Research Open Access

Efficacy of a novel mode of action of an indoor residual spraying product, SumiShield® 50WG against susceptible and resistant populations of Anopheles gambiae (s.l.) in Benin, West Africa

Fiacre R. Agossa 1*, Gil G. Padonou 1,2, Come Z. Kouko 1,3, Jacques Zola-Sahossi 1, Roseric Azondekon 1, Osei K. Akuoko 4, Juniace Ahoga 1, Boris N’dombidje 1, Bruno Akinro 1, Arsene Jacques Y. Fassinou 1,3, Michel Sezonlin 1,2 and Martin C. Akogbeto 1,2

Abstract

Background: Scale-up of the distribution of long-lasting insecticide-treated bed nets and indoor residual spraying with insecticides over the last decade have contributed to the considerable decrease of malaria morbidity and mortality in sub-Saharan Africa. Due to the increasing pyrethroid resistance intensity and the spread of carbamate resistance in Anopheles gambiae (s.s.) mosquitoes and the limited number of insecticides recommended by the WHO for vector control, alternative insecticide formulations for IRS with long-lasting residual activity are required to sustain the gains obtained in most malaria-endemic countries.

Methods: SumiShield 50WG (clothianidin 300 mg ai/m²) developed by Sumitomo Chemical was evaluated alongside deltamethrin 25 mg ai/m² (K-Othrine 250 WG) against a pyrethroid resistant Anopheles gambiae (s.l.) population in experimental huts in Covè, Benin. Residual activity was also tested in cone bioassays with the susceptible An. gambiae “Kisumu” strain and the local wild resistant population.

Results: The results showed very low toxicity from deltamethrin (mortality rates ranged between 1–40%) against host-seeking resistant Anopheles populations. SumiShield in contrast gave an overall mean mortality of 91.7% at the 120 h observation across the eight-month observation period following spraying. The residual activity measured using cone tests was over the 80% WHO threshold for 24 weeks for resistant wild Anopheles population and 32 weeks for the susceptible strain “Kisumu” after the spraying.

Conclusions: SumiShield is a good candidate for IRS in areas of permanent malaria transmission and where Anopheles populations are resistant to other conventional insecticides such as pyrethroids. It would be interesting to complete experimental huts studies by assessing the efficacy and residual effect of SumiShield 50WG at community level (small-scale field testing) in an area where vectors are highly resistant to insecticides.

Keywords: Anopheles gambiae (s.l.), SumiShield 50WG, Clothianidin, Experimental hut, Efficacy, Covè, Benin

* Correspondence: rofargossa@yahoo.fr
1Centre de Recherche Entomologique de Cotonou (CREC), Cotonou, Bénin
Full list of author information is available at the end of the article

© The Author(s). 2018 Open Access This article is distributed under the terms of the Creative Commons Attribution 4.0 International License (http://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made. The Creative Commons Public Domain Dedication waiver (http://creativecommons.org/publicdomain/zero/1.0/) applies to the data made available in this article, unless otherwise stated.
Background
The first lesson learnt from the monitoring and evaluation of indoor residual spraying (IRS) in Africa is the variation in the residual life of the main insecticides used for indoor residual spraying: bendiocarb (carbamate) and pirimiphos methyl (organophosphate). Actellic EC (pirimiphos methyl) and Ficam VC (bendiocarb) have shown a short residual effect in several countries such as Benin, Mali, Rwanda and Equatorial Guinea [1–4]. In previous IRS programs, pirimiphos methyl capsule suspension (CS) showed a longer residual effect in Ghana compared to other countries such as Benin, Ethiopia, Liberia, Mali, Senegal and Zimbabwe [4]. The second problem is the emergence of carbamate and organophosphate resistance [5]. Pyrethroid resistance is widespread in most malaria endemic countries and since carbamate resistance has arisen in some countries, only organophosphates, the remaining class of insecticides recommended by the WHO, is used by National Malaria Control Programmes for indoor residual spraying and for resistance management. For the National Malaria Control Programmes and the international community, it is judicious to prevent the rapid and widespread resistance to carbamates and organophosphates by evaluating new insecticide formulations with different modes of action and a long residual effect.

The solution proposed by the WHO for resistance management [6] appears difficult to implement due to the limited number of insecticides recommended for IRS. The mitigation of the spread of vector resistance is to avoid subjecting Anopheles to the same products or those with the same mode of action over several years or to reduce resistance selection pressures in order to weaken the resistance gene carriers toward these products. In the Atacora, Alibori and Donga areas in Benin where pirimiphos methyl CS is the insecticide used for IRS, the NMCP recommends its usage for not more than two or three years and suggests the implementation strategy for resistance management based on the rotational use of two or three insecticides with different modes of action.

Overall, given the limited number of insecticides recommended for LLINs and IRS since the 1970s and malaria vectors developing resistance to most insecticide classes, formulations of insecticides with new modes of action are needed for resistance management. The Innovative Vector Control Consortium (IVCC) has developed novel active ingredients. These new candidates should be on the market by around 2022 for IRS. Also, new formulations and new classes of insecticides could be recommended for LLINs impregnation.

SumiShield® 50WG (clothianidin) developed by Sumitomo, is a good candidate as needed by all NMCPs in Africa, and has prequalification listing. SumiShield® contains clothianidin, a neonicotinoid insecticide not previously used for vector control. As a result, it is expected that pyrethroid resistant mosquito populations should be killed by SumiShield 50WG. This study was implemented to evaluate the efficacy and residual effect of SumiShield 50WG applied at 300 mg ai/m² against wild pyrethroids resistant Anopheles population in semi-field conditions in experimental huts in Benin, West Africa.

Methods
Study design
The study was conducted in the experimental station at Covè, Southern Benin, over a period of 8 months (October 2015 to May 2016). The huts were built alongside a paddy field which constitutes the permanent breeding sites for Anopheles mosquitoes. Adult volunteers were recruited among the inhabitants of the location (Covè). Pregnant and breast-feeding women were not involved in the study. After having announced through the district for volunteers, selection was done after approval was granted by the traditional head of the study site.

Sleepers (mosquito collectors) were rotated randomly among huts each night following a Latin square design. They entered the huts at dusk (21:00 h) and remained inside until dawn. In the morning at 6:30 h, dead mosquitoes were collected from the floor of the huts and resting mosquitoes from the walls, roof and exit traps using aspirators. Mosquitoes were scored by location as dead or alive and as fed or un-fed. Live mosquitoes were placed in small cups and provided with 10% sugar solution for 24, 48, 72, 96 and 120 h to assess delayed mortality. All wild An. gambiae (s.l.) mosquitoes caught per night and per hut were taken into account. In each month, sleepers collected free-flying host-seeking Anopheles gambiae (s.l.) from Covè during four Latin square weeks per month (24 days). One Latin square week was equal to six days. A sleeper slept once in each of the six huts used during six successive nights and rested on the seventh night. The wild mosquito populations were complemented with the susceptible An. gambiae “Kisumu” strain which were reared in the insectary of the Centre de Recherche Entomologique de Cotonou (CREC) and released in the huts. At least 20 females of An. gambiae “Kisumu” strain aged 5–8 days were released in each hut over one night per month; these numbers were kept reasonably low to prevent sleepers from receiving too many mosquito bites.

The primary parameters measured in these experimental huts according to the duration of the trial were: (i) deterrence: reduction in hut entry relative to the control huts (untreated hut); (ii) induced exophily: the proportion of mosquitoes that exited early and
were found in exit traps; (iii) blood-feeding inhibition: the reduction in blood-feeding compared with that in the control hut; (iv) immediate mortality: the proportion of mosquitoes that were killed at the end of the exposure time; and (v) delayed mortality: the proportion of mosquitoes that were killed after 24, 48, 72, 96 and 120 h.

The huts used were designed to simulate local and typical West African households (Fig. 1). The description and how the host-seeking mosquitoes were captured as described by [7]. Six experimental huts (smooth cement) were used in this study: 2 huts for SumiShield 50WG (contains 50% w/w clothianidin), 2 huts for K-Othrine 250WG (Deltamethrin 250 WG, contains 25% w/w deltamethrin) and 2 untreated (control).

**Insecticide treatment**
The maximum safety instructions and protective measures were observed. The operators who treated the walls wore full protective clothing: long-sleeved shirt and trousers, hat, rubber boots, gloves and a particle-filtering half-mask.

**Dilution process**
Standard nozzles recommended by the WHO for malaria vector control are types 8002 or 8001. A standard nozzle type 8002 was used. This nozzle delivers a volume of 760 ml of insecticide solution / min (for 19 m²). If a sprayer does not have a CFV (control flow valve), an average application rate of 40 ml/m² is assumed. In the present study, the nozzle was accompanied by a red CFV (1.5 bar pressure) which delivers a volume of 570 ml of insecticide solution/ min (for 19 m²) or 30 ml/m².

(i) Dilution of SumiShield 50WG. The capacity of the sprayer used (Hudson Xpert, Chicago, USA) was 6 l. The sprayer was rinsed with clean water after each treatment. For a volume of 6 l, the dilution was: \( Q = \frac{(300 \text{ mg} \times 6000 \text{ ml})}{30 \text{ ml}} = 60,000 \text{ mg} \) of clothianidin in 6 l of water. The SumiShield 50WG contained 50% clothianidin. Therefore 120 g (120,000 mg) of SumiShield 50WG was diluted in 6 l of water to treat 200 m². To manage the remaining diluted insecticide after treatment, each insecticide solution was adjusted to each hut surface area. For hut 1 (17 m²), the dilution was 10,200 mg SumiShield 50WG in 510.0 ml of water. For hut 5 (16.74m²), dilution was 10,044 mg SumiShield 50WG in 502.0 ml of water.

(ii) Dilution of K-Othrine 250WG. For a volume of 6 l of the sprayer (Hudson Xpert) used, the dilution was: \( Q = \frac{(6000 \text{ ml} \times 25 \text{ mg})}{30 \text{ ml}} = 5000 \text{ mg} \) of deltamethrin in 6 l of water. K-Othrine 250WG contained 25% of deltamethrin; therefore, the dilution was 20 g (20,000 mg) of K-250 WG Othrine in 6 l of water to treat 200 m². To manage the rest of diluted insecticide after treatment, each insecticide solution was adjusted to each hut surface area. For hut 3 (16.86 m²), the dilution was 1686 mg of K-250 WG Othrine in 506 ml of water. For hut 4 (16.00 m²), the dilution was 1600 mg of K-250 WG Othrine in 480 ml of water. Huts 2 and 6 were used as control (untreated).

**Measurement of pH of the wall substrates**
The purpose of this activity was to intermittently follow the evolution of the pH of the wall surfaces and the efficacy of the impregnated insecticides as well. A small quantity (5 g of substrate) from the wall surface of each hut was scraped into a Petri dish before, during and at the end of the study. The substrates were dissolved in distilled water for the determination of the pH in CREC.

**Status of resistance of the wild An. gambiae population of the study area**
Susceptibility tests using WHO tubes were performed using 2–5 day-old aged adult female mosquitoes.

![Fig. 1 Experimental hut station at Covè, Benin](image-url)
Detection of Leu-Phe kdr mutation was performed with untested mosquito samples using PCR following the protocol described by [8].

**WHO cone bioassay**

For each month, WHO cone bioassays were performed. A laboratory colony of *An. gambiae* “Kisumu” strain which were fully susceptible to all insecticides and a wild population of *An. gambiae* (s.l.) resistant to pyrethroids sampled around the study site were used. The WHO cone bioassays [9] were carried out in week 1, and each month after spraying. During the whole study, cones were placed at fixed level (0.5, 1, 1.5 and 2 m) on the walls (treated and untreated huts). Each level was randomly selected and labelled to one wall surface of each hut. For each month, at least 10 females of the *An. gambiae* susceptible reference strain “Kisumu” and wild *An. gambiae* (s.l.) mosquitoes aged 3–5 days were introduced per cone for 30 min exposure. After this exposure period, knockdown mosquitoes were recorded and those alive were kept in observation for 24, 48, 72, 96 and 120 h to score delayed mortalities. Mosquitoes exposed to unsprayed substrates were used as controls. When the mortality in the control was between 5 and 20%, corrected mortality was determined using Abbot’s formula.

**Statistical analysis**

The raw data were managed with Microsoft Excel and the statistical analyses were performed using R software. The WHO criteria were used to classify the level of resistance of tested mosquitoes from Covè to insecticides. The WHO bio-efficacy threshold was used for the analyses of the residual effect in time per treatment. Others analyses on the significance between each of the bio-efficacy measured parameters in treated huts were made and compared between treatment and control (untreated) using R software.

**Results**

**Analysis of pH of samples of wall substrates**

Analysis of the pH of wall samples of the huts was done 7 days prior to spraying, and 4 and 8 months after spraying in the huts. The mean pH observed is indicated in Table 1. No significant variation of mean pH was observed between insecticide treatments at the observation times (4 and 8 months after spraying).

**Resistance level of *An. gambiae* (s.l.) population from the experimental huts station**

Prior to spraying, the resistance status of the population of *An. gambiae* (s.l.) from the study site was investigated. The results showed that the *Anopheles* population from the experimental hut station was resistant to deltamethrin 0.05% (mortality rate: 27.45%) and susceptible to pirimiphos methyl 0.25% (mortality rate: 100%). A resistance to bendiocarb 0.1% was also suspected (Fig. 2).

**Resistance mechanisms in *Anopheles* population**

PCR results showed a high frequency (0.95) of knockdown resistance (kdr) and very low frequency (0.03) of acetylcholinesterase insensitive mutation (Ace-1) in the *Anopheles* population from the experimental hut station (Table 2).

**Efficacy of clothianidin 50WG against host-seeking *Anopheles* populations in the experimental huts station**

Over the period of eight months of evaluation of the efficacy of clothianidin 50WG in comparison to that of deltamethrin 250WG, 24,135 host-seeking *An. gambiae* (s.l.) were collected from the six experimental huts used in this study. Among these mosquitoes, 35.7% (8609), 31.4% (7577) and 33.0% (7949) were collected from the untreated huts (control huts), the huts treated with clothianidin and those treated with deltamethrin, respectively. The results of the measured parameters are shown below.

| Table 1 pH measured on walls prior to spraying, 4 and 8 months after spraying |
|-----------------|-----------------|--------|---------|
| Treatment       | Structure ID    | Time   | Respective pH values | Mean pH | SD |
| Control         | H2 and H6       | T0     | 7 ; 7              | 7       | 0  |
| Control         | H2 and H6       | T4     | 7 ; 7              | 7       | 0  |
| Control         | H2 and H6       | T8     | 7 ; 7              | 7       | 0  |
| Deltamethrin    | H3 and H4       | T0     | 7 ; 6              | 6.5     | 0.70 |
| Deltamethrin    | H3 and H4       | T4     | 7 ; 8              | 7.5     | 0.70 |
| Deltamethrin    | H3 and H4       | T8     | 9 ; 8              | 8.5     | 0.70 |
| SumiShield      | H1 and H5       | T0     | 6 ; 6              | 6       | 0  |
| SumiShield      | H1 and H5       | T4     | 8 ; 8              | 8       | 0  |
| SumiShield      | H1 and H5       | T8     | 7 ; 10             | 8.5     | 2.12 |

Abbreviations: H2 and H6 controls 1 and 2, H3 and H4 deltamethrin 1 and 2, H1 and H5 SumiShield 1 and 2, T0 prior to spraying, T4 4 months after spraying, T8 8 months after spraying, SD standard deviation
Exophily
Induced exophily is the proportion of mosquitoes that exited the huts and were thus found in the exit trap veranda. Overall, all treatments induced in the host-seeking *An. gambiae* (s.l.) population an exit behavior from the treated surfaces to the exit trap’s veranda (Table 3). However, significantly higher exophily rates were observed with deltamethrin ($P < 0.0001$).

Blood-feeding
Blood-feeding inhibition describes the reduction in blood-feeding compared with that in the control huts. Irrespective of whether the huts were treated or not, no difference was observed on the blood-feeding of mosquitoes in the experimental huts (Table 4). The blood-feeding rates were over 80% in the treated and control huts.

Toxicity effect
The toxicity or lethal property of deltamethrin was low (ranging between 1 and 40%). The mortality rates recorded with this insecticide decreased after one month (Fig. 3). However, high mortality rates (mean mortality rate of 91.7% at the 120 h observation point) were recorded over a period of eight months on the evaluation with clothianidin. While good initial mortality was observed, mortality continued to increase across observation times to 120 h indicating the additional killing properties of clothianidin.

Efficacy of clothianidin 50WG against laboratory susceptible *Anopheles* populations in the experimental huts station
The results from releasing activities are summarized in Tables 5, 6 and Fig. 4. The exophily and blood-feeding rates observed in the huts treated either with clothianidin or deltamethrin were not significantly higher than those observed in the untreated huts (Tables 5, 6). However, clothianidin induced high mortality rates (about 100%) post-120 h observation time over a period of seven months of the evaluation. The induced mortality rate by deltamethrin decreased to about 80% at month 8. The additional killing effect of clothianidin over time against the susceptible *Anopheles* population released in the huts was also noticed (Fig. 4).

Residual effect of clothianidin 50WG against susceptible strain “Kisumu” and wild in experimental huts
Cone bioassay results
The efficacy of each treatment in terms of mortality rate observed after exposure of mosquitoes to treated walls was compared to the WHO bio-efficacy threshold [9], 80% and to that recorded in the untreated huts. The mortality rates over 5% in control huts were corrected using Abbott’s formula. During the eight months of the evaluation, the huts treated with clothianidin showed the mortality rates over 80% at the 120 h observation point against susceptible *Anopheles* population “Kisumu” (Fig. 5). Concerning the wild *An. gambiae* (s.l.) population from Covè (experimental hut station) the mortality rates over 80% were observed until six months in the huts treated...
Table 3 Exophily rates observed per month and per treatment with wild host-seeking *Anopheles* populations

| Treatment  | Month | Total number | Proportion | 95% CI       | P-value* |
|------------|-------|--------------|------------|--------------|----------|
| Control 1  | 1     | 386          | 33.68      | 28.96–38.39  | –        |
|            | 2     | 563          | 28.60      | 24.86–32.33  | –        |
|            | 3     | 630          | 20.16      | 17.03–23.29  | –        |
|            | 4     | 379          | 30.34      | 25.71–34.97  | –        |
|            | 5     | 411          | 33.82      | 29.25–38.39  | –        |
|            | 6     | 445          | 29.89      | 25.63–34.14  | –        |
|            | 7     | 647          | 32.77      | 29.15–36.38  | –        |
|            | 8     | 802          | 23.19      | 20.27–26.11  | –        |
| Control 2  | 1     | 531          | 30.51      | 26.59–34.42  | 0.31     |
|            | 2     | 731          | 29.96      | 26.64–33.28  | 0.59     |
|            | 3     | 722          | 25.62      | 22.44–28.81  | 0.02     |
|            | 4     | 523          | 30.78      | 26.83–34.74  | 0.89     |
|            | 5     | 481          | 27.86      | 23.85–31.87  | 0.05     |
|            | 6     | 427          | 28.57      | 24.29–32.86  | 0.67     |
|            | 7     | 475          | 34.32      | 30.05–38.59  | 0.59     |
|            | 8     | 456          | 36.84      | 32.41–41.27  | < 0.0001 |
| SumiShield 1| 1   | 379          | 33.51      | 28.76–38.26  | 0.96     |
|            | 2   | 453          | 33.33      | 28.99–37.67  | 0.10     |
|            | 3   | 683          | 20.35      | 17.33–23.37  | 0.93     |
|            | 4   | 426          | 26.53      | 22.33–30.72  | 0.23     |
|            | 5   | 365          | 26.58      | 22.04–31.11  | 0.03     |
|            | 6   | 424          | 28.54      | 24.24–32.84  | 0.66     |
|            | 7   | 484          | 34.71      | 30.47–38.95  | 0.49     |
|            | 8   | 614          | 36.64      | 32.83–40.46  | < 0.0001 |
| SumiShield 2| 1   | 317          | 33.12      | 27.94–38.30  | 0.88     |
|            | 2   | 513          | 29.24      | 25.30–33.18  | 0.82     |
|            | 3   | 500          | 31.40      | 27.33–35.47  | < 0.0001 |
|            | 4   | 370          | 32.70      | 27.92–37.48  | 0.49     |
|            | 5   | 439          | 34.62      | 30.17–39.07  | 0.80     |
|            | 6   | 431          | 31.79      | 27.39–36.18  | 0.54     |
|            | 7   | 562          | 37.01      | 33.02–41.08  | 0.12     |
|            | 8   | 617          | 40.36      | 36.49–44.23  | < 0.0001 |
| Deltamethrin 1| 1  | 450          | 77.56      | 73.70–81.41  | < 0.0001 |
|            | 2   | 523          | 75.33      | 71.64–79.03  | < 0.0001 |
|            | 3   | 454          | 61.67      | 57.20–66.15  | < 0.0001 |
|            | 4   | 482          | 70.75      | 66.69–74.81  | < 0.0001 |
|            | 5   | 405          | 73.09      | 68.77–77.41  | < 0.0001 |
|            | 6   | 469          | 65.25      | 60.94–69.55  | < 0.0001 |
|            | 7   | 560          | 57.50      | 53.41–61.59  | < 0.0001 |
|            | 8   | 652          | 55.21      | 51.40–59.03  | < 0.0001 |
| Deltamethrin 2| 1  | 388          | 79.12      | 75.08–83.17  | < 0.0001 |
|            | 2   | 542          | 75.09      | 71.45–78.73  | < 0.0001 |
|            | 3   | 487          | 62.42      | 58.12–66.72  | < 0.0001 |
|            | 4   | 369          | 78.05      | 73.83–82.27  | < 0.0001 |
|            | 5   | 441          | 74.15      | 70.06–78.24  | < 0.0001 |
|            | 6   | 490          | 64.90      | 60.67–69.12  | < 0.0001 |
|            | 7   | 637          | 61.54      | 57.76–65.32  | < 0.0001 |
|            | 8   | 600          | 58.67      | 54.73–62.61  | < 0.0001 |

Abbreviation: CI confidence interval

*5% significance threshold*
Table 4  Blood-feeding observed with wild host-seeking *Anopheles* populations

| Treatment   | Month | Total number | Proportion | 95% CI      | P-value<sup>a</sup> |
|-------------|-------|--------------|------------|-------------|---------------------|
| Control 1   | 1     | 386          | 91.97      | 89.26–94.68 | –                    |
|             | 2     | 563          | 94.32      | 92.40–96.23 | –                    |
|             | 3     | 630          | 93.02      | 91.03–95.01 | –                    |
|             | 4     | 379          | 88.92      | 85.76–92.08 | –                    |
|             | 5     | 411          | 93.19      | 90.75–95.62 | –                    |
|             | 6     | 445          | 97.98      | 96.67–99.29 | –                    |
|             | 7     | 647          | 89.64      | 87.30–91.99 | –                    |
|             | 8     | 802          | 96.63      | 95.39–97.88 | –                    |
| Control 2   | 1     | 531          | 93.97      | 91.95–95.70 | 0.24                 |
|             | 2     | 731          | 92.48      | 90.56–94.39 | 0.19                 |
|             | 3     | 722          | 84.63      | 81.99–87.26 | < 0.0001             |
|             | 4     | 523          | 92.73      | 90.51–94.96 | 0.05                 |
|             | 5     | 481          | 96.67      | 95.07–98.28 | 0.02                 |
|             | 6     | 427          | 92.27      | 89.74–94.80 | < 0.0001             |
|             | 7     | 475          | 94.95      | 92.98–96.92 | < 0.0001             |
|             | 8     | 456          | 94.08      | 91.91–96.25 | 0.03                 |
| SumiShield 1| 1     | 379          | 94.46      | 92.16–96.76 | 0.17                 |
|             | 2     | 453          | 92.27      | 89.81–94.73 | 0.19                 |
|             | 3     | 683          | 91.36      | 89.25–93.47 | 0.27                 |
|             | 4     | 426          | 96.01      | 94.15–97.87 | < 0.0001             |
|             | 5     | 365          | 96.99      | 95.23–98.74 | 0.02                 |
|             | 6     | 424          | 96.70      | 94.53–98.02 | 0.24                 |
|             | 7     | 484          | 94.63      | 92.62–96.64 | < 0.0001             |
|             | 8     | 614          | 84.36      | 81.49–87.24 | < 0.0001             |
| SumiShield 2| 1     | 317          | 90.85      | 87.68–94.03 | 0.60                 |
|             | 2     | 513          | 90.45      | 87.90–92.99 | 0.02                 |
|             | 3     | 500          | 89.80      | 87.15–92.45 | 0.05                 |
|             | 4     | 370          | 92.62      | 88.80–94.44 | 0.21                 |
|             | 5     | 439          | 97.95      | 96.62–99.28 | < 0.0001             |
|             | 6     | 431          | 95.36      | 93.37–97.35 | 0.03                 |
|             | 7     | 562          | 95.37      | 93.64–97.11 | < 0.0001             |
|             | 8     | 617          | 88.01      | 85.44–90.57 | < 0.0001             |
| Deltamethrin 1| 1    | 450          | 88.00      | 84.67–90.69 | 0.06                 |
|             | 2     | 523          | 93.12      | 90.95–95.29 | 0.41                 |
|             | 3     | 454          | 91.41      | 88.83–93.99 | 0.33                 |
|             | 4     | 482          | 95.64      | 93.82–97.47 | < 0.0001             |
|             | 5     | 405          | 98.02      | 96.67–99.38 | < 0.0001             |
|             | 6     | 469          | 96.16      | 94.42–97.90 | 0.11                 |
|             | 7     | 560          | 89.82      | 87.32–92.33 | 0.92                 |
|             | 8     | 652          | 91.87      | 89.77–93.97 | < 0.0001             |
| Deltamethrin 2| 1    | 388          | 88.66      | 85.50–91.81 | 0.12                 |
|             | 2     | 542          | 90.77      | 88.34–93.21 | 0.02                 |
|             | 3     | 487          | 89.94      | 87.27–92.61 | 0.06                 |
|             | 4     | 369          | 95.39      | 93.25–97.53 | < 0.0001             |
with clothianidin (Fig. 6). The mortality rate decreased a little below 80% from the seventh month (77.9%) to the eighth month (60.3%). Mortality rates increased each month across observation times demonstrating the additional time-dependent killing effect of clothianidin.

**Knockdown effect**

The efficacy of each treatment in terms of knockdown rate obtained after 30 min exposure in cone bioassays of mosquitoes to treated walls was compared to the WHO threshold bio-efficacy [9] which is 95% for LLINs, and to that observed in the untreated huts. Overall, the knockdown rates in the structures treated with clothianidin were very low (< 95% for most) compared with those obtained in the huts treated with deltamethrin (Fig. 7).

**Discussion**

The present study assessed the efficacy and residual effect of a novel mode of action Indoor Residual Spray product, SumiShield 50WG under semi-field conditions in West Africa (Covè, Bénin) where An. gambiae (s.l.) is resistant to pyrethroids. SumiShield was evaluated against a population of An. gambiae characterized by a high frequency (0.95) of knockdown resistance (kdr) and elevated oxidase and glutathione S-transferase (GST) activities. After eight months of evaluation, SumiShield 50WG showed a better efficacy and lasting residual effect compared to deltamethrin. The results were encouraging since deltamethrin was ineffective against a pyrethroid resistant host-seeking Anopheles population from Covè during the whole study period.

The exophily of host-seeking wild An. gambiae (s.l.) recorded with deltamethrin was significantly higher than what was observed in the control huts. This is not unexpected for pyrethroids. During the evaluation period, the blood-feeding rates were high in both treated and control huts. Irrespective of the huts treated or not, the blood-feeding behavior was the same. This is, however, not worrying; many phase II and phase III evaluations implemented in Benin continuously demonstrated that despite the treatment of the houses with insecticides, the majority of mosquitoes successfully enter these treated houses and take their blood meal of their host before resting on the treated walls [10, 11]. Indeed, this is not unexpected.

### Table 4

| Treatment | Month | Total number | Proportion | 95% CI | P-value* |
|-----------|-------|--------------|------------|--------|----------|
| Sumi      | 5     | 441          | 98.19      | 96.94–99.43 | < 0.0001 |
|           | 6     | 490          | 97.96      | 96.71–99.21 | 0.98     |
|           | 7     | 637          | 92.62      | 90.59–94.65 | 0.06     |
|           | 8     | 600          | 93.83      | 91.91–95.76 | 0.01     |

Abbreviation: CI confidence interval

*a*5% significance threshold

---

![Fig. 3](image_url)

**Fig. 3** Mortality rates recorded per observation time and per treatment against free-flying, host-seeking Anopheles population from Covè. SumiImmediate and Sumi24h, Sumi48h, Sumi72h, Sumi96h and Sumi120h refer to the mortality rates observed each month 24, 48, 72, 96 and 120 h observation times in the huts treated with SumiShield, respectively. DeltaImmediate and Delta24h, Delta48h, Delta72h, Delta96h and Delta120h refer to the mortality rates observed each month 24, 48, 72, 96 and 120 h observation times in the huts treated with deltamethrin, respectively.
for IRS treatments except for DDT that has some spatial action as well as contact activity. This finding explains why it was proposed to Benin NMCP to always educate communities who are protected by IRS to additionally sleep under LLINs to supplement and maximize malaria control efforts. Such combination strategy implicates an increase in the cost of malaria prevention, but it is desirable for areas with high levels of malaria transmission. Fortunately, 95% of blood-fed *An. gambiae* (s.l.) die after resting on the treated walls in huts treated with clothianidin 50WG. This will keep them from staying alive to continue

| Treatment       | Month | Total number | Proportion | 95% CI          | P-value* |
|-----------------|-------|--------------|------------|-----------------|----------|
| Control 1       | 2     | 30           | 66.67      | 42.81–90.52     | –        |
|                 | 3     | 27           | 62.96      | 44.75–81.18     | –        |
|                 | 4     | 26           | 26.92      | 9.87–43.97      | –        |
|                 | 5     | 13           | 53.85      | 26.75–80.95     | –        |
|                 | 6     | 32           | 28.13      | 12.55–43.70     | –        |
|                 | 8     | 31           | 38.71      | 21.56–55.86     | –        |
| Control 2       | 2     | 32           | 68.75      | 46.04–91.46     | 0.90     |
|                 | 3     | 44           | 50.00      | 35.23–64.77     | 0.29     |
|                 | 4     | 30           | 50.00      | 32.11–67.89     | 0.08     |
|                 | 5     | 25           | 28.00      | 10.40–45.60     | 0.12     |
|                 | 6     | 49           | 36.73      | 23.24–50.23     | 0.42     |
|                 | 8     | 26           | 57.69      | 38.70–76.68     | 0.15     |
| SumiShield 1    | 2     | 56           | 75.00      | 63.66–86.34     | 0.52     |
|                 | 3     | 25           | 44.00      | 24.54–63.46     | 0.17     |
|                 | 4     | 35           | 45.71      | 29.21–62.22     | 0.13     |
|                 | 5     | 17           | 70.59      | 48.93–92.25     | 0.35     |
|                 | 6     | 27           | 37.04      | 18.82–55.25     | 0.47     |
|                 | 8     | 14           | 64.29      | 39.19–89.39     | 0.11     |
| SumiShield 2    | 2     | 27           | 48.15      | 29.30–67.00     | 0.25     |
|                 | 3     | 12           | 8.33       | 0.30–23.97      | < 0.0001 |
|                 | 4     | 49           | 16.33      | 5.98–26.68      | 0.27     |
|                 | 5     | 24           | 45.83      | 25.90–65.77     | 0.64     |
|                 | 6     | 38           | 63.16      | 47.82–78.50     | < 0.0001 |
|                 | 8     | 22           | 72.73      | 54.12–91.34     | 0.01     |
| Deltamethrin 1  | 2     | 47           | 48.94      | 34.64–63.23     | 0.23     |
|                 | 3     | 40           | 55.00      | 39.58–70.42     | 0.52     |
|                 | 4     | 32           | 46.88      | 29.58–64.17     | 0.12     |
|                 | 5     | 17           | 58.82      | 35.43–82.22     | 0.79     |
|                 | 6     | 13           | 46.15      | 19.05–73.25     | 0.24     |
|                 | 8     | 25           | 44.00      | 24.54–63.46     | 0.69     |
| Deltamethrin 2  | 2     | 21           | 61.90      | 41.13–82.68     | 0.77     |
|                 | 3     | 18           | 33.33      | 11.56–55.11     | 0.05     |
|                 | 4     | 42           | 0          |                 | < 0.0001 |
|                 | 5     | 19           | 47.37      | 24.92–69.82     | 0.72     |
|                 | 6     | 32           | 59.38      | 42.36–76.39     | 0.01     |
|                 | 8     | 22           | 68.18      | 48.72–87.65     | 0.03     |

Abbreviation: CI confidence interval

*5% significance threshold

Table 5: Exophily rates observed per treatment from month 2 to 8 after treatment with laboratory susceptible *Anopheles* populations released in the huts
malaria transmission. Furthermore, community-wide use of SumiShield 50WG in IRS will produce a “mass effect” on the reduction of the density of infective mosquitoes in the area and, consequently, protecting the whole community including those whose houses are not treated.

Given that SumiShield 50WG has demonstrated at least eight months (study period) efficacy in this study, this insecticide could be a good solution for IRS in areas of permanent malaria transmission. Clothianidin is a novel neonicotinoid insecticide acting as an agonist of the nicotinic acetylcholine receptor (nAChR).

### Table 6

| Treatment     | Month | Total number | Proportion | 95% CI     | P-value * |
|---------------|-------|--------------|------------|------------|-----------|
| Control 1     | 2     | 30           | 100        | 92.3–100   | –         |
|               | 3     | 27           | 51.85      | 33.99–69.26| –         |
|               | 4     | 26           | 65.38      | 47.10–83.67| –         |
|               | 5     | 13           | 69.23      | 44.14–94.32| –         |
|               | 6     | 32           | 96.88      | 0.85–98.33 | –         |
|               | 8     | 31           | 90.32      | 75.10–96.65| –         |
| Control 2     | 2     | 32           | 75.00      | 53.78–96.22| 0.04      |
|               | 3     | 44           | 88.64      | 79.26–98.01| < 0.0001  |
|               | 4     | 30           | 63.33      | 46.09–80.58| 0.87      |
|               | 5     | 25           | 92.00      | 75.03–97.78| 0.07      |
|               | 6     | 49           | 97.96      | 89.31–99.64| 0.76      |
|               | 8     | 26           | 57.69      | 38.70–76.68| < 0.0001  |
| SumiShield 1  | 2     | 56           | 80.36      | 69.95–90.76| 0.06      |
|               | 3     | 25           | 64.00      | 45.18–82.82| 0.38      |
|               | 4     | 35           | 48.57      | 32.01–65.13| 0.19      |
|               | 5     | 17           | 82.35      | 64.23–99.20| 0.4       |
|               | 6     | 27           | 88.89      | 77.03–96.30| 0.22      |
|               | 8     | 14           | 42.86      | 16.93–68.78| < 0.0001  |
| SumiShield 2  | 2     | 27           | 51.85      | 33.99–69.26| < 0.0001  |
|               | 3     | 12           | 33.33      | 6.66–60.01 | 0.28      |
|               | 4     | 49           | 30.61      | 17.71–43.52| < 0.0001  |
|               | 5     | 24           | 70.83      | 52.65–89.02| 0.92      |
|               | 6     | 38           | 68.42      | 53.64–83.20| < 0.0001  |
|               | 8     | 22           | 90.91      | 72.19–97.47| 0.94      |
| Deltamethrin 1| 2     | 47           | 19.15      | 7.90–30.40 | < 0.0001  |
|               | 3     | 40           | 57.50      | 42.18–72.82| 0.65      |
|               | 4     | 32           | 53.13      | 35.83–70.42| 0.35      |
|               | 5     | 17           | 64.71      | 41.99–87.42| 0.79      |
|               | 6     | 13           | 38.46      | 12.01–64.91| < 0.0001  |
|               | 8     | 25           | 48.00      | 28.42–67.58| < 0.0001  |
| Deltamethrin 2| 2     | 21           | 9.52       | 3.03–22.08 | < 0.0001  |
|               | 3     | 18           | 16.67      | 5.53–33.88 | 0.02      |
|               | 4     | 42           | 30.95      | 16.97–44.93| 0.01      |
|               | 5     | 19           | 15.79      | 0.61–32.19 | < 0.0001  |
|               | 6     | 32           | 100        | 97.12–100  | 0.31      |
|               | 8     | 22           | 9.09       | 1.53–21.10 | < 0.0001  |

Abbreviation: CI confidence interval

*5% significance threshold
receptor is different from those of the existing recommended insecticide families (organochlorine, pyrethroids, carbamates and organophosphates). So far, good performance of clothianidin against a resistant *Anopheles* population was demonstrated in cement built experimental huts of Malanville, Benin (Corbel, 2012, personal communication). The residual effect observed is superior to that of insecticides recommended by the WHO for indoor residual spraying [8]. The knockdown rates observed with clothianidin were low: this is not unexpected given the mode of action of this insecticide. Additionally, clothianidin has been intensively used in agriculture. Several studies have demonstrated that clothianidin is highly active not only against hemipteran insects but also coleopteran, thysanopteran, dipteran and some lepidopteran pests [12]. Because of its broad spectrum of insecticidal activity, good systemic properties and low mammalian toxicity, clothianidin is a compound that is considered to be compatible with integrated pest management strategies [13].

The use of the new cost-effective, long-lasting IRS insecticides with a new mode of action such as SumiShield for malaria control or elimination in
endemic countries will help support resistance management and the optimization of vectors control strategies. However, NMCPs should recommend the judicious use of these new insecticides in preventing the early selection and development of resistance in these malaria parasite vectors.

The additional killing effect of clothianidin over time against both susceptible and resistant *Anopheles* populations was noticed. Given this, it is important to study the impact of this action on the fertility of female malaria vectors subjected to clothianidin in terms of the number of eggs laid and the viability of the embryo from the egg. Furthermore, as the performance of clothianidin as observed in this study was beyond six months, it would also be important to implement another study at community level (small scale Phase III trial) to assess the efficacy and residual effect of SumiShield 50WG under field conditions.

![Fig. 6](image1.png) **Fig. 6** Residual effect of SumiShield 50WG and deltamethrin 250 WG represented by the mortality rates observed following cone bioassays against wild resistant *Anopheles gambiae* (s.s.) in the experimental hut of Covè. Sumi24h, Sumi48h, Sumi72h, Sumi96h and Sumi120h refer to the mortality rates observed each month 24, 48, 72, 96 and 120 h observation times in the huts treated with SumiShield, respectively. Delta24h, Delta48h, Delta72h, Delta96h and Delta120h refer to the mortality rates observed each month 24, 48, 72, 96 and 120 h observation times in the huts treated with deltamethrin, respectively.

![Fig. 7](image2.png) **Fig. 7** Efficacy represented by knockdown rate at 30 min after WHO cone bioassay per month, per observation time with SumiShield 50WG and deltamethrin 250 WG against susceptible strain "Kisumu" *Anopheles gambiae* (s.s.) 1M, 2M, 3M, 4M, 5M, 6M and 7M refer to 1, 2, 3, 4, 5, 6 and 7 months, respectively.
Conclusions
After eight months evaluation in semi-field conditions, a good efficacy and residual effect of SumiShield 50WG against both susceptible and pyrethroid resistant Anopheles population were observed. This insecticide with a novel mode of action for vector control could be a good alternative for IRS in areas of permanent malaria transmission and where mosquitoes are resistant to other insecticides.

Abbreviations
CPV: Control flow valve; CREC: Centre de Recherche Entomologique de Cotonou; CS: Capsule suspension; DDT: Dichlorodiphenyltrichloroethane; EC: Emulsifiable concentrate; IRS: Indoor residual spraying; IVC: Innovative Vector Control Consortium; KDR: Knockdown resistance; LLIN: Long-lasting insecticidal net; NMCP: National Malaria Control Programme; WG: Water dispersible granules; WHO: World Health Organization

Acknowledgements
We thank anonymous reviewers for their constructive comments on the manuscript. We acknowledge Sumitomo for their support. We also acknowledge all volunteers, mosquito collectors and local authorities for their participation in the study.

Funding
The research leading to these results was financially supported by Sumitomo Chemical Company Ltd. The funding partner did not participate in the design of the study, collection, analysis, interpretation of data or in writing the manuscript

Availability of data and materials
Data supporting the conclusions of this article are included within the article. Raw data will be made available upon request to the corresponding author.

Authors’ contributions
FRA, GGP and MCA designed the study. FRA, GGP, CZK, AJYHF, IZ, JA and BN carried out the experiment. FRA, RZ and BA analyzed the data. FRA drafted the manuscript. FRA, MS, OKA and MCA critically revised the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate
The project received approval (Avis éthique favorable No. 10 du 28 mai 2015, Letter No. 050/MS/DC/SGM/DFR/CNRES/SA DU 15 juin 2015) from the National Ethical Committee for Health Research (CNERS). The mosquito collectors gave their consents before being part of this study.

Funding
The project received approval (Avis éthique favorable No. 10 du 28 mai 2015, Letter No. 050/MS/DC/SGM/DFR/CNRES/SA DU 15 juin 2015) from the National Ethical Committee for Health Research (CNERS). The mosquito collectors gave their consents before being part of this study.

Revised: 6 February 2018 Accepted: 23 April 2018
Published online: 10 May 2018

References
1. Akogbéto MC, Aïkpon RY, Azondékon R, Padonou GG, Ossè RA, Agossa FR, et al. Six years of experience in entomological surveillance of indoor residual spraying against malaria transmission in Benin: lessons learned, challenges and outlooks. Malar J. 2015;14:242.
2. Aïkpon R, Agossa F, Ossè R, Oussou O, Azioun N, Ouk-Agbo F, et al. Bendiocarb resistance in Anopheles gambiae s.l. populations from Atacora department in Benin, West Africa: a threat for malaria vector control. Parasit Vectors. 2013;6:192.
3. Bradley J, Matias A, Schwabe C, Vargas D, Monti F, Nseng G, et al. Increased risks of malaria due to limited residual life of insecticide and outdoor biting venus protection by combined use of nets and indoor residual spraying on Bioko Island, Equatorial Guinea. Malar J. 2012;11:242.
4. Deneje D, Aklilu S, Bradford L, Benjamin J, Kristen G, Allison B, et al. Multi-country assessment of residual bio-efficacy of insecticides used for indoor residual spraying in malaria control on different surface types: results from program monitoring in 17 PMI/USAID-supported IRS countries. Parasit Vectors. 2018;11:71.
5. Edi CVA, Koumou BG, Jones CM, Weetman D, Rashan H. Multiple-Insecticide Resistance in Anopheles gambiae Mosquitoes, Southern Côte d’Ivoire. Emerg Infect Dis. 2012;18:1508–11.
6. WHO. World Health Organization Global Malaria Programme. In: World malaria report 2011. Geneva: World Health Organization; 2012. p. 30–3.
7. Agossa RF, Aïkpon R, Azondékon R, Govoetchan R, Padonou GG, Oussou O, et al. Efficacy of various insecticides recommended for Indoor Residual Spraying: Pirimiphos methyl, potential alternative to bendiocarb for pyrethroid resistance management from Benin, West Africa. Trans R Soc Trop Med Hyg. 2014;108:84–91.
8. Martinez-Torres D, Chandre F, Williamson MS, Dariel F, Bergé JB, Devonshire AL, et al. Molecular characterization of pyrethroid knockdown resistance (kdr) in the major malaria vector Anopheles gambiae s.s. Insect Mol Biol. 1998;7:179–84.
9. WHO. Guidelines for testing mosquito adulticides for indoor residual spraying and treatment of mosquito nets. Geneva: World Health Organization; 2006.
10. Akogbéto MC, Padonou GG, Gbémou D, Irish S, Yadouleton A. Research Bendiocarb, a potential alternative against pyrethroid resistant Anopheles gambiae in Benin, West Africa. Malar J. 2010;9:204.
11. Damien GB, Djènontin A, Rogier C, Corbel V, Bangana SB, Chandre F, et al. Malaria infection and disease in an area with pyrethroid-resistant vectors in southern Benin. Malar J. 2010;9:380.
12. Tomizawa M, Casida JE. Neonicotinoid insecticide toxicology: mechanisms of selective action. Annu Rev Pharmacol Toxicol. 2005;45:247–68.
13. Okawara Y, Akayama A, Matsuoka K, Andersch W. Clothianidin: a novel broad-spectrum neonicotinoid insecticide. Proc Br Crop Prot Coun, Pests Dis. 2002;151–8.