World Health Organization

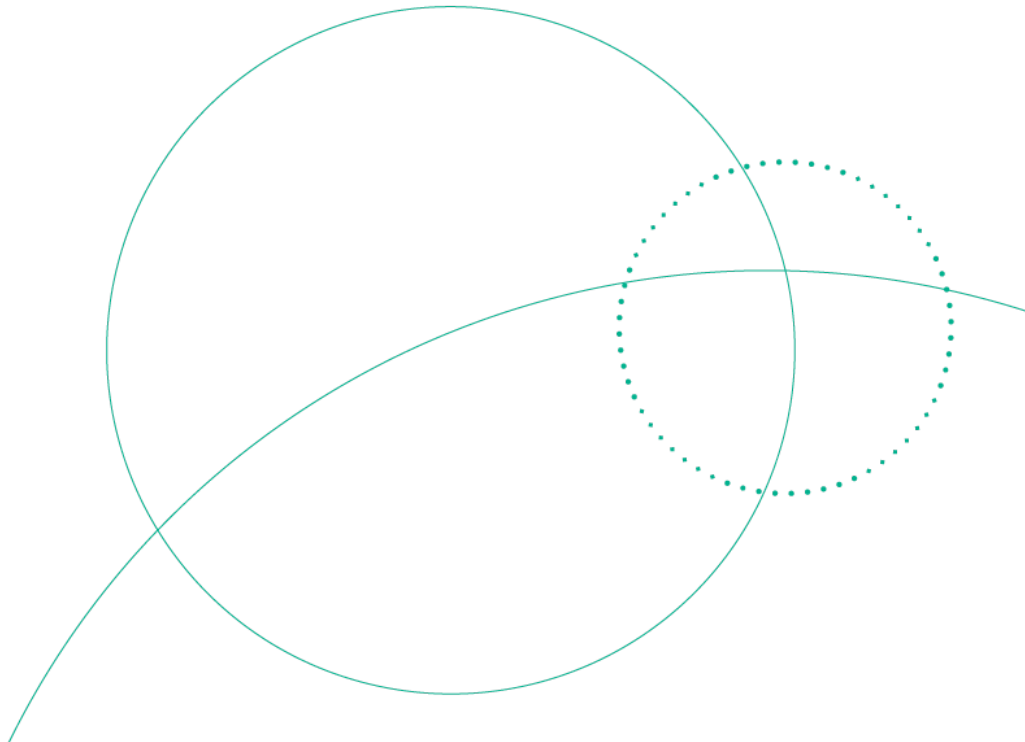
WHO Prequalification Programme / Vector Control Product Assessment

WHO Public Assessment Report: WHOPAR Part 5

Yorkool G3 LN
(Tianjin Yorkool)

021-003

Efficacy Assessment



Contents

1 Introduction 3
2 Semi-field Studies 3
2.1 Experimental hut trials 3
2.2 Chemical characterization 6
2.3 Semi-field studies conclusions 7
3 Efficacy conclusions 7

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.

Yorkool G3 LN is a homogenous ITN incorporated with deltamethrin 120 mg AI/m^2 and the synergist piperonyl butoxide (PBO) 440 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 insecticide and the synergist is that the deltamethrin 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 Yorkool G3 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 Yorkool G3 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 20180515, 20200415 and 20200326.

Two semi-field studies were presented to evaluate the efficacy and wash resistance of Yorkool G3 in Tanzania and in Benin. 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 24-hour mortality. The negative control used in each study was an untreated net and the positive controls were a prequalified product treated with 800 mg AI/m^2 permethrin and 400 mg AI/m^2 PBO, hereafter referred to as PC1 and an ITN treated with 55 mg AI/m^2 deltamethrin, hereafter referred to as PC2.

The product was tested against pyrethroid resistant natural populations of, in Lupiro, Tanzania, *An. arabiensis* with pyrethroid resistance mediated by the over-expression of cytochrome P450 enzymes and observed mortality to the diagnostic doses of deltamethrin, permethrin and alpha-cypermethrin of 48%, 16% and 22%, respectively. Resistance testing with PBO pre-exposure conducted prior to the semi-field trial restored susceptibility to 67% (deltamethrin), 65% (permethrin), and 64% (alpha-cypermethrin). In Covè, Benin, the vector population at the semi-field site was *An. gambiae* s.l., carrying *kdr* gene frequencies of >90% L1014F and metabolic resistance through over-expression of CYP6P3, and observed mortality to the diagnostic doses of deltamethrin and permethrin of 42% and 11%, respectively. Resistance testing with PBO pre-exposure conducted prior to the semi-field trial restored susceptibility to 72% (deltamethrin), and 8% (permethrin).

The results from the free-flying mosquito studies are presented in Table 1. Yorkool G3 ITNs that were washed 20 times using a 1-day wash interval induced 49.3% and 19.3% 24-hour mortality in free-flying *An. gambiae* s.l. mosquitoes in Tanzania and Benin, respectively. Statistical analyses demonstrated that the mortality observed in the 20x Yorkool G3 trial arms was significantly higher than the comparator PC2 (Tanzania: OR 2.46, 95% CI 2.23-2.72, p = <0.0001; Benin: Mortality 19.8% vs 9.7%, P<0.001).

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 Tanzania, the *An. gambiae* s.s. Ifakara strain was used as an insecticide susceptible test system. The pyrethroid resistant test system used was the *An.arabiensis* Kingani strain; the observed mortality from resistance testing to deltamethrin, permethrin, alpha-cypermethrin, lambda-cyhalothrin, etofenprox, and pirimiphos methyl was 25%, 13%, 16%, 4%, 67% and 100%, respectively. In Benin, the *An. gambiae* s.s. Kisumu strain was used as an insecticide susceptible test system. The pyrethroid resistant test system used was *An. gambiae* s.l Covè strain. 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% and 24-hour mortality greater than 80% was observed using unwashed and 20x washed nets, before and after the EHT in Tanzania and Benin. In WHO cone tests using pyrethroid resistant test systems, 60-minute KD greater than 95% was observed using unwashed and 20x washed nets, before and after the EHT in Tanzania. Mortality greater than 80% was observed using unwashed and 20x washed nets after the EHT. In Benin, KD greater than 95% was not observed; mortality greater than 80% was not observed.

In tunnel tests using insecticide susceptible test systems in Benin, 24-mortality greater than 80% and blood feeding inhibition greater than 90% was observed against unwashed and 20x nets. In tests using insecticide resistant test systems, 24-hour mortality greater than 80% and blood feeding inhibition greater than 90% was observed against unwashed and 20x nets.

Table 1. Mortality and Blood Feeding Inhibition of Free-Flying Pyrethroid Resistant *An. gambiae* s.l. in two Experimental Hut Trials				
Product	**Washing condition**	**% M24 (95% CI)**	**% Feeding inhibition (95% CI)**	**Sample Size**
Tanzania (*An.gambiae* s.l)				
Total number of mosquitoes collected = 21,965			Compliant with power calculation? Yes	
Control	Unwashed	11.1 (8.1-14.1)	-	1,277
Yorkool G3	Unwashed	52.4 (47.1-57.8)	94.5 (92.3-96.7)	2,750
	20x washed	49.3 (45.0-53.5)	97.2 (96.4-98.0)	3,584
PC1	Unwashed	43.8 (39.5-48.1)	98.3 (97.6-99.1)	3,154
	20x washed	34.7 (30.4-38.9)	97.7 (97.8-98.6)	4,202
PC2	Unwashed	26.8 (22.9-30.6)	93.8 (91.7-95.8)	2,727
	20x washed	27.6 (24.4-30.8)	95.8 (94.8-96.8)	4,271
Benin (*An.gambiae* s.l.)				
Total number of mosquitoes collected = 2,693			Compliant with power calculation? Yes	
Control	Unwashed	1.4 (0.4-2.4)		518
Yorkool G3	Unwashed	20.6 (15.1-26.1)	61.1 (54.5-67.7)	209
	20x washed	19.3 (14.8-23.8)	64.2 (58.7-69.7)	296
PC1	Unwashed	14.4 (9.7-19)	79.5 (74.1-84.9)	216
	20x washed	8.2 (5.8-10.7)	8.8 (6.3-11.3)	473
PC2	Unwashed	13 (9.6-16.4)	5.8 (3.4-8.2)	377
	20x washed	7.6 (5.5-9.7)	0.8 (0.1-1.5)	604

Table 2. Knockdown and mortality results for insecticide susceptible and pyrethroid resistant laboratory strains using WHO cone bioassays												
	Tanzania						**Benin**					
	An. gambiae Ifakara			*An. arabiensis* Kingani			*An. gambiae* Kisumu			*An. gambiae* Covè		
	n	%KD60 (95% CI)	%M24 (95% CI)	n	%KD60 (95% CI)	%M24 (95% CI)	n	%KD60 (95% CI)	%M24 (95% CI)	n	%KD60 (95% CI)	%M24 (95% CI)
Before hut trial												
UW	300	100	100	300	100	68.7 (63.7-73.6)	50	100	100	49	32.7 (20-46)	10.2 (2-19)
20x washed	300	100	100	300	100	67.0 (62.0-72.0)	53	96.2 (91-100)	100	48	16.7 (6-27)	4.2 (0-10)
After hut trial												
UW	200	100	100	200	100	84.5 (79.4 –89.6)	42	100	100	46	21.7 (10-34)	10.9 (2-20)
20x washed	200	100	100	200	100	94.5 (91.3-97.7)	49	100	100	45	13.3 (3-23)	4.4 (0-10)

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

	Benin					
	An. gambiae Kisumu			*An. gambiae* Covè		
	n	%BFI	%M24 (95% CI)	n	%BFI	%M24 (95% CI)
UW	226	96.1	100 (95-100)	209	99	96 (93-99)
20x washed	240	93.5	100 (95-100)	190	99	88 (83-93)

2.2 Chemical characterization

Data on the deltamethrin and piperonyl butoxide content of sampled pieces of the Yorkool G3 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 20180515 and 20180516 (for Tanzania) and batches 20200326, 20200415, & 20200518 (for Benin). The results are summarized in Table 4.

Table 4. AI content and retention of sampled pieces of Yorkool G3 used in the semi-field studies in Tanzania (batch numbers 20180515, 20180516) and Benin (batch numbers 20200326, 20200415, 20200518)

	Tanzania				Benin			
	Mean deltamethrin content (g/kg)	Deltamethrin retention (per wash)	Mean piperonyl butoxide content (g/kg)	Piperonyl butoxide retention (per wash)	Mean deltamethrin content (g/kg)	Deltamethrin retention (per wash)	Mean piperonyl butoxide content (g/kg)	Piperonyl butoxide retention (per wash)
Before hut trial								
UW	3.08	-	11.26	-	3.10	-	11.11	-
20x washed	2.91	94.48% (99.72%)	8.52	75.67% (98.62%)	2.87	92.58% (99.62%)	8.36	75.25% 98.59%
After hut trial								
UW	3.07	-	11.21	-	3.09	-	11.07	-
20x washed	2.91	94.79% (99.73%)	8.53	76.09% (98.64%)	2.85	92.23% (99.60%)	8.32	75.16% (98.58%)

The mean AI content presented in Table 4 was determined based on 15 net samples belonging to 2 batches (for Tanzania) and 15 net samples belonging to 3 batches (for Benin) 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 $^{n}\sqrt{(t_n/t_0)}$ 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., 3.0 ± 25% g/kg for deltamethrin and 11.0 ± 25% g/kg for piperonyl butoxide).

2.3 Semi-field studies conclusions

The submitted semi-field studies demonstrate the impact of Yorkool G3 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 Yorkool G3 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 Yorkool G3 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
BIT 059	The non-inferiority testing of Yorkool G3 LN in comparison to Olyset Plus LN and Permanet 2.0 LN in the experimental huts against wild mosquitoes in Tanzania.
20-06	WHO/PQ experimental hut evaluation of the efficacy and wash resistance of Yorkool G3 (a PBO and deltamethrin treated LLIN) by Tianjin Yorkool International Trading Co., Ltd against pyrethroid resistant *Anopheles gambiae* sl in Cove, Southern Benin.
BIT030	Chemical content analysis of Yorkool G3 LN at the Ifakara Health Institute (IHI) in Tanzania
Studies that were not used to inform decision making	
	None