REPORT OF THE FOURTEENTH

WHOPES

WORKING GROUP MEETING

WHO/HQ, GENEVA
11—15 APRIL 2011

Review of:

SPINOSAD● EC
LIFENET● LN
MAGNET™ LN
ROYAL SENTRY● LN
YAHE● LN

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1. INTRODUCTION

The fourteenth meeting of the WHOPES Working Group, an advisory group to the WHO Pesticide Evaluation Scheme (WHOPES), was convened at WHO headquarters in Geneva, Switzerland, from 11 to 15 April 2011. The objective of the meeting was to review Spinosad EC (Clarke Mosquito Control, USA) for mosquito larviciding; and LifeNet® (Bayer CropScience, France) for malaria prevention and control. The meeting also assessed the regeneration, wash resistance and efficacy of MAGNet[TM] LN (VKA Polymers, India), Royal Sentry® LN (Disease Control Technologies, USA) and Yahe® LN (Fujian Yamei Industry, China) as part of the requirements for extending WHO specifications to these products.

The meeting also addressed issues and challenges related to procedures, criteria and requirements for testing and evaluation of public health pesticides, and made appropriate recommendations.

The meeting was attended by 11 scientists (see Annex I: List of participants). Professor Dr Marc Coosemans was appointed as Chairman and Dr John Gimnig as Rapporteur. The meeting was convened in plenary and group sessions, in which the reports of the WHOPES supervised trials and relevant published literature and unpublished reports were reviewed and discussed (see Annex II: References). Recommendations on the use of the above-mentioned products were made.

Declaration of interests
All invited experts were requested to complete a WHO Declaration of Interests form prior to the meeting. The following interests were declared:

Dr. Rajendra Bhatt's institute has received prescribed standard fees from six manufacturers of pesticide products (BASF India, Bayer CropScience India, Clarke Mosquito Control USA, Sumitomo Chemicals India, Syngenta Crop Protection India and Vestergaard Frandsen India) in order to meet the costs of product evaluation.

Dr Marc Coosemans' research unit has received long-lasting insecticidal mosquito nets (LNs) free of charge from Sumitomo Chemicals Japan for use in a study on long-lasting insecticidal hammocks in Cambodia and Viet Nam. In addition, his research unit received a grant from UBS Bank for the same research project.

Dr Vincent Corbel's institute has received grants from DART (a joint venture between Vestergaard Frandsen, the Acumen Fund and Richard Allan), for testing and evaluation of its durable wall lining products, and from Vestergaard Frandsen for testing and evaluation of its LNs. In addition, his travel to a malaria meeting in Nairobi in 2009 was paid for by Vestergaard Frandsen.

Dr John Gimnig's research unit has received larvicide for testing from Clarke Mosquito Control, USA, to the value of US$ 800.

Dr Olivier Pigeon's institute has received prescribed standard fees from VKA Polymers and Bayer CropScience in order to meet the costs of testing LNs manufactured by the companies.

Dr Fabrice Chandre's institute has received prescribed standard fees from Sumitomo Chemicals Japan, Bayer CropScience Germany and SPCI France in order to meet the costs of evaluating their respective LNs. In addition, his travel to a malaria meeting in Nairobi in 2009 was paid for by Bayer Environmental Science France.

Dr Mark Rowland's research unit has received grants from Bayer Environmental Sciences and Vestergaard Frandsen for testing and evaluation of their LNs, as well as from Innovative Vector Control Consortium (IVCC) for laboratory and field testing of different insecticide products.

The interests declared by the experts were assessed by the WHO Secretariat. With the exception of Dr Chandre's declared personal interest and Dr Mark Rowland's declared interest on the part of his research unit, the declared interests were not found to be directly related to the topics under discussion at the meeting; the discussion was aimed at evaluating the pesticide products manufactured by Bayer CropScience, France; Clarke Mosquito

Control, USA, Disease Control Technologies, USA; Fujian Yamei Industry, China; and VKA Polymers, India. It was, therefore, decided that all of the above-mentioned experts (with the exception of Dr Chandre and Dr Rowland) could participate in all evaluations, subject to the public disclosure of their interests.

In view of his declared personal interest, Dr Chandre did not participate in the evaluation of Bayer's LifeNet LN.

In view of the declared interest on the part of his research unit, Dr Rowland did not participate in the evaluation of Bayer CropScience's LifeNet LN.

2.   REVIEW OF SPINOSAD EC

Spinosad 20.6% (240 g AI/L [active ingredient per litre]) emulsifiable concentrate (EC) contains a complex of the active ingredients spinosyn A and D. The product is manufactured by Clarke Mosquito Control, USA, and uses spinosad technical material (TC) of Dow AgroSciences, which has served as a reference profile for developing WHO specification 636/TC February 2007. Spinosad EC is intended for dilution with water in a spray tank and to be used as a mosquito larvicide, except for container-breeding mosquitoes for which a more suitable formulation of spinosad has already been developed.

Spinosad is a natural product produced by fermentation technology that employs the bacterium *Saccharopolyspora spinosa* (Actinomycetales) from which it is obtained by extraction and purification of the whole broth. The Insecticide Resistance Action Committee of CropLife International has classified spinosad under group 5, describing its mode of action as nicotinic acetylcholine receptor (nAchR) allosteric activators.[1] Spinosad GR (granule); SC (suspension concentrate) and DT (tablet for direct application) have previously been evaluated by WHOPES for mosquito larviciding. A WHO safety assessment of spinosad and recommendations[2,3] for its use, as well as WHO specifications[4] for quality control of the named products, have previously been published. The assessment concluded that spinosad is a mosquito larvicide that poses no

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[1] *Prevention and management of insecticide resistance in vectors of public health importance*, 2nd ed. Insecticide Resistance Action Committee, CropLife International, 2010 (also available at: http://www.afpmb.org/sites/default/files/whatsnew/2011/irac_manual.pdf).
[2] *Report of the tenth WHOPES Working Group Meeting, WHO/HQ, Geneva, 11–14 December 2006.* Geneva, World Health Organization, 2007 (document WHO/CDS/NTD/WHOPES/2007.1; also available at http://www.who.int/whopes/recommendations/wgm/en/).
[3] *Report of the eleventh WHOPES Working Group Meeting, WHO/HQ, Geneva, 10–13 December 2007*. Geneva, World Health Organization, 2008 (document WHO/HTM/NTD/WHOPES/2008.1; also available at http://www.who.int/whopes/recommendations/wgm/en/).
[4] http://www.who.int/whopes/quality/newspecif/en/.

undue threat to the health of users or to the environment. The assessment is limited to spinosad with the equivalent impurity profile of that used for development of WHO specifications.

The current review assesses the efficacy of spinosad 20.6% EC in comparison with spinosad 11.6% SC formulation, for which WHO recommendations have previously been published.

2.1 Efficacy – WHOPES supervised trials

Cuddalore, Tamil Nadu, India

Sadanandane et al. (2010) carried out small-scale field trials of spinosad 20.6% EC in an urban area against *Culex quinquefasciatus* breeding prolifically in cesspits and open drains receiving wastewater from houses and disused wells containing highly organic matter. Cesspits are dug outside the houses while cement-lined U-shaped drains are often found choked with debris and silt in the absence of proper cleaning. Four dosages of spinosad EC (25, 50, 100 and 150 mg AI/m^2) were evaluated to determine optimum field application dosage, compare efficacy with spinosad SC applied at 50 mg AI/m^2 and determine the residual activity. From each type of habitat and each dose, five replicates were run. An equal number of replicates of each type were taken as untreated controls. As the drains were choked with silt and debris, every 10 m linear segment was taken as a replica. However, while applying the formulations, the entire length of the drain was treated. Separate drains were selected for each dosage/formulation as well as for control. For each formulation, habitats of each type were made into six groups with comparable pre-treatment larval and pupal densities.

The groups were assigned randomly to five treatment dosages (four for spinosad EC and one for spinosad SC) and one control.

Both the formulations were applied as an aqueous spray using a hand-operated compression sprayer. Sampling from cesspits and drains was done using a dipper (300 ml capacity) twice a week for 1–2 weeks before treatment and then every 2–3 days after treatment, and continued until density in treated sites reached that

of the control or pre-treatment level. Sampling from wells was done using an iron bucket of 3 L capacity. Three dips were taken in each replicate, and larvae (first + second instars; third + fourth instars) and pupae were counted. The percentage reduction in treated habitats was based on the population of late instars (third + fourth) and pupae. The reduction of larval and pupal densities on post-treatment days was estimated by comparing the pre- and post-treatment densities in the treated habitats with the corresponding densities in the untreated habitats using Mulla's formula (Mulla et al., 1971).[1] The effective duration was taken as the period for which the lower limit of the 95% confidence interval (CI) exceeded 80% reduction in larval density.

In cesspits, spinosad EC reduced the pupal density by 80–100% for 7–10 days at 25 and 50 mg AI/m^2 (Table 1). The reduction was 71–80% on day 14; thereafter, the effect declined gradually. At 100 and 150 mg AI/m^2, the pupal density was reduced by 68% on day 1 and 95–100% up to day 14. The reduction of density of late-instar larvae ranged from 97% to 100% for one week post-treatment and from around 55% to 65% on day 14 at 25 and 50 mg AI/m^2. At 100 and 150 mg AI/m^2, the reduction of late-instar was from 97% to 100% up to day 10. Although the mean percentage reduction of late-instar was >80% on day 14, its lower limits of 95% CI for the means were <80%. Spinosad SC reduced pupal density by 95–100% for 4–10 days, while density of late instar larvae was reduced by 97–100% for 7 days.

In drains, the pupal density was suppressed by 85–100% and 79–88% up to 10 and 14 days respectively at 25 and 50 mg AI/m^2. At 100 and 150 mg AI/m^2, the pupal density was reduced by 87–100% up to 14–17 days. The density of late-instar larvae was reduced by 95–100% for 7–10 days at 25, 50 and 100 mg AI/m^2, declining to 50% on day 14. The dose of 150 mg AI/m^2 yielded >90% reduction up to day 14 and declined to around 35% on day 21. Spinosad SC reduced pupal density by 89–100% and that of late-instar larvae by >90% for 10 days.

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[1] Mulla MS et al (1971). Control of chironomid midges in recreational lakes. *Journal of Economic Entomology*, 62:300–307.

In disused wells, the pupal density was suppressed by >90% up to days 14–17 at 25 and 50 mg AI/m^2. At 100 and 150 mg AI/m^2, the reduction in pupal density was 98–100% for 30 days. The reduction in late-instar larvae was >90% for 11–17 days at 25 and 50 mg AI/m^2 and for 24–28 days at 100 and 150 mg AI/m^2. Spinosad SC reduced pupal density by almost 100% up to day 17 and that of late-instar larvae by 100% for 11 days.

At 50 mg AI/m^2, both EC and SC formulations were equally effective in suppressing larval and pupal densities of *Cx. quinquefasciatus* in cesspits, drains and disused wells. The effective duration of control provided by 100 and 150 mg AI/m^2 of spinosad EC was 1.4–2 times greater compared with that by 25 and 50 mg AI/m^2 dosages.

Mbita, Kenya
Fillinger (2011) evaluated spinosad 20.6% EC in comparison with spinosad 11.6% SC against *Anopheles gambiae s.s.*, *An. arabiensis* and *Cx. quinquefasciatus* in the laboratory, in small-scale standardized field tests and in natural habitats in western Kenya.

Laboratory experiments were carried out in a netting-screened greenhouse at ambient temperature and photoperiod. Batches of 25 insectary-reared third- to fourth-instar *An. gambiae* s.s., *An. arabiensis* and *Cx. quinquefasciatus* were placed separately in 200 ml chlorine-free filtered tap water in disposable cups and exposed to five concentrations of spinosad 20.6% EC (0.0021–0.0329 mgAI/L) and spinosad 11.6% SC (0.0012–0.0186 mgAI/L) formulations, each replicated four times. These concentrations were determined after doing preliminary range-finding tests. An equal number of controls were set up simultaneously with untreated tap water. The concentrations were made from 1% stock solution of the formulations prepared in distilled water. The larvae were exposed for 3 days and provided with larval food after 24 hours and 48 hours. Mortality was recorded after 1, 2 and 3 days. Moribund larvae were counted and added to dead larvae for calculating mortality. Each test was repeated three times on different days using freshly prepared stock solutions and dilutions

and different batches of larvae. When the control mortality was between 5% and 20%, the mortality of the treatment group was corrected according to Abbott's formula.[1] Data from all rounds and replicates were pooled, and LC_{50} and LC_{90} values were calculated using log-probit dose-response regression analyses.

From the laboratory bioassays, the LC_{50} and LC_{90} values for 24 hours for *An. gambiae* s.s. were calculated as 0.013 and 0.032 mg AI/L for spinosad EC, and 0.0064 and 0.018 mg AI/L for spinosad SC. For *An. arabiensis*, the LC_{50} and LC_{90} values were 0.041 and 0.232 mg AI/L for spinosad EC, and 0.024 and 0.133 mg AI/L for spinosad SC. For *Cx. quinquefasciatus*, the LC_{50} and LC_{90} values were 0.013 and 0.038 mg AI/L for spinosad EC, and 0.005 and 0.013 mg AI/L for spinosad SC. The LC_{50} and LC_{90} values decreased significantly over the test period of 3 days for both formulations and the three mosquito species tested (Table 2). After comparison of LC50 values at 24 hours, it was observed that spinosad 11.6% SC was generally more effective than the spinosad 20.6% EC formulation. The LC values did not vary significantly between 48-hours and 72-hours post-exposure. This emphasizes the need to make observations after 24-hour and 48-huor intervals.

Simulated field tests were performed in 20 circular plastic tubs of 40 cm diameter buried into an open sunlit field with the rim of the tub 2 cm above the ground level. The bottom of each tub was filled with soil to simulate the field conditions. Tubs were filled with de-chlorinated tap water to a depth of 20–30 cm. Batches of 20 late third-instar insectary-reared *An. gambiae s.s.* larvae were exposed in each tub using small-screened floating 2-L plastic containers. Each container had four 2 x 1 cm screened portholes 2 cm above the bottom to allow the movement of water and larval food into the container. The number of larvae surviving in the container was counted daily until all larvae had either pupated and emerged or died. A new batch of larvae was introduced to evaluate any residual effect until no difference in mortality/emergence inhibition was observed between treated and untreated controls. Three

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[1] Abbott WS (1925). A method of computing the effectiveness of an insecticide. *Economic Entomology*, 18:265–267.

experiments were set up to test three sets of dosages. Spinosad 20.6% EC was tested at 2.0 and 3.6 mg AI/m^2 as well as at 0.5, 1.5 and 2 times these dosages (Table 3). Spinosad 11.6% SC was tested at 1.0 and 1.8 mg AI/m^2 as well as at 0.5, 1.5 and 2 times these dosages. The dosages were selected based on laboratory studies. Both the tests were replicated once (two test rounds). The percentage inhibition of adult emergence (IE%) was calculated on day 3 post-exposure. A second batch of larvae was introduced on 3 days post-treatment, and emergence inhibition was calculated on days 7 or 9 post-treatment.

In experiment 1, after 3 days of exposure to both the dosages of the two formulations tested, an average 95.5–97.7% of the third-instar *An. gambiae* s.s. larvae had failed to emerge into adults (Table 3). A fresh batch of larvae introduced on day 3 post-treatment required another 4 days (day 7 post-treatment) to either die or emerge into adults. The larvicide had no further effect on the development of larvae (corrected emergence inhibition, 0–14.6%).

In experiment 2, the efficacy of the half-dosages showed an IE% of 45.1–74.4% after 3 days of exposure. The difference in survival between the two formulations and various dosages was not significant. The second batch of larvae introduced after 3 days post-treatment took another 6 days to either die or emerge into adults. The IE% ranged from 7.8% to 14.2%. The increase of dosages to 1.5 and 2 times the maximum recommended dosages in experiment 3 did not yield any significant improvement in efficacy of the two formulations (IE range 85.3–96.3%, which was similar to those achieved with the recommended minimum and maximum dosages). No extended residual effect was achieved by the higher concentrations.

Field studies were carried out in a small highland valley in the Western Province of Kenya. Based on the results of the simulated field trial, 0.01ml/m^2 (2 mg AI/m^2) dose of spinosad EC was found to be optimum for further evaluation in natural habitats. Two habitat types were included in the field trial in natural habitats: (i) drains dug by farmers for small-scale maize and fish farming; and (ii) borrow pits that were dug for various uses. A total of 62 habitats (29 borrow pits and 33 drains) containing *Anopheles* larvae were

selected for the trial. Some habitats also contained *Culex* larvae. The three study arms (control, spinosad EC and spinosad SC) were allocated randomly. Spinosad formulations were applied with the help of a hand-held sprayer. Larval densities were recorded in both experimental and control sites 4 and 2 days prior to larvicide application and on day 0 and 1, 2, 3 days post-treatment and every 2 days thereafter. Immatures of anophelines and culicines were sampled by taking 10 dips from each habitat, and counted, recorded and returned to the respective sites. There were scanty rains during the trial period. Re-application with the same dose was required at weekly intervals based on residual efficacy. The number of habitats decreased with each cycle (rounds 2 and 3 included 60 and 59 habitats respectively). The percentage reduction in larval densities was estimated using Mulla's formula (Mulla, 1971).

Anopheline larvae were more common than culicine larvae. A sample of 100 late-instar *Anopheles* larvae collected during the pre-treatment period were identified as *An. gambiae* s.s. by polymerase chain reaction (PCR) analysis. *Culex* included several species, including *Cx. quinquefasciatus*. The spinosad EC formulation provided effective control of *Anopheles* larvae in all habitats for 3–5 days (Figure 1). After a drastic reduction in the density of immature stages, especially of late instars and pupae, in the first 3–5 days post-treatment, habitats were quickly re-colonized by larvae. Therefore, taking into account the accumulation of late instars, re-application was necessary at weekly intervals to effectively control mosquito breeding. The residual action of spinosad formulations (>80% reduction) on *Culex* larvae was 3–4 days (Figure 2). The densities of late-instar anopheline and *Culex* larvae remained low throughout the 21-day experiment in the treated habitats compared with the untreated control habitats.

2.2 Conclusions and recommendations

The bio-efficacy of spinosad 20.6% EC was tested against *Cx. quinquefasciatus* in cesspits, street drains and disused wells at dosages of 25, 50, 100 and 150 mg AI/m^2. When applied at dosages of 25 and 50 mg AI/m^2, the formulation suppressed the larval population by 80–100% for 7–10 days in cesspits and drains, and for 11–17 days in disused wells. At 50 mg AI/m^2, the efficacy of the two spinosad formulations (that is, EC and SC) was comparable. Therefore, for effective control of *Cx. quinquefasciatus* in cesspits and drains, application of spinosad EC is recommended at 25–50 mg AI/m^2 at weekly intervals, and in disused wells at fortnightly intervals. Although increasing the dose to 100 or 150 mg AI/m^2 provided some added residual efficacy, it was not sufficient to recommend these increased doses at longer application intervals.

In the simulated small-scale field trial, 2.0 mg AI/m^2 and 3.0 mg AI/m^2 dosages of spinosad EC as well as 0.9 mg AI/m^2 and 1.7 mg AI/m^2 dosages of spinosad SC formulations were tested against *An. gambiae* s.s. After 3 days of exposure, an average 95.5–98% of the introduced larvae had died. Treatment at half the recommended dosage showed greatly reduced larval mortality. Increasing dosages by 1.5 and 2 times resulted in 85–96% mortality showed no significant improvement in efficacy compared with the recommended doses. At 7 or 9 days, percentage emergence inhibition had declined significantly for both formulations at all doses tested. Although the volume of application for the two formulations was similar, a higher concentration of active ingredient of spinosad 20.6% EC was needed to achieve a level of control similar to that of spinosad 11.6% SC formulation.

In the field trial against *An. gambiae* s.s. and associated *Culex spp.* breeding in natural habitats of agricultural drains and borrow pits, application of spinosad 20.6% EC at 2 mg/m^2 and spinosad 11.6% SC at 1.2 mg/m^2 resulted in >80% reduction in larval density for up to 5 days. Given the early appearance of early-instar larvae and the increase in number of late-instar larvae after 4–5 days, weekly applications are necessary for the EC formulation as well as for the

SC formulation. It is concluded that spinosad 20.6% EC will give effective control when applied at weekly intervals.

Noting the safety and efficacy of spinosad 20.6% EC, the meeting recommended:

- the use of spinosad 20.6% EC formulation in open bodies of clean water, such as borrow pits and drains, at the rate of 20 g AI/ha, with an expected duration of efficacy of one week.

- the use of 20.6% EC formulation in open bodies of polluted waters with high organic matter, such as cesspits and street drains, at the rate of 250–500 g AI/ha, with an expected duration of efficacy of 7–10 days.

- the use of spinosad 20.6% EC formulation in disused wells at the rate of 250–500 g AI/ha, with an expected duration of efficacy of 11–17 days.

Table 1. **Efficacy of spinosad formulations tested against mosquito larvae in small-scale field trials in various habitats**

Country and location	Product	Species	Habitat	Dose (active ingredient mg/m^2)	Longevity (days)	% reduction in adult emergence
India, Cuddalore (Tamil Nadu)	20.6% EC	*Cx. quinque-fasciatus*	Cesspits	25	7	81–100
				50	7–10	94–100
				100	10–14	97–100
				150	10–14	95–100
			Street drains	25	7–10	86–100
				50	10	87–100
				100	10–17	89–100
				150	14–17	87–100
			Disused wells	25	11–14	91–100
				50	17	95–100
				100	24–30	88–100
				150	28–30	98–100
	11.6% SC	*Cx. quinque-fasciatus*	Cesspits	50	7–10	95–100
			Street drains	50	10	89–100
			Disused wells	50	11–17	99–100

EC = emulsifiable concentrate; SC = suspension concentrate

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Table 2. **Comparative bioactivity of two formulations of spinosad against third- and fourth-instar mosquito larvae in laboratory studies, Mbita, Kenya**

Product	Hours post-treatment	Activity (mg/L active ingredient) against					
		An. gambiae s.s.		*An. arabiensis*		*Cx. quinquefasciatus*	
		LC_{50} (95% CI)	LC_{90} (95% CI)	LC_{50} (95% CI)	LC_{90} (95% CI)	LC_{50} (95% CI)	LC_{90} (95% CI)
Spinosad 20.6% EC	24h	0.0131 (0.0112-0.0156)	0.0321 (0.0253-0.0451)	0.0414 (0.0286-0.0777)	0.2322 (0.1112-0.9505)	0.0126 0.0109-0.0146	0.0383 (0.0305-0.0519)
	48h	0.0045 (0.0043-0.0049)	0.0097 (0.0088-0.0109)	0.0064 (0.0053-0.0074)	0.0266 (0.0208-0.0373)	0.0072 (0.0064-0.0082)	0.0233 (0.0195-0.0295)
	72h	0.0032 (0.0031-0.0037)	0.0074 (0.0066-0.0084)	0.0035 (0.0031-0.0039)	0.0101 (0.0086-0.0123)	0.0052 (0.0045-0.0058)	0.0175 (0.0146-0.222)
Spinosad 11.6% SC	24h	0.0064 (0.0058-0.0071)	0.0182 (0.0155-0.0221)	0.0242 (0.0194-0.0329)	0.1333 (0.0837-0.2602)	0.0048 (0.0043-0.0053)	0.0135 (0.0112-0.0166)
	48h	0.0009 (0.0007-0.0011)	0.0034 (0.0029-0.0039)	0.0023 (0.0021-0.0027)	0.0073 (0.0062-0.0087)	0.0022 (0.0019-0.0024)	0.0072 (0.0063-0.0085)
	72h	0.0008 (0.0006-0.0009)	0.0022 (0.0019-0.0025)	0.0014 (0.0012-0.0015)	0.0042 (0.0036-0.0049)	0.0016 (0.0014-0.0018)	0.0053 (0.0046-0.0065)

EC = emulsifiable concentrate; SC = suspension concentrate

Table 3. **Percentage emergence inhibition of *Anopheles gambiae s.s.* larvae exposed to two spinosad formulations (emulsifiable concentrate (EC) and suspension concentrate (SC) in simulated field tests, Mbita, Kenya**

No. of experiment	Days post-treatment	Spinosad 20.6% EC		Spinosad 11.6% SC	
Experiment 1 (Dose based on laboratory studies)					
		2.0 mg AI/m^2	3.6 mg AI/m^2	1.0 mg AI/m^2	1.8 mg AI/m^2
	Day 3	97.7	95.5	97.7	96.3
	Day 7*	14.6	9.5	0.0	5.1
Experiment 2 (Half-dosages evaluated in experiment 1)					
		1.0 mg AI/m^2	1.8 mg AI/m^2	0.5 mg AI/m^2	0.09 mg AI/m^2
	Day 3	48.8	74.4	45.1	54.1
	Day 9*	7.8	11.3	14.2	13.5
Experiment 3 (1.5 and 2 times of the maximum dosages evaluated in experiment 1)					
		5.5 mg AI/m^2	7.2 mg AI/m^2	2.8 mg AI/m^2	3.6 mg AI/m^2
	Day 3	85.3	90.4	93.4	96.3
	Day 9*	15.9	38.6	15.1	37.1

*A fresh batch of 3rd instar larvae was introduced on day-3 post-treatment and mortality was observed on day-7 or day-9 post-treatment.
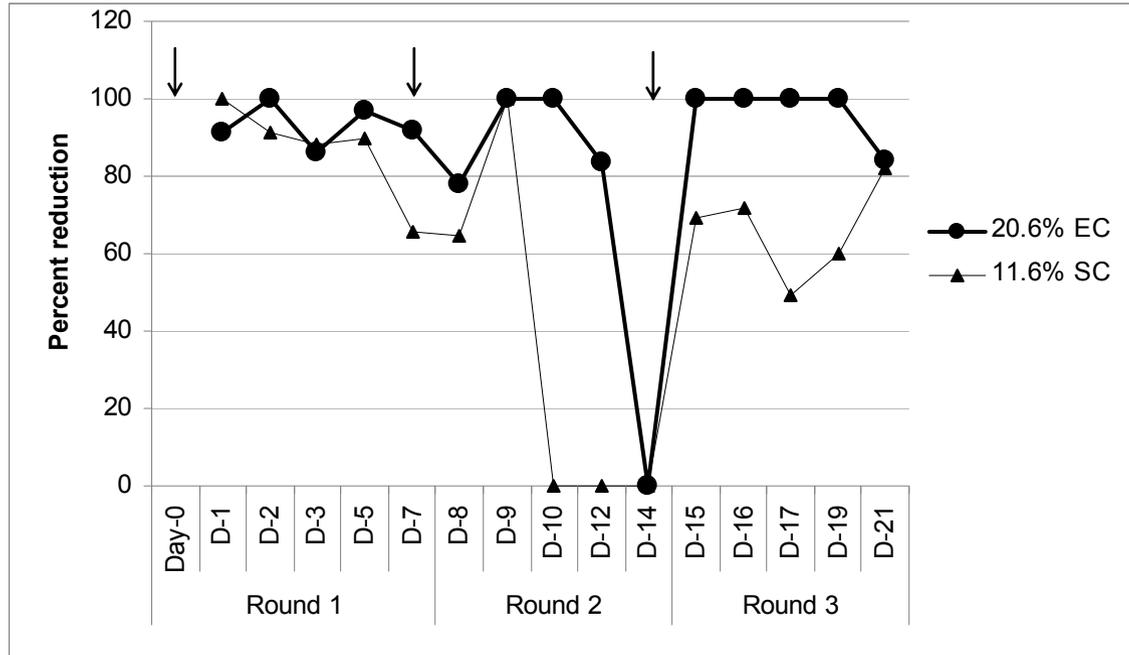
Figure 1. **Percentage reduction of anopheline late-instar larvae resulting from application of spinosad 20.6% emulsifiable concentrate (EC) at 2 mg Al/m^2 and 11.6% suspension concentrate (SC) at 1.2 mg Al/m^2 in natural habitats (all habitats combined); arrows indicate day of application of the formulations**
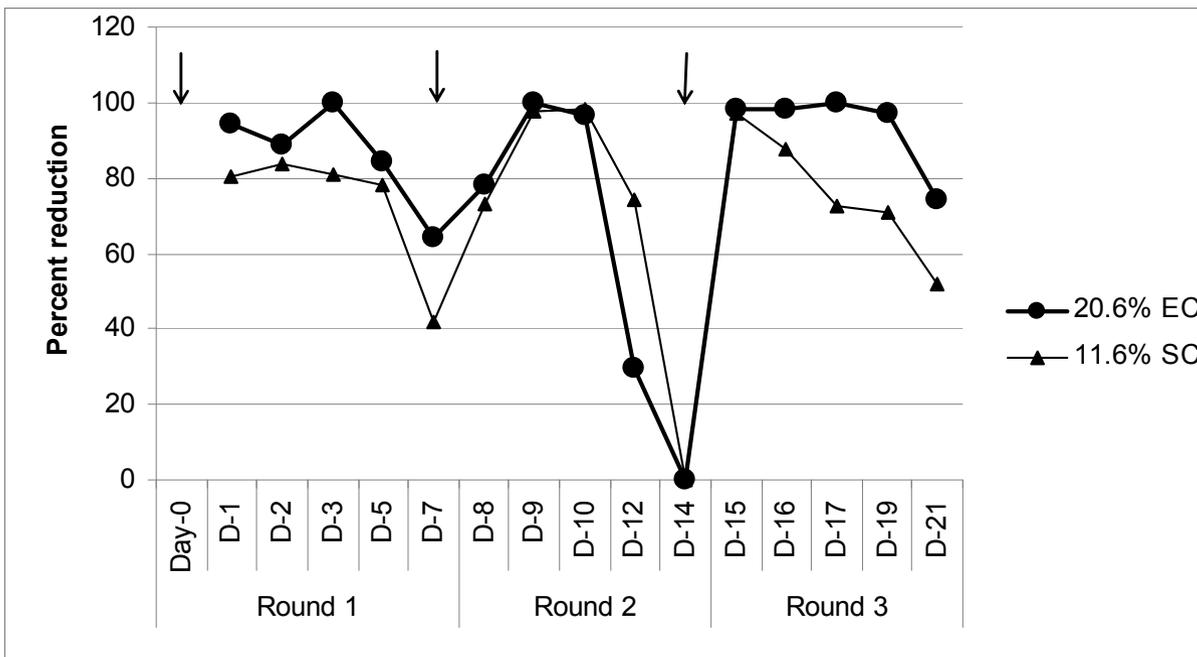
Figure 2. **Percentage reduction of culicine larvae due to application of spinosad 20.6% emulsifiable concentrate (EC) at 2 mg Al/m^2 and 11.6% suspension concentrate (SC) at 1.2 mg Al/m^2 in natural habitats (all habitats combined); arrows indicate day of application of the formulations**

3. REVIEW OF LIFENET®

The LifeNet® is manufactured by Bayer CropScience as a deltamethrin-treated long-lasting (incorporated into filaments) insecticidal [mosquito] net (LN). Technical deltamethrin complying with the requirements of WHO specification 333/TC is incorporated into 100 denier poly-filament polypropylene fibres at the target dose of 8.5 g AI/kg, corresponding to 340 mg of deltamethrin per m^2 LN. The net is available in a minimum mesh size of 21 complete holes per cm^2, with an average of 21–29 holes per cm^2. The minimum bursting strength of the fabric is declared as 450 kPa.

Deltamethrin has previously been evaluated by WHOPES for conventional treatment of mosquito nets, at a target dose of 15–25 mg AI/m^2.[1]

3.1 Safety assessment

The assessment of the risk to humans of washing and sleeping under the LN, provided by the manufacturer, was assessed by the Finnish Institute of Occupational Health (FIOH, 2010) on behalf of WHOPES. The WHO *Generic risk assessment model for insecticide treatment and subsequent use of mosquito nets*[2] was used as a guiding document. The following assumptions were used by the proposer in drafting the assessment:

FIOH concluded that the hazard characterization is based on the hazard data developed by the FAO/WHO Joint Meeting on Pesticide Residues (JMPR) acceptable daily intake (ADI) for sleeping under the net and the acute reference dose (ARfD) for net washing.[3] In assessing the risk of exposure, the default

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[1] http://www.who.int/whopes/Insecticides_ITN_Malaria_ok3.pdf.

[2] *A generic risk assessment model for insecticide treatment and subsequent use of mosquito nets.* Geneva, World Health Organization, 2004 (WHO/CDS/WHOPES/GCDPP/2004.6; also available at http://whqlibdoc.who.int/hq/2004/WHO_PCS_04.1.pdf; accessed 12 July 2011).

[3] *Inventory of IPCS and other WHO pesticide evaluations and summary of toxicological evaluations performed by the Joint Meeting on Pesticide Residues (JMPR) through 2008.* Geneva, World Health Organization (also available at:

assumptions and values of the model are generally applied. The use of alternative values is justified by appropriate experimental data, where the Generic Model defaults are not adopted. This is the case for the following:

I.   maximum 19% of the deltamethrin in LifeNet may be transferred to the washing liquid, which is lower than the default value of 30%;
II.   the default value for dermal absorption (10%) is overestimated and that 0.2% is more realistic;
III. the default value for release of deltamethrin to saliva in direct oral exposure from chewing or sucking the net is overestimated and is a maximum of 0.47%.

The proposer's conclusion that LifeNet, when used as instructed, does not pose undue hazards to the user, is justified.

3.2   Efficacy – background and supporting documents

The regeneration time, wash resistance and efficacy of LifeNet provided by the manufacturer were determined in laboratory (Phase I) studies according to WHOPES guidelines[1] (Rossignol et al., 2010c). Pre-cut LifeNet samples of 30 x 30 cm (n = 24) of one batch of LifeNet were used. To estimate the regeneration time, six net samples were washed and dried three times on one day to deplete the concentration of insecticide on the net surface. After washing, cone bioassays with *An. gambiae* (susceptible Kisumu strain) were carried out after 1, 3 and 5 days. Knock-down and mortality was 100% between day 1 and day 5. The regeneration time for that specific batch of LifeNet was one day.

The wash-resistance study (0, 10, 20, 25, 30 and 35 washes using three netting samples at each wash point) performed on *An. gambiae* (Kisumu strain) showed a KD of 100% up to 35 washes. Mortality rates were 100% up to 25 washes and

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http://www.who.int/ipcs/publications/jmpr/pesticide_inventory_edition10.pdf).

[1] *Guidelines for laboratory and field testing of long-lasting insecticidal mosquito nets.* Geneva, World Health Organization, 2005 (WHO/CDS/WHOPES/GCDPP/2005.11; also available at: http://www.who.int/whopes/guidelines/en/; accessed 12 July 2011).

remained high (95%) after 35 washes. A similar study using the pyrethroid-resistant VK-Per strain of *An. gambiae* obtained a KD effect of 100% up to 20 washes and 97% after 35 washes. The mortality rates were 100% for the unwashed net and maintained high levels between 10 and 25 washes (79–69%). After 30 washes, mortality fell to 7%. The manufacturer concluded that the LifeNet batch fulfilled the criteria required by WHO (mortality $\geq$80% or KD$\geq$95% after 20 washes) for LN.

3.3   Efficacy – WHOPES supervised trials

3.3.1   Laboratory studies

The regeneration time, wash resistance and efficacy of LifeNet were determined in laboratory (Phase I) studies according to WHOPES guidelines[1] against susceptible Kisumu strains of An*. gambiae s.s.* (Duchon et al., 2010).

Chemical analyses were performed on 8 unwashed nets and net samples washed 1, 3, 5, 10, 15, 20, 25, 30 and 35 times (Pigeon, 2010c). Per wash regimen, one piece (25 x 25 cm) from each of 4 nets was analysed to determine the deltamethrin content. The analytical method used for determination of deltamethrin and its R-alpha isomer (non-relevant impurity) was based on the extension of the CIPAC method 333/LN/(M)/3 (deltamethrin in incorporated net) and involved extraction (by heating under reflux with xylene) and chromatographic determination (by gas chromatography with flame ionization detection, GC-FID) using the internal standard calibration.

Results of analysis for AI content and retention (wash curve) are presented in Figure 3. The between-net variation is expressed as the relative standard deviation (RSD) of the insecticide content on the four pieces. Retention is calculated according to Annex 1 of the *Report of the eleventh WHOPES*

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[1] *Guidelines for laboratory and field testing of long-lasting insecticidal mosquito nets.* Geneva, World Health Organization, 2005 (WHO/CDS/WHOPES/GCDPP/2005.11; also available at: http://www.who.int/whopes/guidelines/en/; accessed 12 July 2011).

Working Group Meeting, [1] assuming free migration-stage behaviour.

The average deltamethrin content of 7.02 g AI/kg of the unwashed nets complied with the target dose of 8.5 g AI/kg (± 25%), with a between-net RSD of 2.5% showing good homogeneity of the treatment. The unwashed LifeNet LNs contained, in addition to the deltamethrin content, 0.48 g/kg (or 6.8% of the active deltamethrin content) of the insecticidally inactive R-alpha isomer of deltamethrin; this amount did not change after washing. After 20 washes, the average deltamethrin content was 4.68 g AI/kg (between-net RSD = 6.9%). The overall deltamethrin retention was 65% after 20 washes and 53% after 35 washes, corresponding to an average retention per wash of 98% (Pigeon, 2010c).

After three consecutive washes, the regeneration-time study carried out on susceptible *An. gambiae* Kisumu strains showed that biological activity was maximal (KD, 100%; mortality 100%) after 1 day. The regeneration time was therefore considered to be one day. The median knock-down time (MKDT) was also used to estimate the peak of bioavailability of insecticide on the net using the method described by Skovmand et al., 2008.[2] The MKDT with an unwashed net was 185 seconds (3.08 minutes); similar values were obtained on nets stored during 1–7 days after three consecutive washes, suggesting complete regeneration after 1 day.

The efficacy of net samples was measured by WHO cone bioassays on net samples washed 1, 5, 10, 15, 20, 25, 30 and 35 times. KD rates were 100% until wash 35. Mortality was 100% over 15 washes and remained above 90% until wash 35. It was concluded that the wash resistance of LifeNet exceeded the minimum WHO requirements of 20 standard washes.

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[1] *Report of the eleventh WHOPES working group meeting, WHO/HQ, Geneva 10–13 December 2007.* Geneva, World Health Organization, 2008 (WHO/HTM/NTD/WHOPES/2008.1; also available at http://www.who.int/whopes/recommendations/wgm/en/; accessed 12 July 2011).
[2] Skovmand O et al. Median knock-down time as a new method for evaluating insecticide-treated textiles for mosquito control. *Malaria Journal*, 2008, 7:114.

3.3.2 Experimental hut studies

Three field studies were implemented under WHOPES supervision: one in Moshi (United Republic of Tanzania) on a deltamethrin-susceptible *An. arabiensis* population (Oxborough et al., 2011); one in Malanville (Benin) on a pyrethroid-resistant *An. gambiae s.l.* population (Sidick et al., 2011); and one in India on a deltamethrin-susceptible population of *An. fluviatilis* (Gunasekaran et al., 2011).

In Benin and the United Republic of Tanzania, the west and east African experimental huts respectively were used to evaluate the efficacy of LifeNet in terms of blood-feeding inhibition, deterrence, induced exophily and mortality.[1]

In India, an adapted version of the west African experimental huts was used, whereby mosquitoes can only escape outside to a single veranda trap. The dimension of each hut with a single room is similar to that of the village huts (length 3m, width 3 m, height of walls 2 m, height in the middle 2.5 m), having brick walls with cement plastering and a thatched roof, above which there is a tin sheeted roofing for protecting the thatched roof. There is no space between the two roofs. Each hut is constructed on a concrete platform surrounded by a water-filled gutter acting as an ant trap. Each hut has four windows (0.45 x 0.45 m), two on the front and one on each of the side of the house. Each window was grilled with wooden planks fixed horizontally in a tilted position one above the other, leaving a gap of 1 cm through which mosquitoes could enter but not exit the hut, as confirmed by a release-recapture test before the study. The back of the house has a full-screened veranda for collecting the exophilic fraction of mosquitoes. Before the trial, and using non-treated nets, the attractiveness of these experimental huts, based on indoor resting densities of *An. fluviatilis*, was consistently higher (4 to 20 times) compared with traditional houses. To overcome the high scavenging rate, the rooms, veranda and surroundings of huts were cleaned daily, resulting in declines of scavenging to 0% over the experimental

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[1] *Guidelines for laboratory and field testing of long-lasting insecticidal mosquito nets.* Geneva, World Health Organization, 2005 (WHO/CDS/WHOPES/GCDPP/2005.11; available at: http://www.who.int/whopes/guidelines/en/; accessed 12 July 2011).

period, except on four occasions when scavenging ranged between 5% and 10%.

In each locality, six trap huts were used and six treatment arms were tested, following the WHO guidelines. Per treatment arm, the number of nets tested was equal to one test period (6 nights in Benin and India, 4 nights in Moshi) to allow for one full rotation for each test period. After each test period, the huts were cleaned and ventilated for one night, and assigned to another treatment arm according to a Latin square scheme. The sleepers were rotated randomly among huts each night of the study. Each net was deliberately holed with six holes (4 cm x 4 cm) to simulate a torn net. One additional net per treatment arm was used for chemical analyses and bioassays. Standard WHO procedures[1] for Phase II were used for washing the nets.

A one-day interval between successive washes was used, according to the regeneration study in WHOPES supervised Phase I experiments (Duchon et al., 2010).

The initial design included the following arms: (i) unwashed LifeNet®; (ii) LifeNet® washed 20 times; (iii) LifeNet® washed 30 times; (iv) polyester net conventionally treated (CTN) with deltamethrin at 25 mg AI/m², and washed until just before exhaustion, defined as the last wash providing mortality $\geq$80% or KD $\geq$95%; (v) polyester CTN with deltamethrin at 25 mg AI/m² and washed 20 times; and (vi) untreated polypropylene net.

The analysis of numeric data of hut trials was carried out using negative binomial regression (India and the United Republic of Tanzania) and non-parametric Kruskalis-Wallis test (Benin). Logistic regressions were used for proportional outcomes.

Cone bioassays were carried out using pyrethroid-susceptible colony strains of *An. gambiae* (Kisumu strain) in Benin and the United Republic of Tanzania. In India, fully-fed[2] *An. stephensi*

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[1] *Guidelines for laboratory and field testing of long-lasting insecticidal mosquito nets.* Geneva, World Health Organization, 2005 (WHO/CDS/WHOPES/GCDPP/2005.11; also available at: http://www.who.int/whopes/guidelines/en/; accessed 12 July 2011).
[2] The use of 2–5-day-old unfed mosquitoes is recommended by WHO for cone bioassays.

(colony strain) were tested on unwashed nets before the experimental hut trial, and fully-fed, field-collected *An. fluviatilis* were used just before and after the trial.

The analytical method used to determine concentrations of deltamethrin and its R-alpha isomer (non-relevant impurity) in samples of LifeNet and conventionally treated nets was based on the extension of CIPAC method 333/LN/(M)/3 (deltamethrin incorporated into polyethylene net) and involved extraction (by heating under reflux for 30 minutes with xylene in the presence of dibutyl phthalate) as an internal standard, and determination (by gas chromatography with flame ionization detection, GC-FID).

Moshi, United Republic of Tanzania

The study area is situated in the Lower Moshi Rice Irrigation Zone, an irrigated area surrounded by an upland semi-arid steppe. The main vector is *An.arabiensis*, a more zoophilic and exophilic member of the *An. gambiae* complex. Wild female *An. arabiensis* reared from field-caught larvae show some degree of resistance against permethrin and deltamethrin (10% survival). This pyrethroid resistance was confirmed on F1 progeny from the surviving adult females (survival rate of 40–50%).

Before washing, all treatment arms showed 100% KD and mortality, indicating the full bioavailability of deltamethrin regardless of treatment. The number of washes to just before exhaustion was three for the CTN (KD, 86%; mortality, 96%). At the end of the trial, bioassays of the CTNs fulfilled the WHO criteria for mortality (88%) but not for KD (90%). Before the start of the trial, CTNs washed 20 times induced a KD of 30% and a mortality of 22%. This was respectively 20% and 16% at the end of the trial. All the LifeNet (after 0, 20 and 30 washes) induced 100% KD and mortality after washing and at the end of the trial.

The AI content for four unwashed LifeNet (average 6.70 g AI/kg) complied with the target dose of 8.5 g AI/kg (± 25%), with an average within nets RSD of 2.9% showing good homogeneity of the treatment. The unwashed LifeNet LNs contained, in addition to the deltamethrin content, 1.10 g /kg of the insecticidally inactive R-alpha isomer (non-relevant impurity) of deltamethrin

(corresponding to 17% of the AI content); this amount did not change after washing (Pigeon, 2011a).

After 20 and 30 washes, the average deltamethrin content for LifeNet was 4.20 g AI/kg (within-net RSD, 4.7%) and 3.79 g AI/kg (within-net RSD, 6.1%) respectively. The overall deltamethrin retention was 63% after 20 washes and 57% after 30 washes.

The unwashed CTN contained 3.7–4.0 mg AI/m² (0.09–0.10 g AI/kg; within-net RSD, 66–33%), with deltamethrin representing only 15% of the target dose. The CTN washed to just before exhaustion contained 0.6 mg AI/m² (0.02 g AI/kg) deltamethrin, corresponding to a retention rate of 22%. The amount of deltamethrin on CTNs washed 20 times was below the detection limit (<0.01 g AI/kg) (Pigeon, 2011a).

After the trial, the deltamethrin content in all tested arms did not decrease significantly.

During a 34-night period corresponding to 24 nights of collection, 92 *An. arabiensis* (average of 3.8 per night) were collected in the huts with untreated nets. No significant deterrent effect was induced by any of the LifeNet (0, 20 and 30 washes) and the CTNs washed to just before exhaustion compared with the untreated nets. Exophily in untreated huts was high (87%) as it was with all other treatment arms (78.1–97.4%).

The rate of blood-feeding inhibition was 39.1% for the untreated nets and was significantly higher compared with all other treatment arms. The increase in the blood feeding rates with increasing number of washes of the LifeNets was not significant (0 washes, 20.4%; 20 washes, 23.0%; 30 washes, 28.1%). Blood feeding inhibition induced by LifeNet washed 20 or 30 times was not significantly different from CTNs washed to just before exhaustion.

The mortality rate was 13% for untreated nets and 61.3% for unwashed LifeNets. Mortality observed for LifeNet washed 20 or 30 times (42.5%) was significantly higher than that induced by CTNs washed until just before exhaustion (33%).

Malkangiri District, Orissa State, India

The study village (altitude, 150–200 m above sea level) is located along a stream in a hilly, forested area. Climate is characterized by hot summers (March–June), and rainy (July–October) and cold (November–February) seasons. The village is endemic for *Plasmodium falciparum* malaria transmitted by *Anopheles fluviatilis*. In this area, *An. fluviatilis* is susceptible to deltamethrin.

Before washing, all treatment arms showed 100% mortality using *An.stephensi* (colony strain) in the bioassays, while no mortality was observed with the untreated net. After washing, the nets were bioassayed against *An. stephensi* (50 mosquitoes per net) as well as against wild, blood-fed *An. fluviatilis* (25 mosquitoes per net). After the trial, wild, blood-fed *An. fluviatilis* (25 per net) were again used in the bioassays. The authors used a CTN washed 10 times for the treatment arm ("CTN washed until just before exhaustion"). However, this number of washes was just beyond exhaustion for mortality (82% after 9 washes and 78% after 10 washes); KD values were not reported. Prior to hut evaluation, the CTN washed 20 times induced a mortality of 60%, and 100% with all LifeNet treatments. At the end of the experimental hut trial, the CTN washed to just before exhaustion caused 72% mortality and the CTN washed 20 times caused 56% mortality, while all LifeNets retained their full capacity to kill the mosquitoes (100% mortality).

The deltamethrin content in unwashed LifeNets complied with the target dose of 8.5 g AI/kg (± 25%) for three samples (6.62, 6.47 and 6.64 g AI/kg) and was just below the tolerance lower limit (6.375 g AI/kg) for two samples (6.07 g AI/kg and 6.20 g AI/kg). The unwashed LifeNets contained, in addition to the deltamethrin content, 1.34 g/kg of the insecticidally inactive R-alpha isomer of (non-relevant impurity) deltamethrin (or 21% of the AI content); this amount did not change after washing (Pigeon, 2011b).

The within-net variation, expressed as the relative standard deviation (RSD), of the content found on the five pieces cut from the same net was 7.0, 5.2, 2.0, 1.1 and 4.2% for five samples, showing good homogeneity of the distribution of AI over the net. The average deltamethrin content was 4.02 g AI/kg after 20 washes and 4.04 g AI/kg after 30 washes,

corresponding to an overall deltamethrin retention of 62% for both washes. The AI within-net variation on LifeNet samples washed 20 and 30 times remained low (RSD 10.7% and 7.5% respectively). The unwashed CTN contained 2.3–3.1mg AI/m² (0.05–0.07g AI/kg; within-net RSD, 105–108%) deltamethrin, which was only 11% of the target dose. The deltamethrin content of the CTNs washed to just beyond exhaustion and the CTNs washed 20 times was below the detection limit (<0.01 g AI/kg). The deltamethrin content of the respective nets was similar before and after the trial (Pigeon, 2011b).

During a 42-day period corresponding to 36 nights of collection, 274 *An. fluviatilis* (average of 7.6 per night) were collected in the huts with untreated nets. Compared with the untreated arm, the reduction rate of entry for LifeNet arms was 70.8–78.8%, indicating a marked deterrent effect of LifeNet LNs against *An. fluviatilis*. No difference in entry rate was observed between the LifeNet arms (0, 20 and 30 washes). The reduction of entry for conventionally treated polyester nets was lower than that for LifeNet LNs (from 37.2% to 46.7%), but was not significant.

Exiting of mosquitoes to the veranda was higher (range, 50.7–64.4%) in the treatment arms compared with the control arm (39.1%).

The rate of blood feeding inhibition was 80.3% in the untreated huts and was significantly lower for all other treatments. For unwashed LifeNet LNs, blood feeding decreased to 21%, and was around 28% when washed 20 or 30 times. The blood feeding rate was 52.1% for the CTN washed to just before exhaustion and 68% after 20 washes.

With untreated nets, mortality was 2.2%. The rate of mortality was highest with unwashed LifeNets (98.3%) and those washed 20 times (91.3%). After 30 washes, the observed mortality was significantly higher for the LifeNet (73.8%) compared with the CTN net washed to just beyond exhaustion (39.7%).

Malanville, Benin

In this rice irrigation area in the Sudan-savannah zone of northern Benin, *An. gambiae s.l.* is the main malaria vector, with 95–97% *An. gambiae* s.s. (M form) and 3–5% *An. arabiensis.*

An. gambiae s.l showed susceptibility to most pyrethroids[1,2] but recent findings showed a significant increase of pyrethroid resistance (40% mortality at the discriminative dose of 0.05% deltamethrin) as a result of the co-occurrence of kdr mutations (frequency of 1014F allele, 47%) and enhanced oxidase activity (Vincent Corbel, personal communication).

Before washing, all treated nets caused 100% KD and mortality. LifeNet retained full biological activity after 20 and 30 washes (100% KD and mortality after washing and at the end of the trial in experimental huts). The number of washes to just before exhaustion was 2 for the CTN (KD, 93%; mortality, 83%). After 20 washes of a CTN, KD dropped to 63% and mortality to 60%. After the trial, CTNs washed to just before exhaustion had mortality of 92% and KD of 94%. After the trial, the CTN washed 20 times caused 74% mortality and 94% KD.

The AI content for four unwashed LifeNets (average 6.96 g AI/kg) complied with the target dose of 8.5 g AI/kg (± 25%); with an average within-net RSD of 3.3% showing good homogeneity of the treatment. The unwashed LifeNets contained, in addition to the deltamethrin content, 0.71 g /kg of the insecticidal inactive R-alpha isomer (non-relevant impurity) of deltamethrin (or 10% of the AI content); this amount did not change after washing. After 20 washes, the average deltamethrin content for one net of each was 5.26 g AI/kg (RSD, 2.0%) and 5.44 g AI/kg (RSD, 3.3%) after 30 washes. The overall deltamethrin retention rates were 76% after 20 washes and 78% after 30 washes (Pigeon, 2011c).

The unwashed CTN contained 21.8–25.8 mg AI/m² (0.53–0.63 g AI/kg; within-net RSD, 10–21%) in compliance with the deltamethrin target dose. The CTN washed to just before exhaustion contained 1.6 mg AI/m² (0.04 g AI/kg) deltamethrin,

[1] Corbel V et al. Multiple insecticide resistance mechanisms in Anopheles gambiae and Culex quinquefasciatus from Benin, West Africa. *Acta Tropica*, 2007, 101(3):207–216.
[2] Djogbenou L et al. Insecticide resistance in the *Anopheles gambiae* complex in Benin: a nationwide survey. *Medical and Veterinary Entomology*, 2010 (DOI: 10.1111/j.1365-2915.2010.00925.x; available at: http://onlinelibrary.wiley.com/doi/10.1111/j.1365-2915.2010.00925.x/abstract).

corresponding to a retention rate of 6%. The amount of deltamethrin on CTNs washed 20 times was 1.1 mg AI/m² (0.03 g AI/kg) (Pigeon, 2011c).

The experimental hut trial occurred between 28 November 2010 and 1 April 2011 during two collection periods, each corresponding to a full rotation of a Latin square design with a break during the peak of the dry season. During the 72 nights of collection, 87 *An. gambiae* sensu lato (average of 1.2 females per night) were collected. No deterrent effect was observed with either of the treatments; however, all treatments induced significantly higher exophily (15% for the untreated nets versus 38% with the CTN washed 20 times, and 53–62% for the other treatment arms).

The blood-feeding inhibition rate of *An. gambiae* s.l. was significantly lower for the LifeNet treatments (1–11%) and CTNs washed to just before exhaustion (15%) compared with control huts (38%) and CTNs washed 20 times (30%). The blood feeding inhibition was 96% in presence of a LifeNet washed 20 times, and only 62% for CTN washed to just before exhaustion. Even after 30 washes, LifeNet induced a blood feeding inhibition of 72%, which is similar to CTNs washed to just before exhaustion (62%).

The overall mortality was only 1% for the untreated nets. With LifeNet LNs washed 20 times, mortality was significantly higher (70%) compared with the CTN washed to just before exhaustion (44%). The mortality of a LifeNet washed 30 times was not significantly different (55%) compared with a CTN washed to just before exhaustion (44%).

Taking all mosquito species into consideration (including 10% *Anopheles* spp., 1% *Culex* spp. and 89% *Mansonia* spp.), the unwashed and washed (20 and 30 washes) LifeNet treatments reduced significantly the blood feeding and mortalities were higher compared with CTNs washed to just before exhaustion.

3.4   Conclusions and recommendations

LifeNet[®] is a long-lasting insecticidal net manufactured by Bayer CropScience. The net is made of poly-filament polypropylene fibres, including deltamethrin, at the target dose

of 8.5 g AI/kg, corresponding to 340 mg of deltamethrin per LN m^2.

The WHO assessment of the manufacturer's compliance with the assessment of exposure to and risks of washing and sleeping under a LifeNet® was in line with the WHO generic risk assessment model and, when used as instructed, do not pose undue hazard to the user.

The deltamethrin content in unwashed LifeNet samples tested in Phase I and II studies complied with the target dose of 8.5 g AI/kg (± 25%), except for two samples in the Phase II trial in India, which were just below the tolerance lower limit. The unwashed LifeNet samples tested in Phase II trials in India and the United Republic of Tanzania contained a relatively high amount (average 19%) of the insecticidally inactive deltamethrin R-alpha isomer compared with samples tested in Phase I (7%) and Phase II in Benin (10%). This non-relevant impurity can occur by epimerization of deltamethrin during the manufacturing process or when LNs are exposed to excessive heat or ultraviolet light during storage or use, and must be controlled in order to avoid under-dosage of the AI content. The between-net and within-net variation of the deltamethrin content were within the limits specified by the WHO guidelines[1] and showed good homogeneity of the treatment. The average deltamethrin retention index (98% per wash) in the Phase I trial complied with the specification proposed by the manufacturer (Table 4 and Figure 3).

Laboratory studies of fully susceptible mosquitoes revealed that LifeNet® meets WHOPES Phase I requirements for a LN (mortality >80% or KD >95% after 20 washes). Even after 35 washes, bio-efficacy complied with the WHO criteria. A regeneration time of one day is needed after each wash.

In Phase II trials, a LifeNet washed 20 times should have similar or better efficacy than a net conventionally treated with the WHO-recommended dose of the corresponding insecticide and washed unitl just before exhaustion. In two (India and the

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[1] *Manual on development and use of FAO and WHO specifications for pesticides. Second revision of the 1st edition.* FAO, Rome and WHO, Geneva, 2010 (also available at: http://www.who.int/whopes/quality/en/; accessed 12 July 2011).

United Republic of Tanzania) of the three studies, only 10% of the target dose was used to treat the CTNs. Moreover, in India, the number of washes (10) was to just beyond exhaustion (mortality, 78%). However, in the Tanzanian study, the "CTNs washed to just before exhaustion" arm induced 96% mortality after washing and 88% after the trial in cone bioassays.

In the United Republic of Tanzania, the trial assessed the performance of LifeNet on a population of *An. arabiensis* partially susceptible to deltamethrin characterized by high exophilic behaviour (87% in the controls). Rates of blood-feeding inhibition induced by LifeNets washed 20 and 30 times (41% and 28% respectively) was similar to CTNs washed until just before exhaustion (30%). Mortality induced by LifeNet washed 20 or 30 times (43% for both arms) was nearly twice that observed with the CTN washed until just before exhaustion (23%).

In India (Orissa), the impact of LifeNet was assessed on *An. fluviatilis,* susceptible to deltamethrin and showing to some extent a degree of exophily (39% exit rate in the controls). All LifeNet arms induced significant blood-feeding inhibition (74% with unwashed and around 65% with nets washed 20 and 30 times), which was greater than the CTN washed to just beyond exhaustion (35%). Induced mortality of an unwashed LifeNet and a LifeNet washed 20 times was above 90%. Induced mortality of a LifeNet washed 30 times (73%) was about twice than the one observed with CTNs washed to just beyond exhaustion (10 washes).

In Benin, the trial was conducted on a population of free-flying *An. gambiae* s.l. resistant to deltamethrin and showing relatively low exophilic behaviour (15% in the control). Induced blood-feeding inhibition and mortality of LifeNet LNs washed 20 times (96% and 69% respectively) was higher than that of CTNs washed to just before exhaustion (62% and 43%). The performance of LifeNets washed 30 times was similar to CTNs washed to just before exhaustion on the *An. gambiae* s.l. population and better when considering all mosquitoes entering into the huts.

Field studies demonstrated good efficacy of LifeNet[®] LNs washed 20 or 30 times on mortality and blood-feeding inhibition of prominent malaria vectors (Tables 5 and 6). This confirms

that the LifeNet® fulfils the WHOPES efficacy criteria of Phase II studies for LNs.

Considering the safety, efficacy and wash-resistance of LifeNet in laboratory studies and small-scale field studies, it is recommended:

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

- that WHOPES coordinates large-scale studies (WHOPES Phase III studies) of LifeNet to confirm its long-lasting efficacy, fabric integrity and community acceptability as a requirement for developing full recommendations on the use of the product.

Note: WHO recommendations on the use of pesticides in public health are valid ONLY if linked to WHO specifications for their quality control.[1]

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[1] *WHO specifications for pesticides used in public health*, Geneva, World Health Organization, 2011 (also available at: http://www.who.int/whopes/quality/newspecif/en/; accessed 12 July 2011).

Table 4. **Deltamethrin content (DMC in g AI/kg), within-net relative standard deviation (RSD in %) (Table 4a) and deltamethrin retention (DMR, in percentage of wash zero) of different treated nets (Table 4b)**

a. Before washing

Treatment	United Republic of Tanzania		Benin		India	
	DMC	RSD	DMC	RSD	DMC	RSD
Untreated net	NA	NA	<0.01	–	<0.01	–
LifeNet unwashed	6.78	2.3%	7.16	2.7%	6.62	7.0%
LifeNet 20 washes	6.58	3.3%	6.72	3.5%	6.47	5.2 %
LifeNet 30 washes	6.57	3.7%	7.37	3.7%	6.64	2.0%
CTN exhausted	0.09[a]	65.5%	0.63	20.9%	0.07[a]	107.5%
CTN 20 washes	0.10[a]	32.6 %	0.53	9.5%	0.05[a]	105.2%

CTN, conventionally treated net; NA, not available
[a] In India and the United Republic of Tanzania, the initial dose of deltamethrin on CTNs was 1/10th of the target dose.

b. After washing, before testing

Treatment	United Republic of Tanzania		Benin		India	
	DMC	DMR	DMC	DMR	DMC	DMR
Untreated net	NA	–	0.01	–	<0.01	–
LifeNet unwashed	6.88	–	6.59	–	6.14	–
LifeNet 20 washes	4.20	63%	5.26	76%	4.02	62%
LifeNet 30 washes	3.79	57%	5.44	78%	4.04	62%
CTN exhausted	0.02[a]	22%	0.04	6%	<0.01[b]	–
CTN 20 washes	<0.01[a]	–	0.03	6%	<0.01[b]	–

CTN, conventionally treated net; NA, not available
[a] In India and the United Republic of Tanzania, the initial dose of deltamethrin on CTNs was 1/10th of the target dose.
[b] In India, washed until just beyond exhaustion (10 washes).

c. After testing

Treatment	United Republic of Tanzania		Benin		India	
	DMC	RSD	DMC	RSD	DMC	RSD
Untreated net	NA	nana	< 0.01	–	< 0.01	–
LifeNet unwashed	6.60	2.7	6.46	5.8	6.69	2.5
LifeNet 20 washes	4.33	7.3	4.61	4.8	4.81	4.0
LifeNet 30 washes	3.65	6.7	4.33	2.5	4.04	13.6
CTN exhausted	0.02[a]	50.0	< 0.01	–	<0.01[b]	–
CTN 20 washes	<0.01[a]	–	< 0.01	–	<0.01[b]	–

CTN, conventionally treated net; NA, not available
[a] In India and the United Republic of Tanzania, the initial dose of deltamethrin on CTNs was 1/10th of the target dose.
[b] In India, washed until just beyond exhaustion (10 washes).
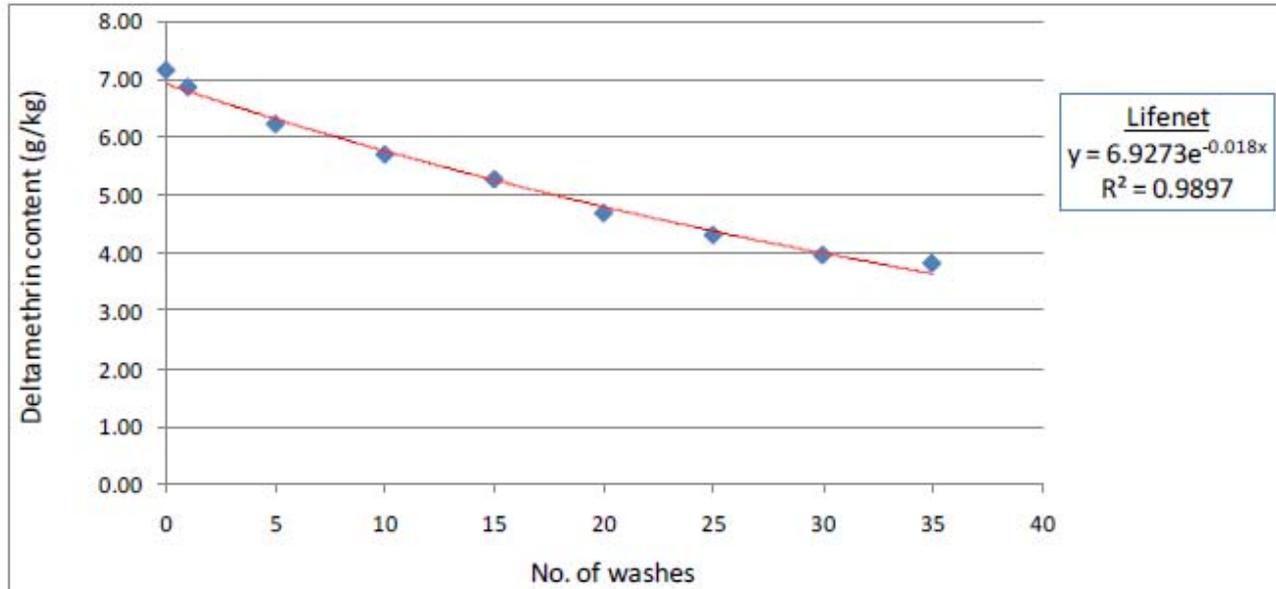
Fig. 3: **Deltamethrin content and retention (wash curve) of LifeNet (WHOPES Phase I study)**

Table 5. **Overview of the blood-feeding (%) and blood-feeding inhibition (% in bold) induced by LifeNet compared with conventionally treated nets (CTNs) washed to just before exhaustion (values in the same row sharing the same superscript letter do not differ significantly (P>0.05))**

Study sites (number of mosquitoes collected in the control hut and species)[1,2]	Pyrethroid Resistance status	Un-treated Net	LifeNet unwashed	LifeNet washed 20 times	LifeNet washed 30 times	CTNs[1,2] washed to just before exhaustion	CTNs[1] washed 20 times
Moshi – United Republic of Tanzania (92 *An. arabiensis*)	Pyrethroid resistance present	39[a]	20[b] **48**	23[b] **41**	28[b,c] **28**	27[b,c] **30**	31[c] **20**
Malanville – Benin (87 *An. gambiae s.l.*)	Kdr mutation Oxidase	38[a]	5[bc] **88**	1[c] **96**	11[b] **72**	15[b] **62**	30[a] **20**
Malanville – Benin (689 Culicidae)*	Unknown	33[a]	1[c] **96**	2[c] **94**	2[c] **93**	5[b] **85**	11[d] **67**
Orissa – India (274 *An. fluviatilis)*	Deltamethrin susceptible	80[a]	21[d] **74**	29[b] **64**	28[b] **66**	52[c] **35**	68[c] **15**

* 10% *Anopheles* spp., 1% *Culex* spp. and 89% *Mansonia* spp.
[1] In India and the United Republic of Tanzania, the initial dose of deltamethrin on CTNs was 1/10th of the target dose.
[2] In India, washed until just beyond exhaustion (10 washes).

Table 6. **Overview of the mortality (%) and corrected mortality (% in bold) induced by LifeNet compared with conventionally treated nets (CTNs) washed to just before exhaustion (values in the same row sharing the same superscript letter do not differ significantly (P>0.05))**

Study sites (number of mosquitoes collected in the control hut and species) [1,2]	Pyrethroid Resistance status	Un-treated Net	LifeNet unwashed	LifeNet washed 20 times	LifeNet washed 30 times	CTNs[1,2] washed to just before exhaustion	CTNs[1] washed 20 times
Anopheles sp.							
Moshi – United Republic of Tanzania	Pyrethroid resistance present	13[a]	61[b]	50[c]	50[c]	33[d]	31[d]
(92 *An. arabiensis*)			**56**	**43**	**43**	**23**	**21**
Malanville – Benin (87 *An. gambiae s.l.*)	*kdr* mutation Oxidase+	1[a]	75[c]	70[cd]	55[bd]	44[b]	26[e]
			75	**69**	**55**	**43**	**25**
Malanville – Benin (689 Culicidae)*	Unknown	11[a]	97[c]	94[d]	92[d]	83[b]	60[e]
			97	**93**	**91**	**81**	**54**
Orissa – India (274 *An. fluviatilis*)	Deltamethrin susceptible	2[a]	98[b]	91[b]	74[c]	40[c]	12[d]
			98	**91**	**73**	**38**	**10**

* 10% *Anopheles* spp., 1% *Culex* spp. and 89% *Mansonia* spp.
[1] In India and the United Republic of Tanzania, the initial dose of deltamethrin on CTNs was 1/10th of the target dose.
[2] In India, washed until just beyond exhaustion (10 washes).

4. REVIEW OF CANDID LNS FOR EXTENSION OF WHO SPECIFICATIONS

The regeneration, wash resistance and efficacy of three candidate long-lasting insecticidal mosquito nets (LNs) were determined, as part of the requirements for extension of WHO specifications. [1,2] These were:

Product	Manufacturer	Extension of WHO specification
MAGNet[TM] LN	V.K.A. Polymers, India	Alpha-cypermethrin long-lasting (incorporated into filaments) insecticidal net, WHO interim specification 454/LN/2 (October 2009)
Royal Sentry® LN	Disease Control Technologies, USA	Alpha-cypermethrin long-lasting (incorporated into filaments) insecticidal net, WHO interim specification 454/LN/2 (October 2009)
Yahe® LN	Fujian Yamei Industry, China	Deltamethrin long-lasting (coated onto filaments) insecticidal net, WHO interim specification 333/LN/1 (netting and net) (September 2010)

The three manufacturers have certified to WHO that the technical material used in manufacturing their LNs is from a source complying with WHO specifications. These are alpha-cypermethrin of Gharda for manufacturing MAGNet LN; alpha-

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[1] *Report of the 13th WHOPES working group meeting – review of Olyset LN, Dawaplus 2.0 LN and Tianjin Yorkool LN*, 28–30 July 2009. Geneva, World Health Organization, 2009 (WHO/HTM/NTD/WHOPES/2009.5; also available at http://whqlibdoc.who.int/publications/2009/9789241598712_eng.pdf; Accessed 12 July 2011).

[2] *Manual on development and use of FAO and WHO specifications for pesticides* [second revision of the first edition]. Rome, FAO and Geneva, WHO, 2010; also available at http://www.who.int/whopes/quality/en/; accessed 12 July 2011).

cypermethrin of Tagros for manufacturing Royal Sentry LN; and deltamethrin of Bayer CropScience for manufacturing Yahe LN.

Performance of the candidate LNs was compared with that of the reference LN for which the WHOPES specification was originally developed (Rossignol et al., 2010 a, b; Rossignol et al., 2011).

The evaluation included the following two steps: (i) to determine whether a period of time is required for regeneration of activity after washing, in comparison with the regeneration curves of the reference LN; and (ii) to determine wash resistance and efficacy of the candidate LNs after 0, 1, 3, 5, 10, 15, 20 and 25 washes, in comparison with the reference LN.

4.1   Materials and methods

Net material
The WHO Collaborating Centre in Montpellier, France (LIN/IRD laboratory) obtained four LN samples from each of the above-mentioned companies. Ten pieces of netting were cut from each of the four LNs. Eight of these pieces (2 per net) were used for the regeneration study, 28 (7 per net) for the wash resistance evaluation and 4 (1 per net) were kept as reserved samples for chemical analysis only. Two LNs of the reference product were used for comparison with each candidate LN.

All net samples used during the evaluation were stored at 4 °C after bio-efficacy testing and sent to the WHO Collaborating Centre for quality control of pesticides in Gembloux, Belgium (CRA-W), for chemical analysis.

Biological material
Non-blood-fed females, 2–5 days old, of *An. gambiae* s.s. Kisumu strain, a standard susceptible strain originating from Kenya, 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 six netting samples (four candidate LNs plus two reference LNs) washed and dried three times on the same day to deplete surface insecticide and then tested for regeneration at 1, 2, 3, 5 and 7 days after the third

wash. Insecticide bio-efficacy curves (24-hour mortality and KD at 60 minutes), as measured by 3-minute exposure in cone bioassays, were established for the six samples washed three times and compared with six unwashed samples. The number of days for efficacy to reach a plateau was considered to be the time required for full regeneration of the net.

Regeneration time studies were supplemented by median knock-down time (MKDT) tests, using the method of Skovmand et al. (2008). [1] Eleven 2–5-day-old, *Anopheles gambiae* mosquitoes were introduced into a circular chamber, 10 cm wide and 1 cm high, in contact with the netting sample. The time for knock-down of the sixth mosquito is defined as the MKDT.

Wash resistance
The resistance of the candidate and reference LN 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 bio-efficacy 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 washed 1, 3, 5, 10, 15, 20 and 25 times.

Determination of insecticide content in net samples
For the regeneration time study, chemical analyses were performed on each candidate LN (MAGNet, Royal Sentry and Yahe) and their corresponding reference LN (Duranet or PermaNet 2.0) washed 0 and 3 times (Pigeon, 2010 a, b; Pigeon, 2011d). For the wash resistance study, chemical analyses were performed on each candidate and reference LN samples washed 0, 1, 3, 5, 10, 15, 20 and 25 times. Per wash cycle, 1 piece (25 cm x 25 cm) from each of 4 nets for the candidate product and 1 piece (25 cm x 25 cm) from each of 2 nets of the reference LN were analysed. For Yahe and Permanet 2.0, determination of deltamethrin content (and deltamethrin R-alpha isomer) was done according to the CIPAC method 333/LN/(M)/3, which involved extraction by sonication

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[1] Skovmand O et al. Median knock-down time as a new method for evaluating insecticide-treated textiles for mosquito control. *Malaria Journal*, 2008, 7:114.

and shaking with isooctane/dioxane (80/20, v/v) in the presence of dipropyl phthalate as an internal standard and chromatographic determination by high performance liquid chromatography with UV Diode Array Detection (HPLC-DAD). For MAGNet, Royal Sentry and Duranet, determination of alpha-cypermethrin content was done according to the CIPAC method 454/LN/M/3.2, which involved extraction by heating under reflux for 30 minutes with xylene in the presence of dioctyl phthalate as an internal standard, and determination by gas chromatography with flame ionisation detection (GC-FID) (Pigeon, 2010 a, b; Pigeon, 2011d).

4.2   Results

4.2.1   MAGNet LN

Regeneration time
The regeneration time of MAGNet LN was studied and compared with that of Duranet (Table 7). The mortality and KD were both maximal (100%) for the unwashed MAGNet. Mortality and KD both remained at 100% on the first day after the three consecutive wash–dry cycles, and remained at that level on subsequent days of storage. The time required to reach plateau efficacy – the regeneration time – was therefore 1 day.

Mortality and KD with Duranet were 100% both on unwashed nets and on day 1 after washing, as well as on the 7 subsequent days of storage.

In order to better understand the dynamics of the insecticide on the fibres of MAGNet and Duranet after washing, circular chamber tests were carried out at 1, 2, 3, 5 and 7 days of storage (Table 8). The initial mean MKDT on unwashed MAGNet was 151 seconds. The MKDT showed no significant change after three washes or during the week of storage, indicating no change in efficacy during this period. The MKDT of Duranet was not significantly different from that of MAGNet either before or after washing or during the 7 days of storage. The MKDT study confirms that the efficacy of MAGNet and Duranet is fully restored within one day of the 3 washes and that regeneration time is 1 day.

Wash resistance and efficacy

The results of bioassays carried out on unwashed and washed LNs are presented in Table 9. The KD of MAGNet net after 20 washes was 100% and therefore met the WHO threshold. Over the course of 25 washes, KD was never less than 99%. The mortality of mosquitoes with the unwashed MAGNet net was 100%. From the first wash until 20 washes, the mortality with MAGNet was always higher than the WHO threshold of 80% mortality; it was close to 100% during the first 10 washes and 97% at 20 washes. Therefore, MAGNet retained its efficacy until 20 washes. After 25 washes, mortality was 89%.

For the reference LN, Duranet, the KD and mortality were also very high, showing a very similar trend to MAGNet up to 25 washes, and not significantly different from KD and mortality of MAGNet.

Chemical assays

Summary data are presented in Table 10 and Figure 4. The alpha-cypermethrin (cis II) content (6.08 g AI/kg and 6.07 g AI/kg) in the reserved and the unwashed MAGNet complied with the target dose ± 25% of 5.8 g AI/kg [4.35 g AI/kg – 7.25 g AI/kg]. The between-net variation, expressed as the relative standard deviation (RSD) of samples taken from the four nets, was 1.9% in the reserved MAGNet and 2.5% in the unwashed MAGNet, and showed good homogeneity of the AI content between nets. The AI between-net variation on MAGNet samples washed 1 to 25 times remained low (RSD, 1.2–3.0%). The insecticidally inactive alpha-cypermethrin cis I isomer (non-relevant impurity) content in the unwashed MAGNet sample was 0.30 g /kg, corresponding to 4.9% of the alpha-cypermethrin (cis II) content; this amount did not increase with the number of washes (4.9–5.2% for washes 1–25). The average alpha-cypermethrin (cis II) content was 5.50 g AI/kg after 10 washes and 5.34 g AI/kg after 20 washes. The overall alpha-cypermethrin (cis II) retention after 20 washes was 88.0%, corresponding to an average retention per wash of 99.4%.

The alpha-cypermethrin (cis II) content (4.64 g AI/kg and 4.80 g AI/kg) in the reserved and the unwashed Duranet complied with the target dose (± 25%) of 5.8 g/kg [4.35 g AI/kg – 7.25 g AI/kg]. The between-net variation, expressed as the relative standard deviation (RSD) of the content found on the two pieces, was 2.0% and 9.7% respectively, and showed good homogeneity of

the AI content between the nets. The AI between-net variation on Duranet samples washed 1–25 times remained quite low (RSD, 1.5–16.3%). The insecticidally inactive alpha-cypermethrin cis I isomer (non-relevant impurity) content in the unwashed Duranet sample was 0.27 g/kg, corresponding to 5.6% of the alpha-cypermethrin (cis II) content; this amount did not increase with the number of washes (5.9–6.7% for washes 1–25). The average alpha-cypermethrin (cis II) content was 4.27 g AI/kg after 10 washes and 3.90 g AI/kg after 20 washes. The overall alpha-cypermethrin (cis II) retention after 20 washes was 81.3%, corresponding to an average retention per wash of 99.0%.

Based on the above data, it can be concluded that MAGNet had an alphacypermethrin content within the acceptable limits of the target dose (±25%). Its wash resistance (Figure 4) and alpha-cypermethrin retention index per wash were similar to those of Duranet.

4.2.2   Royal Sentry LN

Regeneration time
The regeneration time of Royal Sentry LN was studied and compared with that of Duranet (Table 11). The mortality and KD were both maximal (100%) for the unwashed Royal Sentry. Mortality and KD both remained at 100% on the first day after the three consecutive wash–dry cycles, and remained at that level on subsequent days of storage. The time required to reach plateau efficacy – the regeneration time – was therefore 1 day.

Mortality and KD with Duranet were 100% both on unwashed nets and on day 1 after washing, as well as after the 7 subsequent days of storage.

In order to better understand the dynamics of the insecticide on the fibres of Royal Sentry and Duranet after washing, circular chamber tests were carried out at 1, 2, 3, 5 and 7 days of storage (Table 12). The initial mean MKDT on unwashed Royal Sentry was 195 seconds. After three washes the MKDT significantly decreased to 121 seconds on day 1, indicating an increase in efficacy after washing (this increase was not obvious in the cone test because KD and mortality were already 100% both before and after washing). During the week of

storage, there was no further change in MKDT. The MKDT of Duranet was not significantly different from that of Royal Sentry either before or after washing or during the 7 days of storage. The MKDT study confirmed that the efficacy of Royal Sentry and Duranet is fully restored within one day of the 3 washes and that regeneration time is 1 day.

Wash resistance and efficacy

The results of bioassays carried out on unwashed and washed LNs are presented in Table 13. The KD of Royal Sentry after 20 washes was 98% and therefore higher than the WHO threshold of 95%. At 25 washes, the KD was 97%. The mortality of mosquitoes with the unwashed Royal Sentry net was 100%. Mortality remained at 100% until 5 washes but declined to 79% mortality at 20 washes. Mortality rates with Royal Sentry nets after 15 and 20 washes were significantly lower (78% and 79%, respectively) than the mortality rates with Duranet (90% and 95% respectively). After 25 washes, mortality of both nets was identical (87%) and higher than the WHO threshold of 80%.

For the reference LN, Duranet, the mortality rates between 10 and 25 washes ranged between 96% and 87%.

Chemical assays

Summary data are presented in Table 14 and Figure 5. The alpha-cypermethrin (cis II) content (5.93 g AI/kg and 5.59 g AI/kg) in the reserved and unwashed Royal Sentry complied with the target dose of 5.8 g AI/kg ± 25% [4.35 g AI/kg – 7.25 g AI/kg]. The between-net variation, expressed as the relative standard deviation (RSD) of pieces taken from the four nets, was 4.2% in the reserved Royal Sentry and 2.5% in the unwashed Royal Sentry, showing good homogeneity of the AI content between nets. The AI between-net variation on Royal Sentry samples washed 1–25 times remained low (RSD, 2.6–6.7%). The (insecticidally inactive) alpha-cypermethrin cis I isomer (non-relevant impurity) content in the unwashed Royal Sentry sample was 0.16 g AI/kg, corresponding to 2.8% of the alpha-cypermethrin (cis II) content; this amount did not increase with the number of washes (2.4–2.7% for washes 1–25). The average alpha-cypermethrin (cis II) content was 5.21 g AI/kg after 10 washes and 5.19 g AI/kg after 20 washes. The overall alpha-cypermethrin (cis II) retention after 20 washes is 92.8%, corresponding to an average retention per wash of 99.6%.

The alpha-cypermethrin (cis II) content (4.64 g AI/kg and 4.80 g/kg) in the reserved and unwashed Duranet complied with the target dose of 5.8 g AI/kg (± 25%) (see details in 3.2.1 above). The overall alpha-cypermethrin (cis II) retention after 20 washes was 81.3%, corresponding to an average retention per wash of 99.0%.

Based on the above data, it can be concluded that Royal Sentry had an alpha-cypermethrin content within the acceptable limits of the target dose (±25%). Its wash resistance (Figure 5) and alpha-cypermethrin retention index per wash were similar to those of Duranet.

4.2.3   Yahe LN

Regeneration time
The regeneration time of Yahe LN was studied and compared with that of PermaNet 2.0 (Table 15). For Yahe LN, the KD was only 67% for the unwashed net, but increased to 97% on the day after 3 consecutive washes and remained at 99% after 7 days of storage. The plateau and highest percentage KD was reached after just 1 day of storage.

Mortality was very low for the unwashed Yahe net (14%). After the 3 washes and 1 day of storage, the mortality rate increased to 79%, and stayed at around 73–80% until 5 days of storage. After 7 days of storage, the net's efficacy decreased to 51%. The plateau and highest mortality rate was obtained from day 1 to day 5 of storage.

For Permanet 2.0, the reference LN, the KD and mortality rates were 100% for unwashed and washed samples on all 7 days of storage.

On the basis of this data, the regeneration time for Yahe net was considered to be 1 day, similar to that of Permanet 2.0. However, the mortality was significantly different between Yahe and Permanet 2.0 when unwashed, on the day after 3 times washing, and on each day of storage.

To examine the dynamics of deltamethrin on Yahe LN and PermaNet 2.0 after washing, circular chamber tests were

carried out at 1, 2, 3, 5 and 7 days of storage (Table 16). Contrary to the cone test results, the MKDT tests did not show differences in performance between the unwashed Yahe LN and the unwashed PermaNet 2.0. The average MKDT on unwashed Yahe was 330 seconds, and after 3 washes the MKDT did not change significantly over 7 days of storage. For PermaNet 2.0, the average MKDT on the unwashed samples was 350 seconds and after 3 washes there were no consistent differences with Yahe LN over the 7 days of storage.

Wash resistance and efficacy
The results of bioassays carried out on unwashed and washed LNs are presented in Table 17. The KD of Yahe net pre-washing was 67%, but between 1 wash and 20 washes was never less than 98% and therefore higher than the WHO threshold of 95%. At 25 washes, the KD was recorded as 100%. The mortality of mosquitoes with the unwashed Yahe net 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%. No tunnel testing of Yahe LN was required as the product met the criteria in cone tests of 95% KD and/or 80% mortality after 20 washes.

For PermaNet 2.0, the KD was always 100%, and the mortality rate gradually decreased from 100% at 5 washes to 89% at 25 washes, but was always above the WHO threshold of 80%.

Chemical assays
Summary data are presented in Table 18 and Figure 6. The deltamethrin content (2.13 and 1.99 g AI/kg) in the reserved and unwashed Yahe LN complied with the target dose (± 25%) of 1.8 g/kg [1.35 g AI/kg – 2.25 g AI/kg]. However, the between-net variation, expressed as the relative standard deviation (RSD) of pieces taken from the four nets, was 30% in the reserved Yahe LN and 18.5% in the unwashed Yahe LN, showing a high heterogeneity of AI content between nets. The AI between-net variation on 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 lower than 1% of the deltamethrin content; this amount did not increase with the number of washes (<1% for washes 1 to 25). The average deltamethrin content was 1.45 g AI/kg after 10 washes and 1.07

g AI/kg after 20 washes. The overall deltamethrin retention after 20 washes was 53.5%, corresponding to an average retention per wash of 96.9%.

The deltamethrin content (2.26 and 2.01 g AI/kg) in the reserved and the unwashed PermaNet 2.0 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 2 pieces, was 6.8% and 12.9% respectively, showing an acceptable homogeneity of the distribution of the active substance between the nets. The AI between-net variation on PermaNet 2.0 samples washed 1 to 25 times was sometimes high (RSD, 3.0–51.0%). The deltamethrin R-alpha isomer content in the unwashed PermaNet 2.0 sample was 3.3% of the deltamethrin content; this amount did not increase with the number of washes (1.5% for wash 1 and <1% for washes 1–25). The average deltamethrin content was 1.10 g AI/kg after 10 washes and 0.91 g AI/kg after 20 washes. The overall deltamethrin retention after 20 washes was 45.3%, corresponding to an average retention per wash of 96.1%.

Based on the above data, it can be concluded that Yahe LN had a deltamethrin content within the acceptable limits of the target dose (±25%). However, the deltamethrin content on different Yahe LNs was highly variable, and the wash resistance of the Yahe LN (Figure 6) differed from that of the PermaNet 2.0.

4.3 Conclusions and recommendations

MAGNet LN and Royal Sentry LN complied with the WHO interim specifications (454/LN/2; October 2009) with reference to total content of alpha-cypermethrin and retention index.

The MAGNet and Royal Sentry nets showed high efficacy against susceptible *Anopheles gambiae* in Phase I laboratory bioassays. KD of mosquitoes remained above 95% and/or mortality above 80% for 20 washes; therefore, both MAGNet and Royal Sentry met the WHO criteria for the Phase I study. Both nets regenerated within 1 day and showed similar wash resistance to the WHO approved reference LN (Duranet).

The Yahe LN complied with the WHO interim specifications (333/LN/1; September 2010) 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, the biological efficacy of the Yahe LN before the first wash did not meet WHO criteria. After the first wash, the Yahe LN net showed KD consistently above the 95% threshold. However, the mortality rates of the Yahe LN never exceeded 86% at any wash point and were always lower than mortality rates of the reference LN. This indicates that the wash resistance of the Yahe LN was different from that of the reference LN (PermaNet 2.0).

Considering the above, the meeting concluded that:

♣ The bio-efficacy and wash resistance of MAGNet and Royal Sentry LNs are comparable to the reference product for which WHO specifications for alpha-cypermethrin long-lasting (incorporated into filaments) insecticidal net have been developed.

♣ The bio-efficacy and wash resistance of Yahe LN are different from those of the reference product for which WHO specifications for deltamethrin long-lasting (coated onto filaments) insecticidal net have been developed.

The meeting recommended:

♣ Extension of WHO specifications for alpha-cypermethrin long-lasting (incorporated into filaments) insecticidal net to MAGNet and Royal Sentry LNs, subject to satisfactory assessment of the physical and chemical properties of the two products by the FAO/WHO Joint Meeting on Pesticide Specifications (JMPS).

♣ 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 an independent product.

Table 7. **Regeneration time as determined by average percentage mortality (%M) and knockdown (%KD) of *Anopheles gambiae* females in bioassays of unwashed and 3-times washed nets stored at 30 °C for 1–7 days, for the MAGNet long-lasting insecticidal mosquito net (LN) in comparison with the reference LN**

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
MAGNet	100	100	100	100	100	100	100	100	100	100	100	100
Duranet	100	100	100	100	100	100	100	100	100	100	100	100

Table 8. **Median knock-down times (average and confidence interval in seconds) of *Anopheles gambiae* females exposed to unwashed and 3-times washed nets stored at 30 °C for 1–7 days, for the MAGNet long-lasting insecticidal mosquito net (LN) in comparison with the reference LN**

LN	Unwashed	3 washes + 1 day	3 washes + 2 days	3 washes + 3 days	3 washes + 5 days	3 washes + 7 days
MAGNet	151 (120–182)	128 (108–148)	163 (146–180)	149 (126–172)	151 (129–173)	139 (118–160)
Duranet	182 (140–222)	123 (106–140)	126 (110–142)	157 (131–183)	142 (125–159)	134 (107–161)

Table 9. **Wash resistance as determined by average percentage mortality (%M) and knockdown (%KD) of *Anopheles gambiae* females in bioassays of unwashed and 1–25-times washed nets, for the MAGNet long-lasting insecticidal mosquito net (LN) in comparison with the reference LN**

LN	Unwashed		1 wash		3 washes		5 washes	
	%M	%KD	%M	%KD	%M	%KD	%M	%KD
MAGNet	100	100	100	100	100	99 ± 1	100	100
Duranet	100	100	100	100	100	100	100	100

LN	10 washes		15 washes		20 washes		25 washes	
	% M	% KD	% M	% KD	% M	% KD	% M	% KD
MAGNet	98 ± 3	100	91 ± 3	100	97 ± 1	100	89 ± 3	99 ± 2
Duranet	96 ± 0.4	100	90 ± 3	97 ± 3	95 ± 3	100	87 ± 8	96 ± 3

Table 10. **Alpha-cypermethrin (AC) content and retention (wash curve) of MAGNet long-lasting insecticidal mosquito net (LN) in a laboratory wash resistance study (WHOPES Phase I) (target dose and tolerance limit for AC in MAGNet LN = 5.8 g/kg ± 25% [4.35 g/kg–7.25 g/kg])**

Wash	AC content (g/kg)	Between-net RSD (%)	AC retention (% of wash 0)	Average AC retention (% at each wash)
0	6.07	2.5	–	–
1	6.01	1.5	99.0	99.0
3	5.93	1.2	97.7	99.2
5	5.77	3.0	95.1	99.0
10	5.50	1.5	90.6	99.0
15	5.48	1.8	90.3	99.3
20	5.34	2.5	88.0	99.4
25	5.23	2.6	86.2	99.4

RSD, relative standard deviation
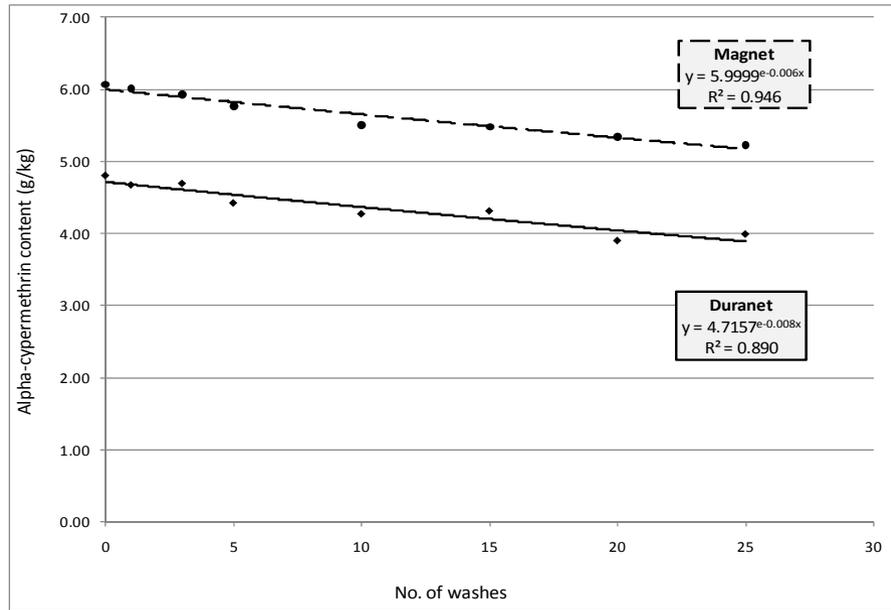
Figure 4. **Alpha-cypermethrin content and retention (wash curve) for MAGNet long-lasting insecticidal mosquito net (LN) and the reference LN (WHOPES Phase I study)**

Table 11. **Regeneration time as determined by average percentage mortality (%M) and knockdown (%KD) of** *Anopheles gambiae* **females in bioassays of unwashed and 3-times washed nets stored at 30 °C for 1–7 days, for the Royal Sentry long-lasting insecticidal mosquito net (LN) in comparison with the reference LN**

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
Royal Sentry	100	100	100	100	100	100	100	100	100	100	100	100
Duranet	100	100	100	100	100	100	100	100	100	100	100	100

Table 12. **Median knock-down times (average and confidence interval in seconds) of *Anopheles gambiae* females exposed to unwashed and 3-times washed nets stored at 30 °C for 1–7 days, for the Royal Sentry long-lasting insecticidal mosquito net (LN) in comparison with the reference LN**

LN	Unwashed	3 washes + 1 day	3 washes + 2 days	3 washes + 3 days	3 washes + 5 days	3 washes + 7 days
Royal Sentry	195 (152-238)	121 (100–142)	145 (128–162)	145 (121–166)	116 (147–135)	147 (133–161)
Duranet	182 (140-222)	123 (106–140)	126 (110–142)	157 (131–183)	142 (125–159)	134 (107–161)

Table 13. **Wash resistance as determined by average percentage mortality (%M) and knock-down (%KD) of *Anopheles gambiae* females in bioassays of unwashed and 1–25-times washed nets, for the Royal Sentry long-lasting insecticidal mosquito net (LN) in comparison with the reference LN**

LN	Unwashed		1 wash		3 washes		5 washes	
	%M	%KD	%M	%KD	%M	%KD	%M	%KD
Royal Sentry	100	100	100	100	100	100	100	100
Duranet	100	100	100	100	100	100	100	100

LN	10 washes		15 washes		20 washes		25 washes	
	%M	%KD	%M	%KD	%M	%KD	%M	%KD
Royal Sentry	94 ± 4	99 ± 1	78 ± 3	100	79 ± 5	98 ± 2	87 ± 6	97 ± 1
Duranet	96 ± 0.4	100	90 ± 3	97 ± 3	95 ± 3	100	87 ± 8	96 ± 3

Table 14. **Alpha-cypermethrin (AC) content and retention (wash curve) of Royal Sentry long-lasting insecticidal mosquito net (LN) in a laboratory wash-resistance study (WHOPES Phase I) (target dose and tolerance limit for AC in Royal Sentry LN = 5.8 g/kg ± 25% [4.35 g/kg–7.25 g/kg])**

Wash	AC content (g/kg)	Between-net RSD (%)	AC retention (% of wash 0)	Average AC retention (% at each wash)
0	5.59	2.5	–	–
1	5.59	6.7	100.0	100.0
3	5.52	5.3	98.7	99.6
5	5.36	3.0	95.9	99.2
10	5.21	5.3	93.2	99.3
15	4.96	3.0	88.7	99.2
20	5.19	2.6	92.8	99.6
25	5.08	4.9	90.9	99.6

RSD, relative standard deviation
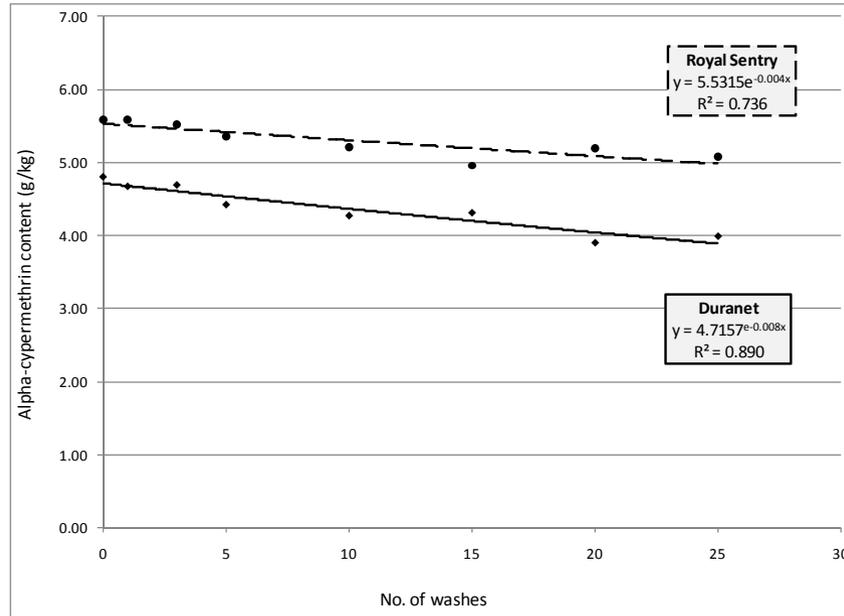
Figure 5. **Alpha-cypermethrin content and retention (wash curve) for Royal Sentry and the reference LN (WHOPES Phase I study)**

Table 15. **Regeneration time as determined by average percentage mortality (%M) and knock-down (%KD) of _Anopheles gambiae_ females in bioassays of unwashed and 3-times washed nets stored at 30 °C for 1–7 days, for the Yahe long-lasting insecticidal mosquito net (LN) in comparison with the reference LN**

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
Yahe LN	14	67	79	97	73	99	80	99	79	99	51	99
	± 5	± 14	± 3	± 1	± 3	± 2	± 7	± 1	± 4	± 1	± 15	± 1
Permanet 2.0	100	100	100	100	100	100	100	100	100	100	100	100

Table 16. **Median knock-down times (average and confidence interval, in seconds) of *Anopheles gambiae* females exposed to unwashed and 3-times washed nets stored at 30 °C for 1–7 days, for the Yahe long-lasting insecticidal mosquito net (LN) in comparison with the reference LN**

LN	Unwashed	3 washes + 1 day	3 washes + 2 days	3 washes + 3 days	3 washes + 5 days	3 washes + 7 days
Yahe LN	330 (286–374)	311 (294–328)	308 (285–331)	274 (236–313)	318 (277–359)	292 (269–315)
Permanet 2.0	350 (304–397)	401 (342–460)	255 (230–280)	262 (229–294)	332 (319–345)	311 (274–347)

Table 17. **Wash resistance as determined by average percentage mortality (%M) and knock-down (%KD) of *Anopheles gambiae* females in bioassays of unwashed and 1–25-times washed nets, for the Yahe long-lasting insecticidal mosquito net (LN) in comparison with the reference LN**

LN	Unwashed		1 wash		3 washes		5 washes	
	%M	%KD	%M	%KD	%M	%KD	%M	%KD
Yahe LN	14 ± 5	67 ± 14	80 ± 13	98 ± 1	75 ± 8	100 ± 1	86 ± 13	100
Permanet 2.0	100	100	100	100	100	100	100	100

LN	10 washes		15 washes		20 washes		25 washes	
	%M	%KD	%M	%KD	%M	%KD	%M	%KD
Yahe LN	86 ± 6	99 ± 2	70 ± 14	98 ± 1	86 ± 6	98 ± 1	62 ± 14	100
Permanet 2.0	99 ± 1	100	93 ± 1	100	95 ± 5	100	89 ± 2	100

Table 18. **Deltamethrin (DM) content and retention (wash curve) of Yahe long-lasting insecticidal mosquito net (LN) in a laboratory wash resistance study (WHOPES Phase I) (target dose and tolerance limit for DM in Yahe LN = 1.8 g/kg ± 25 % [1.35–2.25 g/kg])**

Wash	DM content (g/kg)	Between-net RSD (%)	DM retention (% of wash 0)	Average DM retention (% at each wash)
0	1.99	18.5	–	–
1	1.88	16.0	94.4	94.4
3	1.85	17.6	92.6	97.5
5	1.68	21.3	84.2	96.6
10	1.45	11.1	72.5	96.8
15	1.28	13.3	64.1	97.1
20	1.07	27.9	53.5	96.9
25	1.03	32.3	51.9	97.4

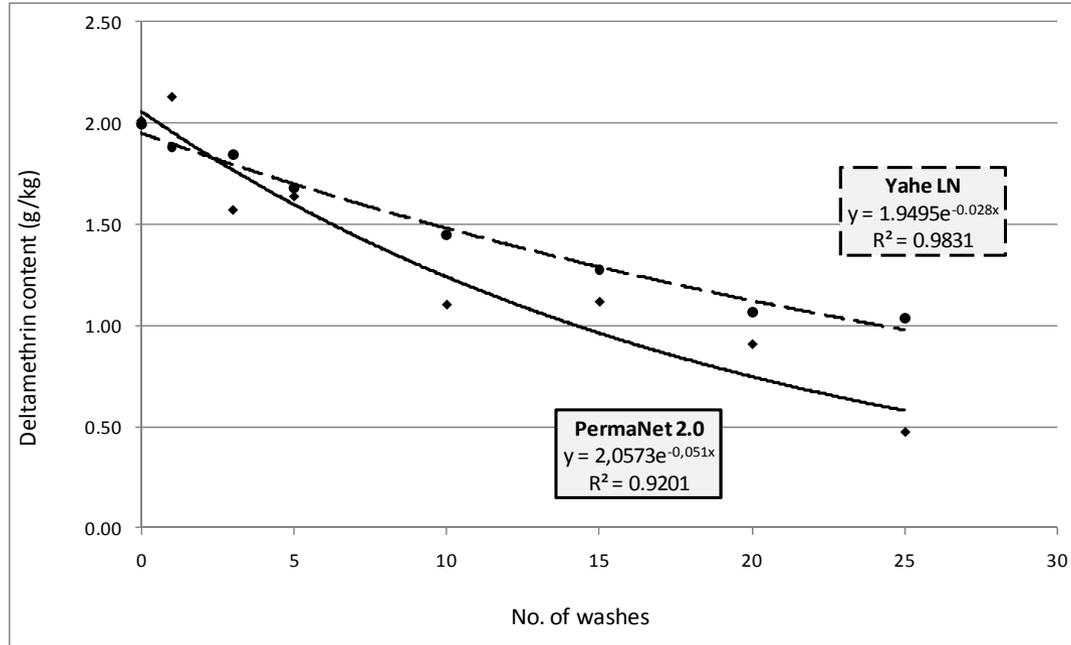RSD, relative standard deviation

Figure 6. **Deltamethrin content and retention (wash curve) for Yahe long-lasting insecticidal mosquito net (LN) and the reference LN (WHOPES Phase I study)**

5.   GENERAL RECOMMENDATIONS

The fourteenth meeting of the WHOPES Working Group discussed the following issues in considerable detail and made the following recommendations. Industry, academia and other stakeholders are invited to provide feedback and suggestions, which will be considered by the next WHOPES Working Group Meeting.

I.   **Definition of knock-down and mortality for adult mosquitoes**

A mosquito is considered knocked down if it is unable to stand or fly in a coordinated way. The holding container may be tapped a few times before a final determination is made. The assessment of knock-down is made within 60 minutes post-exposure.

Usually, mortality is measured at 24 hours post-exposure. A mosquito is classified as dead if it is immobile or unable to stand or fly in a coordinated way.

II.   **Amendments to the existing WHOPES guidelines for efficacy testing of pyrethroid-treated LNs**

The meeting proposed the following amendments to the existing WHOPES guidelines for efficacy testing of LNs based on experience gained in the evaluation of such products.

- Following the publication by Skovmand et al. (2008),[1] WHOPES has studied median knock-down time (MKDT) as a supplementary test to determine regeneration time of washed LNs, including both coating and incorporation technologies (Reports of the 13th and 14th

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[1] Skovmand O et al. Median knock-down time as a new method for evaluating insecticide-treated textiles for mosquito control. *Malaria Journal*, 2008, 7:114.

WHOPES Working Group Meetings [1] and unpublished data). Based on the evidence to date, no additional benefit was found in the determination of the MKDT over %KD or %mortality from the cone bioassay.

- Given the challenges in proper treatment of mosquito nets in the field; the determination of exhaustion point; the experiences gained and information available on WHOPES-recommended LNs; and the desire to better standardize experimental hut studies, it was recommended to use positive controls as a replacement for conventionally treated nets. The positive controls should be WHOPES-recommended LNs, unwashed and washed 20 times. To meet phase II requirements, a candidate LN washed 20 times must perform equal to or better than the positive control washed 20 times in terms of mortality and/or blood-feeding inhibition. It is recommended to standardize the study arms to include as a minimum the following:

    1. Untreated net of the same material as the candidate LN
    2. Unwashed candidate LN
    3. Candidate LN washed 20 times
    4. An unwashed reference positive control (a WHOPES-recommended LN similar to the candidate in terms of fabric, active ingredient and/or treatment technology)
    5. A reference positive control washed 20 times.

The reference LN should be the one used to develop WHO recommendations and specifications. For logistic and practical reasons during testing, the maximum acceptable regeneration time of the reference LN should be 3 days. Additional arms with candidate LNs

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[1] Available at http://www.who.int/whopes/recommendations/wgm/en/; accessed 12 July 2011.

washed according to the manufacturer's claim may also be included.

- It is recommended to conduct phase II studies in areas of pyrethroid susceptibility. However, it is recognized that pyrethroid resistance is expanding rapidly and may be unavoidable in the future. Studies conducted in areas with pyrethroid-resistant mosquitoes can provide equally valuable information, as the comparison is with positive controls. It is essential to present biological assays of insecticide resistance of the local vector population. Where possible, biochemical and molecular characterization of resistance mechanisms within the vector population should also be reported.

- As part of phase II studies, it was recommended that baseline information on hut attractiveness, recapture rates of known numbers of live and dead mosquitoes released in the huts, and contact bioassays on the walls be collected and reported. All mosquitoes collected during the study should be preserved over a desiccant or other medium (e.g. silica gel, ethanol) and labelled according to location of collection in the hut, intervention in place and status at time of collection (dead/alive, blood-fed/unfed) for quality control and/or future studies of genetic markers of insecticide resistance.

- For phase III studies, the design and procedures detailed in WHO guidelines for monitoring durability of LNs under operational conditions[1] should be adopted.

- Based on observations from field trials, shrinkage/compactness of some LNs, particularly polyethylene monofilament products, has been

[1] *Guidelines for monitoring the durability of long-lasting insecticidal mosquito nets under operational conditions.* Geneva, World Health Organization, 2011 (also available at: http://www.who.int/whopes/resources/en/, accessed 12 July 2011).

reported but should be further documented. Measurement of changes in LN dimensions (length and width along the seams and height at the corners) should be included in phase III studies.

- A risk assessment[1] is performed before phase II studies of LNs. Nevertheless, any adverse effects reported by sleepers should be documented during the course of the study to provide medical care to the sleepers if necessary and information provided to WHOPES. It should be noted that phase II studies as well as the phase III studies are not designed to evaluate the safety of the LN products in the field, and separate studies may be required.

- Modifications in the protocol developed for phase I studies are required. In phase I studies performed for new LNs or for extending LN specifications, from each of the 4 nets tested for regeneration time and wash resistance, 5 pieces of 25 cm x 25 cm should be cut according to the WHO specification guideline for LNs, reserved and tested only for chemical analysis to allow accurate estimation of within-net and between-net variations of the AI content. In Phase I studies performed for extension of LN specifications, the same number of replicates (4) should be tested for regeneration time, wash resistance and chemical analysis both for reference and candidate LNs.

- In phase III studies, net samples of the candidate and reference LNs taken before the trial and after 6 months and 1, 2 and 3 years should be ideally analysed for determination of AI content to facilitate the interpretation of bioassays results. Another alternative is to keep the samples taken

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[1] *A generic risk assessment model for insecticide treatment and subsequent use of mosquito nets.* Geneva, World Health Organization, 2004 (WHO/CDS/WHOPES/GCDPP/2004.6; also available at: http://whqlibdoc.who.int/hq/2004/WHO_PCS_04.1.pdf).

at intermediate points under appropriate conditions for further chemical analysis if needed for interpretation of bioassays results.

III. Novel public health pesticides

The massive scale at which malaria control is being applied and the consequent resistance problems arising mean that the demand for new public health pesticides (PHPs) will increase. Novel PHPs may include new active ingredients or mixed formulation insecticides for LNs and indoor residual spraying (IRS) as well as new application technologies. These may include approaches that may fit within existing WHOPES guidelines for evaluation. However, some of these new approaches will require proof of principle and the development of new evaluation guidelines and criteria.

Some of these novel PHPs may have mechanisms of action and performance criteria that are well understood and familiar and, in this case, they may be assessed using established WHOPES methods and criteria (e.g. IRS formulations with longer residual activity; LNs with new fabrics). In other cases, the mechanism of action may be entirely different and the conditions for effectiveness not yet known (e.g. spatial repellents for transmission control; LNs with non-repellent/irritant insecticides). In such cases, proof of principle, including epidemiological evidence, may be required. In yet other cases, there may be new PHPs within established categories that have new intended functions or purposes (e.g. LNs or IRS formulations with mixtures of insecticides to protect against resistant populations). In this case, additional test procedures and criteria will need to be established within the WHOPES scheme.

Most of the new PHPs have been brought to the market to control insecticide-resistant vector populations. WHOPES can assess the entomological effectiveness of different PHPs for protection against geographically defined populations of insecticide-resistant mosquitoes and/or specific resistance mechanisms, although some modifications of existing guidelines may be required.

It should be noted that insecticide resistance management strategies are designed to prevent or delay the spread of insecticide resistance and depend on the biology, ecology and behaviour of the insect species, and on the resistance mechanisms present in field populations. This may be achieved through the use of a combination of tools and approaches. No single product can be labelled as a resistance management tool. The development and implementation of an insecticide resistance management strategy is the responsibility of national programmes.

III.I WHOPES efficacy testing of LNs with insecticide other than pyrethroids

LNs are widely used for the prevention of vector-borne diseases, particularly malaria. Currently, only pyrethroid insecticides are recommended for use on LNs. However, pyrethroid resistance is spreading in the major malaria vectors and threatens to undermine the effectiveness of these tools. Therefore, new products incorporating alternative insecticides with acceptable safety are urgently needed for use on LNs.

LNs are the only PHPs where an interim recommendation is provided by WHO. The working group recommended that interim recommendation be considered for future LN products with alternative insecticides as well. Understanding the precise mechanism of action of the alternative compound on mosquitoes is essential in designing the criteria and requirements for testing and evaluation of alternative products in phase I, phase II and phase III studies. Such understanding is also essential for designing approaches to implementation.

If the primary effect of the alternative insecticide is through contact toxicity similar to pyrethroids (rapid knock-down and mortality), the existing general framework for evaluating LNs will be applicable, although some specific modifications may be required in each phase of testing. Products acting through mortality alone, through repellency alone or through an alternative mechanism on mosquitoes, will require, as proof of principle, epidemiological studies to demonstrate efficacy in reducing malaria transmission and/or disease.

The following modifications are proposed to phase I, phase II and phase III studies:

♣ Phase I testing is designed to assess efficacy, wash resistance and dynamics of the insecticide on the netting. Current guidelines recommend testing against susceptible strains of mosquitoes. As new insecticides are incorporated into LNs, cross-resistance studies should be conducted using standard methods as well as bioassays with the insecticide treated LN.

♣ In phase II, the efficacy of LNs is determined against wild, free-flying mosquitoes susceptible both to pyrethroids and to the insecticide on the candidate LN. Existing guidelines for phase II studies should be followed, but it is recommended that the study arms be standardized to include the following:

  ♣ an untreated net of the same material as the candidate LN ;
  ♣ an unwashed candidate LN;
  ♣ a candidate LN washed 20 times;
  ♣ an unwashed positive control (a WHO-recommended LN);
  ♣ a positive control washed 20 times.

The positive control should preferably be made of the same fabric material and use the same application technology (incorporation or coating) where possible.

A net will be considered to have met the requirements for interim recommendation if the mortality and blood-feeding inhibition of the candidate LN washed 20 times is equal to or better than the positive control washed 20 times. If the candidate LN meets these criteria in a pyrethroid-susceptible population, further tests should be conducted in areas with pyrethroid resistance. It is clear that the vector population should also be susceptible to the compound used in making the candidate LN.

If the candidate LN does not meet the criteria in a pyrethroid-susceptible population, it would still be useful to conduct testing in an area with pyrethroid resistance.

However, in this case, further proof of principle may be required, particularly if the candidate LN has different mechanisms of action on mosquitoes than pyrethroid-treated LNs.

♣ Phase III studies should follow existing WHOPES guidelines, with modifications to include a positive control as recommended above. In basic design and procedures, phase III studies should follow the general guidelines provided for monitoring the durability of LNs under operational conditions.

Novel insecticides may require modification to the laboratory evaluations of these products. For example, some slow-acting insecticides may require observations on mortality at intervals beyond 24 hours. As noted above, candidate LNs treated with insecticides with effects on mosquitoes that differ from the effects of pyrethroid insecticides may require proof of principle.

As new, non-pyrethroid insecticides are brought to the PHP market, it is important to test them against a range of mosquito strains with different resistance mechanisms. It is therefore recommended that new mosquito strains with novel resistance mechanisms be colonized whenever possible.

III.II Efficacy testing of LNs with a mixture of insecticides

It is anticipated that novel LN products will have mixtures of unrelated insecticides and, at a meeting of the WHO Global Malaria Programme, the development of mixtures of insecticides for use on ITNs or in IRS was considered as a research priority. Mixtures refer to products in which two insecticides of different classes are co-formulated in the same product such that an insect would be exposed to both insecticides at the same time. Mosquitoes that are not killed by one insecticide will likely be killed by the second. There are several challenges to the development of mixtures, particularly in formulating products such that the decay rates provide good efficacy for both insecticides and in ensuring the safety of the formulated product to humans. However, research in agriculture and modelling studies indicates that mixtures are one of the most effective approaches to the management of insecticide resistance.

Unless one or both of the elements in a mixture require additional testing due to their mode of action, the basic requirements for phase I studies should be followed. In all cases, studies to determine efficacy, wash resistance and regeneration of the candidate LN should be done on the product as a mixture as well as on the individual components of the product. Phase I testing should be done against both a susceptible strain as well as one or more pyrethroid-resistant strains. The resistant strain should be well characterized according to phenotypic susceptibility in WHO resistance assays, kdr genotype and metabolic enzymes. Determination of regeneration time and selection of washing interval should be based on that of the slowest regenerating compound in the mixture. Therefore, the following treatment arms are recommended for LNs in which both compounds in the mixture LN are active against mosquitoes:

      a. Candidate mixture LN
      b. Candidate LN with compound A only
      c. Candidate LN with compound B only.

For phase II testing, trials should initially be conducted in an area with pyrethroid-susceptible mosquitoes and mosquitoes susceptible to compounds used in the mixture in the candidate LN. If the product is as effective as the reference LN, it should also be tested in an area with pyrethroid-resistant populations which give reduced mortality and blood-feeding inhibition when conventional LNs with pyrethroid are used.

1. Candidate mixture LN, unwashed
2. Candidate mixture LN, washed 20 times
3. Candidate LN with compound A only, unwashed
4. Candidate LN with compound B only, unwashed
5. Candidate LN with compound A only, washed 20 times
6. Candidate LN with compound B only, washed 20 times
7. Positive control (an LN that has received a WHOPES recommendation), unwashed
8. Positive control, washed 20 times (using a regeneration time not exceeding 3 days, as discussed above)
9. Untreated net.

If one of the compounds is a synergist that causes no mortality at operational dos*es* as determined in phase I studies, the treatment arms should include only the candidate mixture LN and the candidate LN with the insecticide only. It is not necessary to test the candidate LN with the synergist only.

Ultimate decision is based on the comparison of the candidate LN (washed 20 times) versus positive control washed 20 times. The candidate LN should have equal or greater efficacy in terms of mortality and blood-feeding inhibition.

As noted above, mosquitoes collected in experimental hut studies should be preserved for quality control and/or future studies of genetic markers of insecticide resistance and their relation to efficacy in the experimental huts.

III.III   Efficacy testing of combination LNs

Combination LNs include two or more different nettings in their manufacture. Each netting has a different specification which may be for different fibres and/or active ingredient(s) with or without synergists.

In phase I, each netting component of the LN must be assessed separately. In phase II, the full product should be studied. Where the netting includes mixtures of insecticides or that of insecticide plus a synergist, the principles for evaluating LNs with mixtures as described above will generally apply.

III.IV   Efficacy testing of mixed formulations for IRS

Mixtures of AIs may be applied as IRS treatments to delay the selection of resistance to each component and to provide improved control.

In phase I testing, the product and its components should be tested on different substrates using both susceptible and resistant strains as recommended by WHOPES guidelines.

For phase II testing the following arms are proposed:

1. Untreated hut
2. Mixture IRS
3. IRS of component 1 at the same dose as in the mixture
4. IRS of component 2 at the same dose as in the mixture
5. IRS of component 1 at recommended rate (optional positive control)
6. IRS of component 2 at recommended rate (optional positive control).

Mosquitoes collected from the experimental huts should be preserved for quality control or future studies of genetic markers of insecticide resistance.

ANNEX I.   LIST OF PARTICIPANTS

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