

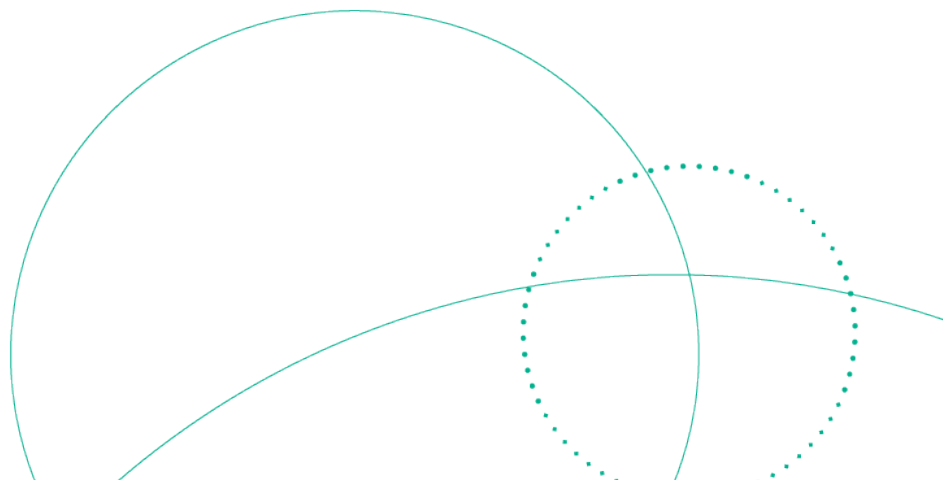
WHO Prequalification Programme / Vector Control Product Assessment

WHO Public Assessment Report: WHOPAR Part 5

PRONet Duo
(V.K.A. Polymers Pvt. Ltd.)

P-12406

Efficacy Assessment



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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.

PRONet Duo is a homogenous ITN incorporated with bifenthrin 7.0 g AI/kg and chlorfenapyr 8.0 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 PRONet Duo 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 Experimental hut trials

Data on the semi-field performance of PRONet Duo in experimental huts 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 VKA-257-1-1, VKA 257-1-2 and VKA-257-1-3.

Two semi-field studies were presented to evaluate the efficacy and wash resistance of PRONet Duo Tanzania and Côte d'Ivoire. Both semi-field studies that were conducted were experimental hut trials (EHT). ITNs used in semi-field studies were prepared using the wash interval of one day determined in laboratory studies. The endpoint used to evaluate bioavailability was 72-hour mortality. The negative control used in each study was an untreated net and the positive controls were a prequalified product coated with 2.4 g AI/kg alpha-cypermethrin and 4.8 g AI/kg chlorfenapyr, hereafter referred to as PC1, and an ITN incorporated with 5.8 g AI/kg alpha-cypermethrin, hereafter referred to as PC2. In non-inferiority analyses conducted on the results of the semi-field studies, PC1 was used as an active comparator and PC2 was used as a standard comparator.

The product was tested against pyrethroid resistant natural populations of, in Tanzania, *An. gambiae* complex, of which >99.9% was *An. arabiensis*. In resistance testing conducted prior to the EHT, 12% 24-hour mortality was observed following exposure to the diagnostic dose of alpha-cypermethrin, using WHO cylinder tests and 100% 120-hour mortality was observed following exposure to the diagnostic dose of chlorfenapyr in bottle bioassays (Table 1).

In the semi-field study conducted in Côte d'Ivoire, the product was tested against pyrethroid resistant natural populations of *An. gambiae* s.l. In resistance testing conducted prior to the commencement of the EHT, pyrethroid resistance to 10 times the diagnostic dose of alpha-cypermethrin and deltamethrin was detected, using WHO cylinder tests. In bottle bioassays using the diagnostic dose and two times the diagnostic dose of chlorfenapyr, 96% 72-hour mortality was observed (Table 1). The LC₅₀ of the local vector population at the experimental hut site is presented in Table 2.

The results from the free-flying mosquito studies are presented in Table 3. PRONet Duo ITNs that were washed 20 times using a 1-day wash interval induced 55% 72-hour mortality in wild free-flying *An. arabiensis* mosquitoes in Tanzania and 80% 72-hour mortality in free-flying *An. gambiae* s.l. mosquitoes in Côte d'Ivoire, respectively. Statistical analyses demonstrated that the mortality observed in the 20x PRONet Duo trial arms was statistically superior to PC1 and superior to PC2 (Table 4).

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. Tunnel tests were the experimental methods used in bioavailability studies. Both sites conducted supplementary bioassays using insecticide susceptible and pyrethroid resistant test systems. In Tanzania, the *An. gambiae* s.s. Ifakara strain was used as the insecticide susceptible test system; in Côte d'Ivoire, the insecticide susceptible test system used was *An. gambiae* s.s. Kisumu. The pyrethroid resistant test systems used were *An. arabiensis* Kingani strain in Tanzania and *An. gambiae* s.l. M'Be in Cote D'Ivoire. The endpoint used to evaluate bioavailability was 72-hour mortality.

The results from supplementary bioassays are presented in Table 5. In tunnel tests using insecticide susceptible and insecticide resistant test systems, 72-hour mortality greater than 85% was observed in all unwashed and washed arms before and after the EHTs.

Table 1. Insecticide resistance characterization of *An. arabiensis* in Tanzania and *An. gambiae* sl M'Be in Côte d'Ivoire using WHO cylinder tests and CDC bottle bioassays

Mosquito species	Test date	WHO cylinder							CDC bottle bioassay	
		Alpha-cypermethrin			Deltamethrin			Alpha-cypermethrin + PBO	Chlorfenapyr	
		0.05%	0.25% (5xDD)	0.5% (10xDD)	0.05%	0.25% (5xDD)	0.5% (10xDD)	0.05% (ac), 4% (PBO)	100µg (1xDD)	200µg (2xDD)
		%M24	%M24	%M24	%M24	%M24	%M24	%M24	%M120 (Tz), %M72 (Cdl)	%M72
<i>An. arabiensis</i> Kingani	March 2023	25						100	100	
<i>An. arabiensis</i> Lupiro	October 2023	12						95	98	
<i>An. gambiae</i> s.l. M'Be	June 2023	2.9	19.4	57.3	6.9	52.9	61.5	31.1	96.0	96.2

Table 2. LC₅₀ of *An. gambiae* sl M'Be vector population in Côte d'Ivoire

Mosquito species	Insecticide	Endpoint	Slope (SE)	LC ₅₀	95% CI
<i>An. gambiae</i> s.s Kisumu	Alpha-cypermethrin		2.12 (0.19)	0.044	0.034 – 0.056
<i>An. gambiae</i> sl M'Be	Alpha-cypermethrin	RR50 = 698.48	1.860 (0.141)	31.125	25.306 – 37.391
<i>An. gambiae</i> s.s Kisumu	Chlorfenapyr		1.194 (0.232)	0.0045	0.0018 – 0.008
<i>An. gambiae</i> sl M'Be	Chlorfenapyr	RR50 = 1.74	0.998 (0.204)	0.008	0.002 – 0.0186

Table 3. Mortality and blood feeding inhibition of free-flying pyrethroid resistant *An. gambiae* s.l. in two experimental hut trials (EHT)

Product	Washing condition	% M72 (95% CI)	% Feeding inhibition (95% CI)	Sample size
Tanzania (<i>An.arabiensis</i>)				
Total number of mosquitoes collected = 9,383			Compliant with power calculation? Yes	
Control	Unwashed	3	-	1,796
PRONet Duo	Unwashed	76 (68,84)	86 (70, 93)	722
	20x washed	55 (46, 65)	90 (81, 99)	651
PC1	Unwashed	52 (44, 61)	91 (84,98)	967
	20x washed	38 (31, 45)	79 (69, 89)	1,540
PC2	Unwashed	42 (34,50)	90 (83, 98)	1,667
	20x washed	28 (21,35)	89 (81,96)	2,040
Côte d'Ivoire (<i>An. gambiae</i> s.l.)				
Total number of mosquitoes collected = 6,338			Compliant with power calculation? Yes	
Control	Unwashed	-	-	804
PRONet Duo	Unwashed	87 (82 - 92)	77 (70 – 84)	544
	20x washed	80 (74 – 85)	83 (77 – 88)	799
PC1	Unwashed	73 (67 – 79)	67 (59 – 75)	580
	20x washed	69 (63 – 74)	53 (45- 61)	1,026
PC2	Unwashed	29 (24 – 35)	55 (47 – 64)	726
	20x washed	23 (19 – 28)	65 (58 – 72)	926

NB. Control-corrected mortality presented for Côte d'Ivoire study

Table 4. Non-inferiority analysis for PRONet Duo in experimental hut studies in Tanzania and Côte d'Ivoire

	Unwashed				20x washed				Pooled			
Indicator and reference	Target outcome	NI margin	OR (95% CI)	Interpretation	Target outcome	NI margin	OR (95% CI)	Interpretation	Target outcome	NI margin	OR (95% CI)	Interpretation
Tanzania												
M72 PC1	Non-inferiority	0.75	3.05 (2.34, 3.99)	Superior	Non-inferiority	0.73	2.41 (1.91, 3.05)	Superior	Non-inferiority	0.75	2.77 (2.31, 3.33)	Superior
M24 PC2	Superiority	NA	8.61 (6.73, 11.02) p <0.0001	Superior	Superiority	NA	6.15 (4.90, 7.73) p <0.0001	Superior	Superiority	NA	7.24 (6.08, 8.62) p <0.0001	Superior
Blood feeding rate PC1	Non-inferiority	2.60	0.67 (0.39, 1.14)	Non-inferior	Non-inferiority	2.21	0.23 (0.12, 0.44)	Superior	Non-inferiority	2.38	0.41 (0.28, 0.61)	Superior
Blood feeding rate PC2	Superiority	NA	1.30 (0.75, 2.24) p = 0.351	Indeterminate	Superiority	NA	0.46 (0.02, 0.23) p = 0.020	Superior	Superiority	NA	0.81 (0.54, 1.22) p = 0.322	Indeterminate
Côte d'Ivoire												
M72 PC1	Non-inferiority	0.71	1.49 (1.06 - 2.08)	Superior	Non-inferiority	0.73	1.58 (1.24 - 2.01)	Superior	Non-inferiority	0.72	1.54 (1.27 - 1.88)	Superior
M24 PC2	Superiority	NA	17.32 (12.74 – 23.53) p <0.0001	Superior	Superiority	NA	15.32 (11.98 - 19.59) p <0.0001	Superior	Superiority	NA	16.40 (13.52 - 19.90) p <0.0001	Superior
Blood feeding rate PC1	Non-inferiority	1.57	0.70 (0.49 - 1.00)	Superior	Non-inferiority	1.43	0.31 (0.23 - 0.41)	Superior	Non-inferiority	1.48	0.42 (0.34 - 0.52)	Superior
Blood feeding rate PC2	Superiority	NA	0.38 (0.27 - 0.53) p <0.0001	Superior	Superiority	NA	0.52 (0.38 - 0.71) p <0.0001	Superior	Superiority	NA	0.40 (0.38 - 0.60) p <0.0001	Superior

Table 5. 72-hour mortality, blood feeding rate (FR, Côte d'Ivoire) and blood feeding inhibition (FI, Tanzania) results for insecticide susceptible and pyrethroid resistant laboratory strains and field-derived test systems using tunnel tests

	Tanzania						Côte d'Ivoire					
	<i>An. gambiae</i> Ifakara			<i>An. arabiensis</i>			<i>An. gambiae</i> Kisumu			<i>An. gambiae</i> s/l M'Bé		
	n	%M72 (95% CI)	%FI (95% CI)	n	%M72 (95% CI)	%FI (95% CI)	n	%M72 (95% CI)	%BFR (95% CI)	n	%M72 (95% CI)	%BFR (95% CI)
Before hut trial												
UW	766	100	99 (97 – 100)	768	100	100	559	100	3.0 (1.6 – 4.5)	470	98.9 (98.0 – 99.9)	0.6 (0.0 – 1.4)
20x washed	750	98 (96 – 99)	99 (98 – 100)	753	87 (79 – 96)	99 (98 – 100)	439	100	2.3 (0.9 – 3.7)	417	96.2 (94.3 – 98.0)	1.0 (0.0 – 1.9)
After hut trial												
UW	465	100	100	461	100	100	366	100	4.6 (2.5 – 6.8)	292	96.9 (94.9 – 98.9)	1.4 (0.0 – 2.7)
20x washed	460	100	100	467	100	100	347	100	2.3 (0.7 – 3.9)	317	94.6 (92.2 – 97.1)	0.3 (0.0 – 0.9)

2.1.1 Chemical characterization

Data on the bifenthrin and chlorfenapyr content of sampled pieces of the PRONet Duo 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 VKA-257-2-1, VKA-257-2-2 and VKA-257-2-3 for Tanzania and for Côte d'Ivoire. The results are summarized in Table 6.

Table 6. AI content and retention of sampled pieces of PRONet Duo used in the semi-field studies in Côte d'Ivoire and Tanzania (batch numbers VKA-257-2-1, VKA-257-2-2 and VKA-257-2-3)								
	Côte d'Ivoire (Study VCPEC-IPR 23/05 / 24142)				Tanzania (Study BIT 103 / 24143)			
	Mean bifenthrin content (g/kg)	Bifenthrin retention (per wash)	Mean chlorfenapyr content (g/kg)	Chlorfenapyr retention (per wash)	Mean bifenthrin content (g/kg)	Bifenthrin retention (per wash)	Mean chlorfenapyr content (g/kg)	Chlorfenapyr retention (per wash)
Before hut trial								
UW	7.12 (7.02-7.26)	-	8.32 (8.17-8.47)	-	7.10 (6.99 - 7.20)	-	8.12 (8.00 -8.24)	-
20x washed	3.98 (3.86-4.11)	55.90% (97.13%)	2.65 (2.56-2.74)	31.85% (94.344%)	4.16 (4.10 -4.28)	58.59% (97.36%)	2.73 (2.64 - 2.80)	33.62% (94.70%)
After hut trial								
UW	7.13 (7.01 -7.21)	-	8.3 (8.17 - 8.41)	-	7.20 (7.11 - 7.28)	-	8.27 (8.10 - 8.54)	-
20x washed	3.97 (3.88 - 4.09)	55.72% (97.12%)	2.65 (2.57 - 2.75)	31.87% (94.44%)	4.03 (3.96 -4.10)	55.97% (97.14%)	2.66 (2.61 - 2.72)	32.16% (94.49%)

The mean AI content presented in Table 6 for Côte d'Ivoire was determined based on 5 net samples belonging to 3 batches for unwashed (UW) product and after 20 washes before hut trial and 4 samples belonging to 2 batches for unwashed (UW) product and after 20 washes after hut trial, indicating ranges to the AI content in parenthesis.

The mean AI content presented in Table 6 for Tanzania was determined based on 5 net samples belonging to 3 batches for unwashed (UW) product and after 20 washes before hut trial and 3 samples belonging to 3 batches for unwashed (UW) product and after 20 washes after hut trial, indicating ranges to the AI content in parenthesis.

AI retention per wash in Table 4 is calculated as:

- AI retention per wash = $100 \times \sqrt[n]{(t_n/t_0)}$ 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., $7.0 \pm 15\%$ g/kg for bifenthrin and $8.0 \pm 15\%$ g/kg for chlorfenapyr.

2.2 Semi-field studies conclusions

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

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 studies submitted to WHO as part of the prequalification dossier

Studies that were relied upon for decision making.	
Study number	Study Title
24142	Chemical Analysis of PRO Net Duo LN Samples Used as an Investigational Item in the Experimental Hut Study VCPEC-IPR 23/05 Conducted at Vector Control Product Evaluation Center (VCPEC) - Institut Pierre Richet (IPR) at Bouake, Ivory Coast PRONet Duo: having Bifenthrin 7.0 g/kg + Chlorfenapyr 8.0 g/kg
24143	Chemical Analysis of PRONet Duo LN ITN samples used as an Investigational item in the Experimental Hut Study BIT 103 conducted at Ifakara Health Institute, Tanzania PRONet Duo: having Bifenthrin 7.0 g/kg + Chlorfenapyr 8.0 g/kg
BIT103	Laboratory and experimental hut evaluation of PRONet Duo Insecticide Treated Net (ITN) in comparison with Interceptor® G2 ITN and MAGNet® ITN against strongly pyrethroid-resistant <i>Anopheles arabiensis</i> and fully susceptible <i>Anopheles gambiae</i> s.s. in Tanzania
VCPEC-IPR 23/05	Efficacy of a novel prototype LLIN (PRONet Duo) manufactured by VKA Polymers Pvt. Ltd., India, against insecticide resistant <i>Anopheles gambiae</i> s.l.: an experimental hut trial at M'Bé field site, central Côte d'Ivoire
Studies that were not used to inform decision making.	
	None