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WHO Prequalification Programme / Vector Control Product Assessment

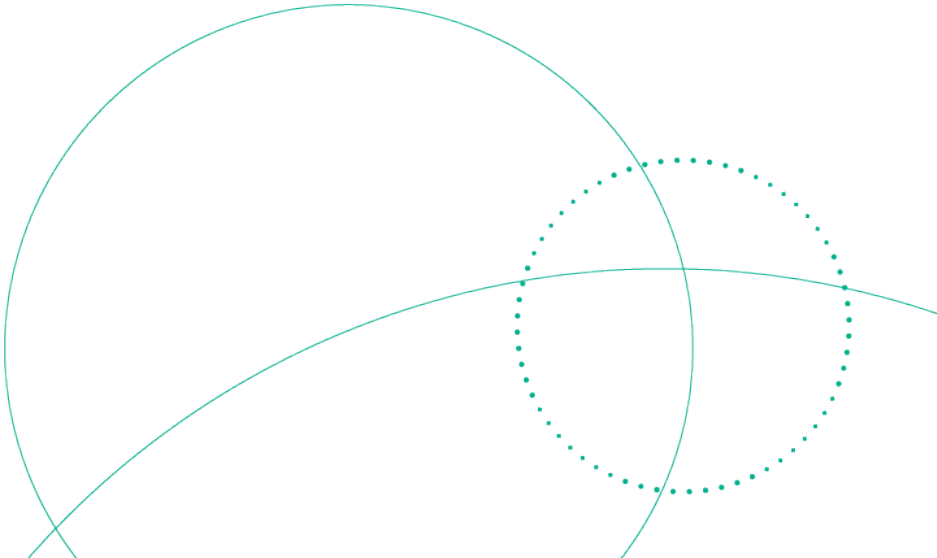
WHO Public Assessment Report: WHOPAR Part 5

Synera DuoForte

(GDM Health Product Ltd.)

P-13215

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.

Synera DuoForte is a homogenous ITN coated with alpha-cypermethrin 3.75 g AI/kg and chlorfenapyr 5.6 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 Synera DuoForte 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 Synera DuoForte 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 1101WT2101001, 1101WT2101002 and 1101WT2101003.

Two semi-field studies were presented to evaluate the efficacy and wash resistance of Synera DuoForte in Benin and Tanzania. 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 product coated with 2.4 g AI/kg alpha-cypermethrin and 4.8 g AI/kg chlorfenapyr, hereafter referred to as PC1; a mosaic ITN fabricated with a roof incorporated with deltamethrin 4 g AI/kg and piperonyl butoxide 25 g AI/kg and sides coated with deltamethrin 2.1 g AI/kg, hereafter referred to as PC2; an ITN incorporated with 20 g AI/kg permethrin and 10 g AI/kg PBO, hereafter referred to as PC3, and an ITN coated with 5 g AI/kg alpha-cypermethrin, hereafter referred to as PC4. All

positive controls used in the studies were prequalified products. In non-inferiority analyses conducted on the results of the semi-field studies, PC1 was used as an active comparator and PC4 was used as a standard comparator.

The product was tested against pyrethroid resistant natural populations of, in Benin, a mixed species complex of *Anopheles coluzzii* and *Anopheles gambiae* which exhibits >200-fold resistance to pyrethroid insecticides, attributed to a high frequency (>90%) of the L1014 *kdr* mutation and overexpression of metabolic enzymes, specifically CYP6P3.In resistance testing conducted prior to the EHT, 42% 24-hour mortality was observed following exposure to the diagnostic dose of alpha-cypermethrin, using WHO cylinder tests and 100% 72-hour mortality was observed following exposure to the diagnostic dose of chlorfenapyr in bottle bioassays (Table 1).

In the semi-field study conducted in Tanzania, the product was tested against a mixed population of *An. gambiae* sl (65.3%) and *Anopheles funestus* (34.7%). The submitted report described both populations as pyrethroid resistant, however no characterization data for the *An. funestus* population was provided. In resistance testing conducted prior to the commencement of the EHT, pyrethroid resistance in the *An. gambiae* population to 10 times the diagnostic dose of deltamethrin and permethrin was detected, using WHO cylinder tests. In bottle bioassays using two times the diagnostic dose of chlorfenapyr, 100% 72-hour mortality was observed (Table 1).

The results from the free-flying mosquito studies are presented in Table 2. Synera DuoForte ITNs that were washed 20 times using a 1-day wash interval induced 73% 72-hour mortality in wild free-flying *An. gambiae* s.l mosquitoes in Benin and 69% 72-hour mortality in the free-flying mixed *An. gambiae* and *An. funestus* mosquitoes in Tanzania, respectively. Statistical analyses demonstrated that the mortality observed in the 20x washed Synera DuoForte trial arms was non-inferior to PC1 and superior to PC2 (Table 3).

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 in the study that was conducted in Benin. The study conducted in Tanzania conducted supplementary bioassays using unwashed and 20x washed nets prior to the EHT only, and therefore the quality of the products used in this study were incompletely quality assured. Cone tests and tunnel tests were the experimental methods used in bioavailability studies. Both sites conducted supplementary bioassays using insecticide susceptible and pyrethroid resistant test systems. *An. gambiae* Kisumu was used as the insecticide susceptible test system in both sites. The pyrethroid resistant test systems used were *An. gambiae* Covè strain in Benin and *An. gambiae* Zeneti in Tanzania. The endpoint used to evaluate bioavailability was 72-hour mortality, with the exception of cone tests conducted in Tanzania using the pyrethroid resistant strain, in which mortality monitoring was conducted at 24 hours post-exposure.

The results from supplementary bioassays are presented in Table 4. In cone tests conducted in Benin and Tanzania using insecticide susceptible test systems, greater than 80% mortality was observed in all tested arms. The observed mortality of the pyrethroid resistant test system for cone tests conducted in Tanzania was less than 25%. In tunnel tests using insecticide susceptible and insecticide resistant test systems, 72-hour mortality greater than 85% was observed in tested arms.

Table 1. Insecticide resistance characterization of *An. gambiae* Covè in Benin and *An. gambiae* sl Zeneti in Tanzania using WHO cylinder tests and CDC bottle bioassays

Mosquito species	Test date	WHO cylinder										CDC bottle bioassay	
		Alpha-cypermethrin	Deltamethrin	Deltamethrin	Deltamethrin	Permethrin	Permethrin	Permethrin	PBO	Alpha-cypermethrin + PBO	Deltamethrin + PBO	Chlorfenapyr	Chlorfenapyr
		0.05%	0.05%	5 x 0.05%	10 x 0.05%	0.75%	5 x 0.75%	10 x 0.75%	4%	0.05% (ACM), 4% (PBO)	0.05% (DM), 4% (PBO)	100µg (1xDD)	200µg (2 x DD)
		%M24	%M24	%M24	%M24	%M24	%M24	%M24	%M24	%M24	%M24	%M72	%M72
An. gambiae sl Covè	Dec 2023	42 (32 – 52)	13 (6 – 20)						2 (1 – 3)	92 (87 – 97)	89 (83 – 95)	100	
An. gambiae Zeneti	June 2023	12 (4 – 20)		80.75 (75 – 87)	88.5 (86- 91)	41 (30 – 53)	83.75 (79- 88)	88.75 (83- 95)					
An. gambiae Zeneti	April 2022												100

Table 2. 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
Benin (*An.gambiae* sl)				
Total number of mosquitoes collected = 10,065			Compliant with power calculation? Yes	
Control	Unwashed	4.42 (3-6)	-	926
Synera DuoForte	Unwashed	76.22 (72-81)	42.22 (37-47)	349
	20x washed	73.36 (71-76)	34.96 (32-38)	1,130
PC1	Unwashed	54.76 (50-60)	30.42 (26-35)	347
	20x washed	50.14 (47-53)	13.85 (12-16)	1,041
PC2	Unwashed	48.75 (45-52)	27.2 (24-30)	718
	20x washed	37.47 (35-40)	7.17 (6-9)	1,249
PC4	Unwashed	38 (35-41)	27.4 (24-31)	771
	20x washed	28.78 (27-31)	10.8 (9-12)	1,529
Tanzania (mixed *An. gambiae* s.l. and *An. funestus*)				
Total number of mosquitoes collected = 1,988			Compliant with power calculation? Yes	
Control	Unwashed	9.7 (6.1 – 13.2)	-	269
Synera DuoForte	Unwashed	66.7 (61.1 – 72.2)	69.5 (64.0 – 7.9)	276
	20x washed	68.6 (62.5 – 74.6)	77.4 (71.9 – 82.8)	229
PC1	Unwashed	54.7 (47.2 – 62.1)	52.9 (45.4 – 60.4)	172
	20x washed	54.6 (48.7 – 60.6)	60.2 (54.4 – 66.1)	269
PC3	Unwashed	37.2 (30.2 – 44.3)	60.4 (53.2 – 67.5)	180
	20x washed	30.1 (24.2 – 36.1)	61.8 (55.5 – 68.1)	229
PC4	Unwashed	33.1 (25.9 – 40.4)	60.2 (52.7 – 67.7)	163
	20x washed	33.3 (26.8 – 39.9)	69.4 (63.0 – 75.7)	201

Table 3. Non-inferiority analysis for Synera DuoForte in experimental hut studies in Benin and Tanzania

	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
Benin												
M72 PC1	Non-inferiority	0.76	2.58 (1.84-3.62)	Non-inferior and Superior	Non-inferiority	0.75	2.65 (2.19-3.20)	Non-inferior and Superior	Non-inferiority	0.75	2.44 (1.84-3.24)	Superior
M72 PC4	Superiority	0.73	6.06 (4.48-8.19)	Superior	Superiority	0.69	7.32 (6.09-8.80)	Superior	Superiority	0.72	7.52 (5.74-9.84)	Superior
Blood feeding rate PC1	Non-inferiority	1.40	0.83 (0.58-1.20)	Non-inferior	Non-inferiority	1.36	0.71 (0.58-0.86)	Non-inferior and superior	Non-inferiority	1.36	0.74 (0.62-0.88)	Non-inferior and superior
Blood feeding rate PC4	Superiority	1.39	0.71 (0.52-0.97)	Superior	Superiority	1.35	0.56 (0.46-0.67)	Superior	Superiority	1.36	0.61 (0.52-0.72)	Superior
Tanzania												
M72 PC1	Non-inferiority	0.76	2.07 (1.32-3.26)	Non-inferior and superior	Non-inferiority	0.76	1.97 (1.31-2.95)	Non-inferior and superior	Non-inferiority	0.75	2.02 (1.49-2.73)	Non-inferior and Superior
M72 PC4	Superiority	0.71	4.46 (2.79-7.14)	Superior	Superiority	0.71	5.63 (3.60-8.79)	Superior	Superiority	0.71	5.11 (3.70-7.06)	Superior
Blood feeding rate PC1	Non-inferiority				Non-inferiority				Non-inferiority	1.67	0.49 (0.30-0.78)	Non inferior and Superior
Blood feeding rate PC4	Superiority				Superiority				Superiority	1.79	0.61 (0.37-1.01)	Not superior

Table 4. Knockdown, 72-hour mortality and blood feeding inhibition results for insecticide susceptible and pyrethroid resistant strains in supplementary cone and tunnel bioassays using Synera DuoForte conducted in Benin and Tanzania.

	Cone tests						Tunnel tests					
Benin												
	An. gambiae Kisumu						*An. gambiae* Kisumu			*An. gambiae sl* Covè		
	n	%KD60 (95% CI)	%M72 (95% CI)				n	%M72 (95% CI)	%BFI (95% CI)	n	%M72 (95% CI)	%BFI (95% CI)
Before hut trial												
UW	80	100 (98-100)	100 (98-100)				164	100 (98-100)	92.15 (87-96)	212	96.23 (93-98)	95.11 (91 – 98)
20x washed	80	97.5 (94-100)	92.5 (87-98)				164	100 (97 – 100)	96.73 (93-99)	216	93.98 (90 – 97)	89.20 (84 – 93)
After hut trial												
UW	80	96.3 (92-100)	90 (83-97)				183	100 (97 – 100)	95.51 (92-98)	216	90.28 (86 – 94)	94.60 (90 – 97)
20x washed	80	87.5 (80-95)	87.5 (80-95)				196	100 (98 – 100)	96.64 (93 – 99)	202	89.60 (85 – 93)	91.66 (87 – 95)
Tanzania												
	An. gambiae Kisumu			*An. gambiae* Zeneti						*An. gambiae* Zeneti		
	n	%KD60 (95% CI)	%M72 (95% CI)	n	%KD60 (95% CI)	%M24 (95% CI)				n	%M72 (95% CI)	%BFI (95% CI)
Before hut trial												
UW	50	100	100	50	0	4 (0 – 9)				100	99 (97 – 100)	96 (92 – 100)
20x washed	50	100	100	50	6 (0 - 13)	20 (9 - 31)				100	94 (89 – 99)	89 (83 - 95)

2.1.1 Chemical characterization

Data on the alpha-cypermethrin and chlorfenapyr content of sampled pieces of the Synera DuoForte 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 1101WT2101001, 1101WT2101002, and 1101WT2101003 for Benin and Tanzania. The results are summarized in Table 6. Whole nets were washed at 1-day intervals in soapy water in bowls and stored at ambient temperature between washes.

Table 6. AI content and retention of sampled pieces of Synera DuoForte used in the semi-field studies in Benin and Tanzania (batch numbers 1101WT2101001, 1101WT2101002, and 1101WT2101003)

	Benin (Study 22-02-/GLP / Study 23561)				Tanzania (Study 2023/001 / Study 24168)			
	Mean alpha-cypermethrin content (g/kg)	Alpha-cypermethrin retention (per wash)	Mean chlorfenapyr content (g/kg)	Chlorfenapyr retention (per wash)	Mean alpha-cypermethrin content (g/kg)	Alpha-cypermethrin retention (per wash)	Mean chlorfenapyr content (g/kg)	Chlorfenapyr retention (per wash)
Before hut trial								
UW	2.90 (2.84-3.02)	-	5.91 (5.65-6.24)	-	2.91 (2.89-2.92)	-	5.49 (5.45-5.53)	-
20x washed	1.64 (1.49-1.90)	56.55% (97.19%)	2.33 (2.16-2.72)	39.42% (95.45%)	1.79 (1.77-1.81)	61.51% (97.60%)	3.30 (3.26-3.34)	60.11% (97.49%)
After hut trial								
UW	2.91 (2.84-3.06)	-	5.84 (5.59-6.08)	-	2.85 (2.80-2.89)	-	5.41 (5.36-5.47)	-
20x washed	1.68 (1.47-1.91)	57.73% (97.29%)	2.48 (2.20-2.76)	42.47% (95.81%)	1.75 (1.73-1.78)	61.40% (97.59%)	3.24 (3.19-3.30)	59.89% (97.47%)

The mean AI content presented in Table 6 for Benin was determined based on 4 net samples belonging to 3 batches for unwashed (UW) product and after 20 washes before and 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 and 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., 3.75 g/kg + 15% and 3.75 g/kg - 25% for alpha-cypermethrin and 5.6 ± 20% g/kg for chlorfenapyr.

2.2 Semi-field studies conclusions

The submitted semi-field studies demonstrate the impact of Synera DuoForte on pyrethroid resistant free-flying mosquitoes and the bioavailability of the treatments on the ITN fabric using cone and tunnel test bioassays and insecticide susceptible and pyrethroid resistant test systems. Based on the submitted studies, the impact of Synera DuoForte 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 Synera DuoForte 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
23561	Chemical analysis of different nets used in the hut study (Phase II trial) of Interceptor LN (Alpha-cypermethrin); Interceptor G2 LN (Alpha-cypermethrin + Chlorfenapyr); PermaNet 3.0 LN [Deltamethrin + Piperonyl butoxide (PBO) - Roof panel & Deltamethrin - Side panel]; Mont Inari Dual 1 (MDI) LN (Alpha-cypermethrin + Chlorfenapyr - Roof panel & Alpha-cypermethrin - Side panel) and Mont Inari Dual 3 (MD3) LN (Alpha-cypermethrin + Chlorfenapyr) before and after subjecting to different washes
24168	Chemical analysis of different nets used in the hut study (Phase II trial) of Interceptor LN (Alpha-cypermethrin 5.0 g/kg); Interceptor G2 LN (Alpha-cypermethrin 2.4 g/kg + Chlorfenapyr 4.8 g/kg); PBO D1 (Piperonyl butoxide 17.5 g/kg + Alpha-cypermethrin 6 g/kg - Roof Panel and Alpha-cypermethrin 5 g/kg - Side Panels); Olyset Plus (Permethrin 20 g/kg + Piperonyl butoxide 10.0 g/kg) and MD3 LN (Alpha-cypermethrin 3.75 g/kg + Chlorfenapyr 5.6 g/kg) before and after subjecting to different washes
2023/001	Phase II experimental hut evaluation of the efficacy and wash resistance of two pyrethroid plus CFP mixture bed nets against wild, pyrethroid-resistant *Anopheles gambiae s.l.* and *Anopheles funestus s.l.* in Tanzania; a non-inferiority assessment
22-02-/GLP	Phase II experimental hut evaluation of the efficacy and wash resistance of two new pyrethroid plus chlorfenapyr mixture bed nets MD1 and MD3 against wild, pyrethroid-resistant *Anopheles gambiae sl* in Covè, southern Benin; a non-inferiority assessment.
Studies that were not used to inform decision making.	
	None