REPORT OF THE EIGHTEENTH
WHOPES
WORKING GROUP MEETING

WHO/HQ, GENEVA
29 JUNE – 1 JULY 2015

Review of:

MIRANET LN
PANDA NET 2.0 LN
YAHE LN
SAFENET LN

World Health Organization

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1. INTRODUCTION

The 18th meeting of the WHOPES Working Group, an advisory group to the World Health Organization Pesticide Evaluation Scheme (WHOPES), was held at WHO headquarters in Geneva, Switzerland from 29 June to 1 July 2015.

The Working Group reviewed four long-lasting insecticidal net products[1] for the prevention and control of malaria: (i) MiraNet LN of A to Z Textile Mills Ltd; (ii) Panda Net 2.0 LN of Life Ideas Textiles Company Ltd[2]; (iii) Yahe LN of Fujian Yamei Industry; and (iv) SafeNet LN of Mainpol GmbH. The Working Group made recommendations on the use of each of these products.

The meeting was opened by Dr Dirk Engels, Director, WHO Department of Control of Neglected Tropical Diseases, who welcomed participants; he noted that WHOPES has entered its 55th year and that reforms to the Scheme are being planned. Quality assurance has gained increasing prominence across public health products, such as medicines and vaccines, and increasingly there is a drive to align evaluation and quality control of pesticides with other product streams. Additionally, WHOPES is implementing procedural changes in response to a growing need of the vector control community, including the manufacturing and procurement sectors. Changes to WHOPES will be a part of a broader package to stimulate innovation in vector control, in collaboration with the WHO Global Malaria Programme and the WHO Prequalification Programme.

Dr Raman Velayudhan, Coordinator, Vector Ecology and Management, briefed the Working Group on the broad objectives of the proposed changes to WHOPES, particularly the move to a standard accreditation system for the testing sites to improve data quality.

Dr Rajpal Yadav, Scientist, WHOPES, summarized the interests declared by the invited experts and explained the objectives and procedures of the meeting. Dr Vincent Corbel, an IRD scientist based at Kasetsart University, Thailand was appointed as Chairperson and Dr John Gimnig, Entomologist, Centers for Disease Control and Prevention, USA as Rapporteur.

The meeting was attended by 11 invited experts, one observer and five WHO Secretariat staff (see Annex 1. List of participants). The Working Group met in both plenary and break-out sessions, in which the reports of the WHOPES-supervised studies, the reports submitted by the manufacturers, and the relevant published literature and unpublished reports were reviewed and discussed (see Annex 2. References).

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[1] In this report, "long-lasting insecticidal net (LN)" refers to a formulated product.
[2] WHO has been advised that as from 10 August 2015, the name of the manufacturer has been changed to Life Ideas Biological Technology Company Ltd. in place of Life Ideas Textiles Company Ltd.

Declarations of interest

All invited experts completed a *Declaration of interests for WHO experts* prior to the meeting for assessment by the WHO Secretariat. The following interests were declared:

Dr Nicole Achee's university has received mosquito repellent products free of charge from SC Johnson & Son, USA for use in a large-scale intervention trial funded by the Bill & Melinda Gates Foundation, USA.

Dr Fabrice Chandre's institute has received prescribed standard fees from Sumitomo Chemical, Japan; Bayer CropScience, Germany; and SPCI, France to meet the costs of product evaluation.

Professor Dr Marc Coosemans' institute has received grants from the Bill & Melinda Gates Foundation for evaluating the impact of repellents on malaria in Cambodia. The institute has also received repellents free of charge from SC Johnson & Son, USA for use in the study.

Dr Vincent Corbel's institute has received prescribed standard fees from Bayer CropScience, Germany and Sumitomo Chemical, Japan to meet the costs of evaluating their respective pesticide products.

Dr Sarah Moore's research unit has received research funding from the United States Agency for International Development and the Research Council of Norway for evaluating various long-lasting insecticidal nets. The unit has also received prescribed standard fees from Bayer CropScience, Germany; Syngenta, Switzerland; and Vestergaard Frandsen, Switzerland for evaluating their long-lasting insecticidal nets.

Professor Dr Mark Rowland's unit has received funding from the Innovative Vector Control Consortium, United Kingdom for the testing and evaluation of various pesticide products. The unit has also received prescribed standard fees from Sumitomo Chemical, Japan; BASF, Germany; and Vestergaard Frandsen, Switzerland to meet the costs of evaluating their respective pesticide products.

Dr Olivier Pigeon's research centre has received prescribed standard fees from Life Ideas Textile Company, China to meet the costs of physico-chemical studies of Panda Net 2.0 LN manufactured by the company.

Dr Juan Arredondo from Universidad Autónoma de Nuevo León, Mexico was invited to attend the meeting as an observer and declared no interest.

The interests declared by the experts and the observer were assessed by the WHO Secretariat. The declared interests were not found to be directly related to the topics under discussion at the meeting. It was therefore decided that all of the above-mentioned experts could participate in all evaluations, subject to the public disclosure of their interests.

2. REVIEW OF MIRANET LN

MiraNet LN is an alpha-cypermethrin long-lasting (incorporated into polyethylene) insecticidal net. Alpha-cypermethrin is incorporated into 130-denier, monofilament HDPE yarn, with the target dose of 4.5 g AI/kg of the fabric weight corresponding to 180 mg of alpha-cypermethrin per square metre. MiraNet LN is manufactured by A to Z Textile Mills Ltd, United Republic of Tanzania.

Alpha-cypermethrin has previously been recommended[1] by WHO as an insecticide product for conventional treatment of mosquito nets for malaria vector control, at the target dose of 20–40 mg/m².

2.1 Safety assessment

The assessment of risk to humans of washing and sleeping under the MiraNet LN was performed on the basis of the data and a report provided by the manufacturer (Aitio, 2014). The revised WHO *Generic risk assessment model for insecticide-treated nets*[2] was used as a guiding document.

The following assumptions were used by the evaluator in drafting the risk assessment:

- the product and the active ingredient comply with the WHO specification;
- the wash resistance index (⩾ 95%) reflects the proportion of the active ingredient available for dermal and oral absorption, and that released during the washing of the net;
- dermal absorption of alpha-cypermethrin is 1% and oral absorption is 60%;
- the translodgeable part of alpha-cypermethrin from the net onto the skin is 6% of the available surface concentration defined from the wash resistance index, in line with the estimate of the United States Environmental Protection Agency for soft surfaces; and
- representative biological half-time (in mother's milk) of alpha-cypermethrin is 25 days.

It was concluded that when used as instructed, sleeping under or washing MiraNet LN does not pose undue hazards to people.

[1] WHO recommended insecticide products for treatment of mosquito nets for malaria vector control [updated 17 November 2014] (http://who.int/whopes/Insecticides_ITN_Malaria_Nov2014.pdf, accessed August 2015).
[2] WHO (2012). A generic risk assessment model for insecticide-treated nets – revised edition. Geneva: World Health Organization (http://whqlibdoc.who.int/publications/2012/9789241503419_eng.pdf, accessed August 2015).

2.2 Efficacy – WHOPES-supervised studies

2.2.1 Laboratory study

The regeneration time, resistance to washing and efficacy against a susceptible *An. gambiae* s.s. strain (Kisumu) of MiraNet LN were determined in a laboratory (Phase I) study (Rossignol et al., 2013a). The four nets used were washed with soap (Savon de Marseille) following the WHOPES guidelines.[1] The chemical analyses of samples of these nets were performed using the CIPAC method 454/LN/M/3.2 (GC-FID) (Pigeon, 2013a).

The average alpha-cypermethrin content measured in the four unwashed MiraNet LN was 5.10 g/kg (range 5.00 to 5.12 g AI/kg), corresponding to 195 to 203 mg AI/m² (Table 2.1). All nets thus complied with the target dose of 4.5 g AI/kg ± 25% (3.375–5.625 g AI/kg). The within-net variation, expressed as the relative standard deviation of the alpha-cypermethrin content found on five pieces cut from each net, ranged from 0.5% to 4.3%, showing an acceptable homogeneity of the distribution of active ingredient in the net. The between-net variation, expressed as the relative standard deviation of the alpha-cypermethrin content found on the four different nets, was 0.8%, showing an acceptable level of homogeneity of the active ingredient between the nets.

The regeneration time for MiraNet LN was estimated using cone bioassays. The knockdown and mortality were found to be 100%. After one cycle of washing and drying three consecutive times on the same day, the knockdown stayed at its initial value for 7 days, while the mortality declined to 80%. On the second day of storage of netting pieces, mortality reached a plateau (97%) and was maintained in tests until day 7. A regeneration time of 2 days was therefore considered after each wash round.

In the wash resistance study the initial knockdown of 100% was maintained until 20 washes and declined after 25 washes to 97%, which was still above the WHOPES threshold of ≥ 95% (Table 2.1). The mortality decreased slightly but stayed above the WHOPES threshold of ≥ 80% until the 15th wash. From 20 to 25 washes, mortality fell below the ≥ 80% threshold. Therefore, based on the knockdown criteria, MiraNet LN fulfilled the WHOPES Phase I efficacy criteria after 20 washes.

After 20 washes, the average alpha-cypermethrin content in MiraNet LN was 4.88 g AI/kg (corresponding to 95.7% of the initial dose) (Figure 2.1, Table 2.1). The wash resistance index per wash as estimated by the exponential regression curve was 99.8%.

2.2.2 Experimental hut studies

Three studies were implemented under WHOPES supervision: the first in M'Be, Côte d'Ivoire on *An. gambiae*; the second in Muheza district, United Republic of Tanzania on *An. funestus* and the third in Mae Sot District, Tak Province, Thailand on *Anopheles* mosquitoes (mainly on *An. minimus*).

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[1] WHO (2013). Guidelines for laboratory and field-testing of long-lasting insecticidal nets. Geneva: World Health Organization (http://who.int/iris/bitstream/10665/80270/1/9789241505277_eng.pdf, accessed August 2015).

In Côte d'Ivoire (N'Guessan et al., 2015) and Thailand (Duchon et al., 2014) the efficacy of MiraNet LN in terms of blood-feeding inhibition, deterrence, induced exophily and mortality was evaluated in the West African style of experimental huts. In the United Republic of Tanzania, efficacy was evaluated in the East African style of experimental huts (Tungu et al., 2015a). MAGNet LN, a WHOPES-recommended LN,[1] was used as the positive control in all three studies. In this net, alpha-cypermethrin is incorporated into the 150-denier, monofilament HDPE yarn, with the target dose of 5.8 g AI/kg.

For the experimental hut study, the following treatment arms were included in the study:

- untreated 100-denier polyester net
- unwashed MiraNet LN
- unwashed MAGNet LN
- MiraNet LN washed 20 times
- MAGNet washed 20 times
- polyester net treated conventionally with alpha-cypermethrin at a target dose of 40 mg/m^2 and washed until just before exhaustion (in M'Be and Muheza sites only).

Alpha-cypermethrin content in the treated nets was determined three times: at baseline (before washing), after washing but before start of the experimental hut study, and after finishing the trial. The CIPAC method 454/LN/M/3.2 was used as the analytical method for determination of alpha-cypermethrin content in samples of MiraNet LN, MAGNet LN, conventionally treated nets and untreated nets. This method involves extraction of alpha-cypermethrin by refluxing for 30 minutes with xylene in the presence of dioctyl phthalate as an internal standard and citric acid, and determination by Gas Chromatography with Flame Ionization Detection (GC-FID). The performance of the analytical method was controlled during the analysis of samples in order to validate the analytical results (Pigeon et al., 2014; 2015a; 2015b).

M'Be, Côte d'Ivoire

The field site at M'Be is located in central Côte d'Ivoire some 40 km south of Bouaké city where the Institute Pierre Richet responsible for the study is located. The site is a large valley where rice is irrigated producing year round *An. gambiae* s.s (M form). Characterization of the insecticide susceptibility of *An. gambiae* s.s. collected from this site in June 2012 showed increased activities of esterases (both esterase alpha and esterase beta), oxidases and glutathione-S-transferase relative to the levels found in susceptible *An. gambiae* Kisumu strain. Mortality of *An. gambiae* s.s. with 0.05% alpha-cypermethrin test papers was 68% and the frequency of individuals carrying the L1014F *kdr* allele was relatively low (0.33).[2] However, analysis of the *kdr* frequency in a subsequent study in 2014 showed a very high frequency (0.94) (F. Chandre, personal communication).

[1] WHO (2011). Report of the 14th WHOPES Working Group meeting. Review of Spinosad® EC, LifeNet® LN, MAGNet™ LN, Royal Sentry® LN, Yahe® LN , 11–15 April 2011. Geneva: World Health Organization (http://whqlibdoc.who.int/publications/2011/9789241502160_eng.pdf, accessed August 2015).
[2] Koffi AA et al. (2013). Insecticide resistance status of *Anopheles gambiae* s.s. population from M'Be: a WHOPES-labelled experimental hut station, 10 years after the political crisis in Côte d'Ivoire. Malar J;12:151.

The efficacy of MiraNet LN against free-flying, wild *An. gambiae* s.s. was evaluated in experimental huts and compared with positive control nets, i.e. unwashed and 20 times washed MAGNet LN and a conventional net treated with alpha-cypermethrin (10% SC; dose 40 mg AI/m²) washed until just before exhaustion (N'Guessan et al., 2015).

To begin with, cone bioassays were performed on nets. For cone bioassays, two mosquito strains were used: a susceptible colony of *An. gambiae* s.s. Kisumu strain and a wild, laboratory-colonized strain of *An. gambiae* from the M'Be site. The results of the cone tests carried out on the conventionally treated net using the Kisumu strain showed that knockdown and mortality rates decreased below the respective WHO thresholds (≥ 95% and ≥ 80%, respectively) after the third wash. Hence, two washes were considered as the number of washes required until just before exhaustion. Before washing, knockdown and mortality rates of the susceptible *An. gambiae* on all treated nets were > 99%. Against the wild pyrethroid-resistant strain from M'Be, unwashed MAGNet LN induced significantly higher knockdown (87% versus 64%) and mortality rates (93% versus 22%) than the unwashed MiraNet LN. After 20 washes, using the susceptible Kisumu strain MiraNet LN did not fulfill the WHOPES criteria for knockdown (92%) and mortality (29%), while MAGNet LN complied with both criteria (99% and 85%, respectively). The conventionally treated net washed until just before exhaustion resulted in knockdown of 90% and mortality of 87%. Rates of knockdown and mortality against the resistant M'Be strain appeared marginally low (< 4%) across all treatments because of insecticide resistance.

The nets were then evaluated in huts. After 6 weeks of field-testing, two of the three nets per treatment arm were randomly selected and tested in cone bioassays again. All treated nets (washed and unwashed) provided high knockdown and mortalities (> 95%) with the Kisumu strain. With the pyrethroid-resistant M'Be strain, mortality (3.9–22%) and knockdown (0.7–32%) were relatively low.

Results of the experimental hut study are presented in Table 2.2. The hut trial took 6 weeks (from 13 October to 22 November 2014), corresponding to 36 night collections per hut, i.e. 6 nights per week (one complete Latin square). A mean number of 137.2 mosquitoes, belonging to different genera and species, was collected per night during the study period. Of these, 85.2% were anophelines and 14.8% were other Culicidae (*Aedes*, *Culex* and *Mansonia* spp.). *An. gambiae* s.s. was abundant, representing 98% of the total anophelines.

During the 6-week period, 1594 *An. gambiae* s.s. were collected in the control hut, representing a mean number of 44 females per night of collection. About 62% of them were blood-fed (983 of 1594). The "natural" exophily was 26.3% and mortality was 8%.

Compared to mosquito entry rate in the huts with an untreated net, 63–84% fewer *An. gambiae* s.s. entered the huts with insecticide-treated nets. The unwashed MiraNet LN caused a significantly higher deterrent effect (84%) than for the other treatment arms (62– 68%). The induced exophily in *An. gambiae* s.s. was higher with both LN washed 20 times (42%) than the conventionally treated net washed until just before exhaustion (27%).

All insecticide-treated nets, except the conventionally treated net, induced higher mortality in *An. gambiae* s.s. than the untreated nets. Across all treatment types, the insecticidal impact against the pyrethroid-resistant *An. gambiae* s.s. was found to be limited (induced mortality ranged from 3% to 24%) (Table 2.2). The unwashed MAGNet LN killed significantly more *An. gambiae* (24%) than the unwashed MiraNet LN (8%). After 20 washes, however, there was a significant loss of insecticidal activity of MAGNet LN (12% mortality), which was similar to that of MiraNet LN after 20 washes (7% mortality) but higher than that of alpha-cypermethrin conventionally treated net washed until just before exhaustion (3% mortality).

Blood-feeding of *An. gambiae* s.s. was inhibited in every hut with insecticide-treated nets but the levels of inhibition were moderate (31–55%) (Table 2.3). Blood-feeding inhibition in huts with unwashed MAGNet LN (55%) was higher than with unwashed MiraNet LN (37%) but after washing LNs or conventionally treated nets 20 times until just before exhaustion no difference was observed in blood-feeding inhibition (around 30%) between the treated nets.

The level of personal protection against *An. gambiae* s.s. was high for all treatments (75–90%). The overall insecticidal effect was compromised by pyrethroid resistance at this site and was not detectable with conventionally treated nets washed until just before exhaustion.

Among other mosquito species (*Culex, Mansonia* and *Aedes*) all treatments induced higher induced mortality (79–95%) than for *An. gambiae* s.l. (3–24%), despite the relatively high proportion of Culicinae dying in the control hut (35%) (Table 2.2). The differences in mortality between the different net types were not significant.

Among other Culicidae blood-feeding was highly inhibited with all treatments (85–95%) compared with the untreated net arm (47%) and was above the levels for *An. gambiae* s.l. (31–55%) (Table 2.3). The differences across LN treatments were not significant.

No adverse events were reported by the sleepers during the study period.

One net of each arm was randomly selected to determine its insecticidal content before washing, and after washing and testing in the 6-week trial.

The alpha-cypermethrin content and within-net variations in unwashed LNs are given in Table 2.4, while the alpha-cypermethrin contect and retention rate are given in Table 2.5.

Both nets complied with the target dose (of 4.5 g AI/kg ± 25% for MiraNet LN and 5.8 g AI/kg for MAGNet LN) with an acceptable within-net variation (RSD < 20%) (Table 2.4). The overall retention rate of active ingredient after 20 washes was around 85% for both the nets. After the field trial the alpha-cypermethrin content did not decrease significantly, as it was 4.62 g AI/kg for the unwashed MiraNet LN and 4.10 g AI/kg for the MiraNet LN washed 20 times (Table 2.5a). Similarly for the MAGNet LN, it was 5.95 and 4.87 g/kg respectively.

The mean alpha-cypermethrin content of the conventionally treated net before washing was 1.50 g AI/kg (46.1 mg/m²) which was 115% above the target dose of 40 mg AI/m² with a within-net variation of 15.5% showing an acceptable homogeneity of the active substance within the net (Table 2.5a). The alpha-cypermethrin content after washing until just before exhaustion decreased to 0.06 g AI/kg (or < 2 mg AI/m²) which was close to the quantification limit.

Muheza District, United Republic of Tanzania

The six experimental huts are situated in Zenet village in Muheza district of the United Republic of Tanzania (5° 13’ S; 38° 39’ E, altitude 193 m). The huts are made to a standard traditional East African hut design with a veranda trap.

Susceptibility tests were done on adult *An. funestus* collected from experimental huts with untreated nets. It was not possible to use newly emerged adults from larval collections because larvae could not be collected in sufficient numbers. *An. funestus* was found to be resistant to deltamethrin and alpha-cypermethrin at the discriminating dose of 0.05%, with mortality rates of 75.3% and 60.3%, respectively, after 1 hour of exposure.

The efficacy of MiraNet LN against free-flying, wild *An. funestus* was evaluated in experimental huts and compared with positive control nets, i.e. unwashed and 20 times washed MAGNet LN and as an additional check a net conventionally treated with alpha-cypermethrin (10% SC; dose 40 mg AI/m²) and washed until just before exhaustion (Tungu et al., 2015a).

Cone bioassays were performed on nets to establish baseline bioefficacy. For cone bioassays, a susceptible *An. gambiae* colony Kisumu strain was used. Cone bioassay tests with *An. gambiae* Kisumu on the conventionally treated net showed that the knockdown effect decreased to < 95% after the second wash and mortality to < 80% after four washes (induced 85% knockdown and 81% mortality). Hence, three washes were considered as the number of washes required until just before exhaustion. The conventionally treated net washed until just before exhaustion showed a mortality of 86% after the trial. Both unwashed LNs induced a knockdown and mortality of 100% before and after the trial. After 20 washes, knockdown and mortality decreased to around 85%. At the end of the trial, knockdown was 100% for both washed LNs and mortality 94% and 84% for washed MiraNet LN and MAGNet LN, respectively.

In the experimental hut study, the six treatment arms were rotated once a week (i.e. 6 nights/week) through the experimental huts according to a Latin square design. Data were collected for 36 nights. Three nets were available per treatment arm and each net was tested for 2 days per week. At the end of each rotation the huts were cleaned and aired for one day before the treatments were moved to the next hut.

During the 6-week period, 136 *An. funestus* were collected in the huts with untreated net (control) (Table 2.2). The “natural” exophily in control huts was 49%, mortality 3.7% (5/136) and blood-feeding 23.5% (Table 2.3). While there were some significant differences between treatments, there was no consistent evidence for insecticide-induced deterrence.

The overall killing effect recorded against *An. funestus* was not significantly different between treatment and untreated control arms (Table 2.2).

The exit rate of *An. funestus* was significantly lower in the huts with treated nets. The exit rates of *An. funestus* from huts with treated nets ranged from 73.1% (conventionally treated net washed until just before exhaustion) to 88.7% (unwashed MiraNet LN). The exit rates recorded with washed and unwashed LN arms were higher (83–89%) than those for the conventionally treated net washed until just before exhaustion (73%).

Blood-feeding inhibition was not significantly different between the treatment arms (washed or unwashed), ranging from 39% to 72% (Table 2.3). Higher personal protection was recorded with unwashed MiraNet LN than with unwashed MAGNet LN (72% versus 47%). Similar levels of personal protection (39–41%) were recorded with MiraNet LN and MAGNet LN washed 20 times and the conventionally treated net washed three times.

The alpha-cypermethrin content and within-net variations in the unwashed LN are given in Table 2.4, while the alpha-cypermethrin content and retention rate are given in Table 2.5a.

Both the unwashed nets complied with the target dose for alpha-cypermethrin (MiraNet LN: 4.5 g AI/kg ± 25%; MAGNet LN 5.8 g AI/kg ± 25%) with an acceptable within-net variation (RSD < 20%) (Table 2.4). The overall active ingredient retention rate rate after 20 washes was around 80% for both the nets. No decrease in active ingredient below the tolerance limit was observed at the end of the trial (Table 2.5a).

The mean alpha-cypermethrin content in the conventionally treated net before washing was 1.33 g AI/kg (42.5 mg/m²), which was 106% of the target dose of 40 mg/m². The within-net variation was 14.3%, showing an acceptable homogeneity of the distribution of the active substance within the net. The alpha-cypermethrin content after washing until just before exhaustion decreased to 0.17 g/kg (5.4 mg/m²) (Table 2.5a).

Mae Sot district, Thailand

The experimental hut trial was conducted in Mae Sot district of Tak province in western Thailand because this province recorded the highest number of malaria cases (*n* = 11 532) in 2013 (source: Bureau of Epidemiology, Ministry of Public Health, www.biophics.org/malariaR10/) (Duchon et al., 2014). The study area is located in Tum Sua village (16° 41' N; 98° 41' E) on the border with Myanmar demarcated by mountain ranges and the river Moei. Fruit orchards and agricultural fields on the east and the intact forest on the west surround three-quarters of the area of the study village. Mosquito collections conducted in Mae Sot between 2008 and September 2010 captured three anopheline species including *An. minimus*, *An. dirus* and *An. maculatus*.[1] Trophic behaviour of *An. minimus* was observed throughout the nights of collection with around 60% mosquitoes collected outdoors and about 40% indoors.

Two sibling species, *An. minimus* s.s. (former species A) and *An. harrisoni* (former species C) belonging to the Minimus Complex[2] (Manguin et al. 2001), were present in sympatry at Mae Sot. Molecular identification of the sibling species was done using allele-specific PCR.[3] In addition, sibling species within the Maculatus group were tested by PCR using the protocols of Walton et al. (2007).[4]

The status of resistance to pyrethroids of *An. minimus* and *An. maculatus* was checked using standard WHO cylinder tests with 0.05% deltamethrin and 0.05% alpha-cypermethrin impregnated papers. Results showed full susceptibility (100% knockdown and mortality) of *An. minimus* (*n* = 59) and *An. maculatus* (*n* = 53) to 0.05% deltamethrin. Regarding alpha-cypermethrin, 100% knockdown and mortality were reported with *An. minimus* (*n* = 72)

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[1] Tisgratog R et al. Host feeding pattern of *Anopheles minimus* (dipteral: cucidae) in a malaria outbreak of Thailand [unpublished report].
[2] Manguin S et al. (2001). Molecular identification of sibling *Anopheles* species: example of the *Anopheles minimus* and *Anopheles dirus* complexes, major malarial vectors in Southeast Asia. Med Trop;61:463–9.
[3] Garros C et al. (2004). Restriction fragment length polymorphism method for the identification of major African and Asian malaria vectors within the *Anopheles funestus* and *An. minimus* groups. Am J Trop Med Hyg;70:260–5.
[4] Walton C et al. (2007). Genetic diversity and molecular identification of mosquito species in the *Anopheles maculatus* group using the ITS2 region of rDNA. Infect Genet Evol;7:93–102.

whereas resistance was suspected in *An. maculatus* (100% knockdown and 95% mortality, *n* = 41). No knockdown and low mortality (7.5%) were recorded in the control batch of mosquitoes (*n* = 40).

The efficacy of MiraNet LN was evaluated and compared with that of MAGNet LN both unwashed and washed 20 times against free-flying, wild anophelines in experimental huts.

Cone bioassays were performed to determine baseline bioefficacy. For cone bioassays, a susceptible *An. minimus* strain colonized since 1987 at Kasetsart University was used. Before any washing, three MiraNet LN failed to meet the WHO criteria for mortality of ≥ 80% (73%, 60% and 50%). One of these failed also for the knockdown criteria > 95% (91%). After 20 washes of one MiraNet LN, mortality was still low (58%) but knockdown above 95%. Three unwashed and one 20 times washed MAGNet LN met both WHO efficacy criteria (i.e. knockdown ≥ 95% and mortality ≥ 80%). After field testing, one net of each arm was tested using cone-bioassays: all LNs fulfilled the criteria for knockdown or mortality.

In a baseline survey using untreated nets in the five experimental huts, no difference in mosquito attractiveness between the huts was observed.

The trial occurred over 125 nights of collections per hut, i.e. 5 nights per week during the 25-week period (from 3 February 2014 to 8 August 2014) or five complete Latin squares. A 2-week break was given between the second and the third Latin square to clean the huts and allow the sleepers to visit their families.

Overall, 897 mosquitoes were collected (including baseline) of which 411 (46%) belonged to *Anopheles* genus. Composition of *Anopheles* was as follows: *An. minimus* s.l. 59% (244/411), *An. maculatus* s.l. 24% (100/411), *An. barbirostris* 4% (17/411), *An. jamesii* 3% (15/411) and *An. dirus* s.l. 2% (7/411); other *Anopheles* species represented 7.5%. Among the specimens of the Minimus complex, all (235/235) were identified to be only *An. minimus* s.s. Regarding the Maculatus group, the composition was 62% *An. maculatus* (45/72), 32% *An. pseudowillmori* (23/72) and 6% *An. dravidicus* (4/72). No PCR was performed on specimens of the Dirus complex due to the low number of specimens collected (2% of total *Anopheles*). Considering the small sample size, all anopheline mosquitoes were grouped for the statistical analyses.

During the 125 nights of collection, 77 *Anopheles* mosquitoes were collected in the control hut with untreated net, i.e. a mean number of 0.62 females were caught per night. No blood-fed mosquitoes were found despite the fact that 17% of those were caught under the nets. The high “natural” exophily recorded in the control hut (61%) is probably explained by the low feeding success of mosquitoes that are more likely to escape the huts to find a blood meal. Conversely, mortality was low (6%), indicating that no contamination with alpha-cypermethrin had occurred during the rotation of treated nets.

The total number of Culicidae collected in the control hut (including *Anopheles* spp., *Culex* spp. and *Aedes* spp.) was 181, i.e. a mean number of 1.45 females were caught per night. Only 2% of them were blood-fed (3/181). The “natural” exophily was high (64%) and mortality was low (7%).

In general, the total number of anophelines and mosquitoes collected in the huts did not differ significantly between the treated and control groups.

The number of *Anopheles* collected in the verandah trap was higher in the untreated net (control) group (61%) than in the treated group (around 30%). The exit rate was low in the treated arms in relation to the control, which is explained by the high mortality (around 90%) of *Anopheles* that entered the huts with treated nets.

After the trial, all treatments caused higher induced mortality (> 87%) than the control (6%) (Table 2.2). The candidate MiraNet LN killed significantly more *Anopheles* mosquitoes (97%) than the reference MAGNet LN (87–88%) regardless of the washing regimen. For all Culicidae, MiraNet LN performed equal to (without washes) or better (after washes) than MAGNet LN.

Blood-feeding rates of *Anopheles* and other Culicidae were low (from 0% to 3%) and not significantly different between treated and control groups (Table 2.3). In four arms (including untreated nets) a similar proportion (from 17% to 25%) of *Anopheles* was found under the net, but this proportion was higher in the MiraNet LN unwashed arm (31%).

After having interviewed the sleepers about potential beneficial and/or side-effects some of them reported adverse effects including itching, sneezing and/or nausea.

The alpha-cypermethrin content in the three baseline MiraNet LN ranged from 5.03 to 5.20 g/kg, corresponding to 219.9 to 235.6 mg/m². All the nets complied with the target dose of 4.5 g/kg ± 25% (3.375–5.625 g/kg). The between-net variation, expressed as the relative standard deviation of the alpha-cypermethrin content found on the three different nets, was 1.73%, showing an acceptable homogeneity of the distribution of active ingredient between the nets.

The alpha-cypermethrin content in the three baseline MAGNet LN ranged from 6.56 to 6.67g/kg, corresponding to 314.6 to 335.1 mg/m². All the nets complied with the target dose of 5.8g/kg ± 25% (4.35–7.25 g/kg). The between-net variation, expressed as the relative standard deviation (RSD) of the alpha-cypermethrin content found on the three different nets, was 0.79%, showing an acceptable homogeneity of the AI between nets.

The alpha-cypermethrin content and within-net variations in unwashed treated nets are given in Table 2.4, while the alpha-cypermethrin content and retention rate are given in Table 2.5b.

Before the trial, the alpha-cypermethrin content in the three unwashed MiraNet LN was 4.81, 4.96 and 4.75 g/kg, complying with the target dose (Table 2.4). The within-net variation, expressed as the relative standard deviation of the alpha-cypermethrin content found on five different pieces cut from each of the three unwashed nets, was 4.4%, 3.6% and 4.1%, within the limits specified by the WHO guidelines of acceptable homogeneity (RSD < 20%). The alpha-cypermethrin content was 4.64 g/kg after 20 washes, corresponding to an overall alpha-cypermethrin retention per wash of 93%. After testing in the field, the alpha-cypermethrin content did not decrease, as it was 5.03 g/kg for the unwashed MiraNet LN and 4.72 g/kg for the MiraNet LN washed 20 times (Table 2.5b).

Before the trial the alpha-cypermethrin content in the three unwashed MAGNet LN was 6.23, 6.43 and 6.18 g/kg (Table 2.4). All the nets complied with the target dose. The within-net variation, expressed as the relative standard deviation of the alpha-cypermethrin content found on five different pieces cut from each of the three unwashed nets, was 0.7%, 0.9% and 0.8%, within the limits specified by the WHO guidelines of acceptable homogeneity

[RSD < 20%]. The alpha-cypermethrin content of MAGNet LN was 5.82 g/kg after 20 washes, corresponding to an overall alpha-cypermethrin retention of 91%. After testing in the field, the alpha-cypermethrin content did not markedly decrease, as it was 6.17 g/kg for the unwashed MAGNet LN and 5.94 g/kg for the MAGNet LN washed 20 times (Table 2.5b).

2.3 Efficacy – background and supporting documents

Non-WHOPES experimental hut study

Moshi District, United Republic of Tanzania

A non-WHOPES experimental hut study was conducted in the Lower Moshi rice irrigation area of Kilimanjaro in northern Tanzania (Kweka et al., 2015a). The study site was chosen because of its high vector abundance and high resistance level against pyrethroids of malaria vectors. The study area is located in Mabogini village (3°21' S; 37°20' E). Some 90% of the study area is covered with rice irrigation activities with both traditional and modern irrigation schemes. Almost all mosquitoes of the Gambiae complex are *Anopheles arabiensis* (98.8%).[1] *An. arabiensis* collected in Lower Moshi between 15 January and 18 February 2015 were found to have phenotypic and biochemical resistance mechanisms against pyrethroids.[2,3] The other main mosquito species in this area was *Culex quinquefasciastus*.

The status of resistance to pyrethroids of *An. arabiensis* was checked using the standard WHO insecticide susceptibility test kit using 0.05% deltamethrin and 0.75% permethrin impregnated papers. Results showed resistance in *An. arabiensis* against deltamethrin (mortality 28%) and permethrin (mortality 32%).

The efficacy of MiraNet LN was compared with that of Duranet LN (the reference LN), both unwashed and washed 20 times, and tested against free-flying, wild *An. arabiensis* in experimental huts. Duranet LN has alpha-cypermethrin incorporated into 150-denier, monofilament, HDPE yarn at 5.8 g AI/kg. It is manufactured by Shobikaa Impex Private Limited, India. This product has been evaluated by WHOPES and received a full recommendation.[4]

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[1] Matowo J et al. (2014). Dynamics of insecticide resistance and the frequency of kdr mutation in the primary malaria vector *Anopheles arabiensis* in rural villages of Lower Moshi, North Eastern Tanzania. J Parasitol Vector Biol;6:31–41.
[2] Matowo J et al. (2010). Biochemical basis of permethrin resistance in *Anopheles arabiensis* from Lower Moshi, north-eastern Tanzania. Malar J;9:193.
[3] Kulkarni M et al. (2006). Occurrence of the leucine-to-phenylalanine knockdown resistance (kdr) mutation in *Anopheles arabiensis* populations in Tanzania, detected by a simplified high-throughput SSOP-ELISA method. Malar J;5:56.
[4] WHO (2014). Report of the 16th WHOPES Working Group meeting – Review of Pirimiphos-methyl 300 CS, Chlorfenapyr 240 SC, Deltamethrin 62.5 SC-PE, Duranet LN, Netprotect LN, Yahe LN, Spinosad 83.3 Monolayer DT, Spinosad 25 Extended Release GR, 22–30 July 2013. Geneva: World Health Organization (http://apps.who.int/iris/bitstream/10665/90976/1/9789241506304_eng.pdf, accessed August 2015).

Untreated polyester nets were used as the negative control. The study was done in five East African type experimental huts.[1,2] Using untreated nets in the huts at the baseline, no difference in the mosquito attractiveness of the huts was observed.

The trial included 25 nights of mosquito collections in each of the five huts during 10 January 2015 and 18 February 2015, i.e. 5 nights per week per treatment (one complete Latin square). Every week (5 nights/week), the treatment arms were rotated among the huts according to a Latin square design. Five nets were used per treatment arm and each of the nets was tested during one night of the week.

For cone bioassays, *An. gambiae* s.s. (Kisumu strain), which was susceptible to deltamethrin and permethrin, was used.

The regeneration time between two consecutive washes was found to be of 2 days for MiraNet LN as claimed by the manufacturer, while for the Duranet LN it was 1 day as earlier reported by WHO.[3] Two MiraNet LN tested before washing provided knockdown of 91% and 90%; thus they both failed to meet the WHO cut-off for knockdown of ≥ 95%. One of these nets produced 78% mortality, meaning it also failed to meet the mortality criteria of ≥ 80%. The two unwashed Duranet LN failed in both WHO criteria (i.e. knockdown 90% and mortality 78%). After 20 washes, one each of MiraNet LN and Duranet LN gave knockdown of 100% but mortality was relatively low (around 60%). Similar results were observed with the unwashed LNs. After testing in huts, bioassays were performed on one of the nets from each of the five arms. Mortality was found to be 100% for unwashed and 20 times washed LNs. All knockdown values were below the threshold of ≥ 95% (i.e. 88–94%).

Overall, 5151 Culicidae mosquitoes were collected from the huts (including baseline) of which 4902 (95.2%) were *An. gambiae* s.l. The *An. gambiae* s.l. comprised 100% *An. arabiensis* as determined by PCR.

During the 25 nights' collections, 406 *An. arabiensis* were collected in the control hut, i.e. a mean number of 16.2 females were caught per night. Only 24% of the *An. arabiensis* were blood-fed. The high "natural" exophily recorded in the control hut (63.5%) is probably explained by the low feeding success of mosquitoes, which are more likely to have escaped the huts to find a blood meal. Conversely, mortality in mosquitoes was low (2.5%), indicating that no contamination with alpha-cypermethrin had occurred during the rotation of treated nets among the huts.

The number of *Anopheles* mosquitoes collected in the treated or control huts (i.e. entry rate) did not differ significantly, suggesting the absence of deterrent effect of the treated nets. The exophily (exit rate) was higher for the huts with LNs (73–83%) than the control huts (64%). All treatments caused > 95% induced mortality (mortality after correcting for that observed in the control) except for the Duranet LN washed 20 times (67%) (Table 2.2). Despite the

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[1] Smith A (1964). A review of the origin and development of experimental hut techniques used in the study of insecticides in East Africa. East Afr Med J;41:361–74.
[2] Mahande A et al. (2007). Feeding and resting behaviour of malaria vector, *Anopheles arabiensis* with reference to zooprophylaxis. Malar J;6:100.
[3] WHO (2008). Report of the 11th WHOPES Working Group meeting – Review of Spinosad 7.48% DT, Netprotect®, Duranet®, Dawaplus®, Icon® Maxx . 10–13 December 2007. Geneva: World Health Organization (http://whqlibdoc.who.int/hq/2008/WHO_HTM_NTD_WHOPES_2008.1_eng.pdf, accessed August 2015).

relatively low blood-feeding in the control arm (24%), blood-feeding was significantly lower in all treated arms (blood-feeding inhibition 62–78%) (Table 2.3).

A total of 86 *Culex* mosquitoes were collected in the control arm, of which 35% were blood-fed. The unwashed and washed MiraNet LN induced greater blood-feeding inhibition (100% and 90%, respectively) than the unwashed and washed Duranet LN (70% and 14%, respectively) (Table 2.2). Both the unwashed LNs performed equally well for induced mortality (around 50%) (Table 2.3) but after washing MiraNet LN induced higher mortality (38%) than Duranet LN (26%).

Some of the study participants interviewed reported certain potential beneficial effects of nets but also side-effects such as itching, sneezing and/or nausea. Certain participants who had slept under the untreated (control) nets also reported similar adverse effects.

The samples of MiraNet LN and Duranet LN were analysed for alpha-cypermethrin content using the CIPAC method 454/LN/M/3.2 by gas chromatography with a flame ionization detector (GC-FID) (Kweka et al., 2015b). The mean alpha-cypermethrin content in three unwashed MiraNet LN was found to be 5.10, 5.28 and 5.02 g AI/kg, complying with the target dose of 4.5 g AI/kg ± 25% (3.375–5.625 g AI/kg) (Table 2.4). The within-net variation for these three nets, expressed as the relative standard deviation of the alpha-cypermethin content found on five pieces per net, was 3.90%, 2.47% and 2.95% respectively, showing an acceptable homogeneity (relative standard deviation, RSD < 20%) of the distribution of active ingredient over the net. In MiraNet LN washed 20 times, the alpha-cypermethrin content in three nets was 4.60, 4.60 and 4.46 g/kg (mean 4.55 g/kg), showing an overall retention rate of 89% (Table 2.5b), while at the end of the trial it was respectively 4.44, 4.30 and 4.12 g/kg (mean 4.29 g/kg). The alpha-cypermethrin content in three unwashed Duranet LN was found to be 5.76, 5.72 and 5.80 g AI/kg (mean 5.76 g/kg), which complied with the target dose of 5.8 g/kg ± 25% (4.35–7.25 g AI/kg) (Tables 2.4 and 2.5b). The average within-net variation for these three nets expressed as the relative standard deviation of the alpha-cypermethrin content found in five pieces was 3.60%, 1.92% and 3.66%, showing an acceptable homogeneity (RSD < 20%) of the distribution of active ingredient in the net. In Duranet LN washed 20 times, the alpha-cypermethrin content in three nets was 5.18, 5.24 and 5.18 g/kg (mean 5.20 g/kg) with an overall retention rate of 90% (Table 2.5b), while at the end of the trial it was 4.72, 4.98 and 4.68 g AI/kg (mean 4.79 g/kg) respectively.

2.4 Conclusions and recommendations

MiraNet LN is a long-lasting insecticidal net manufactured by A to Z Textile Mills Ltd. The net is made of a 130-denier mono-filament HDPE yarn, containing alpha-cypermethrin. The alpha-cypermethrin is incorporated into the filaments, with the target dose of 4.5 g AI/kg corresponding to 180 mg of alpha-cypermethrin per square metre of the fabric.

WHO's assessment of the manufacturer's compliance with the assessment of exposure to and risks of washing and sleeping under a MiraNet LN was in line with its generic risk assessment model; when used as instructed, the net does not pose undue risk to the user. The content of alpha-cypermethrin in unwashed MiraNet LN samples tested in Phase I and II studies complied with the target dose of 4.5 g/kg ± 25% [3.375–5.625 g/kg]. The between-net and within-net variation of the alpha-cypermethrin content was within the limits specified by the WHO specifications and guidelines, showing acceptable homogeneity (RSD < 20% for within-net variation).

In Phase I cone-bioassays, a wash resistance study based on cone bioassays showed a regeneration time of 2 days after three consecutive wash–dry cycles in a single day. The chemical analysis of MiraNet LN washed up to 25 times showed an exponential decay of alpha-cypermethrin as a function of the number of washes with an average wash resistance index of 99.8%. After 20 washes the MiraNet LN contained 96% of the initial alpha-cypermethrin content. The MiraNet LN met the WHO Phase I bioefficacy criteria.

In the Phase II experimental hut studies, the retention of active ingredient after 20 washes of nets ranged from 84% to 93%. At the end of the four trials (three WHOPES-supervised, one non-WHOPES supervised) in experimental huts, all tested MiraNet LN washed 20 times complied with the WHO criteria for bioefficacy (mortality ≥ 80% or knockdown ≥ 95%).

The three Phase II studies performed in Africa were conducted in settings with pyrethroid-resistant malaria vectors. In M'Be (Côte d'Ivoire) and Muheza (United Republic of Tanzania), because of the pyrethroid resistance, low induced mortality was observed in the presence of treated nets so that only blood-feeding inhibition was used to evaluate the performance of MiraNet LN against a reference net (MAGNet LN) or a conventionally treated net washed until just before exhaustion. In both settings, blood-feeding inhibition was similar for MiraNet LN washed 20 times and the reference LN washed 20 times or the conventionally treated net washed until just before exhaustion. However, in M'Be (Côte d'Ivoire), high induced mortality was observed on other mosquitoes for all treatment arms (around 90%).

In a non-WHOPES supervised study in Moshi (United Republic of Tanzania) conducted by the Tropical Pesticides Research Institute, Arusha, despite the deltamethrin and permethrin resistance (around 30%) in *An. arabiensis* reported in the documentation submitted, an unexpectedly high mortality (68% to 100%) was observed with all treatments. However, in the same site in previous studies much lower mortalities (33% to 50%) were consistently observed in *An. arabiensis* with nets conventionally treated with alpha-cypermethrin or other pyrethroid insecticides.[1,2] MiraNet LN washed 20 times (mortality of 100%) was reported to perform better than Duranet LN washed 20 times (mortality of 68%). Moreover, blood-feeding inhibition was similar or slightly higher with MiraNet LN than with Duranet LN (around 70%). Although the non-WHOPES study in Moshi was reported here but because the results were at variance with earlier alpha-cypermethrin trials on *An. arabiensis* run in the same location, the meeting decided to not consider this study in making the final recommendation on MiraNet LN.

In Thailand the main vector *An. minimus* s.s. is fully susceptible against alpha-cypermethrin. In this setting, unwashed and washed MiraNet LN induced higher mortalities (> 95%) than with MAGNet LN (88%) in experimental huts. A few of the anopheline mosquitoes were blood-fed in the presence of either treated or untreated nets.

[1] Mosha FW et al. (2008). Comparative efficacies of permethrin-, deltamethrin- and alpha-cypermethrin-treated nets, against *Anopheles arabiensis* and *Culex quinquefasciatus* in northern Tanzania. Ann Trop Med Parasitol;102:367–76.
[2] Oxborough R et al. (2013). ITN mixtures of chlorfenapyr (pyrrole) and alpha-cypermethrin (pyrethroid) for control of pyrethroid resistant *Anopheles arabiensis* and *Culex quinquefasciatus*. PLoS ONE;8:e55781.

In three WHOPES-supervised experimental hut studies the performance of the MiraNet LN was not different from a reference LN (MAGNet LN) or a conventionally treated net washed until just before exhaustion.

Thus, the MiraNet LN fulfilled the requirements of WHOPES Phase I and Phase II studies for LNs.

Considering the safety, efficacy and resistance to washing of MiraNet LN in laboratory studies and small-scale (Phase II) field studies, the 18th WHOPES Working Group recommended:

- that a time-limited interim recommendation be given for the use of MiraNet LN in the prevention and control of malaria; and

- that WHOPES should coordinate large-scale (Phase III) field studies of MiraNet LN to confirm its long-lasting efficacy and assess its attrition, fabric integrity and community acceptability, as a basis for developing full recommendations on the use of this product.

The meeting also recommended:

- that the national authorities and procurement agencies must ensure that the MiraNet LN complies with WHO specifications as recommended by the Organization according to the quality control procedures outlined in the *Guidelines for procuring public health pesticides*.[1]

Note: WHO recommendations on the use of pesticides in public health are valid ONLY if linked to WHO specifications for their quality control.

[1] WHO (2012). Guidelines for procuring public health pesticides. Geneva: World Health Organization (http://whqlibdoc.who.int/publications/2012/9789241503426_eng.pdf, accessed August 2015).

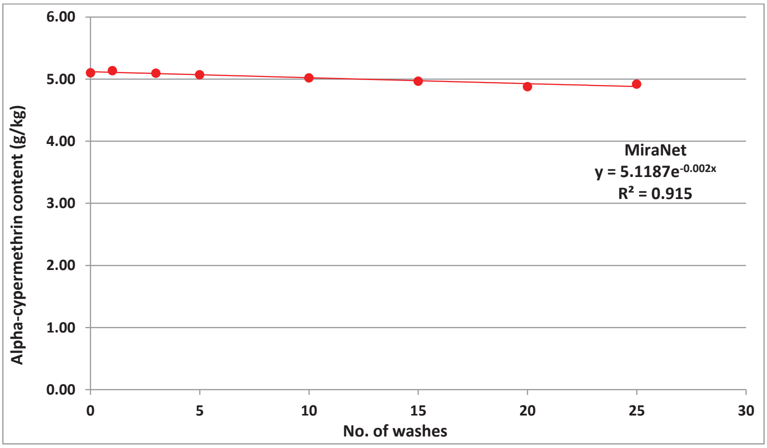

Figure 2.1 Alpha-cypermethrin content and retention (wash curve) for MiraNet (WHOPES Phase I study)

Table 2.1 WHOPES Phase I wash resistance study: alpha-cypermethrin (AI) content and retention of MiraNet LN and corresponding knockdown and mortality in cone bioassays. Target dose and tolerance limit for alpha-cypermethrin content in baseline MiraNet LN = 4.5 g/kg ± 25%. [3.375–5.625 g/kg]

LN	Wash	AI content (g/kg)	Between-net RSD (%)	AI retention (% of wash 0)	AI WRI (% at each wash)	Bioassays	
						Knockdown	Mortality
MiraNet	0	5.10	0.8%	–	–	100%	100%
	1	5.14	1.5%	100.7	100.7	100%	100%
	3	5.10	1.8%	99.9	100.0	100%	95 ± 3%
	5	5.07	2.0%	99.4	99.9	100%	99 ± 1%
	10	5.02	0.8%	98.4	99.8	100%	80 ± 5%
	15	4.97	2.6%	97.4	99.8	100%	88 ± 4%
	20	4.88	0.8%	95.7	99.8	100%	68 ± 6%
	25	4.92	1.3%	96.5	99.9	97 ± 2%	55 ± 7%

AI = active ingredient; RSD = relative standard deviation; WRI = wash resistance index

Table 2.2 MiraNet LN Phase II studies: overview of mortality (%) and corrected mortality (% shown in bold type) in wild mosquitoes. Values in the same row sharing the same superscript letter do not differ significantly

Study sites (number of mosquitoes collected)	Status of pyrethroid resistance	Untreated net	MiraNet LN unwashed	MiraNet LN washed 20 times	MAGNet LN (Duranet LN)[1] unwashed	MAGNet LN (Duranet LN)[1] washed 20 times	CTN washed until just before exhaustion
***Anopheles* spp.**							
M'Be, Côte d'Ivoire	Alpha-cypermethrin resistance/*kdr*[1]	8.0^{c}	$15.2^{b,c}$	13.9^{b}	30.5^{d}	19.2^{c}	$10.6^{a,b}$
An. gambiae s.l. (1594)			**7.8**	**6.5**	**24.4**	**12.3**	**2.9**
Muheza, Tanzania	Deltamethrin+alpha-cypermethrin resistance[2]	3.7^{a}	5.7^{a}	5.8^{a}	5.7^{a}	5.3^{a}	5.0^{a}
An. funestus (136)			**2.1**	**2.2**	**2.1**	**1.6**	**1.4**
Mae Sot, Thailand	Alpha-cypermethrin susceptible *An. minimus*	6.5^{a}	96.8^{b}	97.6^{b}	88.6^{c}	88.3^{c}	
Anopheles spp. (77)			**97**	**97**	**88**	**87**	
Moshi, Tanzania[1]	Deltamethrin+permethrin R[3]	2.5^{a}	97.9^{b}	100^{bc}	99.8^{b}	67.9^{d}	
An. arabiensis (406)			**97.9**	**100**	**99.8**	**67.1**	
Other Culicidae[4]							
M'Be, Côte d'Ivoire		35.0^{a}	91.7^{c}	95.8^{c}	96.6^{c}	90.8^{c}	86.2^{b}
Culicinae (274)			**87.2**	**93.5**	**94.8**	**85.8**	**78.7**
Mae Sot, Thailand		7.2^{a}	96.2^{b}	95.5^{b}	92.0^{b}	88.1^{b}	5.0^{a}
Culicidae (incl. *Anopheles*) (181)			**95.9**	**95.2**	**91.4**	**87.2**	
Moshi, Tanzania[1]		0	51.2^{a}	37.9^{b}	52.9^{a}	26.2^{c}	
Culicinae (86)			**51.2**	**37.9**	**52.9**	**26.2**	

[1] In the non-WHOPES study in Moshi, United Republic of Tanzania, Duranet LN was used as the reference long-lasting insecticidal net (LN).
[2] L1014F *kdr* frequency of 94%; increased esterases (alpha and beta), oxidases and GSTs; 68% mortality at diagnostic dose of alpha-cypermethrin 0.05%.
[3] 60% mortality at diagnostic dose of alpha-cypermethrin 0.05%; 75% mortality at diagnostic dose of deltamethrin 0.05%.
[4] 28% mortality at diagnostic dose of deltamethrin 0.05%; 32% mortality at diagnostic dose of permethrin 0.75%.
CTN = conventionally treated net

Table 2.3 MiraNet Phase II studies: overview of the blood-feeding (%) and blood-feeding inhibition (% shown in bold type) in wild mosquitoes. Values in the same row sharing the same superscript letter do not differ significantly ($P > 0.05$)

Study sites (number of mosquitoes in control huts)	Status of pyrethroid resistance	Untreated net	MiraNet LN unwashed	MiraNet LN washed 20 times	MAGNet LN (Duranet LN)[1] unwashed	MAGNet LN (Duranet LN)[1] washed 20 times	CTN washed until just before exhaustion
***Anopheles* spp.**							
M'Be, Côte d'Ivoire	Alpha-cypermethrin resistance/*kdr*[2]	61.7^{c}	38.9^{b}	42.8^{b}	27.5^{a}	40.8^{b}	41.6^{b}
An. gambiae s.l. (1594)			**36.9**	**30.6**	**55.4**	**33.9**	**32.6**
Muheza, Tanzania	Deltamethrin+alpha-cypermethrin resistance[3]	23.5^{a}	6.6^{b}	19.9^{b}	12.4^{b}	13.8^{b}	$14.3^{a,b}$
An. funestus (136)			**71.9**	**40.9**	**47.2**	**41.3**	**39.1**
Mae Sot, Thailand	Alpha-cypermethrin susceptible *An. minimus*	0^{a}	0^{a}	2.4^{a}	1.3^{a}	0^{a}	
Anopheles spp. (77)			NS	NS	NS	NS	
Moshi, Tanzania[1]	Deltamethrin+permethrin R[4]	24.1^{a}	6.4^{b}	9.1^{b}	5.3^{b}	6.3^{b}	
An. arabiensis (406)			**73.4**	**62.2**	**78.0**	**73.9**	
Other Culicidae							
M'Be, Côte d'Ivoire		46.7^{a}	7.14^{b}	3.4^{b}	3.41^{b}	2.5^{b}	6.92^{b}
Culicinae (274)			**84.7**	**92.8**	**92.7**	**94.6**	**85.2**
Mae Sot, Thailand Culicidae (incl.		1.7^{a}	3.3^{a}	2.2^{a}	2.3^{a}	1.1^{a}	
Anopheles) (181)			NS	NS	NS	NS	
Moshi, Tanzania[1]		34.9	0^{a}	3.2^{b}	10.3^{c}	29.9^{d}	
Culicinae (86)			**100**	**90.1**	**70.5**	**14.3**	

[1] In the non-WHOPES study in Moshi, United Republic of Tanzania, Duranet LN was used as the reference long-lasting insecticidal net (LN).
[2] L1014F *kdr* frequency of 94%; increased esterases (alpha and beta), oxidases and GSTs; 68% mortality at diagnostic dose of alpha-cypermethrin 0.05%.
[3] 60% mortality at diagnostic dose of alpha-cypermethrin 0.05%; 75% mortality at diagnostic dose of deltamethrin 0.05%.
[4] 28% mortality at diagnostic dose of deltamethrin 0.05%; 32% mortality at diagnostic dose of permethrin 0.75%.
CTN = conventionally treated net

Table 2.4 Phase II studies: alpha-cypermethrin average content and within-net variation (RSD), in unwashed MiraNet LN, MAGNet LN and Duranet LN. The compliance of the mean alpha-cypermethrin content with the specification is also presented for each net. Target dose and tolerance limit for alpha-cypermethrin content at baseline are: MiraNet LN = 4.5 g/kg ± 25% [3.375–5.625 g/kg]; MAGNet LN or Duranet LN = 5.8 g/kg ± 25% [4.35–7.25 g/kg]

LN	M'Be, Côte d'Ivoire			Muheza, United Republic of Tanzania			Mae Sot, Thailand		
	AI content (g/kg)	Compliance with WHO specification	AI within-net variation (RSD)	AI content (g/kg)	Compliance with WHO specification	AI within-net variation (RSD)	AI content (g/kg)	Compliance with WHO specification	AI within-net variation (RSD)
MiraNet 1	4.50	Yes	2.1%	4.84	Yes	1.9%	4.81	Yes	4.4%
MiraNet 2	4.79	Yes	8.2%	4.87	Yes	0.9%	4.96	Yes	3.6%
MiraNet 3	–	–	–	4.70	Yes	2.7%	4.75	Yes	4.1%
MAGNet 1	6.43	Yes	2.5%	6.41	Yes	4.5%	6.23	Yes	0.7%
MAGNet 2	6.33	Yes	3.8%	6.36	Yes	6.3%	6.43	Yes	0.9%
MAGNet 3	–	–	–	6.57	Yes	1.7%	6.18	Yes	0.8%

LN	Moshi, United Republic of Tanzania*		
	AI content (g/kg)	Compliance with WHO specification	AI within-net variation (RSD)
MiraNet 1	5.10	Yes	3.90%
MiraNet 2	5.28	Yes	2.47%
MiraNet 3	5.02	Yes	2.95%
Duranet 1	5.76	Yes	3.60%
Duranet 2	5.72	Yes	1.92%
Duranet 3	5.80	Yes	3.66%

*Non-WHOPES supervised trial.
AI = active ingredient; LN = long-lasting insecticidal net; RSD = relative standard deviation; WHO = World Health Organization

Table 2.5a Phase II studies: alpha-cypermethrin content and retention in MiraNet LN and MAGNet (or Duranet) LN. Target dose and tolerance limit for alpha-cypermethrin content in baseline MiraNet = 4.5 g/kg ± 25% [3.375–5.625 g/kg]; target dose and tolerance limit for alpha-cypermethrin content in baseline MAGNet or Duranet LN = 5.8 g/kg ± 25% [4.35–7.25 g/kg]

Treatment	M'be, Côte d'Ivoire				Muheza, United Republic of Tanzania			
	AI content (g/kg) before washing	AI content (g/kg) after washing and before trial	AI retention after washing (% of wash 0)	AI content (g/kg) after trial	AI content (g/kg) before washing	AI content (g/kg) after washing and before trial	AI retention after washing (% of wash 0)	AI content (g/kg) after trial
MiraNet 0 wash	4.50	–	–	4.62	4.84	4.70	–	4.86
MiraNet 20 washes	4.79	4.13	86%	4.10	4.87	4.11	84%	3.75
MAGNet 0 wash	6.43	–	–	5.95	6.41	6.57	–	6.26
MAGNet 20 washes	6.33	5.35	85%	4.87	6.36	5.18	81%	5.07
CTN*	1.50	0.06	4%	0.18	1.33	0.17	13%	< 0.05
Untreated net	< 0.05	–	–	< 0.05	–	–	–	–

AI = active ingredient. *CTN (conventionally treated net) washed until just before exhaustion.

Table 2.5b Phase II studies: alpha-cypermethrin content and retention in MiraNet LN and MAGNet (or Duranet) LN. Target dose and tolerance limit for alpha-cypermethrin content in baseline MiraNet LN = 4.5 g/kg ± 25% [3.375–5.625 g/kg]; target dose and tolerance limit for alpha-cypermethrin content in baseline MAGNet or Duranet LN = 5.8 g/kg ± 25% [4.35–7.25 g/kg]

Treatment	Mae Sot, Thailand				Moshi, United Republic of Tanzania			
	AI content (g/kg) before washing	**AI content (g/kg) after washing and before trial**	**AI retention after washing (% of wash 0)**	**AI content (g/kg) after trial**	**AI content (g/kg) before washing**	**AI content (g/kg) after washing and before trial**	**AI retention after washing (% of wash 0)**	**AI content (g/kg) after trial**
MiraNet 0 wash	4.81	4.75	–	5.03	5.13	–	–	–
MiraNet 20 washes	4.96	4.64	93%	4.72	–	4.55	89%	4.29
MAGNet 0 wash (DuraNet in Moshi, Tanzania)	6.23	6.18	–	6.17	5.76	–	–	–
MAGNet 20 washes (DuraNet in Moshi, Tanzania)	6.43	5.82	91%	5.94	–	5.20	90%	4.79
Untreated net	< 0.05	< 0.05	–	< 0.05	–	–	–	–

3. REVIEW OF PANDA NET 2.0 LN

Panda Net 2.0 LN is a deltamethrin long-lasting (incorporated into polyethylene) insecticidal net. Deltamethrin is incorporated into 120-denier, monofilament HDPE yarn, with the target dose of 1.8 g AI/kg, corresponding to 76 mg AI/m^2 deltamethrin per square metre of the fabric. Panda Net 2.0 is manufactured by Life Ideas Textiles Company Ltd, China.[1]

Deltamethrin has previously been recommended by WHO[2] as an insecticide product for conventional treatment of mosquito nets for malaria vector control, at the target dose of 15–25 mg/m².

3.1 Safety assessment

The assessment of the risk to humans of washing and sleeping under the Panda Net 2.0 LN was performed on the basis of the data provided by the manufacturer (Aitio, 2015a). The revised WHO *Generic risk assessment model for insecticide-treated nets* was used as a guiding document.[3]

The following assumptions were used by the WHO evaluator in drafting the assessment:

- the product and the active ingredient deltamethrin comply with the WHO specification;
- the wash resistance index (⩾ 95%) reflects the proportion of the active ingredient available for dermal and oral absorption, and that released during the washing of the net;
- dermal absorption of deltamethrin is 10% and oral absorption is 75%;
- the translodgeable part of deltamethrin from the net onto the skin is 6% of the available surface concentration defined from the wash resistance index, in line with the estimate of the United States Environmental Protection Agency for soft surfaces; and
- secretion of deltamethrin as such in mother's milk does not exceed that observed in cows multiplied by $10^{0.6}$, i.e. 6.4% of the dose.

It was concluded that when used as instructed, sleeping under or washing Panda Net 2.0 LN does not pose undue hazards to people.

3.2 Efficacy – WHOPES-supervised studies

3.2.1 Laboratory study

A laboratory study was conducted to determine the regeneration time, resistance to washing and efficacy of the Panda Net 2.0 LN (Rossignol et al., 2013b). Four Panda Net 2.0 LN were used for the evaluation. Ten pieces (25 cm x 25 cm) were cut from each net. Eight pieces were used for the regeneration study while 28 pieces were used for the wash resistance evaluation. The remaining four nets were stored at 4 ^{o}C and retained as a reference.

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[1] WHO has been advised that as from 10 August 2015, the name of the manufacturer has been changed to Life Ideas Biological Technology Company Ltd. in place of Life Ideas Textile Company Ltd.
[2] WHO recommended insecticide products for treatment of mosquito nets for malaria vector control [updated 17 November 2014] (http://who.int/whopes/Insecticides_ITN_Malaria_Nov2014.pdf, accessed August 2015).
[3] WHO (2012). A generic risk assessment model for insecticide-treated nets, revised edition. Geneva: World Health Organization (http://whqlibdoc.who.int/publications/2012/9789241503419_eng.pdf, accessed August 2015).

Regeneration time was determined by washing the test nets three times consecutively on day 0 and then performing WHO cone bioassays on the nets at 1, 2, 3, 5 and 7 days after the completion of washing. The number of days required to reach a plateau in mortality was considered the regeneration time. Washing was done by placing individual net samples in 1 L beakers containing 0.5 L of deionized water and 2 g/L of soap (Savon de Marseille, pH = 10–11). The beakers were placed in a water bath at 30 ^{o}C and shaken for 10 minutes at 155 movements per minute. The nets were rinsed twice in deionized water using the same conditions and then dried at room temperature for 2 hours. After washing, nets were stored in aluminium foil at 30 ^{o}C.

WHO cone bioassays were conducted by attaching four plastic WHO cones to each netting piece and introducing five non-bloodfed, 2–5 days old *An. gambiae* Kisumu strain into each cone. The Kisumu strain is susceptible to pyrethroid insecticides and is confirmed to be free of known resistance mechanisms. Mosquitoes were exposed for 3 minutes and then held with access to sugar solution for 24 hours. Knockdown was assessed 60 minutes after exposure and mortality was assessed 24 hours after exposure.

Chemical analysis was done on samples after each washing according to the CIPAC method 333/LN/(M2)/3. Deltamethrin was extracted by refluxing for 30 minutes with xylene with dibutyl phthalate as an internal standard. Deltamethrin content was then measured by high performance liquid chromatography with UV diode array detector (HPLC-DAD) (Pigeon et al., 2013b).

For the determination of regeneration time, knockdown of mosquitoes exposed to the Panda Net 2.0 LN was 97% at baseline. Knockdown was 97% or higher for all bioassays except at 5 days after washing when it was 88%. Baseline mortality of mosquitoes exposed to the Panda Net 2.0 LN was 51%. Mortality declined to 9% after three washes and then rose to 40% after 3 days of storage. Mortality was 61% after 5 days and 42% after 7 days. Based upon mortality, the regeneration time of the Panda Net 2.0 LN was set at 3 days.

In wash resistance tests, knockdown of mosquitoes exposed to the Panda Net 2.0 LN was 97% at baseline and then fell to 86% after one wash (Table 3.1). However, knockdown increased to > 95% through 20 washes and then fell to 58% after 25 washes. Mortality of mosquitoes exposed to the Panda Net 2.0 LN was low even at baseline when it was 51%. Mortality fell to < 30% through fives washes, rose to 46% after 10 washes but then declined again and was less than 20% through 25 washes. Since knockdown remained above 95% through 20 washes, the Panda Net 2.0 LN met the criteria for Phase I testing of long-lasting insecticidal nets and no tunnel tests were therefore required.

For the wash resistance study, the deltamethrin content of the Panda Net 2.0 LN at baseline was 1.83 g AI/kg, which was within specifications (1.35 to 2.25 g AI/kg). After 20 washes, the deltamethrin content had declined to 1.67 g/kg, corresponding to an overall retention rate of 91.1%, and to a wash resistance index per wash of 99.5% (Figure 3.1, Table 3.1).

3.2.2 Experimental hut studies

Muheza, United Republic of Tanzania

An experimental hut study was conducted in Muheza, United Republic of Tanzania, to determine the efficacy of the Panda Net 2.0 LN against a WHOPES-recommended LN (PermaNet 2.0 LN) and a net treated conventionally with deltamethrin and washed until just before exhaustion (Tungu et al., 2015b). Nets were tested in six huts made in the traditional East African style. The huts were made of brick walls plastered with mud on the inside, a wooden ceiling covered with hessian sackcloth and a corrugated iron roof. The huts were constructed on a concrete base with a water-filled moat to exclude scavenging by ants. Each hut had window exit traps and verandas on each side. Two verandas on opposite sides were screened to capture exiting mosquitoes. The other verandas were left open to allow for entry of mosquitoes.

For the experimental hut study, six treatment arms were included in the study as follows:

- untreated polyester net
- unwashed Panda Net 2.0 LN
- unwashed PermaNet 2.0 LN
- Panda Net 2.0 LN washed 20 times
- PermaNet 2.0 LN washed 20 times
- polyester net treated conventionally with deltamethrin at a target dose of 25 mg/m^2 and washed until just before exhaustion.

Nets were washed in 10 L of soap solution at 2 g/L (Savon de Marseille). Nets were agitated by stirring with a wooden pole at 20 rotations per minute for 3 minutes, allowed to soak for 4 minutes and then agitated for another 3 minutes. Nets were rinsed twice in clean water using the same procedure and then allowed to dry between each wash. The interval of time between two washes was 3 days for Panda Net 2.0 LN and 1 day for PermaNet 2.0 LN.

The bioassays were performed on one of the nets from each of the six treatment arms at three intervals: before washing, after washing but before the hut trial, and after completion of the trial. The Both LN were washed 20 times, while the conventionally treated net was washed up to 10 times to determine the point of exhaustion. Bioassays were conducted after each washing by exposing batches of five unfed female *An. gambiae* Kisumu strain in WHO cones fixed to the five different net panels. Ten mosquitoes were exposed at each site for a total of 50 mosquitoes tested for each net. The Panda Net 2.0 LN retained high levels of biological activity up to 20 washes, with mortality consistently > 80%. The conventionally treated net began to lose efficacy after four washes when mortality dropped to 82%. Knockdown fell below 95% and mortality fell below 80% after five washes and therefore, the point just before exhaustion was considered to be four washes.

In the experimental hut study, three nets were available for each arm of the study and were rotated along with the sleepers through each hut in a Latin square so that each net was tested twice during each rotation. At the end of each rotation the huts were cleaned and aired before treatments were moved to the next huts. Data were collected over a single full rotation (i.e. 36 nights).

Deltamethrin content in the treated nets was determined three times: at baseline (before washing), after washing but before start of the experimental hut study, and after finishing the trial. One net from each treatment arm was used for chemical analysis at each time point. For all chemical analyses, five pieces were cut from the net and deltamethrin content determined by HPLC as described above.

Six holes (4 cm x 4 cm) were cut into each net to simulate a torn net following WHO guidelines. Volunteers slept in each hut one night per week according the Latin square rotation. Each morning, mosquitoes were collected from the floor, walls, exit traps, veranda and inside the nets. Each mosquito was scored as dead or alive and as blood-fed or unfed. Live mosquitoes were held for 24 hours to assess delayed mortality. The primary outcomes included deterrence, induced exophily, blood-feeding inhibition and mortality.

WHO susceptibility tests were carried out on live *An. funestus* mosquitoes collected from the huts with untreated nets. The mortality of *An. funestus* exposed to deltamethrin and alpha-cypermethrin impregnated papers (0.05% concentration) was 75.3% and 60.3% respectively. Mortality of F1 *An. gambiae* collected from both control and intervention huts was 73.8% when exposed to deltamethrin, 50.6% when exposed to lambda-cyhalothrin and 81% when exposed to permethrin.

In cone bioassays conducted before net washing, knockdown and mortality of the susceptible *An. gambiae* Kisumu strain were 100% for all treatment arms. After washing, knockdown was 100% for all treatment arms except the unwashed Panda Net 2.0 LN where it was 98%. Mortality was 100% for all treatment arms except the conventionally treated net washed until just before exhaustion where mortality was 84%. After the hut trial, knockdown was 100% for all treatment arms except for the conventionally treated net (knockdown = 92%) while mortality was > 90% for all treatment arms.

In experimental huts *An. funestus* was more abundant than *An. gambiae* but numbers overall were low. A total of 126 *An. funestus* and 29 *An. gambiae* were collected in the huts with untreated nets. Although there were statistically significant differences in the numbers of mosquitoes entering the huts, there was no consistent evidence for deterrence as the huts with untreated nets had the fewest *An. gambiae* and the second fewest *An. funestus*.

Results of the experimental hut trial with free-flying wild mosquitoes are presented in Table 3.2. Blood-feeding rates for *An. funestus* were 28.6% in the huts with untreated nets. Blood-feeding rates were significantly lower in all other treatment arms with the lowest rates observed in huts with the unwashed Panda Net 2.0 LN (6.2%). Results were similar for *An. gambiae* s.l. where 34.5% of mosquitoes captured in the huts with untreated nets were blood-fed. Blood-feeding rates were significantly lower for all other treatment arms except for the PermaNet 2.0 washed 20 times (30.8%).

Mortality of *An. funestus* was significantly higher than that of the control for all treatment arms except the conventionally treated net washed until just before exhaustion. Mortality in huts with the Panda Net 2.0 LN was significantly higher than that for the conventionally treated net but was not significantly different from that of the PermaNet 2.0 washed 20 times. However, corrected mortality did not exceed 20% for any treatment arm. For *An. gambiae*, the mortality rates were significantly higher than the control net for the unwashed Panda Net 2.0 LN and for the unwashed PermaNet 2.0 LN. There were no other differences in mortality.

The deltamethrin content and within-net variations in unwashed LNs are given in Table 3.4. Chemical analysis indicated that before washing, samples of the Panda Net 2.0 LN had relatively high deltamethrin contents but were within specifications. PermaNet 2.0 also complied with its specification limits for deltamethrin content and within-net variation (< 20%).

The deltamethrin content and retention rate are given in Table 3.5. Mean deltamethrin content was 2.24 g AI/kg in the unwashed Panda Net 2.0 and 2.18 g AI/kg in the Panda Net 2.0 LN that was to be washed 20 times. The mean deltamethrin content was 1.42 g AI/kg in the unwashed PermaNet 2.0 LN and 1.41 g AI/kg in the PermaNet 2.0 LN to be washed 20 times. Deltamethrin content on the unwashed conventionally treated net was 0.59 g/kg (24.6 mg AI/m^2; target dose = 25 mg AI/m^2).

After washings were completed, the unwashed Panda Net 2.0 LN had 2.17 g AI/kg and the Panda Net 2.0 LN washed 20 times had 1.54 g AI/kg of deltamethrin, corresponding to an overall deltamethrin retention of 71% (Table 3.5). The deltamethrin content of the unwashed PermaNet 2.0 LN was 1.42 g AI/kg and that of the PermaNet 2.0 LN washed 20 times was 0.69 g AI/kg, corresponding to an overall deltamethrin retention of 49%. The deltamethrin content of the conventionally treated net washed four times was 0.03 g/kg (1 mg/m^2). After the hut trial the deltamethrin content on the unwashed Panda Net 2.0 LN was 2.10 g AI/kg while that of the Panda Net 2.0 LN washed 20 times was 1.62 g AI/kg. For the PermaNet 2.0 LN, the deltamethrin content was 1.14 g AI/kg on the unwashed net and 0.63 g AI/kg on the net washed 20 times. Deltamethrin content on the conventionally treated net was < 0.01 g/kg (< 0.4 mg AI/m^2) (Pigeon et al., 2015c).

M'be, Côte d'Ivoire

A Phase II trial of the Panda Net 2.0 LN was carried out in M'be in Côte d'Ivoire (Koffi et al., 2015a). The study area is in a rice growing region in central Côte d'Ivoire. The mosquito population comprised *An. gambiae* s.l. (primarily *An. coluzzii*), *An. funestus*, and *Culex* spp. and *Mansonia* spp. *An. coluzzii* has been reported to be resistant to pyrethroids, organochlorines and carbamates. Resistance mechanisms include both the *kdr* mutation (L1014F) at over 90% frequency as well as metabolic resistance.

The experimental huts were made of concrete bricks with a corrugated iron roof, a ceiling of polyethylene sheeting and a concrete base with a water-filled moat to prevent the entry of ants. Mosquitoes entered the huts through four window slits constructed of metal at an angle to create a funnel with a 1 cm gap. A single veranda trap made of polyethylene sheeting and screened mesh extended from the back of the hut to capture exiting mosquitoes.

The following comparison arms were tested:

- untreated polyester net
- unwashed Panda Net 2.0 LN
- unwashed PermaNet 2.0 LN
- Panda Net 2.0 LN washed 20 times
- PermaNet 2.0 LN washed 20 times
- polyester net treated conventionally with deltamethrin at a target dose of 25 mg/m^2 and washed until just before exhaustion.

To estimate the number of washes required for the polyester net treated conventionally and washed until just before exhaustion, nets were washed and WHO cone bioassays conducted on the five net panels by exposing susceptible *An. gambiae* Kisumu mosquitoes. Knockdown fell below 95% after two washes while mortality fell below 80% after four washes. Therefore, the conventionally treated nets were washed three times for the experimental hut study. The interval of time between washes was 3 days for Panda Net 2.0 LN and 2 days for PermaNet 2.0 LN (note that according to the WHO specifications of PermaNet 2.0 LN the regeneration time is 1 day).

The nets were washed in non-plastic bowls containing 10 L of water and 2 g/L of soap (Savon de Marseille). Each net was agitated for 3 minutes, left to soak for 4 minutes and then agitated for 3 more minutes. The nets were agitated by stirring with a pole at 20 rotations per minute. The nets were rinsed twice in clean water using the same conditions and then dried horizontally in the shade and stored at ambient temperature between washes.

WHO cone bioassays were run on two nets per treatment arm before and after washing. At the end of the hut trial, two nets per treatment arm were tested using WHO cone bioassays. Five cones were placed on each net with one cone per net panel. Five female *An. gambiae* Kisumu strain mosquitoes were introduced into each cone for 3 minutes. Bioassays were repeated so that a total of 50 mosquitoes were exposed per net. Additional bioassays were performed using wild *An. gambiae* from M'be. For all bioassays, knockdown was recorded 60 minutes after exposure and mortality was recorded 24 hours after exposure. A seventh net was used in each treatment arm for determination of the deltamethrin content. One piece (30 cm x 30 cm) was taken from each of five locations on the net before and after washing. After the hut trial, one net from each arm was randomly sampled for chemical analysis. The net pieces were placed in aluminium foil in sealed bags and stored at 4 ^{o}C until they were analysed by HPLC as described above.

In the experimental hut study, the treatment arms were randomly allocated to the huts and rotated each week. Six nets were used for each treatment arm with a different net used each night. Each net had six holes (4 cm x 4 cm) cut in it to simulate a torn net. At the end of each rotation the huts were thoroughly cleaned and aired for a day to remove potential contamination. The trial was run through two complete rotations (i.e. 72 nights) to collect enough mosquitoes for statistical analysis.

Volunteers were recruited to sleep inside the huts. The sleepers entered the huts at 20:00 and remained inside until 06:00. The sleepers rotated randomly through the huts each night of the study. Mosquitoes were collected in the morning from inside the nets, inside the room and inside the exit traps and veranda. All mosquitoes were scored as dead or alive and as blood-fed or unfed. Live mosquitoes were held in small cups with access to sugar solution and held for 24 hours to assess delayed mortality. The primary outcomes included deterrence, induced exophily, blood-feeding, and immediate and delayed mortality.

In cone bioassays conducted before washing the nets, corrected knockdown and mortality of the susceptible *An. gambiae* Kisumu strain was almost 100% for all treatment arms (Table 3.6). After washing but before field testing, knockdown fell to 32% for the conventionally treated net, 95% for the Panda Net 2.0 LN and 77% for the PermaNet 2.0 LN while mortality fell to 81%, 97% and 87%, respectively. After the completion of the hut trial, knockdown was greater than 90% for all treatment arms except the Panda Net 2.0 washed 20 times where knockdown was 80%. However, mortality was > 90% for all treatment arms. Knockdown and mortality of mosquitoes exposed to the untreated net were ≤ 3%.

In cone bioassays conducted with the wild M'be population of *An. gambiae*, knockdown was 81.5% for the Panda Net 2.0 LN and 53.1% for the PermaNet 2.0 LN before washing while mortality was 61% and 33%, respectively (Table 3.7). After washing but before field testing, mortality and knockdown of mosquitoes were ≤ 3 % for both the Panda Net 2.0 LN and the PermaNet 2.0 LN. After the hut trial, knockdown was 0% for the conventionally treated net washed until just before exhaustion and for the PermaNet 2.0 washed 20 times. Knockdown ranged between 30% and 56% for the other treatment arms. Mortality was < 10% for the washed nets while it was 39% for the unwashed Panda Net 2.0 LN and 26% for the unwashed PermaNet 2.0 LN, confirming a high level of pyrethroid resistance of M'Be population of *An. gambiae*.

Overall, 1014 *An. gambiae* were collected from the control huts. A significant reduction in hut entry compared to the control huts was observed for the unwashed Panda Net 2.0 LN, the unwashed PermaNet 2.0 LN and the Panda Net 2.0 LN washed 20 times (Table 3.2). Compared to the untreated nets, exit rates and blood-feeding rates were significantly lower for all treatment arms except the conventionally treated nets washed until just before exhaustion. Mortality of *An. gambiae* was significantly higher in huts with the unwashed Panda Net 2.0 LN, the unwashed PermaNet 2.0 and the Panda Net 2.0 LN washed 20 times compared to the other three study arms.

Similar results were observed for other Culicidae captured in the huts where evidence for deterrence was observed in all treatment arms except for the conventionally treated nets washed until just before exhaustion and where exiting rates were significantly higher for all treatment groups relative to the untreated control nets (Table 3.3). In the control huts, 35.5% of mosquitoes were blood-fed. This was significantly higher than all other treatment arms. Mortality of mosquitoes captured in all treatment arms was significantly higher than in the control huts.

Chemical testing of nets before washing indicated that the deltamethrin content of the Panda Net 2.0 LN was high but within specifications (2.24 g AI/kg) (Table 3.4 and 3.5). Similarly, the deltamethrin content of the PermaNet 2.0 LN was within specifications (1.36 g AI/kg) while the deltamethrin content of the conventionally treated net was 1.06 g/kg (31.3 mg/m^2). After washing, the deltamethrin content of the Panda Net 2.0 LN fell to 1.76 g AI/kg corresponding to an overall deltamethrin retention of 79%. The deltamethrin content of the PermaNet 2.0 LN washed 20 times fell to 0.24 g AI/kg corresponding to an overall deltamethrin retention of 18%. The deltamethrin content of the conventionally treated net fell to 0.02 g/kg (0.6 mg/m^2) after washing. After the hut trial, the deltamethrin content was 2.14 g AI/kg on the unwashed Panda Net 2.0 LN and 1.55 g AI/kg on the Panda Net 2.0 LN washed 20 times (Table 3.5). The deltamethrin content was 1.24 g AI/kg on the unwashed PermaNet 2.0 LN and 0.17 g AI/kg on the PermaNet 2.0 LN washed 20 times. The deltamethrin content on the conventionally treated net washed until just before exhaustion was 0.02 g/kg (0.5 mg/m^2) after the completion of the hut trial (Pigeon et al., 2015d).

Mae Sot, Thailand

An experimental hut study was conducted with the Panda Net 2.0 LN in Mae Sot, Thailand (Duchon et al., 2015). The study area is in western Thailand near the Myanmar border. In previous studies in the area, the predominant anopheline mosquitoes of the region were identified as members of the Minimus group, the Dirus group and the Maculatus group. *An. minimus* was reported to bite throughout the night from 18:00 to 06:00 with around 60% collected outdoors. However, *An. minimus* was collected more frequently from human dwellings than from cattle shelters, indicating the species is largely anthropophilic.

Five experimental huts were available for the study. The hut design was adapted from the West African style huts. The walls were concrete with the inner side lined with white polyethylene sheeting. Mosquitoes could enter the huts through eight window slits that were 1 cm wide and designed to prevent the exit of mosquitoes from the huts. The backside of the hut included a screened veranda trap to capture exiting mosquitoes. The huts were constructed on a concrete base with a water-filled moat to exclude scavenging ants.

The following comparison arms were tested:

- untreated polyester net
- unwashed Panda Net 2.0 LN
- unwashed PermaNet 2.0 LN
- Panda Net 2.0 LN washed 20 times
- PermaNet 2.0 LN washed 20 times.

The nets were washed in aluminium bowls containing 10 L of water and 2 g/L of soap (Savon de Marseille). Each net was agitated for 3 minutes, left to soak for 4 minutes and then agitated for 3 more minutes. Agitation was done by stirring the nets with a pole at 20 rotations per minute. The nets were rinsed twice in clean water using the same conditions and then dried horizontally in the shade and stored at ambient temperature between washes. The Panda Net 2.0 LN were held for 3 days between each washing to allow for full regeneration of insecticidal activity while the PermaNet 2.0 LN were held for 1 day between washes.

Nets were randomly allocated to one of the five huts. Five nets were used for each treatment arm with a different net used each night. Each net had six holes (4 cm x 4 cm) cut in it to simulate a torn net following WHO guidelines. After 5 nights of collection, the huts were thoroughly cleaned and aired for a day to remove potential contamination and the treatments were rotated to a different hut. The trial was run through four complete rotations (i.e. 20 weeks) to ensure adequate mosquitoes for statistical analysis.

One net from each comparison arm was subjected to WHO cone bioassays before washing, after washing and after the completion of the hut trial. Five cones were placed on each net with one cone per net panel. Five female *An. minimus* mosquitoes were introduced into each cone for 3 minutes. Bioassays were repeated so that a total of 50 mosquitoes were exposed per net. An additional coded net from each treatment arm that was not tested in the experimental huts was used for determination of the deltamethrin content of each net. One piece (30 cm x 30 cm) was taken from each of the five panels of the net before and after washing. After the hut trial, one net from each arm was randomly sampled for chemical analysis. The net pieces were placed in aluminium foil in sealed bags and stored at 4 ^{o}C until they were analysed by HPLC as described above.

Adult volunteers were recruited to sleep inside the huts. The sleepers rotated randomly through the huts each night of the study. Mosquitoes were collected in the morning from inside the nets, inside the room and inside the veranda. All mosquitoes were scored as dead or alive and as fed or unfed. The primary outcomes included deterrence, induced exophily, blood-feeding, and immediate and delayed mortality.

In cone bioassays conducted before washing, knockdown of *An. minimus* was > 96% for all treatment arms. After washing, knockdown was > 94% for all treatment arms while after the hut trial, knockdown was 100% for all treatment arms. Mortality for all treatment arms was 100% before washing, after washing and after the hut trial. Knockdown and mortality of mosquitoes exposed to the untreated net were < 5%.

Mosquitoes collected in the experimental hut trial included the Minimus group (63% of anophelines collected), the Maculatus group (24%), *An. barbirostris*, (8%), *An. jamesii* (3%) and *An. dirus* (0.9%) and others (1.1%). By PCR analysis, *An. minimus* was the only member of the Minimus group while members of the Maculatus group included *An. maculatus* (67% of Maculatus group), *An. dravidicus* (28%) and *An. sawadwongporni* (5%). WHO susceptibility tests conducted on *An. minimus* and *An. maculatus* showed full susceptibility to deltamethrin (0.05% dose) and, therefore, the anopheline species collected in the hut study were combined for analysis.

Results of the experimental hut study are presented in Table 3.2. For the *Anopheles* spp., there was no evidence of a deterrent effect or blood-feeding inhibition due to the treated nets. Exophily was significantly higher in the control arm. However, this may have been due to the very high mortality observed in the *Anopheles* spp. collected inside the huts. Mortality, after adjusting for control mortality, was > 90% for all the treated huts. Similar results were obtained for other Culicidae species (Table 3.3).

Chemical testing of nets before washing indicated that the deltamethrin content of the Panda Net 2.0 LN was within specification (2.15 g AI/kg for both the unwashed Panda Net 2.0 LN and the Panda Net 2.0 LN to be washed 20 times) (Tables 3.4 and 3.5). The mean deltamethrin content of the unwashed PermaNet 2.0 LN was 1.34 g AI/kg while the deltamethrin content of the PermaNet 2.0 LN to be washed 20 times was 1.19 g AI/kg. After washing, the deltamethrin content of the unwashed Panda Net 2.0 LN was 2.15 g AI/kg while the deltamethrin content of the Panda Net 2.0 LN washed 20 times fell to 1.63 g AI/kg, corresponding to an overall deltamethrin retention of 76%. The deltamethrin content of the unwashed PermaNet 2.0 LN was 1.30 g/kg, while for PermaNet 2.0 LN washed 20 times was 0.81 g/kg, corresponding to an overall deltamethrin retention of 68%. After the hut trial, the deltamethrin content of the unwashed Panda Net 2.0 LN and the Panda Net 2.0 LN washed 20 times was 1.85 g AI/kg and 1.49 g AI/kg, respectively. The deltamethrin content of the unwashed PermaNet 2.0 LN was 1.10 g AI/kg and that of the PermaNet 2.0 LN washed 20 times was 0.89 g/kg (Table 3.5) (Pigeon et al., 2015e).

3.3 Conclusions and recommendations

Panda Net 2.0 LN is a long-lasting deltamethrin (incorporated into filaments) insecticidal net manufactured by Life Ideas Textiles Company Ltd, China[1]. The net is made of 120-denier monofilament high density polyethylene fibres. Deltamethrin is incorporated into the fibres at a target dose of 1.8 g AI/kg, corresponding to 76 mg AI/m^2.

In Phase I laboratory study, the deltamethrin content of the unwashed Panda Net 2.0 LN was 1.83 g AI/kg, which was within the acceptable tolerance limits (1.8 g AI/kg ± 25%). The chemical analysis of the Panda Net 2.0 LN washed up to 25 times showed an exponential decay of deltamethrin as a function of the number of washes, with an average wash resistance index of 99.5%. After 20 washes, the Panda Net 2.0 LN contained 91% of the initial deltamethrin content.

The regeneration time of the Panda Net 2.0 LN was estimated at 3 days based upon mortality. In WHO cone bioassays, mortality of mosquitoes exposed to the unwashed Panda Net 2.0 LN was only 51% and mortality remained low after repeated washings. However, knockdown of *An. gambiae* mosquitoes exposed to the Panda Net 2.0 LN was high and the product met the requirements for Phase I testing based upon knockdown in the WHO cone test.

Experimental hut trials were conducted in Côte d'Ivoire, Thailand and the United Republic of Tanzania to demonstrate whether the Panda Net 2.0 LN meets the criteria of a long-lasting insecticidal net by comparison with a WHOPES-recommended reference LN (PermaNet 2.0 LN). In Côte d'Ivoire and the United Republic of Tanzania, the Panda Net 2.0 LN was also compared to a conventionally treated net washed until just before exhaustion.

In the Phase II studies, deltamethrin content in unwashed Panda Net 2.0 LN complied with the target dose and was uniformly distributed within the net. The retention of active ingredient after 20 washes in Phase II studies ranged from 71% to 79%. At the end of the three trials in experimental huts, all tested Panda Net 2.0 LNs washed 20 times met the WHO criteria for bioefficacy based upon cone bioassays (mortality $\geq$ 80% or knockdown $\geq$ 95%).

The Panda Net 2.0 LN washed 20 times had significantly lower blood-feeding and significantly higher mortality of wild mosquitoes compared to the conventionally treated net washed until just before exhaustion in both the Côte d'Ivoire and United Republic of Tanzania trials. In the United Republic of Tanzania, blood-feeding and mortality rates of *An. funestus* were similar for the Panda Net 2.0 LN washed 20 times and the PermaNet 2.0 LN washed 20 times, while blood-feeding rates of *An. gambiae* were lower for the Panda Net 2.0 LN washed 20 times. In Côte d'Ivoire, blood-feeding rates were lower and mortality rates were higher for *An. gambiae* in the huts with the Panda Net 2.0 LN washed 20 times compared to huts with the PermaNet 2.0 washed 20 times. In Thailand, the Panda Net washed 20 times performed similarly to the PermaNet 2.0 LN washed 20 times in terms of blood-feeding and mortality of wild *Anopheles* and other Culicidae.

[1] WHO has been advised that as from 10 August 2015, the name of the manufacturer has been changed to Life Ideas Biological Technology Company Ltd. in place of Life Ideas Textiles Company Ltd.

Considering the safety, efficacy and resistance to washing of Panda Net 2.0 LN in laboratory studies and small-scale (Phase II) field studies, the 18th WHOPES Working Group recommended:

- that a time-limited interim recommendation be given for the use of Panda Net 2.0 LN in the prevention and control of malaria; and
- that WHOPES should coordinate large-scale (Phase III) field studies of Panda Net 2.0 LN to confirm its long-lasting efficacy and assess its attrition, fabric integrity and community acceptability, as a basis for developing full recommendations on the use of this product.

The meeting also recommended:

- that the national authorities and procurement agencies must ensure that the Panda Net 2.0 LN complies with WHO specifications as recommended by the Organization according to the quality control procedures outlined in the *Guidelines for procuring public health pesticides*.[1]

Note: WHO recommendations on the use of pesticides in public health are valid ONLY if linked to WHO specifications for their quality control.

[1] WHO (2012). Guidelines for procuring public health pesticides. Geneva: World Health Organization (http://whqlibdoc.who.int/publications/2012/9789241503426_eng.pdf, accessed August 2015).

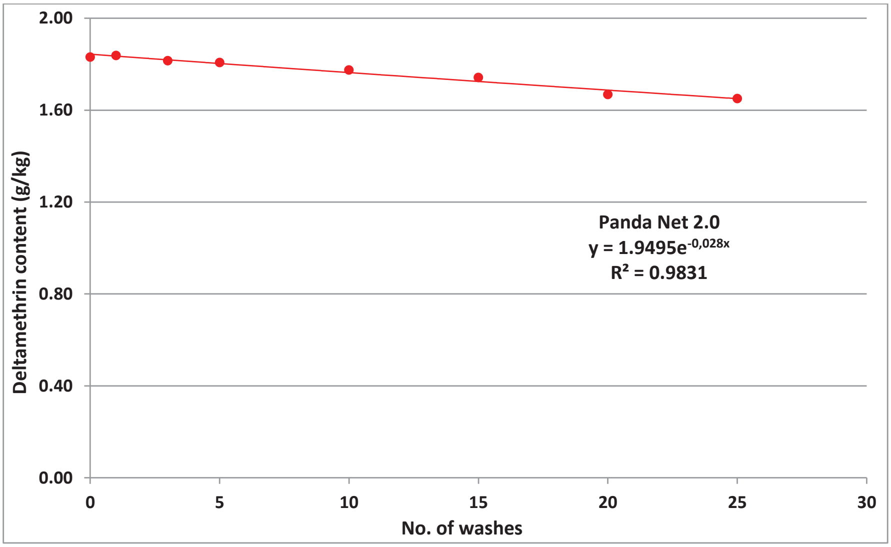

Figure 3.1 Deltamethrin (active ingredient) content and retention (wash curve) for Panda Net 2.0 LN (WHOPES Phase I study)

Table 3.1 Wash resistance test: knockdown and mortality (%) of *An. gambiae* in relation to deltamethrin content and retention of Panda Net 2.0 LN (WHOPES Phase I wash resistance study). Target dose and tolerance limit for deltamethrin in baseline Panda Net 2.0 LN = 1.8 g/kg ± 25% [1.35–2.25 g/kg]

Number of Washes	% Knockdown	% Mortality	Deltamethrin content (g/kg)	Between-net RSD (%)	Deltamethrin retention (% of wash 0)	Deltamethrin WRI (% at each wash)
0	97	51	1.83	0.9	–	–
1	86	15	1.84	0.3	100.4	100.4
3	99	29	1.81	1.3	99.1	99.7
5	97	12	1.81	0.7	98.7	99.7
10	98	46	1.77	2.0	97.0	99.7
15	96	8	1.74	0.7	95.2	99.7
20	96	15	1.67	0.7	91.1	99.5
25	58	12	1.65	0.2	90.2	99.6

RSD = relative standard deviation; WRI = wash resistance index

Table 3.2 Phase II studies: summary results obtained for free-flying, wild *Anopheles* mosquitoes in experimental huts in the United Republic of Tanzania, Côte d'Ivoire and Thailand. Values in the same row sharing the same letter superscript do not differ significantly ($P > 0.05$)

Treatment	Site	Species	Untreated net	Panda Net 2.0 LN unwashed	PermaNet 2.0 LN unwashed	Panda Net 2.0 LN washed 20 times	PermaNet 2.0 LN washed 20 times	CTN washed until just before exhaustion
Females/night	Tanzania	*An. funestus*	1.2^{a}	1.3^{a}	1.0^{a}	1.2^{a}	$1.4^{a,b}$	2.0^{b}
		An. gambiae	$0.2^{a,b}$	0.2^{b}	$0.5^{a,c}$	$0.4^{a,b,c}$	$0.3^{a,b,c}$	0.6^{c}
	Côte d'Ivoire	*An. gambiae*	14.1^{d}	2.9^{a}	6.7^{b}	7.8^{c}	19.3^{d}	15.3^{d}
	Thailand	*Anopheles* spp.	0.6^{a}	0.4^{a}	0.5^{a}	0.5^{a}	0.4^{a}	–
Exophily (%)	Tanzania	*An. funestus*	51.6^{a}	84.1^{b}	87.8^{c}	93.5^{d}	88.5^{b}	67.7^{e}
		An. gambiae	69.0^{a}	93.1^{b}	88.5^{b}	94.2^{b}	92.3^{b}	65.8^{a}
	Côte d'Ivoire	*An. gambiae*	28.4^{a}	66.0^{c}	60.0^{c}	58.8^{c}	38.5^{b}	32.0^{a}
	Thailand	*Anopheles* spp.	52^{a}	19^{b}	13^{b}	29^{b}	12^{b}	–
Blood fed (%)	Tanzania	*An. funestus*	28.6^{a}	6.2^{b}	23.6^{c}	11.6^{d}	12.2^{d}	22.7^{e}
		An. gambiae	34.5^{a}	6.9^{b}	14.8^{b}	11.5^{b}	$30.8^{a,c}$	27.4^{c}
	Côte d'Ivoire	*An. gambiae*	59.6^{c}	22.0^{a}	25.0^{a}	23.2^{a}	50.8^{b}	62.0^{c}
	Thailand	*Anopheles* spp.	8.6^{a}	2.8^{a}	2.2^{a}	6.1^{a}	4.8^{a}	–
Mortality (%)	Tanzania	*An. funestus*	7.9^{a}	26.2^{b}	$24.4^{b,c}$	20.0^{d}	$18.2^{c,d}$	5.1^{e}
		An. gambiae	10.3^{a}	17.2^{b}	16.4^{b}	7.7^{a}	$7.7^{a,b}$	5.5^{a}
	Côte d'Ivoire	*An. gambiae*	8.4^{a}	50.2^{c}	22.5^{b}	25.1^{b}	9.7^{a}	9.2^{a}
	Thailand	*Anopheles* spp.	7^{a}	94^{b}	98^{b}	100^{b}	100^{b}	–

Table 3.3 Phase II studies: summary results obtained for free-flying, wild Culicidae (excluding anopheline) mosquitoes in experimental huts in Côte d'Ivoire and Thailand. Values in the same row sharing the same letter superscript do not differ significantly (*P* > 0.05).

Treatment	Site	Untreated net	Panda Net 2.0 LN unwashed	PermaNet 2.0 LN unwashed	Panda Net 2.0 LN washed 20 times	PermaNet 2.0 LN washed 20 times	CTN washed until just before exhaustion
Females/night	Côte d'Ivoire	14.1^{d}	$6.3^{a,b}$	5.6^{a}	9.9^{c}	$8.7^{b,c}$	$11.6^{c,d}$
	Thailand	0.7^{a}	0.5^{a}	0.5^{a}	0.6^{a}	0.5^{a}	–
Exophily (%)	Côte d'Ivoire	37.1^{a}	48.7^{b}	$54.2^{b,c}$	$51.8^{b,c}$	56.7^{c}	55.5^{c}
	Thailand	50^{a}	27^{b}	13.2^{b}	27^{b}	19^{b}	–
Blood fed (%)	Côte d'Ivoire	35.5^{c}	12.3^{b}	5.2^{a}	6.6^{a}	7.8^{a}	13.9^{b}
	Thailand	7^{a}	4^{a}	2^{a}	5^{a}	6^{a}	–
Mortality (%)	Côte d'Ivoire	30.8^{a}	99.3^{f}	96.0^{e}	90.6^{d}	72.6^{c}	59.6^{b}
	Thailand	6^{a}	96^{a}	98^{b}	98^{b}	100^{b}	–

CTN = conventionally treated net

Table 3.4 Deltamethrin (DM) average content, within-net variations, expressed as the relative standard deviation, in unwashed Panda Net 2.0 LN and PermaNet 2.0 LN tested in WHOPES Phase II studies. The compliance of the mean deltamethrin content with the specification is also presented for each net. Target dose and tolerance limit for deltamethrin in baseline Panda Net 2.0 LN of 120 denier = 1.8 g/kg ± 25% [1.35–2.25 g AI/kg]; target dose and tolerance limit for deltamethrin in baseline PermaNet 2.0 LN of 100 denier = 1.4 g AI/kg ± 25% [1.05–1.75 g AI/kg]

LN samples		United Republic of Tanzania			Cote d'Ivoire			Thailand		
		DM content (g AI/kg)	Compliance with specification	DM within-net variation (RSD)	DM content (g AI/kg)	Compliance with specification	DM within-net variation (RSD)	DM content (g AI/kg)	Compliance with specification	DM within-net variation (RSD)
Panda Net 2.0	1	2.24	Yes	2.8%	2.24	Yes	7.2%	2.15	Yes	1.3%
Panda Net 2.0	2	2.18	Yes	1.0%	–	–	–	2.15	Yes	1.5%
Panda Net 2.0	3	2.17	Yes	1.4%	–	–	–	2.15	Yes	1.9%
PermaNet 2.0	1	1.42	Yes	11.4%	1.36	Yes	4.4%	1.34	Yes	15.1%
PermaNet 2.0	2	1.41	Yes	11.5%	–	–	–	1.19	Yes	22.4%
PermaNet 2.0	3	1.42	Yes	16.4%	–	–	–	1.3	Yes	18.0%

AI = active ingredient; RSD = relative standard deviation

Table 3.5 Deltamethrin (DM) content and retention in Panda Net 2.0 LN and PermaNet 2.0 LN tested in WHOPES Phase II studies. Target dose and tolerance limit for deltamethrin in baseline Panda Net 2.0 LN of 120 denier = 1.8 g AI/kg ± 25% [1.35–2.35 g AI/kg]; target dose and tolerance limit for deltamethrin in baseline PermaNet 2.0 LN of 100 denier = 1.4 g AI/kg ± 25% [1.05–1.75 g AI/kg]

Treatment	United Republic of Tanzania				Côte d'Ivoire				Thailand			
	DM content (g AI/kg) before washing	DM content (g AI/kg) after washing	DM retention after washing (% of wash 0)	DM content (g AI/kg) after hut trial	DM content (g AI/kg) before washing	DM content (g AI/kg) after washing	DM retention after washing (% of wash 0)	DM content (g AI/kg) after hut trial	DM content (g AI/kg) before washing	DM content (g AI/kg) after washing	DM retention after washing (% of wash 0)	DM content (g AI/kg) after hut trial
Panda Net LN 0 wash	2.24	2.17	–	2.10	–	–	–	2.14	2.15	2.15	–	1.85
Panda Net LN 20 washes	2.18	1.54	71%	1.62	2.24	1.76	79%	1.55	2.15	1.63	76%	1.49
PermaNet 2.0 0 wash	1.42	1.42	–	1.14	–	–	–	1.24	1.34	1.30	–	1.10
PermaNet 2.0 20 washes	1.41	0.69	49%	0.63	1.36	0.24	18%	0.17	1.19	0.81	68%	0.89
CTN	0.59	0.03		< 0.01	1.06	0.02	–	0.02	–	–	–	–
Untreated net	–	–	–	–	< 0.01	–	–	< 0.01	< 0.01	< 0.01	–	< 0.01

CTN = conventionally treated net washed until just before exhaustion

Table 3.6 Knockdown (% KD) and mortality (% Mort.) of *An. gambiae* Kisumu strain (pyrethroid susceptible) in WHO cone bioassays in Côte d'Ivoire. N is the sample size for each set of bioassays

Treatment	Before washing			After washing, before field testing			After field testing		
	% KD	% Mort.	N	% KD	% Mort.	N	% KD	% Mortality	N
Untreated polyester net	1	1	155	0	2	50	0	3	103
CTN washed until just before exhaustion	100	100	106	32	81	100	91	91	106
Panda Net LN, unwashed	100	100	202	–	–	–	100	100	109
Panda Net LN, washed 20 times	–	–	–	95	97	205	80	94	102
PermaNet 2.0 LN, unwashed	100	100	156	–	–	–	100	100	158
PermaNet 2.0 LN, washed 20 times	–	–	–	77	87	208	100	98	152

CTN = conventionally treated net washed until just before exhaustion

Table 3.7 Knockdown (% KD) and mortality (% Mort.) of *An. gambiae* M'be population (pyrethroid resistant) in WHO cone bioassays in Côte d'Ivoire. N is the sample size for each set of bioassays

Treatment	Before washing			After washing, before field testing			After field testing		
	% KD	% Mort.	N	% KD	% Mort.	N	% KD	% Mort.	N
Untreated polyester net	0	0	52	0	2	201	0	1	102
CTN washed until just before exhaustion	2	1	149	–	–	–	0	0	107
Panda Net LN, unwashed	82	61	156	–	–	–	56	39	100
Panda Net LN, washed 20 times	–	–	–	3	3	403	30	8	103
PermaNet 2.0 LN, unwashed	53	33	160	–	–	–	31	26	106
PermaNet 2.0 LN, washed 20 times	–	–	–	1	0	100	0	2	103

4. REVIEW OF YAHE LN

Yahe LN is a deltamethrin long-lasting (coated onto filaments) insecticidal net. Deltamethrin is coated onto 75-denier, knitted multifilament polyester fibres, at a target dose of 1.85 g AI/kg, corresponding to 55.5 mg of deltamethrin per square metre of fabric. Yahe is manufactured by Fujian Yamei Industry, China. Deltamethrin has previously been recommended by WHO[1] as an insecticide product for conventional treatment of mosquito nets for malaria vector control, at the target dose of 15–25 mg/m².

Yahe LN was initially submitted to WHOPES for extension of WHO specifications for deltamethrin (coated onto filaments) nets (333/LN/1).[2] The outcomes of the Phase I laboratory studies for regeneration, resistance to washing and efficacy of Yahe LN were published as part of the requirements for extension of WHO specifications.[3] The 14th WHOPES Working Group Meeting had noted that Yahe LN complied with the WHO interim specifications with reference to total content of deltamethrin and retention index. However, there was high variability in the deltamethrin content in the netting samples tested and the mortality rates with Yahe LN never exceeded 86% at any wash point and were always lower than mortality rates of the reference LN (PermaNet 2.0). The Meeting thus concluded that Yahe LN did not meet WHO requirements for extension of specifications and should be subjected to standard WHOPES Phase II studies as a requirement for obtaining WHOPES interim recommendations on its use in malaria prevention and control. The Meeting also advised WHOPES to invite the manufacturer to provide supporting data on homogeneity of deltamethrin content in Yahe LN.

Two WHOPES supervised Phase II (experimental hut) studies comparing the Yahe LN with the reference LN (PermaNet 2.0) and conventionally treated nets were conducted in Thailand and the United Republic of Tanzania. The results of these studies were published in the report of the 16th WHOPES Working Group Meeting.[4] In Phase II studies, candidate LN that perform as well as or better than a conventionally treated net washed until just before exhaustion or a reference LN washed 20 times in terms of blood-feeding inhibition and mortality may be given an interim recommendation for use. As part of both Phase I and Phase II studies, LN should be within the manufacturer's specification or, if available, WHO specifications for insecticide content and homogeneity.

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[1] WHO recommended insecticide products for treatment of mosquito nets for malaria vector control [updated 17 November 2014] (http://who.int/whopes/Insecticides_ITN_Malaria_Nov2014.pdf?ua=1, accessed August 2015).

[2] WHO (2014). WHO specifications and evaluations for public health pesticides – Deltamethrin long-lasting (coated onto filaments) insecticidal nets (http://who.int/whopes/quality/Deltamethrin_coated_LN_specs_eval_WHO_Dec_2014.pdf, accessed August 2015).

[3] WHO (2011). Report of the 14th WHOPES Working Group meeting. Review of Spinosad® EC, LifeNet® LN, MAGNet™ LN, Royal Sentry® LN, Yahe® LN, 11–15 April 2011. Geneva: World Health Organization (http://whqlibdoc.who.int/publications/2011/9789241502160_eng.pdf, accessed August 2015).

[4] WHO (2013). Report of the 16th WHOPES Working Group meeting. Review of Pirimiphos-methyl 300 CS, Chlorfenapyr 240 SC, Deltamethrin 62.5 SC-PE, Duranet LN, Netprotect LN, Yahe LN, Spinosad 83.3 Monolayer DT, Spinosad 25 Extended Release GR, 22–30 July 2013. Geneva: World Health Organization (http://who.int/iris/bitstream/10665/90976/1/9789241506304_eng.pdf, accessed August 2015).

In the Thailand study the mortality of *An. minimus* in the huts with a Yahe LN washed 20 times was not significantly different from that in huts with a PermaNet 2.0 LN washed 20 times. In the United Republic of Tanzania study the blood-feeding inhibition and the mortality of *An. gambiae* s.l. in huts with the Yahe LN washed 20 times was not significantly different than that of the reference LN washed 20 times or the conventionally treated net washed until just before exhaustion. However, in chemical analysis of the Yahe LN, four of the six unwashed nets were outside of the manufacturer's proposed specifications and one net had high within-net variability of deltamethrin content that exceeded the maximum acceptable tolerance limit. The 16th Meeting therefore recommended that the manufacturer be asked to provide the evidence on acceptable within-net variation in deltamethrin content and supporting information on quality assurance of Yahe LN. The meeting also advised that WHOPES conduct a minimum of one additional Phase II study with the Yahe LN complying with the manufacturer's proposed product specifications, as a requirement for an interim recommendation.

The present assessment presents a summary of the safety assessment of Yahe LN, a summary review of the Phase I laboratory studies from the 14th WHOPES Working Group report, a summary review of two Phase II experimental hut studies from the 16th WHOPES Working Group report, and a full review of the results of the third WHOPES-supervised Phase II (experimental hut) study, as a requirement for an interim WHO recommendation.

4.1 Safety assessment

The assessment of the risk to humans of washing and sleeping under Yahe LN was performed based on the data provided by the manufacturer (Aitio, 2015b). The revised WHO *Generic risk assessment model for insecticide-treated nets* was used as a guiding document.[1]

The following assumptions were used by the evaluator in drafting the assessment:

- the product and the active ingredient deltamethrin comply with the WHO specification;
- the concentration of deltamethrin in the net does not exceed the default WHO specification upper limit of nominal concentration + 25% = 69.4 mg/m²;
- based on the limited data submitted, the wash-resistance index of deltamethrin in Yahe LN (mean value of wash resistance index minus three times of standard deviation (SD) ≥ 93.5%), and the surface concentration available for dermal contact, mouthing and washing is thus $\leq$ 6.5%;
- dermal absorption of deltamethrin is 10% and oral absorption is 75%;
- the translodgeable part of deltamethrin from the net onto the skin is 6% of the available surface concentration, in line with the estimate of the United States Environmental Protection Agency for soft surfaces; and
- secretion of deltamethrin as such in mother's milk does not exceed that observed in cows multiplied by $10^{0.6}$, i.e. 6.4% of the dose.

[1] WHO (2012). A generic risk assessment model for insecticide-treated nets – revised edition. Geneva: World Health Organization (http://whqlibdoc.who.int/publications/2012/9789241503419_eng.pdf, accessed August 2015).

It was concluded that when used as instructed, sleeping under or washing Yahe long-lasting insecticidal polyester net does not pose undue hazards to people.

4.2 Efficacy – WHOPES-supervised studies

4.2.1 Laboratory study

Regeneration time

The regeneration time of Yahe LN was studied and compared with that of the reference LN (PermaNet 2.0) (Rossignol et al., 2011). For Yahe LN, the knockdown was only 67% for the unwashed net, but increased to 97% on the first day after three consecutive washes and remained at 99% during 7 days of storage and testing. The plateau and highest percentage knockdown were therefore reached after just 1 day of storage. Mortality was low for the unwashed Yahe net (14%). After the three washes and 1 day of storage, the mortality rate increased to 79%, and stayed at 73–80% until 5 days of storage and then decreased to 51% after 7 days of storage. For the reference LN, PermaNet 2.0, the knockdown and mortality rates were 100% for unwashed and washed samples on all 7 days of storage. The regeneration time for Yahe LN was considered to be 1 day, similar to that of the reference LN. However, it was noted that the mortality for Yahe LN was significantly less than that for the reference LN when unwashed, on the day after 3 times washing, and on each day of storage.

Wash resistance and efficacy

The knockdown of Yahe LN before washing was 67%, but between 1 wash and 20 washes it was never less than 98% and therefore was higher than the WHO threshold of ≥ 95%. At 25 washes, the knockdown was recorded as 100%. The mortality with the unwashed Yahe LN was only 14%; after 1 wash, mortality increased to 80%, and between 3 washes and 20 washes mortality varied between 70% and 86% with quite wide confidence intervals. The mortality rate at 25 washes was 62%. The Yahe LN thus met the Phase I efficacy criteria in cone tests of ≥ 95% knockdown after 20 washes.

For PermaNet 2.0 LN the knockdown was always 100%; the mortality rate gradually decreased from 100% at 5 washes to 95% at 20 washes and 89% at 25 washes but was always above the WHO threshold of ≥ 80%.

Chemical assays

The deltamethrin content in the reserved and unwashed Yahe LN samples (respectively 2.13 g AI/kg and 1.99 g AI/kg) complied with the target dose of 1.8 g AI/kg ± 25% [1.35–2.25 g AI/kg]. However, the between-net variation, expressed as the relative standard deviation (RSD), was 30% in the reserved Yahe LN and 18.5% in the unwashed Yahe LN, showing a high heterogeneity of active ingredient content between nets. The between-net variation of active ingredient in Yahe LN samples washed 1–25 times remained high (RSD, 11.1–32.3%). The deltamethrin *R*-alpha isomer (insecticidally inactive, non-relevant impurity) content in the unwashed Yahe LN sample was less than 1% of the total deltamethrin content and did not increase with washing and storage. The overall deltamethrin retention after 20 washes was 53.5%, corresponding to an average retention per wash of 96.9%.

The deltamethrin content in the reserved and the unwashed reference LN (PermaNet 2.0) samples (respectively 2.26 g AI/kg and 2.01 g AI/kg) complied with the target dose of 1.8 g AI/kg (± 25%). The between-net variation, expressed as the RSD of the content found on the two pieces, was 6.8% and 12.9% respectively, showing an acceptable homogeneity between the nets. The between-net variation of active ingredient on PermaNet 2.0 LN samples washed 1 to 25 times was sometimes high (RSD, 3.0–51.0%). The overall deltamethrin retention after 20 washes was 45.3%, corresponding to an average retention per wash of 96.1%.

From the Phase I study, it was concluded that Yahe LN had a deltamethrin content within the acceptable limits of the target dose (± 25%). However, the deltamethrin content on different Yahe LN was highly variable, and the wash resistance index of the Yahe LN differed from that of the PermaNet 2.0 LN.

Conclusion of laboratory testing

The Yahe LN complied with the WHO interim specifications (333/LN/1) with reference to total content of deltamethrin and retention index. However, there was high variability in the deltamethrin content in the netting samples tested. In contrast to the reference LN (PermaNet 2.0), the biological efficacy of the Yahe LN before the first wash did not meet WHO efficacy criteria. After the first wash, the Yahe LN showed knockdown consistently ≥ 95% threshold. However, the mortality rates for the Yahe LN never exceeded 86% at any wash point and were always lower than mortality rates for the reference LN. This indicated that the wash resistance of the Yahe LN was different from that of the reference LN.

Considering the above, the 14th Working Group meeting concluded that the bioefficacy and wash resistance of Yahe LN were different from those of the reference product for which WHO specifications had been developed.[1] The 14th meeting recommended to not extend WHO specifications for deltamethrin long-lasting (coated onto filaments) insecticidal net to Yahe LN, to invite the manufacturer to provide supporting data on homogeneity of deltamethrin content, and to submit the product for WHOPES Phase II testing as a reference product instead. This therefore led to the Phase II studies of Yahe as a reference LN, the outcomes of which are summarized below.

4.2.2 Experimental hut studies

Three Phase II experimental hut studies have been undertaken on Yahe LN. The two undertaken in Thailand and the United Republic of Tanzania were reported in full in the 16th WHOPES Working Group Report[2] and are also summarized below. The third study was undertaken in Côte d'Ivoire and is reported in full below.

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[1] WHO (2011). Report of the 14th WHOPES Working Group meeting. Review of Spinosad® EC, LifeNet® LN, MAGNet™ LN, Royal Sentry® LN, Yahe® LN, 11–15 April 2011. Geneva: World Health Organization (http://whqlibdoc.who.int/publications/2011/9789241502160_eng.pdf, accessed August 2015).

[2] WHO (2013). Report of the 16th WHOPES Working Group meeting. Review of Pirimiphos-methyl 300 CS, Chlorfenapyr 240 SC, Deltamethrin 62.5 SC-PE, Duranet LN, Netprotect LN, Yahe LN, Spinosad 83.3 Monolayer DT, Spinosad 25 Extended Release GR, 22–30 July 2013. Geneva: World Health Organization (http://who.int/iris/bitstream/10665/90976/1/9789241506304_eng.pdf, accessed August 2015).

Mae Sot District, Thailand

Washed and unwashed Yahe LN were evaluated in experimental huts in western Thailand to determine their effects on free-flying, wild *An. minimus* mosquitoes and their ability to deter entry, repel or drive mosquitoes out of houses, induce mortality and inhibit blood-feeding (Duchon et al., 2013). The PermaNet 2.0 LN that has a full WHOPES recommendation was used as a reference product.

WHO susceptibility tests on *An. minimus* indicated full susceptibility to deltamethrin.

Six experimental huts constructed in the West African design were used in the study. Six treatment arms were tested: (i) an untreated polyester net; (ii) unwashed Yahe LN; (iii) Yahe LN washed 20 times; (iv) unwashed PermaNet 2.0 LN; (v) PermaNet 2.0 LN washed 20 times; and (vi) polyester net conventionally treated with deltamethrin at a target dose of 25 mg AI/m^2. Six nets were used per treatment arm and, to simulate torn nets, all nets had six holes (4 cm x 4 cm) cut in them. The nets were washed according to WHOPES-recommended procedures with a one-day interval between washes.

Blood-feeding was consistently low in all treatment arms, ranging from 6% to 13%, and there were no statistically significant differences among the treatment arms. Mortality in the treatment arms ranged from 32% in huts with the unwashed PermaNet 2.0 LN to 57% in the huts with the unwashed conventionally treated net. Mortality was significantly higher in the huts with the conventionally treated net compared with the unwashed PermaNet 2.0 LN, but no other statistically significant differences were detected. In terms of mortality, the Yahe LN washed 20 times performed as well as either a PermaNet 2.0 LN washed 20 times or an unwashed conventionally treated net.

The average deltamethrin content in two of the three unwashed Yahe LN was above the maximum tolerance limit of 2.31 g AI/kg.

The authors concluded that the Yahe LN performed less well than the PermaNet 2.0 LN in terms of mortality in WHO cone bioassays. However, the unwashed Yahe LN and the Yahe LN washed 20 times performed as well as the PermaNet 2.0 LN and unwashed conventionally treated net in the experimental hut study in terms of mortality. Furthermore, the experimental hut studies should be interpreted with caution due to the low numbers of mosquitoes captured during the course of the study. The results also may have been affected by the higher than acceptable levels of deltamethrin content on some of the unwashed Yahe LN.

Muheza, United Republic of Tanzania

The efficacy of the Yahe LN net was evaluated in veranda trap experimental huts of the east African design in Muheza, United Republic of Tanzania, against wild, free-flying, host-seeking *An. gambiae* (Tungu et al, 2013). The *An. gambiae* population included 93% *An. gambiae* s.s. and 7% *An. arabiensis*. The *An. gambiae* s.s. were 24% heterozygous and 41% homozygous for the 1014S *kdr* allele.

Six experimental huts with six treatment arms were included in the study as follows: (i) untreated polyester net; (ii) unwashed Yahe LN; (iii) Yahe LN washed 20 times; (iv) unwashed PermaNet 2.0 LN; (v) PermaNet 2.0 LN washed 20 times; and (vi) polyester net, conventionally treated with deltamethrin at 25 mg AI/m², washed until just before exhaustion. Three nets were used for each treatment arm. Six holes were cut in each net, which were washed according to WHOPES procedures with a one-day interval between washes.

In the untreated control huts, 25.8% of *An. gambiae* were blood-fed. Significantly more mosquitoes had fed in the untreated control huts compared with the treatment arms. No significant differences were detected between the Yahe LN washed 20 times (17.3%), the PermaNet 2.0 LN washed 20 times (12.4%) and the conventionally treated net washed until just before exhaustion (12.8%).

Mortality was lowest in the untreated control huts and was significantly lower compared with all other treatment arms. Mortality was significantly higher in the huts with unwashed Yahe LNs compared to all other treatment arms. No significant differences in mortality among the different treatment arms were detected.

The Yahe LN washed 20 times performed as well as either a PermaNet 2.0 LN washed 20 times or a conventionally treated net washed until just before exhaustion as measured by blood-feeding inhibition and mortality.

The average deltamethrin content in three unwashed Yahe LNs was 2.28, 2.42 and 2.70 g AI/kg, two of which were above the maximum tolerance limit of 2.31 g AI/kg. Furthermore, the within-net variability of one of these nets exceeded the maximum recommended limit of 20%.

The Yahe LN washed 20 times showed similar efficacy in terms of blood-feeding inhibition and mortality of *An. gambiae* compared with a conventionally treated net washed until just before exhaustion. However, these results must be interpreted with caution as deltamethrin content in several Yahe LNs was above the manufacturer's specifications and one Yahe LN exhibited unacceptably high within-net variation.

M'Be, Côte d'Ivoire

The 16th Working Group recommended that WHOPES should conduct one additional Phase II study with the Yahe LN within the product specifications, as a requirement for an interim recommendation.

The efficacy of the Yahe LN was evaluated in veranda trap experimental huts in M'Be valley, a rice irrigation valley north of Bouaké in central Côte d'Ivoire, against wild, free-flying, host-seeking *An. coluzzii* (formerly named *An gambiae* s.s. M molecular form) and Culicine mosquitoes (principally *Culex* spp. and *Mansonia* spp.) to determine their ability to deter entry, repel or drive mosquitoes out of houses, induce mortality and inhibit blood-feeding (Koffi et al., 2015b). *An. coluzzii* was the main anopheline present and was resistant to DDT, carbamates and pyrethroids. The frequency of L104F *kdr* allele was recorded as 0.33 in

2012 and 0.94 in a 2014 survey; metabolic mechanisms were also implicated in the pyrethroid resistance (Koffi et al., 2013[1]; Koffi et al. unpublished data).

Six experimental huts of the West African design were used. Mosquitoes entered the huts through four entry slits that funnelled mosquitoes into the room and greatly limited exiting through the same aperture. A single screened veranda projected from the back wall of the hut to collect exiting mosquitoes.

Six treatment arms were tested: (i) an untreated polyester net; (ii) unwashed Yahe LN; (iii) Yahe LN washed 20 times; (iv) unwashed PermaNet 2.0 LN; (v) PermaNet 2.0 LN washed 20 times; and (vi) polyester net conventionally treated with deltamethrin at a target dose of 25 mg AI/m^2. Six nets were used per treatment arm and to simulate torn nets all nets had six holes (4 cm x 4 cm) deliberately cut into the sides, with two holes on each long side and one on each short side. The nets were washed at the Institute Pierre Richet (Bouaké) in bowls containing 10 L of water and 2 g/L of Savon de Marseille soap as per WHOPES procedures. Each net was manually agitated for 3 minutes, allowed to soak for 4 minutes and agitated again for 3 minutes and then rinsed twice in clean water. The interval between each wash was 3 days for the Yahe LN and 2 days for the PermaNet 2.0 LN.

The point of exhaustion was determined by washing a conventionally treated net (treated at 25 mg AI/m^2) according to the above protocol and conducting bioassays after each wash cycle. The point of exhaustion was defined as the maximum number of washes a net could withstand before mortality of *An. gambiae* (Kisumu strain) exposed in standard WHO cone assays fell below 80% and knockdown fell below 95%. Knockdown fell below 95% after three washes and mortality fell below 80% after five washes. The point of exhaustion for the conventionally treated net was therefore set at four washes.

Before the start of the study, baseline cone bioassays were carried out on three nets in each treatment arm. After completing the required number of washes, bioassays were again done on nets from each treatment arm approximately 3 days after the final wash. The nets were then used in the hut study. At the end of the study further bioassays were done on nets from each treatment arm. Bioassays were carried out using the *An. gambiae* Kisumu susceptible strain. Supplementary bioassays were performed using the wild *An. coluzzii* strain from the M'Be site. The cone bioassays were carried out on five positions of each net.

The six treatments were randomly assigned to huts for testing. On each night for 6 consecutive nights a different net of the same treatment type was tested in the huts. The six sleepers rotated through each hut over the 6 nights. At the end of the 6 nights, the huts were cleaned and ventilated and the treatment was then rotated to a different hut. Two complete rotations of the treatments were made so that the trial lasted for a total of 12 weeks.

Each night, volunteers would sleep in the huts from 20:00 to 06:00. Each morning, the dead mosquitoes were collected from inside the nets and from the floor of the hut and the veranda trap. Resting mosquitoes were collected using aspirators from the nets, hut and veranda trap.

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[1] Koffi AA et al. (2013). Insecticide resistance status of *Anopheles gambiae* s.s. population from M'Bé: a WHOPES-labelled experimental hut station, 10 years after the political crisis in Côte d'Ivoire. Malar J;12:1–8.

Mosquitoes were recorded as dead or alive and as blood-fed or unfed. Live mosquitoes were provided with sugar solution to assess delayed mortality after 24 hours.

The primary outcomes of the trial were deterrence (the reduction in hut entry relative to the control huts), induced exophily (the proportion of mosquitoes captured in the trap), blood-feeding inhibition (the reduction in blood-feeding of mosquitoes compared with the control huts) and immediate mortality (the proportion of mosquitoes found dead in the morning).

A seventh net from each arm was used for the chemical assays. Before washing, five pieces (30 cm x 30 cm) were removed from these nets for baseline determination of content. After washing was completed, a further five pieces were removed from each net. After the trial was completed, five pieces were taken from one net per treatment arm used in the hut trial. The samples were sealed in aluminium foil, stored at 4 °C and sent to the WHO Collaborating Centre at Gembloux, Belgium for chemical analysis using the CIPAC method 333/LN/(M)/3.

At baseline before washing, the Yahe LN, the PermaNet 2.0 LN and the conventionally treated net washed until just before exhaustion each met the WHOPES threshold in cone bioassays for mortality. Knockdown and mortality were, respectively, 93% and 97% for Yahe LN, 100% and 100% for the PermaNet 2.0 LN, and 99% and 100% for the conventionally treated net. After 20 washes of the LNs and 4 washes of the conventionally treated net, knockdown and mortality were, respectively, 84% and 91% for the Yahe LN, 77% and 86% for the PermaNet 2.0 LN and 34% and 74% for the conventionally treated net. Therefore, both types of LN passed the WHOPES thresholds on mortality criteria but not on knockdown criteria, and the conventionally treated net washed until just before exhaustion failed on both criteria. Post trial, knockdown and mortality of the washed treatments were 68% and 68% for the Yahe LN, 100% and 98% for the PermaNet 2.0 LN, and 96% and 98% for the conventionally treated net. Therefore, compared to post-washing, the post-trial data for Yahe LN indicated a diminishment of performance whereas that for the PermaNet 2.0 LN and the conventionally treated net washed until just before exhaustion indicated an improvement in performance. Care should therefore be taken not to over-interpret these results or trends. The nets tested in bioassay after washing were not the same as the nets tested post-trial and testing was done at different times. Both types of unwashed LN, post trial, produced bioassay mortality that met the WHO threshold of ≥ 80%. It should be emphasized that Phase II criteria are based on experimental hut results and not on supplementary cone bioassay results.

The bioassay results with the M'Be strain of *An. coluzzii* differed markedly from the bioassay results with the susceptible *An. gambiae* Kisumu. With the unwashed nets, knockdown and mortality were 1% and 3% for Yahe LN, 53% and 33% for PermaNet 2.0 LN, and 11% and 7% for the conventionally treated net. After washing, knockdown and mortality of the three treatment types were never greater than 2%. Post-trial knockdown and mortality were consistent with pre-trial results; 10% mortality for unwashed Yahe LN, 25% mortality for unwashed PermaNet and < 4% mortality for the washed LNs and the conventionally treated net washed until just before exhaustion (Table 4.1).

In the experimental hut trial, the average number of *An. coluzzii* collected from the huts was 14 per night for the untreated net, which was not significantly different from the 20 times washed Yahe LN (14%), 20 times washed PermaNet 2.0 LN (19.3%) and washed conventionally treated net (19%). The average number collected from huts with the unwashed Yahe LN (6.6%) and PermaNet 2.0 LN (6.4%) was significantly less than from huts with the washed treatments. Both types of LN recorded statistically significant deterrence when unwashed (50–60%) and still significant but reduced deterrence when washed 20 times (34–39%). Deterrence was non-significant for the conventionally treated net (28%). The level of deterrence was not significantly different between the 20 times washed Yahe LN, the 20 times washed PermaNet 2.0 and the conventionally treated net.

Exiting rates ranged from 28% in the huts with the untreated net to 28–60% in the huts with treated nets (Table 4.2). Exiting rates differed significantly between the 20 times washed Yahe LN, the 20 times washed PermaNet 2.0 and the conventionally treated net.

Blood-feeding rates were lower in all treatment arms (25–37%) compared to the huts with the untreated net (60%) with the exception of the washed PermaNet 2.0 LN (51%) and conventionally treated net (57%). The unwashed PermaNet 2.0 LN recorded a lower blood-feeding rate (25%) than the unwashed Yahe LN (37%). Importantly, there were no statistically significant differences in blood-feeding rates between the 20 times washed Yahe LN (49%) and the 20 times washed PermaNet 2.0 LN (51%); the rate of blood-feeding inhibition for both types of LN after 20 washes was < 20%.

Mortality was significantly higher in the huts with the unwashed treated nets compared to the untreated nets. Mortality was highest with the unwashed PermaNet 2.0 LN (23%) compared to the unwashed Yahe LN (14%), but there were no significant differences between the 20 times washed Yahe LN (10%), the 20 times washed PermaNet 2.0 LN (10%) and the washed conventionally treated net (8%) (Table 4.2). In terms of mortality, the Yahe LN washed 20 times performed as well as the PermaNet 2.0 LN washed 20 times or a washed conventionally treated net.

The results for free-flying Culicidae should be interpreted with caution because these were not recorded separately by genus or species. Percentage mortality ranged from 68% to 96% and therefore the majority of these mosquitoes were more likely to be *Mansonia* spp. than *Culex quinquefasciatus* which tends to be highly pyrethroid-resistant across West Africa. Mortality was higher with unwashed PermaNet 2.0 LN (96%) than with unwashed Yahe LN (68%). Mortality rates were not significantly different between the 20 times washed Yahe LN, the 20 times washed PermaNet 2.0 and the washed conventionally treated net.

Only one unwashed Yahe LN and one PermaNet 2.0 LN were provided by the Phase II study in Côte d'Ivoire for chemical analysis. The mean deltamethrin content in the unwashed Yahe LN was 2.00 g/kg, which complied with the target dose of 1.85 g/kg ± 25% for 75-denier yarn (Table 4.3, 4.4). The within-net variation, expressed as the relative standard deviation of the deltamethrin content on five pieces cut from the unwashed net, was 3.6%, showing acceptable homogeneity within the net. The deltamethrin content was 1.39 g/kg after 20 washes, corresponding to a retention rate of 69%. The deltamethrin R-alpha isomer content was low and did not increase after washing or hut trial. The mean deltamethrin content in the unwashed PermaNet 2.0 LN was 1.43 g/kg, which complied with the target dose of 1.4 g/kg ± 25% for 100-denier yarn. The within-net variation was 4.9%, showing acceptable homogeneity. The deltamethrin content was 0.22 g/kg after 20 washes, corresponding to a retention rate of 15%. The mean deltamethrin content in the conventionally treated net before washing was 0.84 g/kg (28.1 mg/m²), which was close to the target dose of 25 mg/m². The deltamethrin content after washing until just before exhaustion was 0.06 g/kg (2.0 mg/m²) (Pigeon et al., 2015f).

A limitation of this study was that the interval between washes was erroneously set at 3 days for Yahe LN and at 2 days for PermaNet 2.0 LN instead of at the 1-day regeneration time interval established in WHOPES Phase I evaluation at the WHO collaborating centre IRD in Montpellier. This may have led to a greater level of removal of active ingredient from the nets after 20 washes than a 1-day regeneration time would have achieved. Comparison of chemical content between the Ivorian trial and the previous Thai and Tanzanian trials shows that the deltamethrin retention rate in the Ivorian trial (69%) was intermediate between that observed in the Thai trial (77%) and the Tanzanian trial (61%) and therefore that the extended regeneration time for a coated LN might not be so critical as for an incorporation LN. However, against this line of argument are data for PermaNet 2.0 LN where the deltamethrin retention rate was 64% in the Thai trial, 38% in the Tanzanian trial and 15% in the Ivorian trial, indicating that a 2-day interval instead of 1-day between washes may have led to greater removal of active ingredient from PermaNet 2.0 LN, although this could be possibly due to a batch of PermaNet 2.0 LN that might have had a higher bleed rate. Therefore, judgement about Yahe LN in comparison with PermaNet 2.0 LN in the Ivoirian trial should be made cautiously. The conventionally treated net washed until just before exhaustion could be used as an additional positive control and comparison arm. This net produced efficacy results in the Ivorian hut trial that were not significantly different from the 20 times washed Yahe LN.

Conclusion of experimental hut studies

In Mae Sot, Thailand, the Yahe LN was compared with PermaNet 2.0 LN and a conventionally treated net. It was not possible to conclude on blood-feeding inhibition due to low mosquito densities and low rates of blood-feeding. However, the mortality of *An. minimus* in the huts with a Yahe LN washed 20 times (53%) was not significantly different from the mortality in huts with a PermaNet 2.0 LN washed 20 times (48%) or an unwashed conventionally treated net (57%).

In Muheza, United Republic of Tanzania, the Yahe LN was compared with PermaNet 2.0 LN and a conventionally treated net washed until just before exhaustion. The rate of blood-feeding by *An. gambiae* s.l. in huts with the Yahe LN washed 20 times (17.3%) was not significantly different from the 12.4% blood-feeding recorded for PermaNet 2.0 LN washed 20 times and the 12.8% blood-feeding recorded for the conventionally treated net washed until just before exhaustion. Mortality of *An. gambiae* s.l. in huts with the Yahe LN washed 20 times (30.3%) was not significantly different from the 29.7% mortality recorded for the PermaNet 2.0 LN washed 20 times and the 26.8% mortality recorded for the conventionally treated net washed until just before exhaustion.

In M'Be, Côte d'Ivoire, the Yahe LN was compared with the PermaNet 2.0 LN and a conventionally treated net washed until just before exhaustion. The rate of blood-feeding by *An. coluzzii* in huts with the Yahe LN washed 20 times (49%) was not significantly different from the blood-feeding recorded for the PermaNet 2.0 LN washed 20 times (51%). Mortality of *An. gambiae* s.l. in huts with the Yahe LN washed 20 times (10%) was not significantly different to mortality recorded for the PermaNet 2.0 LN washed 20 times (10%) and the conventionally treated net washed until just before exhaustion (8%).

4.3 Conclusions and recommendations

Yahe LN is a long-lasting deltamethrin (coated onto filaments) insecticidal net manufactured by Fujian Yamei Industry, China. Deltamethrin is coated onto 75-denier, knitted, multi-filament polyester fibres at the target dose of 1.85 g AI/kg netting material, corresponding to 55.5 mg of deltamethrin per square metre of the fabric.

Yahe LN has been subject to WHOPES review for extension of WHO interim specification 333/LN/1. The outcome of the regeneration, wash resistance and efficacy studies of Yahe LN in laboratory studies was published as part of the requirements for extension of WHO specifications in the Report of the 14th WHOPES Working Group Meeting. The meeting noted that Yahe LN complied with the WHO interim specifications with reference to total content of deltamethrin and retention index. However, there was unacceptable heterogeneity in the deltamethrin content in the netting samples tested and the mortality rates of Yahe LN were always lower than those of the reference LN. The 14th WHOPES Working Group Meeting concluded that Yahe LN did not meet WHO requirements for extension of specifications and should be subjected to standard WHOPES Phase II studies as a requirement for obtaining WHO recommendations on its use in malaria prevention and control. The Meeting also advised WHOPES to invite the manufacturer to provide supporting data on homogeneity of deltamethrin content.

The results of two WHOPES-supervised Phase II (experimental hut) studies were published in the report of the 16th WHOPES Working Group Meeting (Geneva, 22–30 July 2013). In Phase II studies, candidate LN that perform as well as or better than a conventionally treated net washed until just before exhaustion or a reference LN washed 20 times in terms of blood-feeding inhibition and mortality may be given an interim recommendation provided LNs are within specifications for insecticide content and homogeneity.

Two WHOPES-supervised experimental hut studies comparing the Yahe LN with the reference LN, PermaNet 2.0, and conventionally treated nets were conducted in Thailand and the United Republic of Tanzania. In the Thailand study, the mortality of *An. minimus* in the huts with a Yahe LN washed 20 times was not significantly different from the mortality in huts with a PermaNet 2.0 LN washed 20 times. In the Tanzanian study, the blood-feeding inhibition and the mortality of *An. gambiae* s.l. in huts with the Yahe LN washed 20 times was not significantly different compared to the reference LN washed 20 times or the conventionally treated net washed until just before exhaustion. However, in chemical analysis of the Yahe LN, four of the six unwashed nets were outside specification for AI content and one net had unacceptable AI within-net variability.

The 16th WHOPES Working Group recommended that the manufacturer provide the evidence on acceptable within-net variation in deltamethrin content and supporting information on quality assurance of Yahe LN. The meeting also advised that WHOPES conduct a minimum of one additional Phase II study with the Yahe LN within the product specifications, as a requirement for an interim recommendation.

In the third experimental huts study carried out in M'Be, Côte d'Ivoire, the Yahe LN was compared with the PermaNet 2.0 LN and a conventionally treated net washed until just before exhaustion. The rate of blood-feeding by *An. coluzzii* in huts with the Yahe LN washed 20 times (49%) was not significantly different to the blood feeding recorded for the PermaNet 2.0 LN washed 20 times (51%). Blood feeding in the untreated control net was 60% and there was significant blood-feeding inhibition with treated arms. Mortality of pyrethroid resistant *An. coluzzii* in huts with the Yahe LN washed 20 times (10%) was not significantly different to the mortality recorded for the PermaNet 2.0 LN washed 20 times (10%), the conventionally-treated net washed until just before exhaustion (8%) or the untreated net (8%). The nets tested for deltamethrin content were within specification for deltamethrin content and showed acceptable homogeneity.

Considering the safety, efficacy and resistance to washing of Yahe LN in laboratory studies and small-scale (Phase II) field studies, the 18th WHOPES Working Group recommended:

- that a time-limited interim recommendation be given for the use of Yahe LN in the prevention and control of malaria;

- that WHOPES should coordinate large-scale (Phase III) field studies of Yahe LN to confirm its long-lasting efficacy and assess its attrition, fabric integrity and community acceptability, as a basis for developing full recommendations on the use of this product.

Following a review of the available evidence, and noting the variability in chemical content between netting samples in the Phase I laboratory study and between nets in two of the experimental hut studies, the meeting also recommended:

- that within and between-net variability in deltamethrin content should be measured at baseline as part of the Phase III studies of Yahe LN;

- that the manufacturer be urged to monitor the variability of deltamethrin content in routine production of Yahe LN to ensure that it remains in conformity with the limits proposed by WHO.

The meeting further recommended:

- that the national authorities and procurement agencies must ensure that the Yahe LN complies with WHO specifications as recommended by the Organization according to the quality control procedures outlined in the *Guidelines for procuring public health pesticides*.[1]

Note: WHO recommendations on the use of pesticides in public health are valid ONLY if linked to WHO specifications for their quality control.

[1] WHO (2012). Guidelines for procuring public health pesticides. Geneva: World Health Organization, (http://whqlibdoc.who.int/publications/2012/9789241503426_eng.pdf, accessed August 2015).

Table 4.1 Knockdown (% KD) and mortality (% Mort.) of *An. gambiae* Kisumu (susceptible) and M'Be (resistant) in WHO cone bioassays in Côte d'Ivoire. N is the sample size for each set of bioassays

Kisumu Treatment	Before field testing			After field testing		
	% KD	% Mort.	N	% KD	% Mort.	N
Untreated polyester net	0	6	51	0	2	100
Yahe LN, unwashed	93	97	152	83	86	104
Yahe LN, washed 20 times	84	91	203	68	68	103
PermaNet 2.0 LN, unwashed	100	100	156	100	100	158
PermaNet 2.0 LN, washed 20 times	77	86	208	100	98	152
Conventionally-treated net*	34	74	100	96	98	108

*The conventionally-treated net was washed until just before exhaustion (4 times).

M'Be Treatment	Before field testing			After field testing		
	% KD	% Mort.	N	% KD	% Mort.	N
Untreated polyester net	0	0	50	0	0	104
Yahe LN, unwashed	1	3	50	0	10	99
Yahe LN, washed 20 times	0	2	100	7	3	152
PermaNet 2.0 LN, unwashed	53	33	160	31	25	106
PermaNet 2.0 LN, washed 20 times	1	0	100	0	2	103
Conventionally-treated net*	11	7	150	0	2	103

*The conventionally-treated net was washed until just before exhaustion (4 times).

Table 4.2 Summary results obtained for free-flying, wild *Anopheles* in experimental huts in Thailand, the United Republic of Tanzania and Côte d'Ivoire. Values in the same row sharing the same letter superscript do not differ significantly ($P > 0.05$)

Treatment	Site	Un-treated net	Yahe LN unwashed	Yahe LN washed 20 times	PermaNet 2.0 LN unwashed	PermaNet 2.0 LN washed 20 times	CTN washed*
Females/night	Thailand	0.5^{a}	0.5^{a}	0.5^{a}	0.4^{a}	0.5^{a}	0.7^{a}
	Tanzania	12^{a}	$11^{b,c}$	15^{a}	8^{c}	15^{a}	$13^{a,b}$
	Côte d'Ivoire	14^{b}	7^{a}	14^{b}	6^{a}	19^{b}	19^{b}
Exophily (%)	Thailand	24^{a}	$32^{a,b}$	24^{a}	$35^{a,b}$	48^{b}	26^{a}
	Tanzania	74^{a}	$91^{b,c}$	88^{c}	94^{b}	$91^{b,c}$	$89^{b,c}$
	Côte d'Ivoire	28^{a}	50^{d}	34^{b}	60^{e}	39^{c}	28^{a}
Blood fed (%)	Thailand	6^{a}	3^{a}	6^{a}	13^{a}	11^{a}	6^{a}
	Tanzania	26^{a}	13^{b}	17^{b}	6^{c}	12^{b}	13^{b}
	Côte d'Ivoire	60^{d}	37^{b}	49^{c}	25^{a}	51^{c}	57^{d}
Mortality (%)	Thailand	6^{a}	$46^{b,c}$	$53^{b,c}$	32^{b}	$48^{b,c}$	57^{c}
	Tanzania	10^{a}	40^{b}	30^{c}	32^{c}	30^{c}	27^{c}
	Côte d'Ivoire	8^{a}	14^{b}	10^{a}	23^{c}	10^{a}	8^{a}

*For the Thai trial the conventionally treated net (CTN) was unwashed, whereas for the Tanzanian and Ivoirian trials the CTN was washed until just before exhaustion.

Table 4.3 Deltamethrin (DM) average content, within-net and between-net variations, expressed as the relative standard deviation (RSD), in unwashed Yahe LN and PermaNet 2.0 LN tested in WHOPES Phase II studies. The compliance of the mean deltamethrin content with the specification is also presented for each net. Target dose and tolerance limit for deltamethrin in baseline Yahe LN of 75 denier = 1.85 g AI/kg ± 25% [1.39–2.31 g AI/kg]; target dose and tolerance limit for deltamethrin in baseline PermaNet 2.0 LN of 100 denier = 1.4 g AI/kg ± 25% [1.05–1.75 g AI/kg]

LN		United Republic of Tanzania			Thailand			DM between-net variation (RSD)
		DM content (g AI/kg)	Compliance with specification	DM within-net variation (RSD)	DM content (g AI/kg)	Compliance with specification	DM within-net variation (RSD)	
Yahe LN	1	2.28	Yes	16.3%	2.21	Yes	10.4%	
Yahe LN	2	2.42	No	13.1%	2.46	No	9.2%	6.57%
Yahe LN	3	2.70	No	30.6%	2.33	No	9.4%	
PermaNet 2.0	1	1.52	Yes	11.5%	1.31	Yes	8.4%	
PermaNet 2.0	2	1.47	Yes	11.5%	1.29	Yes	9.0%	7.49%
PermaNet 2.0	3	1.56	Yes	12.7%	1.34	Yes	10.4%	

LN		Côte d'Ivoire		
		DM content (g AI/kg)	Compliance with specification	DM within-net variation (RSD)
Yahe LN	1	2.00	Yes	3.6%
PermaNet 2.0	1	1.43	Yes	4.9%

Table 4.4 Deltamethrin (DM) content (in g AI/kg) and retention in Yahe LN and PermaNet 2.0 LN tested in WHOPES phase II studies. Target dose and tolerance limit for deltamethrin in baseline Yahe LN of 75 denier = 1.85 g AI/kg ± 25% [1.39–2.31 g AI/kg]; target dose and tolerance limit for deltamethrin in baseline PermaNet 2.0 LN of 100 denier = 1.4 g AI/kg ± 25% [1.05–1.75 g AI/kg]

Treatment	United Republic of Tanzania				Thailand				Côte d'Ivoire			
	DM content before washing	DM content after washing	DM retention after washing (% of wash 0)	DM content after testing	DM content before washing	DM content after washing	DM retention after washing (% of wash 0)	DM content after testing	DM content before washing	DM content after washing	DM retention after washing (% of wash 0)	DM content after testing
Yahe LN 0 wash	2.28	2.70	–	2.35	2.21	2.33	–	1.91	–	–	–	1.98
Yahe LN 20 washes	2.42	1.47	61%	1.66	2.46	1.88	77%	1.27	2.00	1.39	69%	1.47
PermaNet 2.0 0 wash	1.52	1.56	–	1.08	1.31	1.34	–	1.19	–	–	–	1.32
PermaNet 2.0 20 washes	1.47	0.56	38%	0.36	1.29	0.82	64%	0.58	1.43	0.22	15%	0.17
CTN*	0.42	0.02	95%	0.03	0.45	0.51	–	0.74	0.84	0.06	93%	0.03
Untreated net	–	–	–	–	< 0.01	< 0.01	–	< 0.01	< 0.01	–	–	< 0.01

* Conventionally-treated polyester (CTN) net washed until just before exhaustion (in Thailand, the CTN was unwashed).

5. REVIEW OF SAFENET LN

SafeNet LN is an alpha-cypermethrin long-lasting (coated onto filaments) insecticidal net. Alpha-cypermethrin is coated onto 75-denier or 100-denier, multifilament polyester fibres, with the target dose of 6.7 g AI/kg or 5.0 g AI/kg, respectively, corresponding to 200 mg of alpha-cypermethrin per square metre of fabric. Mainpol GmbH, Germany, manufactures SafeNet LN. Interceptor LN, the net used as the reference in this study, is manufactured by BASF, Germany. The net is available as 75-denier or 100-denier polyester filament coated with alpha-cypermethrin at a target dose of 6.7 g AI/kg or 5.0 g AI/kg, respectively, corresponding to 200 mg of alpha-cypermethrin per square metre of fabric.[1]

Alpha-cypermethrin has previously been recommended as an insecticide product by WHO[2] for conventional treatment of mosquito nets for malaria vector control, at the target dose of 20–40 mg/m².

The specifications of SafeNet LN are the same as those of Interceptor LN earlier recommended for use by WHOPES. The safety assessment of Interceptor LN had earlier shown that washing or sleeping under the net does not pose undue hazards to the exposed adults, children or newborns.[3] Therefore, no separate WHO risk assessment of SafeNet has been conducted.

5.1 EFFICACY – WHOPES-SUPERVISED STUDY

5.1.1 Laboratory study

A laboratory study was conducted to determine the regeneration time, resistance to washing and efficacy of the SafeNet LN (Chateau et al., 2014).

Materials and methods

Net material

The WHO Collaborating Centre in Montpellier, France (LIN/IRD laboratory) received four 75-denier LNs from two different production batches provided by Mainpol GmbH. Fourteen pieces of netting were cut from each of the four LNs: total 56 net samples were taken. Eight of these pieces (2 from each of four nets) were used for the regeneration study, 28 (7 from each of four nets) for the wash resistance evaluation and 20 samples (5 from each of four nets) were stored at 4 °C for within-net and between-net variability of insecticidal content.

Two reference nets (Interceptor LN 100-denier from BASF) were tested in parallel with the SafeNet samples. Fourteen pieces of netting were cut from each of the two reference LNs,

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[1] WHO specification for alpha-cypermethrin coated onto filaments LN (http://who.int/whopes/quality/Alphacypermethrin_Interceptor_specs_eval_WHO_Oct_2009.pdf, accessed August 2015).

[2] WHO recommended insecticide products for treatment of mosquito nets for malaria vector control insecticide products for treatment of mosquito nets for malaria vector control [updated 17 November 2014] (http://who.int/whopes/Insecticides_ITN_Malaria_Nov2014.pdf?ua=1, accessed August 2015).

[3] WHO (2006). Report of the 10th WHOPES Working Group Meeting, Review of Spinosad 0.5% GR and 12% SC, Lambda-cyhalothrin 10% CS, K-O TAB 1-2-3®, Interceptor®, 11–14 December, 2006. Geneva: World Health Organization (http://whqlibdoc.who.int/hq/2007/WHO_CDS_NTD_WHOPES_2007_1_eng.pdf, accessed August 2015).

so a total of 28 samples were taken. Four of these pieces (2 from each of the two nets) were used for the regeneration study, 14 (7 from each of the two nets) for the wash resistance evaluation after bioefficacy testing and 10 samples (5 from each of two nets) were stored at 4 °C for testing within-net and between-net variability of insecticidal content.

At the end of the study all net samples used in the bioassays or stored at 4 °C from test (SafeNet LN) and reference (Interceptor LN) nets were sent to the WHO Collaborating Centre for quality control of pesticides in Gembloux, Belgium (CRA-W) for chemical analysis (Pigeon et al., 2015g).

For all existing coated polyester nets, 75- and 100-denier share the same WHO specification range for wash resistance index. Therefore, the wash resistance index is not expected to differ significantly according to the denier number.

Biological material

Non-blood-fed females, 2–5 days old, of *An. gambiae* s.s. Kisumu strain, a standard susceptible strain originating from Kenya, and regularly checked to ensure they have maintained susceptibility to pyrethroid insecticides were used during the evaluation.

Regeneration time and initial efficacy

The time required for full regeneration of biological efficacy was measured using WHO cone tests on 12 netting samples (eight candidate SafeNet LN plus four reference Interceptor LN) washed and dried three times on the same day using standard washing procedures to deplete surface insecticide and held between washes at 30 °C. Nets were tested for regeneration at 1, 2, 3, 5 and 7 days after the third wash. Insecticide bioefficacy curves (24-hour mortality and knockdown at 60 minutes), measured by 3-minute exposure in cone bioassays, were established for the six samples washed three times and compared with six unwashed samples (four SafeNet LN and two Interceptor LN for each). The number of days for efficacy to reach a plateau was considered to be the time required for full regeneration of the net i.e. the regeneration time value.

Wash resistance

The resistance of the candidate and reference LNs to washing was determined by cone bioassay tests carried out on netting samples subjected to WHO standardized washing at intervals corresponding to the regeneration time (as determined above). Samples were dried and held at 30 °C between consecutive washes. The bioefficacy evaluation recorded the percentage knockdown of mosquitoes at 60-minutes post-exposure (KD60) and the percentage mortality after 24 hours on samples that were unwashed and samples that were washed 1, 3, 5, 10, 15, 20 and 25 times. Each bioassay was done after the regeneration time and just before the next wash although it should be noted that for practical reasons, LNs were not washed during the weekend but were stored at 30 °C until the next wash on a week day. Data collected were percentage mortality and knockdown for each number of washes. The number of washes providing mortality and/or knockdown above the cut-off point (≥ 80% mortality after 24 hours or ≥ 95% knockdown after 60 minutes post-exposure) was reported.

Determination of insecticide content in net samples

Twenty samples from the SafeNet LN and 10 samples from the Interceptor LN were analysed for insecticidal content to determine within-net and between-net variations. Eight SafeNet LN samples and four Interceptor LN samples were analysed from the regeneration time study (0 and three washes). Twenty-eight samples of SafeNet LN and 14 Interceptor LN reference samples were analysed for the wash resistance study (0, 1, 3, 5, 10, 15, 20 and 25 washes).

The analytical method used for determination of alpha-cypermethrin in samples of SafeNet LN and Interceptor LN is the CIPAC method 454/LN/M/3.1. This method involves extraction of alpha-cypermethrin by refluxing net samples for 5 minutes with tetrahydrofuran in presence of dioctyl phthalate as internal standard, adding of citric acid and determination by Gas Chromatography with Flame Ionization Detection (GC-FID). The performance of the analytical method was controlled during the analysis of samples in order to validate the analytical results.

Results

Regeneration time

The regeneration time of SafeNet LN was compared with that of Interceptor (reference LN) and each result represents a mean of 10 replicates on four SafeNet LN samples and two Interceptor LN samples (Table 5.1). Both the KD60 and mortality were high (99.5–100%) for the unwashed SafeNet and remained so for seven consecutive wash–dry cycles. For Interceptor LN, knockdown and mortality both remained at 99–100% for unwashed nets and after the three consecutive wash–dry cycles. Control mortality throughout the experiment was below 10% and therefore satisfactory. There was no significant difference between the percentage mortality for SafeNet LN and Interceptor LN samples during the whole regeneration time study. No reactivation after washing was necessary for either LN. Data demonstrate that the regeneration time of SafeNet LN based on mortality data was 1 day.

Wash resistance and efficacy

The results of bioassays carried out on unwashed and washed LNs are presented in Table 5.2. Each result represents a mean of 10 replicates on four SafeNet LN samples and two Interceptor LN.

The KD60 of the SafeNet and Interceptor nets remained at 100% until 3 washes and started to decrease at 5 washes. KD60 of both nets was above the WHO threshold (≥ 95%) at 15 and 20 washes, declining to below the threshold at 25 washes. The knockdown of the SafeNet LN and Interceptor LN was not significantly different except at 10 washes (Fisher test, $P = 0.01$) and 25 washes (Fisher test, $P < 0.0001$) where knockdown was lower for Interceptor LN than SafeNet LN.

The percentage mortality of SafeNet LN and Interceptor LN both decreased from 100% to below the WHO threshold (80%) after the 5th wash. The mortality for both net types remained below the WHO threshold from the 5th until the 25th wash. The two net types were not significantly different except at 20 washes where mortality was lower for Interceptor LN than SafeNet LN (Fisher test, $P = 0.001$). Control mortality throughout the experiment was satisfactory (< 10%).

The assessment of efficacy of SafeNet LN and Interceptor LN nets using tunnel tests was not necessary, according to the results in cone tests obtained with knockdown.

Chemical assays

The average alpha-cypermethrin content in unwashed SafeNet LN ranged from 7.38 to 7.85 g/kg, corresponding to 236 to 259 mg AI/m^2. All the nets complied with the target dose of 6.7 g/kg ± 25% for 75-denier yarn [5.025–8.375 g/kg]. The within-net variation, expressed as the relative standard deviation of the alpha-cypermethrin content found on the five pieces of the net, ranged from 5.0% to 9.0% showing acceptable homogeneity of the active ingredient distribution over the net. The between-net variation, expressed as the relative standard deviation of the alpha-cypermethrin content found on four different nets, was 2.9% showing acceptable homogeneity of the active ingredient between the nets.

Similarly, Interceptor LN had an average alpha-cypermethrin content in unwashed samples ranging from 4.27 to 4.51 g/kg, corresponding to 181 to 193 mg/m^2. All the nets complied with the target dose of 5 g/kg ± 25% for 100-denier yarn [3.75–6.25 g/kg]. The within-net variation (RSD), ranged from 3.7% to 4.1% showing acceptable homogeneity of the active ingredient distribution over the net. The between-net variation (RSD) was 3.9% showing acceptable homogeneity of the active ingredient.

After 20 washes, the average alpha-cypermethrin content in SafeNet LN and Interceptor LN was 3.63 g AI/kg and 0.99 g AI/kg, respectively (Table 5.3). The wash resistance index as estimated by exponential regression curve was 97.2% and 92.6% per wash for the SafeNet Ln and Interceptor LN, respectively (Figure 5.1). The wash resistance index of alpha-cypermethrin from the netting in both the SafeNet LN and Interceptor LN products is within the WHO specifications 454/LN/1 for alpha-cypermethrin (coated) LNs.[1]

5.2 Conclusions and recommendations

SafeNet LN complied with the WHO specifications for alpha-cypermethrin (coated) LN (454/LN/1) in terms of active ingredient content and wash resistance index.

The SafeNet LN nets showed acceptable efficacy against susceptible *An. gambiae* in Phase I laboratory bioassays. Knockdown of mosquitoes was above 95% at 20 washes; therefore, SafeNet LN met the WHO criteria for the Phase I study. Mortality declined below 80% after 3 washes. The nets regenerated within 1 day.

Considering the efficacy and resistance to washing of SafeNet LN in laboratory (Phase I) studies, the 18th WHOPES Working Group concluded:

- that the wash-resistance and bioefficacy of SafeNet LN are comparable to those of the reference product (Interceptor LN) for which WHO specifications are already available.

[1] WHO specification for alpha-cypermethrin coated onto filaments LN (http://who.int/whopes/quality/Alphacypermethrin_Interceptor_specs_eval_WHO_Oct_2009.pdf, accessed August 2015).

The meeting recommended:

- that WHO specifications for alpha-cypermethrin long-lasting (coated onto filaments) insecticidal net be extended to SafeNet LN.

The meeting also recommended:

- that the national authorities and procurement agencies must ensure that the SafeNet LN complies with WHO specifications as recommended by the Organization according to the quality control procedures outlined in the *Guidelines for procuring public health pesticides*.[1]

Note: WHO recommendations on the use of pesticides in public health are valid ONLY if linked to WHO specifications for their quality control.

[1] WHO (2012). Guidelines for procuring public health pesticides. Geneva: World Health Organization, 2012 (http://whqlibdoc.who.int/publications/2012/9789241503426_eng.pdf, accessed August 2015).

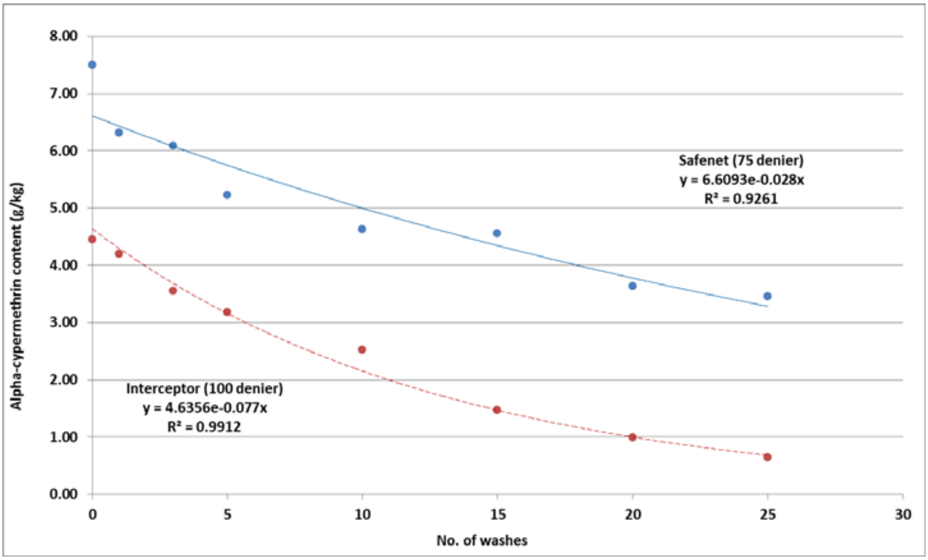

Figure 5.1 Alpha-cypermethrin content and retention (wash curve) for SafeNet LN (75 denier) and Interceptor LN (100 denier) (WHOPES Phase I)

Table 5.1 Regeneration time as determined by average percentage mortality (%M) and knockdown (%KD) with 95% confidence intervals (CI) of *Anopheles gambiae* (Kisumu) females in bioassays of unwashed and 3-times washed nets stored at 30 °C for 1–7 days, for the SafeNet LN in comparison with the reference LN (Interceptor). N indicates the total number of mosquitoes exposed

LN	Unwashed		3 washes + 1 day		3 washes + 2 days		3 washes + 3 days		3 washes + 5 days		3 washes + 7 days	
	%M	%KD	%M	%KD	%M	%KD	%M	%KD	%M	%KD	%M	%KD
SafeNet	100	99.5	99.5	100	100	100	100	100	100	100	99.5	100
95% CI	NA	± 0.9	± 0.9	NA	NA	NA	NA	NA	NA	NA	± 0.9	NA
N	213		214		211		211		231		218	
Interceptor	100	100	99	100	100	100	100	100	–		–	
95% CI	NA	NA	± 1.9	NA	NA	NA	NA	NA				
N	111		102		103		107					

NA = not applicable

Table 5.2 Wash resistance as determined by average percentage mortality (%M) and knockdown (%KD) with 95% confidence intervals (CI) of *Anopheles gambiae* (Kisumu) females in bioassays of unwashed and 1–25-times washed nets, SafeNet LN in comparison with the reference LN (Interceptor). N indicates the total number of mosquitoes exposed

LN	Unwashed		1 wash		3 washes		5 washes		10 washes		15 washes		20 washes		25 washes	
	%M	%KD	%M	%KD	%M	%KD	%M	%KD	%M	%KD	%M	%KD	%M	%KD	%M	%KD
SafeNet	100	100	99.5	100	86.7	100	65.1	98.0	50.5	88.5	33.2	98.5	49.2	97.1	21.8	91.5
95% CI	± 0	± 0	± 1.0	± 0	± 4.6	± 0	± 6.5	± 1.9	± 6.9	± 4.4	± 6.5	± 1.6	± 6.8	± 2.3	± 5.7	± 3.8
N	213		198		207		200		200		206		209		201	
Interceptor	100	100	100	100	79.8	100	63.6	94.0	51.6	76.8	31.3	97.1	28.2	97.0	13.6	62.0
95% CI	± 0	± 0	± 0	± 0	± 7.8	± 0	± 9.3	± 4.7	± 9.8	± 8.3	± 9.1	± 3.2	± 9.1	± 3.3	± 8.8	± 3.3
N	111		104		98		100		99		103		100		100	

Table 5.3 Alpha-cypermethrin content and retention of SafeNet LN (WHOPES Phase I wash resistance study). Target dose and tolerance limit for alpha-cypermethrin content in baseline SafeNet LN = 6.7 g/kg ± 25% for 75 denier yarn [5.025–8.375 g/kg]. Target dose and tolerance limit for alpha-cypermethrin content in baseline Interceptor LN = 5 g/kg ± 25 % for 100 denier yarn [3.75–6.25 g/kg]

LN	Wash	AI content (g/kg)	Between-net RSD (%)	AI retention (% of wash 0)	AI WRI (% at each wash)
SafeNet 75 denier (target dose = 200 mg AI/m^2)	0	7.50	2.5%	–	–
	1	6.32	6.5%	84.2	84.2
	3	6.08	9.6%	81.1	93.3
	5	5.22	11.7%	69.6	93.0
	10	4.63	13.7%	61.8	95.3
	15	4.56	15.8%	60.8	96.7
	20	3.63	14.8%	48.5	96.4
	25	3.46	7.4%	46.1	97.0
Interceptor 100 denier (target dose = 200 mg AI/m^2)	0	4.44	5.0%	–	–
	1	4.19	8.4%	94.3	94.3
	3	3.55	1.6%	79.8	92.8
	5	3.17	9.8%	71.4	93.5
	10	2.52	0.3%	56.7	94.5
	15	1.48	8.6%	33.2	92.9
	20	0.99	23.4%	22.2	92.8
	25	0.65	27.6%	14.6	92.6

AI = alpha-cypermethrin; RSD = relative standard deviation; WRI = wash resistance index

6. GENERAL DISCUSSION

Phase II experimental hut studies are increasingly limited in the testing and evaluation of pyrethroid-only long-lasting insecticidal nets due to low malaria vector densities, low biting rates and/or increasing insecticide resistance in malaria vectors against pyrethroids. Consequently, it is increasingly difficult to conclude on the performance of pyrethroid-only nets on the basis of both of the criteria used for experimental hut studies (i.e. mortality and blood-feeding inhibition). Given these limitations, several experimental hut sites with different vector characteristics (e.g. resistance profile, biting behaviour) are required to be employed so that the overall performance of such nets can be evaluated on both outcome criteria. Alternative solutions (e.g. expansion of the number of experimental hut sites in South-East Asia and Latin America and the use of semi-field systems) are being explored by WHOPES.

New Mountain Innovations, Inc., USA has submitted Larvasonic™ SD Mini to WHOPES for evaluation as an acoustic larvicide device for control of mosquitoes in container habitats. Since the current WHOPES *Guidelines for laboratory and field testing of mosquito larvicides*[1] do not provide guidance on evaluation of such a device, the manufacturer was invited to make a presentation on the device and its claim before the Working Group so that the Group can assist WHOPES in development of a suitable test protocol.

[1] WHO (2005). Guidelines for laboratory and field testing of mosquito larvicides. Geneva: World Health Organization (http://apps.who.int/iris/bitstream/10665/69101/1/WHO_CDS_WHOPES_GCDPP_2005.13.pdf, accessed August 2015)

ANNEXES

ANNEX 1. LIST OF PARTICIPANTS

Dr Nicole Achee, University of Notre Dame, Notre Dame, IN, USA.

Dr Fabrice Chandre, Laboratoire de Lutte contre les Insectes Nuisibles (LIN/IRD), Montpellier, France.

Professor Dr Marc Coosemans, Institute of Tropical Medicine, Antwerp, Belgium.

Dr Vincent Corbel, Department of Entomology, Kasetsart University, Bangkok, Thailand.

Dr Dirk Engels, Department of Control of Neglected Tropical Diseases, World Health Organization, Geneva, Switzerland.

Dr John Gimnig, Centers for Disease Control and Prevention, Atlanta, GA, USA.

Professor Dr Juan Arredondo Jiménez, Universidad Autónoma de Nuevo León, Mexico (Observer).

Dr Sarah Moore, Ifakara Health Institute, Bagamoyo, United Republic of Tanzania.

Dr Pie Müller, Swiss Tropical and Public Health Institute, Basel, Switzerland.

Dr Richard Oxborough, London, UK.

Dr Olivier Pigeon, Walloon Agricultural Research Centre (CRA-W), Gembloux, Belgium.

Professor Dr Mark Rowland, London School of Hygiene & Tropical Medicine, London, United Kingdom.

Dr Anna Drexler, Vector Ecology and Management, Department of Control of Neglected Tropical Diseases, World Health Organization, Geneva, Switzerland.

Dr Abraham Mnzava, Entomology and Vector Control, Global Malaria Programme, World Health Organization, Geneva, Switzerland.

Dr Emmanuel Temu, Entomology and Vector Control, Global Malaria Programme, World Health Organization, Geneva, Switzerland.

Dr Raman Velayudhan, Vector Ecology and Management, Department of Control of Neglected Tropical Diseases, World Health Organization, Geneva, Switzerland.

Dr Rajpal Yadav, Vector Ecology and Management, Department of Control of Neglected Tropical Diseases, World Health Organization, Geneva, Switzerland.

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