were found repaired either by stitches, knots or patches in all cohort surveys. Observation of physical aspects of nets revealed that 62.2%, 49.2%, 56.2% and 61.1% nets were found clean in successive cohort surveys from 6 to 36 months

period in PermaNet 3.0 arm, whereas it was 84.25, 61.2%, 69.3% and 75.9% in PermaNet 2.0 arm. Results of hole index and hole area data of both arms are presented in Table 10. Hole index was calculated on hole size 2, 3, and 4 whereas hole area was calculated on hole size 1,2,3 and 4. During 6 month cohort survey, the hole index was very high (mean: 935.78) as some of the nets developed large holes due to mishandling by users, which came to 207.98 in 12 month survey of the PermaNet 3.0 nets. It had gradually increased up to 594.29 in 36 month period. However, hole index in PermaNet 2.0 increased from 286.78 to 396.01 from 6 to 36 month period. Similarly, mean hole area in PermaNet 3.0 nets was1149.03 in 6 month but in 12 month survey it came down to 267.06 and 786.84 in 36 month. After 6 month cohort survey, IEC activity was carried out with net users for the protection of nets and its importance. It reflected in coming surveys. Mean hole area in PermaNet 2.0 nets varied from 355.3 to 524.3 in these four cohort surveys.

6.6.2 Withdrawn nets

Results of the physical condition and aspects of withdrawn nets in different time periods are presented in Table 11. In the first withdrawal after 6 month use, 23.3% and 10% nets from PermaNet 3.0 and PermaNet 2.0 arms developed holes. Gradually, the per cent increased from 46.7 to 90 in PermaNet 3.0 nets, whereas it was ranged from 30 to 88 % in PermaNet 2.0 nets. In PermaNet 3.0 nets most of the holes of all size were found in seam part whereas in PermaNet 2.0 nets it were in lower part of nets. Some of holes were found repaired by stitched (range 0-93.3%), knotted (range 0-33.3%) and patched (range 0-8.3%) in PermaNet 3.0. Similarly, in PermaNet 2.0, it was 0-100% stitches, 0-14.5% and 0.7.4% patched work. During each withdrawal, all nets were checked for its cleanliness status. It ranged from 30.0 to 56.7% in the PermaNet 3.0 arm, whereas it was 23.3 to 80% in the PermaNet 2.0 arm. Table 12 presents the fabric integrity of withdrawal nets measured in different time intervals. Mean value of hole index varied from 29.71 728.67 from 6 month to 36 month withdrawals. Hole area varied from 373.7-2716 in PermaNet 3.0 nets. Similarly, in PermaNet 2.0 arm, mean value of hole index ranged from 99.78 to 1771.67 and hole area ranged from 0 to 69%. Results of individual withdrawn nets of different time intervals are presented as Annexure (I-VI).

7. Chemical assay

Thirty net samples of each arm were sent for chemical analysis to Walloon Agriculture Research Centre (CRA-W), Belgium in July 2014 as baseline samples. All net pieces were wrapped in aluminium foil before sending for chemical analysis. Plate 6 shows details of packing of sub samples of each net for chemical assay. The analytical method used for determination of deltamethrin in PermaNet 2.0 and PermaNet 3.0 lower and upper side samples is based on the CIPAC method 333/LN/ (M)/3. Extraction of deltamethrin was done by isooctane/dioxane (80/20, v/v) or heptanes and for roof sample hexane was used as extracting solvent. Dipropyl phthalate or dicyclohexyl phthalate were used as internal standard. Analysis was done by High Performance Liquid Chromatography with UV diod Array detection

(HPLC-DAD). The performance of the analytical method was controlled during the analysis of samples in order to validate the analytical results.

Results of deltamethrin content in PermaNet 3.0 and PermaNet 2.0 are presented in Table 13. The mean deltamethrin in PermaNet 2.0 at baseline was 1.32 g/kg. All the samples of baseline PermaNet 2.0 among 30 samples analysed comply with target dose of 1.4 g/kg±25% for 100 denier yarn (1.05-1.75) except one sample (1.01 g/kg). The between net variation, expressed as relative standard deviation (RSD) of the deltamethrin content found in 30 different nets, was 10.7% showing an acceptable homogeneity in the active ingredient distribution between the nets. In samples collected after 1, 2 and 3 years use, the mean deltamethrin content was 0.99 g/kg,60 g/kg and 0.45 g/kg corresponding to a loss of 25%, 55 and 66% of the original dose, respectively.

The mean deltamethrin content in PermaNet 3.0 lower side at baseline was 2.86 g/kg. All the samples of baseline PermaNet 3.0 lower side among 30 samples analysed comply with the target dose of 2.8 g/kg±25%. The relative standard deviation between 30 nets was 9.2% showing an acceptable homogeneity of the active ingredient distribution among the nets. In samples collected after 1, 2 and 3 years of household use, the mean deltamethrin content was 1.79 g/kg,1.17 g/kg and 0.88 g/kg corresponding to loss of 37%, 59% and 69% of the original dose, respectively.

The mean deltamethrin content in PermaNet 3.0 upper side at baseline was 2.69 g/kg. All the samples of baseline PermaNet 3.0 lower side among 30 samples analysed comply with the target dose of 2.8 g/kg±25%. The relative standard deviation between 30 nets was 5.8% showing an acceptable homogeneity of the active ingredient distribution among the nets. In samples collected after 1, 2 and 3 years of household use, the mean deltamethrin content was 1.88 g/kg, 1.09 kg and 0.79 g/kg corresponding to loss of 30%, 59% and 71% of the original dose, respectively.

The mean deltamethrin content in PermaNet 3.0 roof at baseline was 4.32 g/kg. All the samples of baseline PermaNet 3.0 lower side among 30 samples analysed comply with the target dose of 4.0 g/kg±25%. The between net variation, expressed as the relative standard deviation between 30 nets was 3.1% showing an acceptable homogeneity of the active ingredient distribution among the nets. In samples collected after 1, 2 and 3 years of household use, the mean deltamethrin content was 3.34/kg, 2.8 g/kg and 2.3 g/kg corresponding to loss of 23%, 35% and 47% of the original dose, respectively.

The analytical method used for determination of Piperonyl butoxide in PermaNet 3.0 roof samples was based on the CIPAC method 33/LN/(M)/3. The Gas Chromatography method was used to determine the contents of Piperonyl butoxide using Xylene as extracting solvent and Octadecane or dicyclohexyl phthalate as internal standard. The mean Piperonyl butoxide in roof at baseline was 25.54 g/kg. The entire baseline samples of roof analysed comply with the target dose of 25 g/kg±25% except one sample whose content was lower than the lowest limit (17.4 g/kg) and the RSD value of

Piperonyl butoxide was 6.7% showing an acceptable homogeneity of the ingredient distribution among the nets. In samples collected after 1 , 2 years and 3 years of house hold use, the contents of Piperonyl butoxide were 11.46 g/kg, 6.8 g/kg and 5.1 g/kg corresponding to a loss of 55%, 73 % and 80% of the original dose, respectively (Table 14).

8. Biological Assay

Cone-bioassays were carried out at baseline on net samples PermaNet 2.0 on positions 1 to 5. Whereas, bioassay tests on PermaNet 3.0 nets were conducted on 2, 3, 7 and 8 (different sides; upper and lower) and position 10 at the roof. Plate 8 shows the cone-bioassay tests performed in the laboratory on PermaNet 3.0 and PermaNet 2.0 nets withdrawn at different time intervals. All the nets (baseline) of both arms met the WHOPES criterion of ≥80% mortality. In the subsequent bioassays, positions 2 to 5 were selected in PermaNet 2.0 for the test. In PermaNet 3.0 nets bioassay tests were conducted on the same positions. In subsequent withdrawal of nets (6, 12, 18, 24, 30 and 36 months), both nets met WHOPES criterion of ≥80% mortality. Tunnel tests were conducted on different parts of PermaNet 3.0 and whole net of PermaNet 2.0 which could not meet WHOPES criteria, of 24, 30 month withdrawal. Only four PermaNet 3.0 nets failed in tunnel tests. Results of proportion of nets meeting WHOPES criteria in bioassay and tunnel tests are presented in Table 15.The proportion of passing rate of PermaNet 3.0 (in all parts) nets ranged from 86.7 to 100% in bioassays and tunnel tests combined, whereas, it ranged from 93.3 to 100% in PermaNet 2.0 (whole net). Results of each individual net of both arms of different withdrawal periods are presented in Annexure (VII-XIII).

9. Conclusion and recommendation

This study evaluated the performances of PermaNet 3.0, as interim recommended LN, using a WHOPES recommended PermaNet 2.0, LN as positive control for three years under house hold conditions. PermaNet 3.0 as a candidate LN was considered to meet the efficacy criteria for Phase III studies. After three years, more than 80% nets met WHOPES criteria (≥95% knockdown and ≥85% mortality) in the cone bioassay test. This trial provided unique opportunity to compare the performance of nets of two different fabric types and two chemicals in one LN technology i.e. coating and incorporation.

The trial showed that in both types of nets distributed, survivorship was 98.9% for PermaNet 3.0 and 99.1% for PermaNet 2.0 after 6 months use which declined from 98.9% to 85.1 % in the PermaNet 3.0 arm and 99.1% to 92.9 % in the PermaNet 2.0 arm over three year period. From the period between the 6 months and 36 months surveys, the attrition (complete loss of nets) increased from 1.1 to 5.7% with PermaNet 3.0 nets and 0.9 to 3% with PermaNet 2.0 nets. During the cohort survey, it was found that some of the nets from both arms were taken out side of the study area for use (student hostels, agriculture farms and working places). This showed that House holders are well familiar about the importance of nets. Hole index increased in subsequent cohort surveys and varied from 935.8 to 594.29 in PermaNet 3.0

and 286.8 to 396.6 in PermaNet 2.0. Some of the holes were either repaired by knotting or stitching and missing part of nets were repaired with patched work. This showed that nets users care for their nets. During the cohort survey, it was observed that HHs stitched together two to three damaged nets after proper repairing. They appreciated our contribution for providing this type of nets for their use.

Year-round use of PermaNet 3.0 ranged from 76.2 to 88.1% and 74.9 to 81.9% with PermaNet 2.0. The main reason of high frequency of net use in the study area is presence of big water infested with vegetation. All the study villages are situated around this reservoir. House holders are even protecting their bovine population with mosquito nets.

High frequency of net washing was noted during the survey in spite of instructions at the time of net distribution. The main reason of net washing was, most of the sleeping places situated near the kitchen and HHs using the nets regularly in houses and in agricultural fields. Most of the net users preferred detergent powder for washing. During six month cohort survey, it has been observed that people preferred to dry net in the sunlight (> 80%) which was decreased up to 8% to 9.2 % in 36 month surveys. IEC activity was started in the study area to educate the nets users about drying nets under shade. Net users responded positively. Similar results were also found in nets withdrawn in different time intervals.

From the observations, it can be concluded that PermaNet 3.0 has met WHOPES efficacy criteria after the prescribed period of three years of household use under prevailing mosquitogenic conditions in the highly irrigated agricultural areas of Anand district, Gujarat, India.

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Tables

Table 1: Results of the population census and other relevant information

	Parameters	Rail (Old)		Rail (New)		Kaneval		Valli		Total	
		No.	%	No.	%	No.	%	No.	%	No.	%
Type of houses	**Pucca**	142	55.3	19	16.4	53	21.9	91	47.9	305	37.9
	Kuchcha	55	21.4	79	68.1	92	38.0	45	23.7	271	33.7
	Semi-Pucca	60	23.3	18	15.5	97	40.1	54	28.4	229	28.5
	Total	257	100.0	116	100.0	242	100.0	190	100.0	805	100.0
Population	**Male**	518	49.5	243	51.3	542	53.8	463	51.3	1766	51.5
	Female	529	50.5	231	48.7	466	46.2	440	48.7	1666	48.5
	Total	1047	100.0	474	100.0	1008	100.0	903	100.0	3432	100.0
Education	**Illiterate**	496	47.4	91	19.2	472	46.8	264	29.2	1323	38.5
	Literate	551	52.6	383	80.8	536	53.2	639	70.8	2109	61.5
Occupation	**Farming**	378	36.1	164	34.6	350	34.7	317	35.1	1209	35.2
	Business/service	21	2.0	15	3.2	72	7.1	23	2.5	131	3.8
	Student	112	10.7	132	27.8	321	31.8	199	22.0	764	22.3
	Household	218	20.8	131	27.6	242	24.0	273	30.2	864	25.2
	None (Children/ handicapped)	318	30.4	32	6.8	23	2.3	91	10.1	464	13.5
Choice of Nets	**Nylon**	11	4.3	4	3.4	27	11.2	36	18.9	78	9.7
	Polyester	78	30.4	88	75.9	93	38.4	9	4.7	268	33.3
	None	168	65.4	24	20.7	122	50.4	145	76.3	460	57.1

Table 2: Village-wise distribution of PermaNet 3.0 and PermaNet 2.0 nets

Village	Population	Houses	PermaNet 3.0	PermaNet 2.0
Rail (New)	474	116	219	225
Rail (Old)	1047	257	124	85
Kanewal	1008	242	171	253
Valli	903	190	175	202
Total	3432	805	689	765

Table 3: Assessment of adverse effects among PermaNet 3.0 and PermaNet 2.0 users

Name of the village	PermaNet 3.0			PermaNet 2.0		
	No. of houses selected	No. of interviewers responded after 15 Days	No. of interviewers responded after 30 Days	No. of houses selected	No. of interviewers responded after 15 Days	No. of interviewers responded after 30 Days
Rail (New)	50	50	53	34	34	55
Rail (Old)	48	48	56	66	66	82
Kanewal	49	49	57	50	50	57
Valli	53	53	43	50	54	48
Total	200	200	209	200	204	242

Table 4 : Information of Cohort Nets surveyed for attrition and fabric integrity

Arm	Interval of sampling in month	Survey Nets	Actual Net surveyed	Per cent net surveyed	Out station nets/lock	Net loss	% loss	% Net Survive
PermaNet 3.0	6	350	291	83.1	55	4	1.1	98.9
	12	346	308	89.0	32	6	1.7	97.2
	24	340	243	71.5	72	24	7.1	90.3
	36	316	270	85.4	28	18	5.7	85.1
PermaNet 2.0	6	350	298	85.1	49	3	0.9	99.1
	12	347	319	91.9	21	7	2.0	97.1
	24	340	262	77.1	73	5	1.5	95.7
	36	335	261	77.9	64	10	3.0	92.9

Table 5: Survivorship, pattern of usages and storage practices (per cent) in different cohort surveys

Parameters	Time of follow up in months of cohort nets (350 nets in each arm							
	PermaNet 3.0				PermaNet 2.0			
	6	12	24	36	6	12	24	36
A. Net survivorship	98.9	97.2	90.3	85.1	99.1	97.1	95.7	92.9
B. Sleeping pattern (Usages)								
1. How many people slept in your household last night?								
Adult >15 Year	61.0	59.6	85.0	88.5	67.2	65.7	73.5	83.8
5-15 Year	24.6	25.1	5.3	6.9	17.4	18.1	11.9	9.5
<5 Year	14.4	15.4	9.7	4.5	15.3	16.1	14.5	6.7
2. Has this net ever been used for sleeping under?								
Yes	86.6	83.7	93.6	94.8	78.2	84.1	89.3	91.2
No	13.4	16.3	6.4	5.2	21.8	15.9	10.7	8.8
3. Was this net used last night to sleep under?								
Yes	86.6	83.7	93.6	91.1	78.2	84.1	84.4	87.7
No	13.4	16.3	6.4	8.9	21.8	15.9	15.6	12.3
4. In the past week, how often was the net used?								
Every night	83.8	62.9	72.9	61.9	73.2	61.2	71.1	69.7
Most night	7.6	14.7	10.8	27.4	4.4	19.0	11.1	17.2
Some night	2.4	8.5	10.8	4.8	2.7	6.4	6.7	6.5
Not used at all	3.4	13.0	5.6	5.9	15.8	11.9	11.1	6.5
Don't know	2.7	1.0	0.0	0.0	4.0	1.5	0.0	0.0
5. Number of persons used the Nets last night								
Slept under this net(>15 yr)last night	65.0	64.2	83.9	86.8	63.7	62.4	70.0	79.2
Slept under net (5-15) yr last night	24.5	24.7	7.0	8.5	24.5	24.9	8.7	5.5
Slept under net(<5) yr last night	10.5	11.1	9.1	4.7	11.7	12.7	21.3	15.3
6. During which period of the year is this net used to sleep under?								
All year	81.1	76.2	86.9	88.1	76.8	74.9	81.9	80.8
Only rainy season	6.5	13.7	8.4	8.1	10.1	13.8	8.5	11.5
Only dry season	6.9	5.5	2.8	2.6	6.4	5.2	5.2	2.3
Don't know	5.5	4.6	2.0	1.1	6.7	6.1	4.4	5.4
7. Has this net ever been used over the following types of sleeping places?								
Reed mat	5.2	3.3	0.0	1.1	8.4	4.6	0.0	0.0
Wooden Bed frame	7.6	17.3	9.6	15.6	16.8	10.1	13.3	15.7
Wooden bed frame(stick)	82.8	69.1	82.9	75.6	65.4	75.2	73.0	77.0
Metal bed frame	2.4	6.2	1.6	1.1	4.0	4.3	2.2	2.7
Bare floor or ground	2.1	4.2	6.0	6.7	5.4	5.8	11.5	4.6
8. Do you tuck net?								
Yes	83.5	53.4	39.8	51.9	60.7	44.0	42.2	48.7
No	14.1	45.9	59.8	48.1	37.6	55.4	55.9	50.2
Don't know	2.4	0.7	0.4	0.0	1.7	0.6	1.9	1.1

C. Storage of nets and sleeping places								
1. How is the net found?								
Hanging loose over sleeping place	39.9	33.2	36.7	33.0	32.6	32.7	30.7	37.9
Hanging tied in knot	12.0	15.3	11.6	23.0	13.1	18.7	11.1	12.3
Hanging folded	22.3	19.2	14.7	17.0	20.5	18.0	10.7	15.7
Visible but not hung up	2.4	11.4	10.0	12.2	10.7	10.7	8.1	8.8
Stored away	23.4	20.8	27.1	14.8	23.2	19.9	39.3	25.3
2.. What type of sleeping place is the net hanging over?(observe)								
Reed mat	5.2	2.0	0.0	0.0	3.4	3.1	0.0	0.0
Foam mattress	0.3	1.0	0.0	0.7	3.4	1.2	0.0	0.0
Wooden Bed frame	17.5	16.9	7.6	17.8	10.4	14.7	13.6	13.4
Wooden bed frame(stick)	67.7	54.1	80.5	75.2	57.0	56.6	68.3	75.5
Metal bed frame	7.6	6.2	2.0	1.1	9.4	4.3	2.3	1.1
Nothing	1.4	19.9	10.0	5.2	9.7	19.6	15.8	10.0
Don't know	0.3	0.0	0.0	0.0	6.7	0.6	0.0	0.0

Table 6: Survivorship, pattern of usages and storage practices (per cent) in different net withdrawal surveys

Parameters	Time of follow up (in months) of withdrawal nets (30 nets up to 30 months and 50 in 36 months in each arm)											
	PermaNet 3.0						PermaNet 2.0					
	6	12	18	24	30	36	6	12	18	24	30	36
A. Sleeping pattern (Usages)												
1. How many people slept in your household last night?												
Adult >15 Year	70.9	75.0	56.3	79.6	84.4	93.0	70.8	66.7	56.3	86.0	84.4	91.2
5-15 Year	6.3	2.8	15.0	11.1	8.9	5.3	21.5	2.3	15.0	9.3	8.9	7.4
<5 Year	22.8	22.2	28.8	9.3	6.7	1.8	7.7	31.0	28.8	4.7	6.7	1.5
2. Has this net ever been used for sleeping under?												
Yes	96.7	100.0	93.3	100.0	93.3	98.0	90	93.333	90	100	80	100
No	3.3	0.0	6.7	0.0	6.7	2.0	10.0	6.7	10.0	0.0	20	0.0
3. Was this net used last night to sleep under?												
Yes	90.0	100.0	93.3	100.0	93.3	98.0	83.3	93.3	90.0	100.0	80.0	100.0
No	10.0	0.0	6.7	0.0	6.7	2.0	16.7	6.7	10.0	0.0	20.0	0.0
4. In the past week, how often was the net used?												
Every night	90.0	93.3	73.3	93.3	76.7	68.0	80.0	83.3	56.7	83.3	60.0	74.0
Most night	3.3	3.3	13.3	0.0	13.3	20.0	16.7	3.3	16.7	10.0	16.7	24.0
Some night	6.7	3.3	3.3	6.7	3.3	10.0	3.3	13.3	10.0	6.7	6.7	0.0
Not used at all	0.0	0.0	10.0	0.0	6.7	2.0	0.0	0.0	13.3	0.0	16.7	2.0
Don't know	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	3.3	0.0	0.0	0.0
5. Number of persons used the Nets last night												
Slept under this net (>15 yr) last night	73.0	72.6	53.1	84.0	83.7	76.3	70.8	71.1	61.4	100.0	82.2	94.6
Slept under net (5-15 yr) last night	8.1	5.5	22.2	8.0	7.0	20.3	21.5	2.6	28.4	0.0	6.7	5.4
Slept under net (<5 yr) last night	18.9	21.9	24.7	8.0	9.3	3.4	7.7	26.3	10.2	0.0	11.1	0.0

6. During which period of the year is this net used to sleep under?												
All year	66.7	93.3	76.7	96.7	80.0	90.0	80.0	83.3	73.3	96.7	80.0	94.0
Only rainy season	16.7	0.0	3.3	0.0	13.3	6.0	3.3	6.7	0.0	0.0	6.7	4.0
Only dry season	16.7	6.7	16.7	3.3	3.3	4.0	10.0	10.0	26.7	3.3	3.3	0.0
Don't know	0.0	0.0	3.3	0.0	3.3	0.0	6.7	0.0	0.0	0.0	10.0	2.0
7. Has this net ever been used over the following types of sleeping places?												
Reed mat	3.3	3.3	6.7	0.0	0.0	0.0	6.7	0.0	3.3	0.0	0.0	0.0
Wooden Bed frame	10.0	6.7	16.7	10.0	0.0	6.0	3.3	10.0	13.3	13.3	6.7	14.0
Wooden bed frame(stick)	60.0	80.0	60.0	53.3	100.0	74.0	10.0	73.3	56.7	60.0	93.3	72.0
Metal bed frame	6.7	10.0	10.0	0.0	0.0	14.0	80.0	16.7	3.3	0.0	0.0	0.0
Bare floor or ground	20.0	0.0	6.7	36.7	0.0	6.0	0.0	0.0	23.3	26.7	0.0	14.0
8. Do you tuck net?												
Yes	86.7	66.7	43.3	60.0	26.7	54.0	86.7	53.3	40.0	43.3	26.7	42.0
No	13.3	33.3	56.7	40.0	73.3	46.0	6.7	46.7	60.0	56.7	73.3	58.0
Don't know	0.0	0.0	0.0	0.0	0.0	0.0	6.7	0.0	0.0	0.0	0.0	0.0

B. Storage of nets and sleeping places												
1. How is the net found?												
Hanging loose over sleeping place	63.3	53.3	30.0	53.3	40.0	46.0	33.3	43.3	36.7	46.7	30.0	40.9
Hanging tied in knot	10.0	23.3	20.0	6.7	10.0	16.0	20.0	13.3	10.0	10.0	20.0	13.6
Hanging folded	16.7	13.3	30.0	26.7	13.3	18.0	23.3	33.3	20.0	16.7	20.0	25.0
Visible but not hung up	6.7	0.0	3.3	6.7	10.0	14.0	6.7	0.0	0.0	6.7	3.3	9.1
Stored away	3.3	10.0	16.7	6.7	26.7	6.0	16.7	10.0	33.3	20.0	26.7	11.4
2. What type of sleeping place is the net hanging over? (observe)												
Reed mat	13.3	0.0	3.3	0.0	0.0	0.0	10.0	0.0	3.3	0.0	0.0	0.0
Foam mattress	0.0	0.0	3.3	0.0	0.0	0.0	10.0	0.0	0.0	3.3	0.0	0.0
Wooden Bed frame	20.0	6.7	13.3	10.0	0.0	8.0	3.3	10.0	10.0	10.0	10.0	13.6
Wooden bed frame(stick)	50.0	80.0	56.7	60.0	96.7	90.0	0.0	76.7	43.3	70.0	90.0	77.3
Metal bed frame	16.7	13.3	13.3	0.0	0.0	0.0	73.3	13.3	6.7	0.0	0.0	9.1
Nothing	0.0	0.0	10.0	30.0	3.3	2.0	3.3	0.0	36.7	16.7	0.0	0.0
Don't know	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

Table 7: Washing behavior (per cent) of cohort nets

Parameters	Time of follow up in months of cohort nets (350 nets in each arm							
	PermaNet 3.0				PermaNet 2.0			
	6	12	24	36	6	12	24	36
1. Has the net ever been washed?								
Yes	40.5	80.5	91.6	97.0	25.2	72.8	90.4	100.0
No	59.5	17.9	8.0	3.0	74.8	27.2	9.6	0.0
Dont know	0.0	1.6	0.4	0.0	0.0	0.0	0.0	0.0
2. When was the last time you washed the net?								
1 week ago	8.5	17.8	7.8	12.2	4.0	11.3	11.9	12.6
1 week to 1 month ago	11.9	19.0	34.3	30.2	2.7	23.5	29.1	31.4
1-3 months ago	19.5	33.2	38.7	37.0	17.3	32.8	39.3	39.1
3-6 months ago	30.5	19.8	13.5	14.5	46.7	18.5	16.4	13.8
>6 months ago	26.3	9.7	5.7	6.1	29.3	13.9	1.6	2.3
Dont know	3.4	0.4	0.0	0.0	0.0	0.0	1.6	0.8
3. Type of soap used								
None	20.3	5.7	4.8	7.3	24.0	3.8	2.9	3.8
Local bar soap	32.2	33.6	33.9	38.9	30.7	26.5	25.8	36.0
Detergent powder	44.9	42.9	47.4	38.5	38.7	50.8	55.3	50.6
Mix	0.0	17.8	13.5	14.1	0.0	18.5	16.0	9.6
Dont know	2.5	0.0	0.4	3.0	6.7	0.4	0.0	0.0
4. How long did the net soak for?								
Did not soak	88.1	85.0	96.5	79.8	100.0	89.1	95.1	93.9
<1h	5.9	13.8	3.5	18.3	0.0	10.1	3.7	5.4
>1h	0.8	0.8	0.0	0.8	0.0	0.0	0.4	0.0
Don't know	5.1	0.4	0.0	1.1	0.0	0.8	0.8	0.8

5. Was the net scrubbed hard or beaten?								
Yes	5.1	19.4	10.4	7.6	10.7	27.7	5.3	8.4
No	89.8	80.2	88.7	90.8	88.0	72.3	93.9	91.2
Don't know	5.1	0.4	0.9	1.5	1.3	0.0	0.8	0.4
6. Where was the net dried?								
Outside in the sun	84.7	55.9	14.3	9.2	96.0	60.1	11.5	8.0
Outside in the Shade	9.3	43.7	85.7	90.5	4.0	39.9	86.5	90.4
Inside	5.9	0.4	0.0	0.4	0.0	0.0	2.0	1.5

Table 8: Washing behaviour of withdrawn nets

Parameters	Time of follow up (in months) of cohort nets (30 nets up to 30 months and 50 in 36 months in each arm)											
	PermaNet 3.0						PermaNet 2.0					
	6	12	18	24	30	36	6	12	18	24	30	36
1. Has the net ever been washed?												
Yes	40.0	76.7	83.3	96.7	90.0	96.0	30.0	53.3	80.0	83.3	66.7	92.0
No	46.7	23.3	16.7	3.3	10.0	4.0	63.3	46.7	20.0	16.7	33.3	8.0
Dont know	13.3	0.0	0.0	0.0	0.0	0.0	6.7	0.0	0.0	0.0	0.0	0.0
2. When was the last time you washed the net?												
1 week ago	41.7	17.4	4.0	0.0	0.0	14.6	0.0	12.5	0.0	6.7	5.0	10.9
1 week to 1 month ago	0.0	4.3	28.0	16.7	26.9	37.5	0.0	25.0	12.5	30.0	15.0	26.1
1-3 months ago	8.3	56.5	28.0	43.3	53.8	39.6	22.2	56.3	62.5	40.0	40.0	47.8
3-6 months ago	41.7	8.7	20.0	33.3	19.2	8.3	77.8	6.3	12.5	6.7	40.0	13.0
>6 months ago	8.3	8.7	20.0	3.3	0.0	0.0	0.0	0.0	12.5	3.3	0.0	2.2
Dont know	0.0	4.3	0.0	3.3	0.0	0.0	0.0	0.0	0.0	13.3	0.0	0.0
3. Type of soap used												
None	0.0	8.7	4.0	13.3	0.0	8.3	44.4	0.0	8.3	10.0	0.0	4.3
Local bar soap	41.7	30.4	44.0	6.7	19.2	39.6	33.3	43.8	20.8	6.7	40.0	23.9
Detergent powder	58.3	47.8	32.0	46.7	65.4	29.2	22.2	50.0	54.2	50.0	40.0	52.2
Mix	0.0	13.0	16.0	30.0	15.4	22.9	0.0	6.3	16.7	20.0	20.0	19.6
Dont know	0.0	0.0	4.0	3.3	0.0	0.0	0.0	0.0	0.0	13.3	0.0	0.0
4. How long did the net soak for?												
Did not soak	83.3	78.3	100.0	56.7	88.5	81.3	66.7	56.3	95.8	43.3	90.0	91.3
<1h	16.7	21.7	0.0	40.0	11.5	18.8	22.2	43.8	4.2	40.0	10.0	8.7
>1h	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	3.3	0.0	0.0

Don't know	0.0	0.0	0.0	3.3	0.0	0.0	11.1	0.0	0.0	13.3	0.0	0.0
5. Was the net scrubbed hard or beaten?												
Yes	8.3	26.1	8.0	20.0	7.7	6.3	0.0	12.5	12.5	10.0	0.0	13.0
No	91.7	73.9	92.0	76.7	92.3	93.8	100.0	87.5	87.5	76.7	100.0	87.0
Don't know	0.0	0.0	0.0	3.3	0.0	0.0	0.0	0.0	0.0	13.3	0.0	0.0
6. Where was the net dried?												
Outside in the sun	100.0	95.7	72.0	46.7	7.7	0.0	100.0	100.0	54.2	40.0	15.0	6.5
Outside in the Shade	0.0	4.3	28.0	50.0	92.3	100.0	0.0	0.0	45.8	46.7	85.0	93.5
Inside	0.0	0.0	0.0	3.3	0.0	0.0	0.0	0.0	0.0	13.3	0.0	0.0

Table 9: Physical condition and physical aspects (per cent) of nets

Parameters	Time of follow up in months of cohort nets (350 nets in each arm							
	PermaNet 3.0				PermaNet 2.0			
	6	12	24	36	6	12	24	36
A. Physical condition of Nets								
1. Net hole								
Nets having holes	9.3	39.4	85.7	88.1	3.0	37.0	74.1	70.5
Nets having no holes	90.7	60.6	14.3	11.9	97.0	63.0	25.9	29.5
2.1 Size of holes (0.5-2 cm)								
Roof	18.6	11.2	11.0	11.4	18.2	26.2	25.2	21.6
Upper	52.5	23.5	25.3	21.0	40.9	31.9	25.9	31.3
Lower	11.9	24.6	26.4	28.0	40.9	39.4	49.0	47.1
Seams	16.9	40.6	37.3	39.7	0.0	0.0	0.0	0.0
2.2 Size of holes (2-10 cm)								
Roof	17.9	6.9	7.1	14.5	33.3	18.0	18.5	18.5
Upper	44.0	18.9	16.8	22.7	66.7	33.0	35.3	34.0
Lower	10.1	31.5	28.4	26.2	0.0	49.0	44.9	47.5
Seams	28.0	42.7	47.7	36.7	0.0	0.0	1.2	0.0
2.3. Size of holes (10-25 cm)								
Roof	0.0	9.6	7.7	29.2	13.3	25.2	7.7	26.8
Upper	7.0	11.4	38.5	12.2	20.0	21.3	38.5	21.0
Lower	74.4	30.7	30.8	21.0	66.7	53.5	53.8	52.2
Seams	18.6	48.2	23.1	37.7	0.0	0.0	0.0	0.0
2.4. Size of holes (>25 cm)								
Roof	0.0	.0	0.0	.0	100.0	64.0	28.6	16.7

Upper	53.3	50.0	10.0	24.0	0.0	20.0	28.6	46.7
Lower	6.7	50.0	20.0	16.0	0.0	16.0	23.8	36.7
Seams	20.0	0.0	60.0	52.0	0.0	0.0	19.0	0.0
2.5 Hole repaired								
Stitched	20.0	91.2	83.3	87.0	100.0	72.1	100.0	89.1
Knotted	40.0	8.8	8.3	12.3	0.0	24.2	0.0	10.1
Patched	40.0	0.0	8.3	0.7	0.0	3.7	0.0	0.7
B. Physical aspect of net								
Clean	62.2	49.2	56.2	61.1	84.2	61.2	69.3	75.9
Dirty	29.9	41.0	35.9	30.7	15.8	30.0	23.3	19.5
Very dirty	7.9	9.8	8.0	8.1	0.0	8.9	7.4	4.6

Table 10. Results of fabric integrity of cohort nets measured in PermaNet 3.0 and PermaNet 2.0 nets

	PermaNet 3.0					PermaNet 2.0				
		Hole index*		Hole area**			Hole index*		Hole area**	
Survey (Month)	% nets with atleast 1 hole	Mean (SD)	Median (IQR)	Mean (SD)	Median (IQR)	% nets with atleast 1 hole	Mean (SD)	Median (IQR)	Mean (SD)	Median (IQR)
0	0	0	0	0	0	0	0	0	0	0
6	9.28	935.78 (1374.77)	576 (1175)	1149.03 (1687.75)	706.95 (1142.23)	3.02	286.78 (574.63)	0 .0 (219)	355.27 (703.65)	4.92 (267.61)
12	39.41	207.61 (411.49)	23 (219)	267.06 (513.45)	59.02 (274.19)	37	374.48 (864.82)	46 (242)	474.77 (1072.52)	61.48 (318.03)
24	61.89	270.98 (570.93)	69 (265)	355.75 (630.26)	92.22 (322)	57.14	330.69 (894.88)	34.5 (276)	430.29 (1136.29)	53.49 (351.22)
36	75.32	594.29 (1423.34)	138 (553)	786.84 (1792.95)	213.35 (780.03)	54.93	396.01 (880.56)	103.50 (349.75)	524.33 (1092.31)	176.45 (460.47)

*: Hole index caculated on hole size 2,3, and 4

**: Hole area calculated on hole Size 1,2,3and 4

Table 11: Physical condition and physical aspects (per cent) of withdrawn nets

Parameters	Time of follow up (in months) of cohort nets (30 nets up to 30 months and 50 in 36 months in each arm)											
	PermaNet 3.0						PermaNet 2.0					
	6	12	18	24	30	36	6	12	18	24	30	36
A. Physical condition of Nets												
1. Net hole												
Nets having holes	23.3	46.7	73.3	90.0	86.7	86.0	10.0	30.0	56.7	70.0	53.3	88.0
Nets having no holes	76.7	53.3	26.7	10.0	13.3	14.0	90.0	70.0	43.3	30.0	46.7	12.0
2.1 Size of holes (0.5-2 cm)												
Roof	15.4	26.9	6.2	9.0	9.2	9.1	72.0	11.0	15.4	27.1	29.8	23.8
Upper	20.5	15.8	25.9	24.6	22.6	29.6	24.0	45.1	28.1	26.7	24.2	28.0
Lower	15.4	26.3	24.1	25.4	27.0	25.3	4.0	43.9	56.6	46.2	46.0	48.1
Seams	48.7	31.0	43.8	41.0	41.2	36.0						
2.2 Size of holes (2-10 cm)												
Roof	27.0	0.0	4.7	5.9	2.9	13.9	54.0	4.5	13.6	35.3	11.4	21.3
Upper	23.0	41.2	23.4	19.8	24.4	27.1	46.0	36.4	27.3	5.9	56.8	29.3
Lower	0.0	41.2	30.5	15.8	23.3	19.3	0.0	59.1	59.1	58.8	31.8	49.3
Seams	0.0	17.6	41.4	58.4	49.4	39.7						
2.3. Size of holes (10-25 cm)												
Roof	0.0	0.0	0.0	0.0	7.7	12.0	0.0	0.0	13.3	14.3	7.7	28.6
Upper	50.0	14.3	7.0	16.7	38.5	24.1	100.0	50.0	20.0	7.1	38.5	21.4
Lower	40.0	57.1	74.4	33.3	30.8	12.0	0.0	50.0	66.7	78.6	53.8	50.0
Seams	10.0	28.6	18.6	50.0	23.1	51.8						
2.4. Size of holes (>25 cm)												
Roof	0.0	0.0	0.0	0.0	0.0	42.9	0.0	0.0	0.0	100.0	0.0	50.0

Upper	0.0	0.0	0.0	0.0	100.0	0.0	0.0	0.0	0.0	0.0	50.0	25.0
Lower	0.0	0.0	0.0	33.3	0.0	14.3	0.0	0.0	100.0	0.0	0.0	25.0
Seams	0.0	7.0	100.0	66.7	0.0	42.9						
2.5 Hole repaired												
Stitched	0.0	66.7	93.0	91.7	83.3	85.2	0.0	93.3	85.7	85.2	100.0	90.4
Knotted	0.0	33.3	7.0	8.3	8.3	13.9	0.0	6.7	14.3	7.4	0.0	9.6
Patched	0.0	0.0	0.0	0.0	8.3	0.9	0.0	0.0	0.0	7.4	0.0	0.0
B. Physical aspect of net												
Clean	56.7	30.0	40.0	30.0	33.3	42.0	23.3	50.0	53.3	56.7	80.0	56.0
Dirty	40.0	43.3	46.7	56.7	63.3	48.0	73.3	33.3	40.0	33.3	20.0	36.0
Very dirty	3.3	26.7	13.3	13.3	3.3	10.0	3.3	16.7	6.7	10.0	0.0	8.0

Table 12: Results of fabric integrity of withdrawn nets measured in PermaNet 3.0 and PermaNet 2.0 nets

Survey (Month)	PermaNet 3.0					PermaNet 2.0				
	% nets with at least 1 hole	Hole index*		Hole area**		% nets with at least 1 hole	Hole index*		Hole area**	
		Mean (SD)	Median (IQR)	Mean (SD)	Median (IQR)		Mean (SD)	Median (IQR)	Mean (SD)	Median (IQR)
0	0	0	0	0	0	0	0	0	0	0
6	23.33	29.71 (772.76)	0.00 (23)	373.70 (949.17)	11.07 (19.06)	10.00	1771.67 (3068.62)	0.00 (2657.50)	2197.02 (3800.02)	4.92 (3291.84)
12	46.66	441.86 (1119.47)	34.5 (155.25)	557.45 (1369.36)	66.40 (199.80)	30	99.78 (178.84)	23 (23)	133.79 (229.67)	28.28 (28.28)
18	73.33	543.09 (2244.72)	23 (69)	682.17 (2774.14)	36.27 (87.92)	56.67	266.35 (412.28)	69 (380)	343.02 (520.95)	90.99 (543.06)
24	90	429.26 (728.77)	138 (310.5)	544.31 (915.09)	177.06 (398.38)	70	259 (589.1)	46 (196)	332.62 (733.15)	56.56 (236)
30	86.66	35.92 (42.73)	14 (59.75)	2716.82 (3334.63)	413.14 (5410.09)	53.33	294.5 (486.95)	23 (417.75)	386.65 (610.20)	57.18 (506.74)
36	86	718.67 (1527.43)	138 (478)	504.44 (1007.03)	191.83 (304.63)	88	277.89 (637.25)	46 (316.75)	376.58 (807.40)	65.79 (398.87)

**: Hole area calculated on hole size 1,2,3 and 4; *: Hole index calculated on hole size 2,3, and 4;

Table 13. Deltamethrin contents in PermaNet 3.0 and PermaNet 2.0 nets

Net withdrawal period	Type of Nets	Parts of net	Deltamethrin contents (g/kg) Mean	SD	RSD (%)	CI*	Target dose of deltamethrin contents in baseline net samples (g/kg)	% loss of deltamethrin contents
Baseline	PermaNet 3.0	Lower	2.86	0.26	9.2	0.1	2.80	-2.14
		Upper	2.69	0.16	5.8	0.06	2.8	3.93
		Roof	4.32	0.13	3.1	0.05	4.00	-8.00
	PermaNet 2.0	Whole	1.32	0.14	10.7	0.05	1.40	5.71
12 Month	PermaNet 3.0	Lower	1.79	0.76	42.2	0.28	2.80	36.07
		Upper	1.88	0.69	36.8	0.26	2.8	32.86
		Roof	3.34	0.54	16.3	0.2	4.00	16.50
	PermaNet 2.0	Whole	0.99	0.42	42.5	0.16	1.40	29.29
24 Month	PermaNet 3.0	Lower	1.17	0.81	69	0.30	2.80	58.21
		Upper	1.09	0.78	72	0.29	2.8	61.07
		Roof	2.8	0.5	18	0.2	4.00	30.00
	PermaNet 2.0	Whole	0.6	0.33	55	0.13	1.40	57.14
36 Month	PermaNet 3.0	Lower	0.88	0.83	94	0.20	2.80	69
		Upper	0.79	0.77	98	0.20	2.8	71
		Roof	2.3	0.6	25	0.2	4.00	47

PermaNet 2.0	Whole	0.45	0.40	88	0.10	1.40	66.0

*: Confidence Interval

Table 14 . Piperonyl butoxide contents in roof part of PermaNet 3.0

Duration of net withdrawal	Piperonyl butoxide contents (g/kg)				Target dose of piperonyl butoxide content in baseline net samples (g/kg)	% loss of piperonyl butoxide contents
	Mean	SD	RSD (%)	Confidence interval		
Baseline	25.54	1.7	6.7	0.64	25.00	-2.16
12 Month	11.46	6.04	52.7	2.25	25.00	54.16
24 Month	6.8	5	74	1.9	25.00	72.8
36 Month	5.1	4.9	95	1.4	25.00	80.0

Table 15: Proportion of nets meeting the WHOPES criteria in cone bioassays and tunnel tests

	PermaNet 3.0									PermaNet 2.0		
	Lower			Upper			Roof			Whole net		
Net sampling in months	Cone bioassays	Tunnel tests	Cone bioassay & tunnel tests combined	Cone bioassays	Tunnel tests	Cone bioassay & tunnel tests combined	Cone bioassay	Tunnel tests	Cone bioassay & tunnel tests combined	Cone bioas say	Tunnel tests	Cone bioassay & tunnel tests combined
0	100	ND	100	100	ND	100	100	ND	100	96.7	ND	96.7
6	100	ND	100	100	ND	100	93.3	ND	93.3	93.3	ND	93.3
12	100	ND	100	90	ND	90	100	ND	100	100	ND	100
18	100	ND	100	100	ND	100	93.3	ND	93.3	93.3	ND	93.3
24	73.3	26.7	100	76.7	23.3	100	73.3	26.7	100	83.3	16.7	100
30	80	10	90	76.7	10	86.7	83.3	16.7	100	100	ND	100
36	96	ND	96	92	ND	92	98	ND	98	100	ND	100

ND : Not done

Plates

Plate 1: Village census form is being filled

Plate 2 and 3. Net coding is being carried out

Plate 4. PermaNet 2.0 is being inspected for fabric integrity

Plate 5. PermaNet 3.0 is being inspected for fabric integrity

Plate 6: Withdrawal net samples of PermaNet 3.0 and PermaNet 2.0 are being packed for chemical analysis

Plate 7: Cone-bioassay tests are being conducted on PermaNet 3.0 and PermaNet 2.0 nets

Plate 8: Net users converted two PermaNet 3.0 nets into single net by stitching together and repaired the damaged parts.