World Health Organization

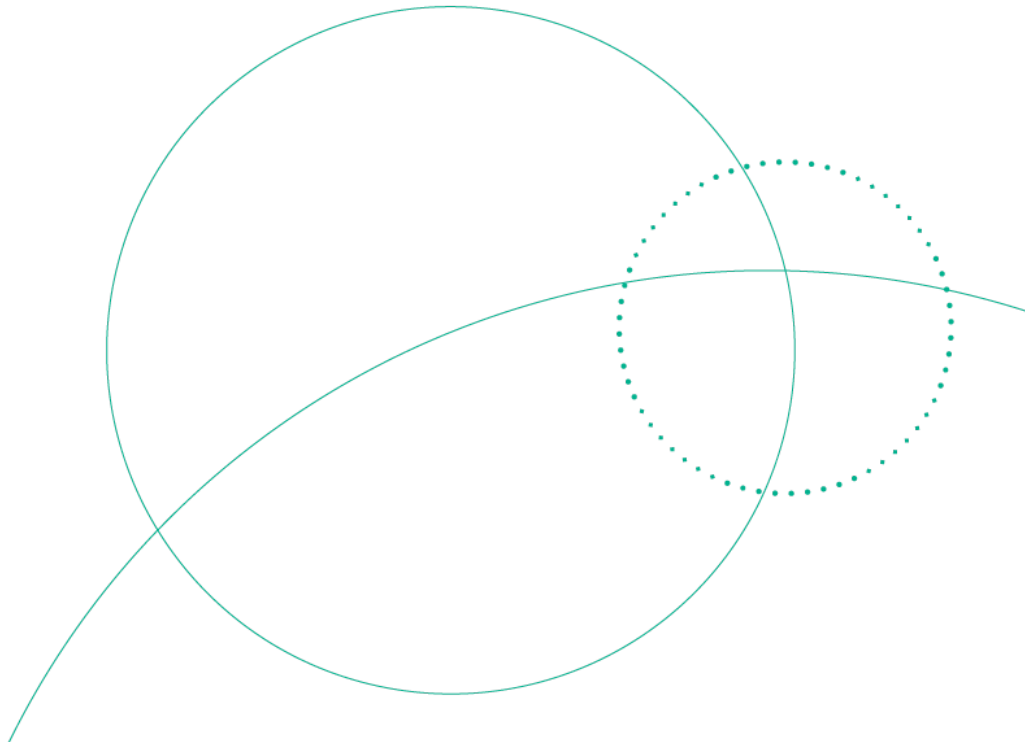
WHO Prequalification Programme / Vector Control Product Assessment

WHO Public Assessment Report: WHOPAR Part 5

GreenNet LN
(Shobikaa Impex Private Limited)

P-00320

Efficacy Assessment



Contents

1 Introduction

The primary purpose for the use of a pesticide is the control of a pest, including disease transmitting vectors. Vector control tools, including formulated pesticides, which provide effective management or control of vectors, may be used as part of a resistance management programme. Vector control products for use in public health are a component of Integrated Vector Management (IVM), which is a programme that relies on a suite of diverse interventions and implementations of best practices to manage the vector and chemical/behavioural resistance.

GreenNet LN is a homogenous ITN coated with deltamethrin 1.4 g AI/kg that is intended to provide personal and community protection from Anopheline mosquitoes as part of malaria control programmes.

Semi-field studies to characterize the performance of GreenNet LN against free-flying mosquitoes with supplementary bioassays to characterize the availability of active ingredients and insecticidal effect of the fabric of the ITN on Anopheline mosquito species were submitted to WHO as part of the prequalification dossier.

2 Semi-field studies

Studies conducted in semi-field settings often include the investigation of endpoints other than mortality, knockdown and blood-feeding inhibition. Examples of these include entry rate, exit rate, and deterrence, as well as analyses for non-standardized calculations of “personal protection”. Based on the existing requirements and established decision framework, mosquito mortality and knockdown are considered the primary endpoints for assessment. Therefore, results for these are included within the summaries of these studies. Calculations of blood feeding inhibition were also included for further characterization of the entomological impact of the product.

2.1 Semi-field studies

Data on the semi-field performance of GreenNet LN were provided. These data were obtained from studies conducted according to established standards and/or Good Laboratory Practices (GLP). These summary results are based on ITNs drawn from batches GN01W1219, GN02W1219, GN03W1219, GN02W0723, GN03W0723 and GN04W0823.

Two semi-field studies were presented to evaluate the efficacy and wash resistance of GreenNet LN in Benin and India. The semi-field study conducted in Benin was an experimental hut trial (EHT) and the semi-field study conducted in India was an ambient chamber test (IACT) study. ITNs used in semi-field studies were prepared using a wash interval of one day that was selected based on the results from the Module 3 laboratory studies. The endpoint used to evaluate bioavailability was 24-hour mortality. The negative control used in each study was an untreated net and the positive control was a prequalified ITN product treated with 1.4 g AI/kg deltamethrin, hereafter referred to as PC1.

The product was tested against pyrethroid resistant natural populations of, in Covè, Benin, *An. coluzzi* and *An. gambiae* s.s, with the relative proportions estimated at 77% and 23%, respectively. The pyrethroid resistance is mediated by *kdr,* with the *An. gambiae* s.l population carrying *kdr* gene frequencies of >90% L1014F and an intensity of resistance of greater than 200-fold the diagnostic dose, and metabolic resistance through over-expression of CYP6P3 enzymes. In resistance testing conducted during the experimental hut trial, 6% and 8% mortality were observed following exposure to the diagnostic doses of permethrin and alpha-cypermethrin, respectively. In the IACT semi-field study conducted in India, the product was tested against insecticide susceptible colonized *An. stephensi* mosquitoes. In insecticide susceptibility testing conducted on the colony, 100% mortality was observed 24 hours after exposure to the diagnostic dose of deltamethrin.

The results from the free-flying mosquito studies are presented in Table 1. GreenNet ITNs that were washed 20 times using a 1-day wash interval induced 11% 24-hour mortality in wild free-flying *An. gambiae* s.l. mosquitoes in Benin and 100% 24-hour mortality in free-flying *An. stephensi* mosquitoes in India, respectively. Statistical analyses demonstrated that the mortality observed in the 20x washed GreenNet study arms was not significantly different to PC1 (Benin: Mortality 11% vs 14%, P=0.081; India: Mortality 100% vs 100%, P = 0.999).

Supplementary bioassays were used to characterize the bioavailability of the active ingredients on the surface of the ITN pre- and post-hut studies using unwashed and 20x washed nets. WHO cone tests and tunnel tests were the experimental methods used in bioavailability studies. In Benin, the *An. gambiae* s.s. Kisumu strain was used as an insecticide susceptible test system. Thresholds of ≥95% knockdown and/or ≥80% mortality in WHO cone tests and ≥80% mortality or ≥90% blood feeding inhibition in tunnel tests were used as the efficacy criteria for bioavailability. The endpoint used to evaluate bioavailability was 24-hour mortality.

The results from supplementary bioassays are presented in Tables 2 and 3. In WHO cone tests using insecticide susceptible test systems, 60-minute KD greater than 95% was not observed using unwashed and 20x washed nets, before the EHT in Benin. After the EHT in Benin, greater than 95% 60-minute KD was observed in cone tests using unwashed nets but not using 20x washed nets. Mortality greater than 80% was not observed using unwashed and 20x washed nets, both before and after the EHT. In tunnel tests using insecticide susceptible test systems in Benin, 100% and 88% 24-mortality, and 100% and 50% blood feeding inhibition were observed against unwashed and 20x washed nets, respectively.

In India, in WHO cone tests using insecticide susceptible test systems, 100% 60-minute KD and 24-hour mortality were observed using unwashed nets, before and after the IACT study. In cone tests conducted using 20x washed nets, 100% 60-minute KD and 24-hour mortality were observed prior to the IACT study, and 96% 60-minute KD and 92% 24-hour mortality were observed after the IACT study.

Table 1. Mortality and Blood Feeding Inhibition of Wild, Free-Flying Pyrethroid Resistant *An. gambiae* s.l. in an Experimental Hut Trial in Benin, and Free-Flying Insecticide Susceptible *An. stephensi* in India				
Product	**Washing condition**	**% M24 (95% CI)**	**% Feeding inhibition (95% CI)**	**Sample Size**
Benin (*An.gambiae* s.l)				
Total number of mosquitoes collected = 1,547			Compliant with power calculation? N/A	
Control	Unwashed	3 (1 – 6)	-	181
GreenNet	Unwashed	16 (10 – 22)	21 (14 – 27)	146
	20x washed	11 (7 – 15)	12 (9 – 15)	157
PC1	Unwashed	18 (13 – 23)	11 (7 – 15)	173
	20x washed	14 (11 – 17)	0 (0 – 1)	170
India (*An.stephensi*)				
Total number of mosquitoes collected = 6,250			Compliant with power calculation? Yes	
Control	Unwashed	5.6	-	1,250
GreenNet LN	Unwashed	100	91.8 (90.7 - 93.0)	1,250
	20x washed	100	89.9 (86.6 - 91.2)	1,250
PC1	Unwashed	100	90.8 (90.5 - 91.1)	1,250
	20x washed	100	88.7 (87.4 - 90.0)	1,250

Table 2. Knockdown and mortality results for insecticide susceptible laboratory strains using WHO cone bioassays						
	Benin			**India**		
	An. gambiae Kisumu			*An. stephensi*		
	n	%KD60 (95% CI)	%M24 (95% CI)	n	%KD60 (95% CI)	%M24 (95% CI)
Before hut trial						
UW	54	94.4 (88.3 – 100)	79.6 (68.9 – 90.4)	50	100	100
20x washed	57	40.4 (27.7 – 53.1)	29.8 (17.9 – 41.7)	50	100	100
After hut trial						
UW	55	98.2 (94.7 – 100)	74.5 (62.9 – 86)	50	100	100
20x washed	51	70.6 (58.1 – 83.1)	52.9 (39.2 – 66.6)	50	96 (90.0 - 102.0)	92 (84.6 - 99.4)

Table 3. Mortality and blood feeding inhibition results for an insecticide susceptible laboratory strain using tunnel tests

	Benin		
	An. gambiae Kisumu		
	n	%BFI	%M24 (95% CI)
UW	236	100 (95 – 100)	100 (95 – 100)
20x washed	212	50 (43 – 57)	88 (82 – 94)

2.2 Chemical characterization

Data on the deltamethrin content of sampled pieces of the GreenNet LN product used in the semi-field studies were provided. These data were obtained from studies conducted according to established standards and/or Good Laboratory Practices (GLP). These summary results are based on ITNs drawn from batches GN01W1219 and GN02W0120. The results are summarized in Table 4.

Table 4. AI content and retention of sampled pieces of GreenNet LN used in the semi-field studies in Benin and Cameroon (batch numbers GN01W1219 and GN02W0120- used in both locations)

	Benin		Cameroon	
	Mean deltamethrin content (g/kg)	Deltamethrin retention (per wash)	Mean deltamethrin content (g/kg)	Deltamethrin retention (per wash)
Before hut trial				
UW	1.49	-	1.47	-
20x washed	0.62	41.61% (95.71%)	0.62	42.18% (95.78%)
After hut trial				
UW	1.46	-	1.46	-
20x washed	0.62	42.47% (95.81%)	0.61	41.78% (95.73%)

The mean AI content presented in Table 4 was determined based on 10 replicates of 4 net samples of 2 batches for unwashed (UW) product and after 20 washes before hut trial and after hut trial.

AI retention per wash in Table 4 is calculated as:

- AI retention per wash = 100 x $\sqrt[n]{(t_n/t_0)}$ where:
  - t_n = total active ingredient content after n washing cycles
  - t_0 = total active ingredient content before washing
  - n = number of washes.

The chemical analysis performed on samples of ITNs used in the semi-field studies confirmed that the test samples conformed with the batch analysis data presented in Module 3 and that the test samples were appropriate for use in the study.

The AI content for the unwashed product complied with the target dose interval limits in the specification (i.e., 1.4 ± 25% g/kg for deltamethrin).

2.3 Semi-field studies conclusions

The submitted semi-field studies demonstrate the impact of GreenNet LN on free-flying mosquitoes and the bioavailability of the treatments on the ITN fabric using WHO cone bioassays and tunnel tests. Based on the submitted studies, the impact of GreenNet LN ITNs that have been prepared using a one-day wash interval can be sustained against pyrethroid resistant and insecticide susceptible free-flying mosquitoes up to 20 washes. The bioavailability of the treatments on the GreenNet fabric was sustained up to 20 washes against insecticide susceptible test systems of the *An. gambiae* complex and *An. stephensi*.

3 Efficacy conclusions

Based on the studies and information provided, all data requirements for the prequalification assessment of product efficacy have been satisfied. These data have been relied upon to assess the bioavailability and the impact on free-flying mosquitoes of the proposed product for the purpose of characterising the fabric of the product and establishing the duration of biological impact using products prepared with a defined wash interval.

The efficacy component of the dossier is considered complete, and the assessment of the submitted information on efficacy supports prequalification of the product.

Table 5. List of efficacy studies submitted to WHO as part of the prequalification dossier

Studies that were relied upon for decision making	
Study number	Study title
23 443	Evaluation of GreenNet - Deltamethrin 1.4 g/kg ± 25% Coated onto Polyester Filaments of Long - Lasting Insecticidal Net (LLIN) - Determination of Efficacy and Wash Resistance against Susceptible Mosquito Strain *Anopheles stephensi* under Ambient Chamber Test (ACT) Condition - IIBAT, Padappai, Tamil Nadu, India
1913	Experimental hut evaluation of the efficacy and wash resistance of GreenNet LN (Deltamethrin treated net) by Shobikaa Impex Private Ltd: a multi-centre study against pyrethroid resistant malaria vectors in West and Central Africa (note: data from Benin was used for decision making; data from Cameroon were not used for decision making)
20128	GreenNet - Deltamethrin 1.4 g/kg coated onto polyester filament of Long-Lasting Insecticidal Net: Regeneration, Wash Resistance and Efficacy against Mosquito, *Anopheles stephensi*
Studies that were not used to inform decision making	
	None