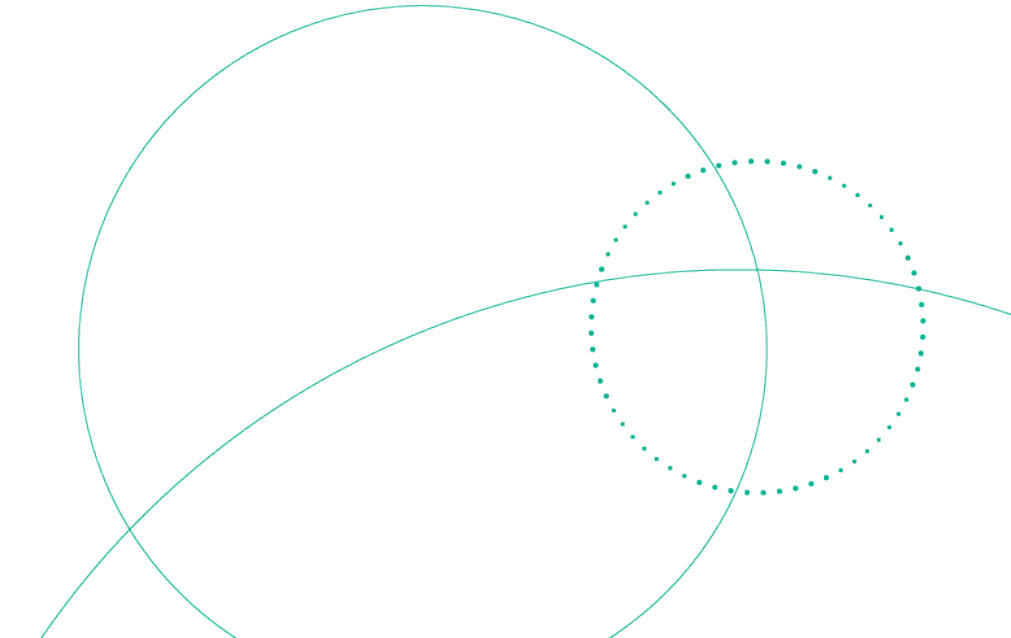
World Health Organization

WHO Prequalification Programme / Vector Control Product Assessment

# WHO Public Assessment Report: WHOPAR Part 5

## Vector Guard
## (Disease Control Technologies LLC)
## P-09284

## Efficacy Assessment



# Contents

# 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.

Vector Guard 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 Vector Guard 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 Vector Guard 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 RS120W-APBO120R, RS121W-APBO121R, RS122W-APBO122R.

Two semi-field studies were presented to evaluate the efficacy and wash resistance of Vector Guard in African settings, 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 for the Vector Guard ITNs and the wash interval determined in previous studies for the control ITNs. The endpoint used to evaluate efficacy and bioavailability was 24-hour mortality, and in free-flying studies Vector Guard was considered to

meet the WHO requirements for prequalification if it performed as well as or better than the positive control in terms of its capacity to kill or inhibit mosquito feeding after 20 washes using standardized wash methods.

The negative control used in each study was an untreated net and the positive controls were a prequalified product treated with 20 g AI/kg permethrin and 10 g AI/kg PBO, hereafter referred to as PC1, and an ITN treated with 5.8 g AI/kg alpha-cypermethrin, hereafter referred to as PC2.

The product was tested against pyrethroid resistant natural populations of: i) *An. arabiensis* in Lupiro, Tanzania, that have an upregulation of CYP450, and ii) *An. gambiae s.l.* in Cove, Benin, carrying *kdr* gene frequencies of >90% L1014F and metabolic resistance through over-expression of CYP6P3. The insecticide resistance of mosquitoes reared from larvae collected at each of the experimental hut sites was characterized using WHO cylinder tests. The population in Tanzania showed restoration of susceptibility following pre-exposure to PBO; pre-exposure to PBO restored partial susceptibility to alpha-cypermethrin to the population in Benin (Table 1).

**Table 1. Insecticide resistance profiles of field strains of *An. gambiae* s.l. in Tanzania and Benin using WHO cylinder tests**

| M24 (%) | Permethrin (0.75%) | Deltamethrin (0.05%) | Alpha-cypermethrin (0.05%) | Permethrin (0.75%) + PBO (4%) | Deltamethrin (0.05%) + PBO (4%) | Alpha-cypermethrin (0.05%) + PBO (4%) |
|---|---|---|---|---|---|---|
| **Tanzania** | | | | | | |
| *An. arabienisis* (Lupiro) | **51** | **18** | **12** | **99** | **100** | **98** |
| **Benin** | | | | | | |
| *An. gambiae* (Cove) | **2.1** | **-** | **4** | **2** | **-** | **34** |

The results from the free-flying mosquito studies are presented in Table 2. Vector Guard ITNs that were washed 20 times using a two-day wash interval induced 20% and 17.23% 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 Vector Guard trial arms was significantly higher than the comparators PC1 (Tanzania: 20% vs 15%, OR 1.50, 95%CI 1.21,1.86, p=<0.001 (superiority analysis); Benin: 17.2% vs 8.8%, p=<0.001) and PC2 (Tanzania: 20% vs. 10%, OR 2.30, 95%CI 1.85, 2.86, p<0.001; Benin: 17.2% vs. 9.9%, p<0.001).

Vector Guard ITNs that were washed 20 times using a two-day wash interval induced 90% and 54.39% blood-feeding inhibition in Tanzania and Benin, respectively. Statistical analyses demonstrated that the blood-feeding success for Vector Guard was inferior to PC1 and not statistically significantly different to PC2 in Tanzania (PC1 superiority analysis: 0.5% vs 0.4%. OR 1.65, 95%CI 1.03, 2.63, p=0.037; PC2 superiority analysis: 0.5% vs 0.7%, OR 0.68, 95%CI 0.45, 1.03, p 0.066); in Benin, the blood feeding success for Vector Guard was significantly lower than feeding success for PC1 (27.3% vs 46.5%, p<0.05) and PC2 27.63% vs. 44.9%, p<0.05).

| Table 2. 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 = 46,835 | | | Compliant with power calculation? Yes | |
| Control | Unwashed | 0.7 (0.4-1.1) | - | 7,654 |
| Vector Guard | Unwashed | 40 (34-45) | 89 (82-93) | 3,513 |
| | 20x washed | 20 (1-24) | 90 (85-93) | 6,092 |
| PC1 | Unwashed | 22 (19-26) | 97 (94-98) | 5,343 |
| | 20x washed | 15 (13-17) | 93 (90-96) | 7,795 |
| PC2 | Unwashed | 21 (19-25) | 91 (86-94) | 7,484 |
| | 20x washed | 10 (8-12) | 85 (79-90) | 8,954 |
| **Benin (*An. gambiae* s.l.)** | | | | |
| Total number of mosquitoes collected = 6,825 | | | Compliant with power calculation? Yes | |
| Control | Unwashed | 1.04 (0.4-1.7) | - | 1,055 |
| Vector Guard | Unwashed | 36.42 (33.2-39.7) | 74.20 (71.2-77.2) | 832 |
| | 20x washed | 17.23 (14.8-19.7) | 54.39 (51.2-57.6) | 923 |
| PC1 | Unwashed | 17.55 (15.1-19.9) | 75.16 (72.4-77.9) | 957 |
| | 20x washed | 8.77 (7.1-10.5) | 23.17 (20.7-25.7) | 1,083 |
| PC2 | Unwashed | 27.24 (24.4-30.1) | 59.78 (56.6-62.9) | 936 |
| | 20x washed | 9.91 (8.1-11.7) | 25.95 (23.9-28.6) | 1,039 |

### 2.1.1 Non-inferiority analysis

Non-inferiority analyses were conducted on the results from the free-flying mosquito trials, using 24-hour mortality as the primary endpoint and blood-feeding success as a secondary endpoint (Table 3). For the mortality endpoint, in Tanzania the estimated odds ratios for the effect of Vector Guard compared to PC1 for unwashed, 20x washed and unwashed and 20x washed results combined were all in the direction of higher mortality for Vector Guard ITNs. In Benin, the estimated odds ratio for the mortality effect of Vector Guard compared to PC1 was 2.584 (95% CI 2.176-3.069), demonstrating non-inferiority of the Vector Guard product. Vector Guard therefore demonstrated non-inferiority compared to PC1 for the primary 24-hour mortality endpoint, as determined using current WHO criteria.

For the blood-feeding endpoint, Vector Guard did not demonstrate non-inferiority to PC1 in Tanzania but did demonstrate non-inferiority in Benin (Table 3).

**Table 3. Non-inferiority analysis of free-flying pyrethroid resistant *An. gambiae* s.l. in two experimental hut trials**

| | Unwashed | | 20x washed | | Pooled | |
|---|---|---|---|---|---|---|
| Indicator and reference | OR (95% CI) | Interpretation | OR (95% CI) | Interpretation | OR (95% CI) | Interpretation |
| Tanzania (*An. gambiae* s.l.) | | | | | | |
| M24 [PC1] | 2.37 (1.89 - 2.95) | Not non-inferior and superior | 1.50 (1.21 - 1.86) | Not non-inferior and superior | 1.88 (1.60 – 2.19) | Not non-inferior and superior |
| BF [PC1] | 4.87 (2.60 - 9.10) | Not non-inferior and inferior | 1.65 (1.03 – 2.63) | Not shown to be non-inferior | 2.47 (1.69 – 3.59) | Not non-inferior and inferior |
| Benin (*An. gambiae* s.l.) | | | | | | |
| M24 [PC1] | - | - | - | - | 2.584 (2.176 – 3.069) | Non-inferior |
| BF [PC1] | - | - | - | - | 0.546 (0.469 – 0.635) | Non-inferior |

## 2.2 Supplementary bioassays

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. Both sites conducted supplementary bioassays using insecticide susceptible and pyrethroid resistant test systems. The *An. gambiae* s.s. Ifakara strain was used as the insecticide susceptible test system in Tanzania and *An. gambiae* s.s. Kisumu was used as the insecticide susceptible test system in Benin. In Tanzania, the pyrethroid resistant *An. arabiensis* Kingani test system which carries pyrethroid resistance at 1x the diagnostic dose was used (Table 4); in Benin, the pyrethroid resistant test system used was *An. gambiae s.l* strain (Cove) carrying *kdr* gene frequencies of >90% L1014F and metabolic resistance through over-expression of CYP6P3. 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.

**Table 4. Insecticide resistance profile of laboratory reared *An. gambiae* s.s. Ifakara, *An. arabiensis* Kingani, and *An. gambiae* Kisumu test systems using WHO cylinder tests.**

| M24 (%) | Permethrin (0.75%) | Deltamethrin (0.05%) | Alpha-cypermethrin (0.05%) | Permethrin (0.75%) + PBO (4%) | Deltamethrin (0.05%) + PBO (4%) | Alpha-cypermethrin (0.05%) + PBO (4%) |
|---|---|---|---|---|---|---|
| **Tanzania** | | | | | | |
| *An. gambiae* s.s. Ifakara | **100** | **100** | **100** | **100** | **100** | **100** |
| *An. arabiensis* Kingani | **11** | **22** | **17** | **99** | **100** | **100** |
| **Benin** | | | | | | |
| *An. gambiae* s.s. Kisumu | **100** | | **100** | | | |

The results from supplementary bioassays are presented in Table 5. 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 EHTs in Tanzania and Benin. In WHO cone tests using pyrethroid resistant strain in Tanzania, 60-minute KD greater than 95% was demonstrated against unwashed nets before and after the EHT, and against 20x washed nets after the EHT; mortality greater than 80% was observed only in unwashed nets before and after the EHT.

In tunnel tests using insecticide resistant test systems in Tanzania and Benin, 24-hour mortality greater than 80% was not observed against 20x nets after the hut trial. Blood-feeding inhibition was greater than 90%.

**Table 5. Knockdown and mortality results for insecticide susceptible and pyrethroid resistant laboratory strains using WHO cone bioassays**

| | Washing condition | Tanzania | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | *An. gambiae* Ifakara | | | | | | *An. arabiensis* (Kingani) | | | | | |
| | | Cone bioassays | | | Tunnel tests | | | Cone bioassays | | | Tunnel tests | | |
| | | n | %KD60 (95% CI) | %M24 (95% CI) | n | %BFI | %M24 (95% CI) | n | %KD60 (95% CI) | %M24 (95% CI) | n | %BFI | %M24 (95% CI) |
| Vector Guard roof | Before hut trial | | | | | | | | | | | | |
| | UW | 180 | 100 | 100 | - | - | - | 180 | 100 | 100 | - | - | - |
| | 20x washed | 180 | 100 | 95.0 (91.5-98.9) | - | - | - | 180 | 85.0 (76.7-93.3) | 15.0 (9.5-20.5) | | 100 | 71.3 (68.0-74.6) |
| | After hut trial | | | | | | | | | | | | |
| | UW | 120 | 100 | 100 | - | - | - | 120 | 100 | 100 | - | - | - |
| | 20x washed | 120 | 100 | 100 | - | - | - | 120 | 100 | 78.3 (67.8-88.9) | - | - | - |
| Vector Guard sides | Before hut trial | | | | | | | | | | | | |
| | UW | 240 | 100 | 100 | - | - | - | 240 | 100 | 100 | - | - | - |
| | 20x washed | 240 | 100 | 100 | - | - | - | 240 | 92.5 (87.3-97.3) | 31.3 (24.0-38.5) | - | - | - |
| | After hut trial | | | | | | | | | | | | |
| | UW | 160 | 100 | 100 | - | - | - | 160 | 100 | 95.0 (90.3-99.7) | - | - | - |
| | 20x washed | 160 | 100 | 100 | - | - | - | 160 | 100 | 93.1 (88.9-97.3) | - | - | - |
| | | Benin | | | | | | | | | | | |
| | | *An. gambiae* Kisumu | | | | | | *An. gambiae* (Cove) | | | | | |
| Vector Guard roof | Before hut trial | | | | | | | | | | | | |
| | UW | 25 | 100 | 100 | - | - | - | - | - | - | 208 | 100 | 99 (97.7 – 100) |
| | 20x washed | 24 | 100 | 100 | - | - | - | - | - | - | 197 | 93.78 | 72.1 (65.8 – 78.3) |
| | After hut trial | | | | | | | | | | | | |
| | UW | 24 | 100 | 100 | - | - | - | - | - | - | - | - | - |
| | 20x washed | 26 | 100 | 100 | - | - | - | - | - | - | - | - | - |
| Vector Guard sides | Before hut trial | | | | | | | | | | | | |
| | UW | 32 | 100 | 100 | - | - | - | - | - | - | - | - | - |
| | 20x washed | 35 | 100 | 100 | - | - | - | - | - | - | - | - | - |
| | After hut trial | | | | | | | | | | | | |
| | UW | 34 | 99.1 (91-100) | 100 | - | - | - | - | - | - | - | - | - |
| | 20x washed | 36 | 100 | 100 | - | - | - | - | - | - | - | - | - |

## 2.3 Semi-field studies conclusions

The submitted semi-field studies demonstrate the impact of Vector Guard 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 Vector Guard ITNs that have been prepared using a two-day wash interval can be sustained against pyrethroid resistant free-flying mosquitoes up to 20 washes. The bioavailability of the treatments on the Vector Guard fabric was sustained up to 20 washes against insecticide susceptible and pyrethroid resistant test systems of the *An. gambiae* complex.

## 2.4 Chemical characterization

Data on the alpha-cypermethrin and piperonyl butoxide content of sampled pieces of the Vector Guard 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 RS120W-APBO120R, RS121W-APBO121R, and RS122W-APBO122R (for Tanzania) and batches RS120W-APBO120R, RS121W-APBO121R (for Benin). The results are summarized in Table 6.

**Table 6. AI content and retention of sampled pieces of Vector Guard used in the semi-field studies (RS120W-APBO120R, RS121W-APBO121R, and RS122W-APBO122R)**

| | Tanzania | | | | Benin | | | |
|---|---|---|---|---|---|---|---|---|
| | 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 hut trial | | | | | | | | |
| Roof | | | | | | | | |
| UW | 5.75 | - | 18.70 | - | 5.23 | - | 16.1 | - |
| 20x washed | 4.08 | 70.96% (98.30%) | 7.09 | 37.91% (95.27%) | 5.39 | 103.06% (100.15%) | 13.4 | 83.23% (99.09%) |
| Sides (120D) | | | | | | | | |
| UW | 5.37 | - | - | - | 5.16 | - | - | - |
| 20x washed | 4.45 | 82.87% (99.06%) | - | - | 4.90 | 94.96% (99.74%) | - | - |
| After hut trial | | | | | | | | |
| Roof | | | | | | | | |
| UW | 5.62 | - | 16.20 | - | 5.71 | - | 15.5 | - |
| 20x washed | 4.59 | 81.67% (98.99%) | 10.12 | 97.7 | 5.08 | 88.97% (99.42%) | 13.7 | 88.39% (99.38%) |
| Sides (120D) | | | | | | | | |
| UW | 5.03 | - | - | - | 5.18 | - | - | - |
| 20x washed | 4.63 | 92.05% (99.59%) | - | - | 4.99 | 96.33% (99.81%) | - | - |

For Tanzania, the mean AI content presented in Table 6 was determined based on 12 net samples (for sides) and 9 net samples (for roof) for unwashed (UW) product and after 20 washes before hut trial and 8 net samples (for sides) and 6 net samples (for roof) for unwashed (UW) product and after 20 washes after hut trial, all belonging to 3 batches (RS120W-APBO120R, RS121W-APBO121R, and RS122W-APBO122R).

For Benin, the mean AI content presented in Table 6 was determined based on 4 net samples (for sides) and 3 net samples (for roof) for unwashed (UW) product and after 20 washes before hut trial and 4 net samples (for sides) and 3 net samples (for roof) for unwashed (UW) product and after 20 washes after hut trial, all belonging to 2 batches (RS120W-APBO120R, RS121W-APBO121R).

AI retention per wash in Table 6 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., 5.8 ± 25% g/kg for alpha-cypermethrin - for the sides -, and 5.8 ± 25% g/kg for alpha-cypermethrin and 20.3 ± 25% g/kg for piperonyl butoxide – for the roof).

# 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 7. 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** |
| BIT063 | Laboratory and experimental hut evaluation of Vector Guard® Insecticide Treated Net (ITN) in comparison with Olyset® Plus ITN and Royal Sentry® 2.0 ITN against strongly pyrethroid-resistant Anopheles arabiensis and fully susceptible Anopheles gambiae s.s. in Tanzania |
| 20-08/GLP | Phase II experimental hut evaluation of the efficacy and wash resistance of Vector Guard® (an alpha-cypermethrin and PBO incorporated net) by Disease Control Technologies, LLC, USA against pyrethroid resistant Anopheles gambiae sl in Cove, Southern Benin |
| 25517 | Chemical analysis of net samples from the Phase II experimental hut study of Vector Guard® compared to Olyset® Plus and Royal Sentry® 2.0 in Tanzania |
| 25518 | Chemical analysis of net samples from the Phase II experimental hut study of Vector Guard® compared to Olyset® Plus and Royal Sentry® 2.0 in Benin |
| **Studies that were not used to inform decision making** | |
| | **None** |