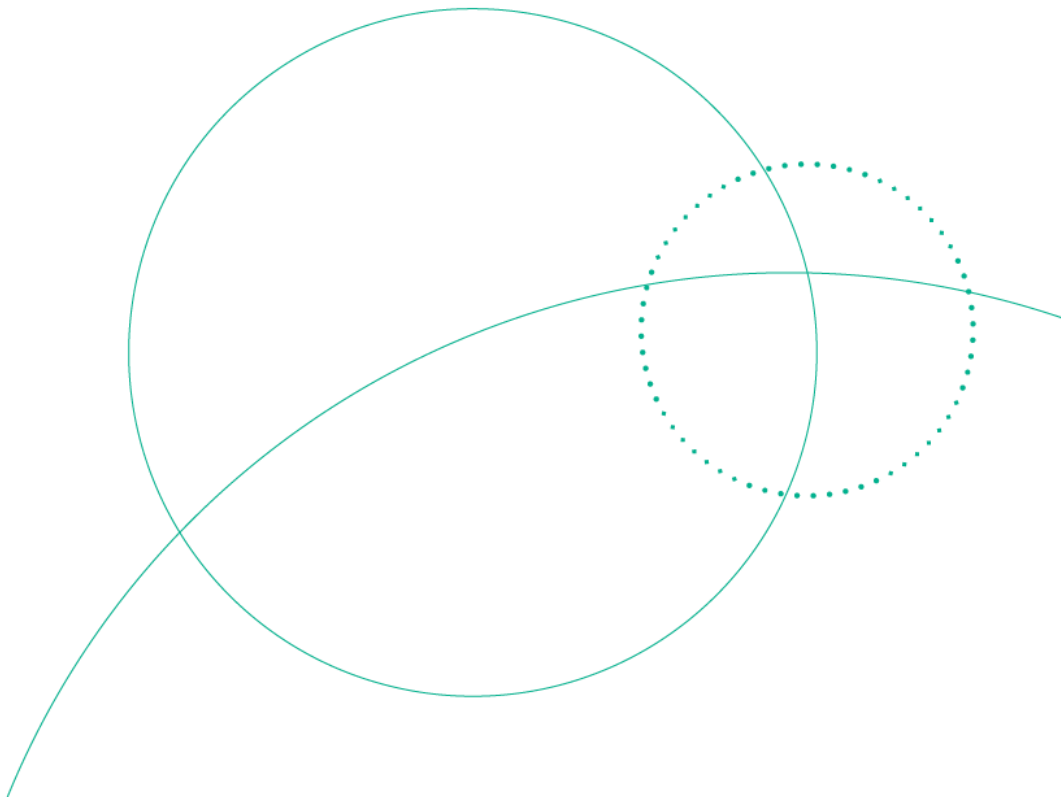
World Health Organization

WHO Prequalification Programme / Vector Control Product Assessment

WHO Public Assessment Report: WHOPAR Part 5

PermaNet Dual (Vestergaard Sarl) P-03228

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.

PermaNet Dual is a homogenous ITN coated with deltamethrin 84 mg AI/m^2 and chlorfenapyr 200 mg AI/m^2 that is intended to provide personal and community protection from Anopheline mosquitoes as part of malaria control programmes. The premise of the combination of the two insecticides is that the dual insecticidal activity provides knockdown and/or kill of mosquitoes including pyrethroid resistant *Anopheles spp.* malaria vectors.

Semi-field studies to characterize the performance of PermaNet Dual 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 PermaNet Dual 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 100720-57, 100720-89, 062220.

Two semi-field studies were presented to evaluate the efficacy and wash resistance of PermaNet Dual in West Africa, in Benin and Côte d'Ivoire. Both semi-field trials were experimental hut trials (EHT). ITNs used in semi-field studies were prepared using the wash interval 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 treated with 100 mg AI/m^2 alpha-cypermethrin and 200 mg AI/m^2 chlorfenapyr, hereafter referred to as PC1; an ITN treated with 84 mg AI/m^2 (sides) or 120 mg AI/m^2

(roof) deltamethrin and 800 mg AI/m^2 PBO, hereafter referred to as PC2, and an ITN treated with 56 mg AI/m^2 of deltamethrin, hereafter referred to as PC3.

The product was tested against pyrethroid resistant natural populations of: i) *An. gambiae s.l.* in Cove, Benin, carrying *kdr* gene frequencies of >90% L1014F and metabolic resistance through over-expression of CYP6P3; ii) *Anopheles gambiae s.l.* in Tiassalé, Côte d'Ivoire, carrying *kdr* gene frequencies of 83% L1014F and *ace-1* frequencies of 44%.

The results from the free-flying mosquito studies are presented in Table 1. PermaNet Dual ITNs that were washed 20 times using a 1-day wash interval induced 74% and 89 72-hour mortality in free-flying *An. gambiae* s.l. mosquitoes in Benin and Côte d'Ivoire, respectively. Statistical analyses demonstrated that the mortality observed in the 20x PermaNet Dual trial arms was significantly higher than the comparators PC2 and PC3 ($p = <0.05$) and not significantly different to PC1 ($p = 0.177$).

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. The *An. gambiae* s.s. Kisumu strain was used as an insecticide susceptible test system in all sites. Pyrethroid resistant test system *An. gambiae s.l* strain (Cove) carrying *kdr* gene frequencies of >90% L1014F and metabolic resistance through over-expression of CYP6P3 was used in Benin; in Côte d'Ivoire, mosquitoes reared from collections at the experimental hut site in Tiassale were used. 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 72-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 observed using unwashed and 20x washed nets, before and after the EHT in Côte d'Ivoire and 72-hour mortality greater than 80% was observed in one unwashed arm after the EHT and in 20x washed nets before and after the hut trial. In Benin, KD greater than 95% and 72-hour mortality greater than 80% was not observed. Mortality greater than 80% was not demonstrated against insecticide resistant test systems using WHO cone tests.

In tunnel tests using insecticide susceptible test systems in Côte d'Ivoire, 72-hour mortality greater than 80% was observed against unwashed and 20x nets, before and after the hut trial. Blood-feeding inhibition was greater than 90% against unwashed nets tested after the hut trial. In tests using insecticide resistant test systems, 72-hour mortality greater than 80% was observed against unwashed and 20x washed nets, before and after the hut trials in Benin and Côte d'Ivoire. Blood-feeding inhibition greater than 90% was observed against unwashed nets after the hut trial and 20x washed nets before and after the hut trial in Benin, and against 20x washed nets after the EHT in Côte d'Ivoire.

Table 1. Mortality and blood feeding inhibition of free-flying pyrethroid resistant *An. gambiae* s.l. in two experimental hut trials

Product	Washing condition	% M72 (95% CI/SEM)	% Feeding inhibition (SEM)	Sample size
Benin (*An.gambiae* s.l.)				
Total number of mosquitoes collected = 5,967			Compliant with power calculation? Yes	
Control	Unwashed	2 (1-4)	-	541
PermaNet Dual	Unwashed	75 (71-79)	45	459
	20x washed	74 (71-78)	20	796
PC1	Unwashed	86 (83-89)	58	623
	20x washed	77 (74-80)	35	669
PC2	Unwashed	56 (52-60)	73	591
	20x washed	30 (27-33)	38	895
PC3	Unwashed	23 (19-27)	21	490
	20x washed	14 (12-16)	-17	903
Côte d'Ivoire (*An. gambiae* s.l.)				
Total number of mosquitoes collected = 3,867			Compliant with power calculation? Yes	
Control	Unwashed	0.1	-	519
PermaNet Dual	Unwashed	93.6 (0.2) 83.3 (0.9)	42.5 (6.8) 41.4 (6.9)	421 381
	20x washed	88.7 (0.2)	43.7 (4.8)	612
PC2	Unwashed	37.5 (2.9)	51 (5.7)	326
	20x washed	14.8 (3.9)	9.8 (3.6)	558
PC3	Unwashed	7.4 (5.1)	12.8 (4.3)	381
	20x washed	11.9 (3.4)	-13.0 (3.6)	669

Table 2. Knockdown and mortality results for insecticide susceptible and pyrethroid resistant laboratory strains using WHO cone bioassays

	Benin				Côte d'Ivoire				Côte d'Ivoire			
	An. gambiae Kisumu				*An. gambiae* Kisumu				*An. gambiae* Tissale			
	n	%KD60 (95% CI)	%M24 (95% CI)	%M72 (95% CI)	n	%KD60 (95% CI)	%M24 (95% CI)	%M72 (95% CI)	n	%KD60 (95% CI)	%M24 (95% CI)	%M72 (95% CI)
Before hut trial												
UW	50	90 (82-98)	44 (30-58)	46 (32-60)	50 50	100 (0.0) 98.0 (2.0)		70.0 (7.1) 76.0 (8.1)	50 50	28.0 (5.8) 32.0 (3.7)		14.0 (6.8) 24.0 (11.2)
20x washed	51	53 (39-67)	16 (6-26)	25 (14-37)	50	100.0 (0.0)		100.0 (0.0)	50	42.0 (8.6)		38.0 (7.0)
After hut trial												
UW	52	71 (59-83)	46 (33-60)	50 (36-64)	50 50	90.0 (3.2) 98.0 (2.0)		34.0 (7.5) 86.0 (5.1)	50 50	12.0 (3.7) 14.0 (7.5)		14.0 (6.0) 24.0 (10.3)
20x washed	52	62 (48-75)	40 (27-54)	56 (42-69)	50	100 (0.0)		94.0 (6.0)	50	18.0 (3.7)		10.0 (3.2)

Table 3. Mortality and blood feeding inhibition results for insecticide susceptible and pyrethroid resistant laboratory strains using tunnel tests

	Benin				Côte d'Ivoire				Côte d'Ivoire			
	An. gambiae Cove				*An. gambiae* Kisumu				*An. gambiae* Tissale			
	n	%BFI	%M24 (95% CI)	%M72 (95% CI)	n	%BFI	%M24 (95% CI)	%M72 (95% CI)	n	%BFI	%M24 (95% CI)	%M72 (95% CI)
Before hut trial												
UW	216	88	98 (96-100)	98 (96-100)	500 500	86.7 (3.9) 85.3 (2.1)		100.0 (0.0) 100.0 (0.0)	500 500	74.7 (4.1) 82.5 (5.9)		95.0 (1.3) 86.0 (4.1)
20x washed	224	91	100 (99-100)	100 (99-100)	500	86.92 (3.2)		100.0 (0.0)	500	91.3 (1.1)		86.2 (4.7)
After hut trial												
UW	229	93	100	100	500 500	92.4 (2.6) 91.6 (3.2)		100.0 (0.0) 100.0 (0.0)	500 500	51.3 (7.8) 50.2 (13.3)		85.0 (4.2) 87.0 (2.7)
20x washed	238	90	99 (97-100)	99 (97-100)	500	87.9 (2.4)		100 (0.0)	500	67.9 (6.4)		88.0 (1.5)

2.1.1 Chemical characterization

Data on the deltamethrin and chlorfenapyr content of sampled pieces of the PermaNet Dual 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 100720-57, 100720-89, 062220. The results are summarized in Table 4.

Table 4. AI content and retention of sampled pieces of PermaNet Dual used in the semi-field studies (batch numbers 100720-57, 100720-89, 06220)

	Benin				Côte d'Ivoire			
	Mean deltamethrin content (g/kg)	Deltamethrin retention (per wash)	Mean chlorfenapyr content (g/kg)	Chlorfenapyr retention (per wash)	Mean deltamethrin content (g/kg)	Deltamethrin retention (per wash)	Mean chlorfenapyr content (g/kg)	Chlorfenapyr retention (per wash)
Before hut trial								
UW	2.30	-	5.58	-	2.41	-	4.98	-
20x washed	1.05	45.7% (95.7%)	1.72	30.8% (93.3%)	1.04	43.2% (95.9%)	1.84	36.9% (95.1%)
After hut trial								
UW	2.27	-	4.76	-	2.47	-	5.01	-
20x washed	0.87	38.3% (95.3%)	0.87	18.3% (91.9%)	1.23	49.8% (96.6%)	2.24	44.7% (96.1%)

The mean AI content presented in Table 4 was determined based on 5 net samples belonging to 3 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 = $100 \times \sqrt[n]{(t_n/t_0)}$ 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., 2.1 ± 25% g/kg for deltamethrin and 5.0 ± 25% g/kg for chlorfenapyr.

2.2 Semi-field studies conclusions

The submitted semi-field studies demonstrate the impact of PermaNet Dual 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 PermaNet Dual 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 PermaNet Dual fabric was sustained up to 20 washes against insecticide susceptible and pyrethroid resistant test systems of the *An. gambiae* complex.

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
20-07-B/GLP	WHO/PQ Phase II experimental hut evaluation of PermaNet P191 (a deltamethrin and chlorfenapyr mixture net) by Vestergaard Sàrl against wild, pyrethroid-resistant *Anopheles gambiae s.l.* in Covè, southern Benin.
VCL-012-18	Determination of active ingredient content in PermaNet 2.0, PermaNet 3.0, PermaNet P191 and Interceptor IG2 from laboratory experimental hut studies in Benin.
CSRS-20/02	Field evaluation of P191 LLIN against natural populations of Anopheles gambiae in comparison with PermaNet 2.0 and PermaNet 3.0 in Côte d'Ivoire: an experimental hut trial.
VCL-012-18	P191 Chemical content of the nets used in the experimental hut trial, Côte d'Ivoire.
Studies that were not used to inform decision making.	
	None