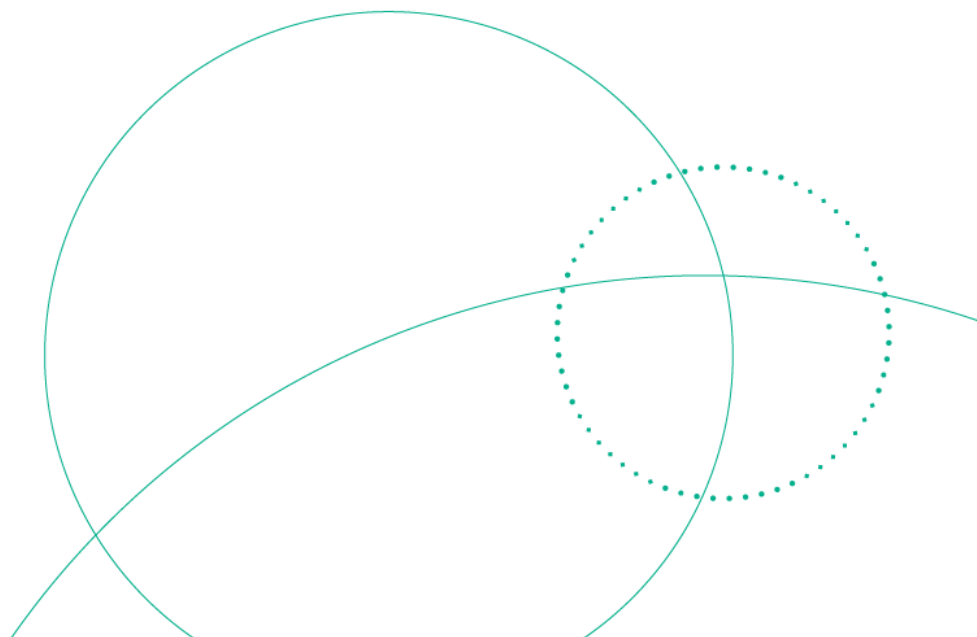


WHO Prequalification Programme / Vector Control Product Assessment

WHO Public Assessment Report: WHOPAR Part 5

YAHE 4.0
(Fujian Yamei Industry & Trade Co. Ltd)
P-04983
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.

YAHE 4.0 is a homogenous ITN incorporated with alpha-cypermethrin 6.25 g AI/kg and PBO 2.25 g AI/kg 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 pyrethroid insecticide with the PBO synergist is that the alpha-cypermethrin insecticidal activity provides knockdown and/or kill of mosquitoes and the PBO inhibits mixed function oxidases implicated in resistance in pyrethroid resistant *Anopheles spp.* malaria vectors.

Semi-field studies to characterize the performance of YAHE 4.0 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 and ambient chamber studies

Data on the semi-field performance of YAHE 4.0 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 T202102011, T202102012, T202102013, 2023052001A, 2023052002B and 2023052002B.

Two semi-field studies were presented to evaluate the efficacy and wash resistance of YAHE 4.0 in Tanzania (EHT) and India (IACT). ITNs used in semi-field studies were prepared using the wash interval determined in 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 controls were a prequalified product incorporated with 5.8 g AI/kg alpha-cypermethrin, hereafter referred to as PC1, and a prequalified product incorporated with 6.0 g AI/kg alpha-cypermethrin, and 2.2 g AI/kg PBO, hereafter referred to as PC2.

The product was tested against a pyrethroid resistant natural population of *Anopheles arabiensis* in Lupiro, Tanzania, which carries metabolic resistance which is mediated by the overexpression of P450 monooxygenases and esterases. In India, the product was tested against a colonized strain of *Anopheles stephensi* which carries metabolic resistance mediated by the overexpression of P450 monooxygenases. Susceptibility testing with and without pre-exposure to PBO using WHO cylinder bioassays was conducted using 2 - 5 days old F1 female mosquitoes emerging from larvae collected from breeding sites close to the experimental huts at each study site in Tanzania. Susceptibility testing without pre-exposure to PBO using WHO cylinder bioassays was conducted using 2 – 5 days old colonized female mosquitoes in Tanzania and India. The results of the susceptibility testing are presented in Table 1.

The results from the free-flying mosquito studies are presented in Table 2. YAHE 4.0 ITNs that were washed 20 times using a 1-day wash interval induced 28.1% 24-hour mortality in free-flying *An. arabiensis* mosquitoes in Tanzania. In the ambient chamber tests conducted using colonized *An. stephensi* mosquitoes, 20x washed YAHE 4.0 ITNs induced 91.2% 24-hour mortality. Statistical analyses demonstrated that the mortality observed in the 20x washed YAHE 4.0 trial arm in Tanzania was significantly higher than in the pyrethroid-only control arm ($p = 0.000$); no statistical analyses of the performance of 20x washed YAHE 4.0 against the positive control were provided for the study in India.

Supplementary bioassays were used to characterize the bioavailability of the active ingredients on the surface of the ITN pre- and post-semi-field studies using unwashed and 20x washed nets. WHO 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. The selected insecticide susceptible test system used in Tanzania was *An. gambiae* Ifakara strain; in India, *An. stephensi* susceptible. The selected pyrethroid resistant test systems were *An. arabiensis* Kingani strain in Tanzania and *An. stephensi* resistant in India. Thresholds of $\geq 95\%$ knockdown and/or $\geq 80\%$ mortality in WHO cone tests and $\geq 80\%$ mortality or $\geq 90\%$ 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 3 and 4. In WHO cone tests using insecticide susceptible test systems, 24-hour mortality greater than 80% was observed in all unwashed and 20x washed arms before and after the EHTs. In WHO cone tests using pyrethroid resistant test systems, 24-hour mortality greater than 80% was observed in all unwashed arms before and after the EHTs. Mortality greater than 80% was not observed for 20x washed ITNs, excepting tests conducted after the EHT in Tanzania.

Table 1. Phenotypic insecticide resistance status of colonized test systems used in bioassays and IACT studies and F1 adults reared from larval collections conducted at the EHT site in Lupiro, Tanzania using WHO cylinder tests (%M24)

Mosquito species/ strain	Permethrin (0.75%)	Deltamethrin (0.05%)	Alpha-cypermethrin (0.05%)	Alpha-cypermethrin (0.30%)	Permethrin (0.75%) + PBO (4%)	Deltamethrin (0.05%) + PBO (4%)	Alpha-cypermethrin (0.05%) + PBO (4%)
<i>An. arabiensis</i> (EHT site)	16	48	22	-	65	67	64
<i>An. gambiae</i> Ifakara strain	100	100	100	-	-	-	-
<i>An. arabiensis</i> Kingani strain	3	46	12	-	-	-	-
<i>An. stephensi</i> susceptible	-	-	100	-	-	-	-
<i>An. stephensi</i> resistant	-	-	-	56	-	-	-

Table 2. Mortality and blood feeding inhibition of free-flying, pyrethroid resistant *An. arabiensis* in an experimental hut trial and free-flying, pyrethroid resistant *An. stephensi* in one ambient chamber study

Product	Washing condition	% M24 (95% CI)	% Feeding inhibition (95% CI)	Sample size
Tanzania (<i>An. arabiensis</i>)				
Total number of mosquitoes collected = 4,572			Compliant with power calculation? Yes	
Control	Unwashed	9.4 (4.3 – 14.5)	-	619
YAHE 4.0	Unwashed	27.5 (21.8 – 33.3)	78.9 (61.1 – 96.7)	908
	20x washed	28.1 (20.6 – 35.5)	93.3 (86.4 – 100)	800
PC1	Unwashed	24.6 (19.7 – 29.6)	68.8 (54.3 – 83.4)	1,103
	20x washed	18.1 (13.6 – 22.5)	69.7 (54.8 – 84.6)	1,144
India (<i>An. stephensi</i>)				
Total number of mosquitoes released = 6,250			Compliant with power calculation? Yes	
Control	Unwashed	6.4 (5.90 – 6.74)	-	1,250
YAHE 4.0	Unwashed	100 (100 - 100)	90.7 (90.35 – 91.08)	1,250
	20x washed	91.2 (89.93 – 92.47)	90.2 (89.77 – 90.78)	1,250
PC2	Unwashed	98.8 (98.46 – 99.30)	76.8 (75.44 – 78.30)	1,250
	20x washed	67.5 (65.55 – 69.49)	67.8 (64.38 – 71.26)	1,250

Table 3. Knockdown and mortality results for insecticide susceptible and pyrethroid resistant laboratory strains using WHO cone bioassays

	Tanzania					
	<i>An. gambiae</i> Ifakara			<i>An. arabiensis</i> Kingani		
	n	%KD60 (95% CI)	%M24 (95% CI)	n	%KD60 (95% CI)	%M24 (95% CI)
Before hut trial						
UW	300	100	94.7 (92.0 – 97.3)	300	99.7 (99.0 – 100)	80.0 (75.4 – 84.6)
20x washed	300	100	99.3 (98.4 – 100)	300	100	45.0 (38.3 – 51.7)
After hut trial						
UW	200	100	100	200	100	99.5 (98.5 – 100)
20x washed	200	100	100	200	100	96.5 (93.1 – 100)
	India					
	<i>An. stephensi</i> susceptible			<i>An. stephensi</i> resistant		
	n	%KD60 (95% CI)	%M24 (95% CI)	n	%KD60 (95% CI)	%M24 (95% CI)
Before IACT						
UW	50	100	100	50	100	100
20x washed	50	98 (92.45 – 100)	92 (81.61 – 100)	50	56 (39.34 – 72.66)	46 (31.84 – 60.16)
After IACT						
UW	50	100	100	50	96 (89.20-100)	88 (74.40-100)
20x washed	50	96 (89.20-100)	88 (74.40-100)	50	46 (22.89-45.11)	40 (31.22 -72.66)

Table 4. Knockdown and mortality results for insecticide susceptible and pyrethroid resistant laboratory strains using tunnel tests

	India				
	<i>An. stephensi</i> resistant				
	n	%M24 (95% CI)	Corrected %M24	% Blood fed	BFI (%)
Before hut trial					
20x washed	100	86.75 (83.22 – 90.28)	85.74 (81.75 -89.73)	11.25 (8.86 – 13.64)	84.24 (80.72 -87.77)
After hut trial					
20x washed	100	84.50 (82.45 – 86.55)	83.43 (81.42-85.44)	17.84 (14.45 – 20.55)	75.29 (71.71-78.88)

2.1.1 Chemical characterization

Data on the alpha-cypermethrin and piperonyl butoxide content of sampled pieces of the YAHE 4.0 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 T202102011, T202102012, and T202102013 for Tanzania and batches 2023052001A, 2023052002B, 2023052003C for India. The results are summarized in Table 5.

Table 5. AI content and retention of sampled pieces of YAHE 4.0 used in the semi-field studies in Tanzania (batch numbers T202102011, T202102012, and T202102013) and India (batch numbers 2023052001A, 2023052002B, 2023052003C)

	India (ACT study conducted by IIBAT)				Tanzania (conducted by IHI)			
	Mean alpha-cypermethrin content (g/kg)	Alpha-cypermethrin retention (per wash)	Mean PBO content (g/kg)	PBO retention (per wash)	Mean alpha-cypermethrin content (g/kg)	Alpha-cypermethrin retention (per wash)	Mean PBO content (g/kg)	PBO retention (per wash)
Before ACT (India)/ Before hut trial (Tanzania)								
UW	7.35		2.72		7.11	-	2.54	-
20x washed	5.37	73.06% (98.44%)	2.02	74.26% (98.52%)	5.5	77.36% (98.72%)	1.83	72.05% (98.37%)
Before ACT (India) / After hut trial (Tanzania)								
UW	6.70		2.66		6.85	-	2.36	-
20x washed	5.35	79.85% (98.88%)	2.09	78.57% (98.80%)	5.42	79.12% (98.84%)	1.74	73.73% (98.49%)

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, 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., $6.25 \pm 25\%$ g/kg for alpha-cypermethrin and $2.2 \pm 25\%$ g/kg for piperonyl butoxide.

2.2 Semi-field studies conclusions

The submitted semi-field studies demonstrate the impact of YAHE 4.0 on free-flying mosquitoes and the bioavailability of the treatments on the ITN fabric using WHO cone bioassays and insecticide susceptible test systems. Based on the submitted studies, the impact of YAHE 4.0 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 YAHE 4.0 fabric was sustained up to 20 washes against insecticide susceptible *An. gambiae* and *An. stephensi* test systems but not against pyrethroid resistant 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 6. List of studies related to efficacy submitted to WHO as part of the prequalification dossier

Studies that were relied upon for decision making.	
Study number	Study Title
BIT072	Phase II experimental hut trial of PBO Yahe® 4.0 in comparison to a standard pyrethroid MAGNet® LN
21498	Chemical analysis of different nets used in the hut study (Phase II trial) of Yahe 4.0 LN (Alpha-cypermethrin 250 mg/m ² and Piperonyl Butoxide 90 mg/m ²) before and after subjecting to different washes
23192	Evaluation of YAHE 4.0 LLIN - Alpha-cypermethrin 250mg/m ² ± 25% + Piperonyl butoxide (PBO) 90mg/m ² ± 25% incorporated into polyethylene filaments Determination of Efficacy and Wash Resistance against Pyrethroid Resistant Mosquito Strain <i>Anopheles stephensi</i> under Ambient Chamber Test (ACT) Condition - IIBAT, Padappai, Tamil Nadu, India.
Studies that were not used to inform decision making.	
CSRS21203	CSRS21203- YAHE 4.0 LLIN - Alphacypermethrin 250 mg/m ² ±25% + Piperonylbutoxide 90 mg/m ² ± 25% Incorporated into polyethylene filaments: Experimental hut evaluation of YAHE 4.0 against wild Pyrethroid-Resistant <i>Anopheles gambiae</i> in Côte d'Ivoire