Laboratory and experimental hut trial evaluation of VECTRON™ T500 for indoor residual spraying (IRS) against insecticide resistant malaria vectors in Burkina Faso [version 1; peer review: 2 approved, 1 approved with reservations]

Koama Bayili¹, Hyacinthe D. Ki¹, Bazoma Bayili¹,², Bazoumana Sow¹,², Abdoulaye Ouattara¹, Graham Small³, Roch K. Dabire¹, Abdoulaye Diabate¹,²

¹Entomologist, Institut de Recherche en Sciences de la Santé, Bobo-dioulasso, 545, Burkina Faso
²Entomologist, Université Nazi Boni, Bobo-Dioulasso, Burkina Faso
³Senior Technical Manager, Innovative Vector Control Consortium, Liverpool, Liverpool L3 5QA, UK

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1. Johnson J. Matowo, Kilimanjaro Christian Medical University College, Moshi, Tanzania
2. Emmanuel Mbuba, Ifakara Health Institute, Bagamoyo, Tanzania
3. Rosemary S. Lees, Liverpool School of Tropical Medicine, Liverpool, UK

Katherine Gleave, Liverpool School of Tropical Medicine, Liverpool, UK
Giorgio Praulins, Liverpool School of Tropical Medicine, Liverpool, UK
George Parsons, Liverpool School of Tropical Medicine, Liverpool, UK

Abstract

Background: Malaria cases in some areas could be attributed to vector resistant to the insecticide. World Health Organization recommended insecticides for vector control are limited in number. It is essential to find rotational partners for existing Indoor Residual Spraying (IRS) products. VECTRON™ T500 is a novel insecticide with broflanilide as active ingredient. It has a mode of action on mosquitoes completely different to usually used. The aim of this study was to determine the optimum effective dose and efficacy of VECTRON™ against susceptible and resistant strains of Anopheles in Burkina Faso.

Methods: VECTRON™ was sprayed at 50, 100 and 200 mg/m² doses onto mud and concrete blocks using Potter Spray Tower. The residual activity of broflanilide was assessed through cone bioassays 1 week and then monthly up to 14 months post spraying. Its efficacy was evaluated at 100 and 150 mg/m² against wild free-flying mosquitoes in experimental huts on both substrates. Actellic 300CS was applied at 1000 mg/m² as reference product. Cone assays were conducted monthly, using susceptible and resistant mosquito strains.

Results: In the laboratory, VECTRON™ showed residual efficacy (≥80% mortality) on An. gambiae Kisumu up to 12 and 14 months, respectively, on concrete and mud blocks. Similar results were found with 100 and 200 mg/m² using An. coluzzii pyrethroid resistant strain. In experimental huts, a total of 19,552 An. gambiae s.l. were collected. Deterrence, blood-feeding inhibition and exophily with VECTRON™ treated huts were very low. At 100 and 150 mg/m², mortality of wild An. gambiae s.l. ranged between 55% and 73%. Monthly cone bioassay
mortality remained >80% up to 9 months.

**Conclusions:** VECTRON™ shows great potential as IRS formulation for malaria vector control. It can be added to the arsenal of IRS products for use in rotations to control malaria and manage mosquito insecticide resistance.

**Keywords**
Malaria, Anopheles gambiae, VECTRON™ T500, Insecticide Residual Spray (IRS), Pyrethroid resistance, Residual efficacy
Introduction

Malaria remains one of the most critical public health problems in Africa, despite intense national and international efforts to control it. According to the World Health Organization (WHO), malaria caused 409,000 deaths out of 229 million cases registered in 2019. A parasitic disease, malaria is caused by a hemotrophic protozoan of the genus *Plasmodium*. This pathogen is transmitted to humans during the bite of an infected *Anopheles* female mosquito. Current measures to control malaria are based on early detection and appropriate treatment of human cases, but also on prevention based primarily on mosquito vector control. Prevention is based mainly on the use of Long-Lasting Insecticidal Nets (LLINs) and indoor residual spraying (IRS), which aims to reduce vector densities and human-vector contact. Widespread deployment of LLINs and IRS by countries has played a crucial role in the reduction of malaria incidence and mortality in sub-Saharan Africa in the last 20 years. It was estimated that 1.5 billion malaria cases and 7.6 million malaria deaths have been averted during the period of 2000 to 2019 due to malaria control policy put in place by countries. These policies include mass distribution of insecticide-treated nets, mass use of IRS, prompt malaria cases management, and use of drugs to prevent malaria.

Indoor residual spraying (IRS) is one of the main vector control methods used for preventing malaria in many malaria-endemic countries. IRS can reduce malaria transmission by reducing female mosquito density and longevity when the IRS product is applied inside residential houses. The residual insecticide on the potential resting surfaces such as internal walls, eaves and ceilings is effective against female mosquitoes that contact these surfaces and are killed. Historically, IRS was the principal tool of the global malaria eradication campaign that allowed malaria elimination from Europe and several countries in the Americas and the Caribbean during the 1950s and 1960s. Reduction in malaria morbidity and mortality was observed in endemic countries in Africa and Asia that increased significantly the coverage of IRS during the last 20 years. Unfortunately, the success of malaria control programs is being compromised by the emergence and spread of insecticide resistance in major mosquito vector species.

This has led in recent years to the combination of IRS and LLIN in some African countries to increase the impact of vector control. The Global Plan for Insecticide Resistance Management (GPIRM) has recommended a rotation of non-pyrethroid insecticides with different modes of action for IRS in countries where IRS and LLINs are combined. The two non-pyrethroid insecticides currently formulated into IRS products which have been listed by WHO Prequalification Unit Vector Control Product Assessment Team (WHO PQT/VCP) are clothianidin (a neonicotinoid insecticide; available as SumiShield 50WG and coformulated with deltamethrin as Fludora Fusion) and pirimiphos-methyl (an organophosphorus insecticide formulated as Actellic 300CS). However, to properly implement an insecticide resistance management strategy based on the rotation of insecticides with different modes of action, IRS products containing at least 3 different insecticides will be required. Therefore, finding additional alternative insecticides with novel modes of action to vector control has become a priority. VECTRON™ T500, containing the active ingredient broflanilide (N-[2-bromo-4-(perfluoropropan-2-yl)-6-((trifluoromethyl)phenyl)-2-fluoro-3-(N-methylbenzamido)benzamid]), is a novel insecticide formulation developed by Mitsui Chemicals Agro, Inc., (MCAG; Tokyo, Japan) for IRS use to control malaria vectors or other pests. It has the potential to control mosquitoes that have become resistant to pyrethroids and other known classes of conventional insecticides. Broflanilide has been categorized as a member of a new group, Group 30: GABA-gated chloride channel allosteric modulators, by the Insecticide Resistance Action Committee (IRAC). It targets the GABA-receptor of chloride channels in the nervous system of insects. Broflanilide is a meta-diamide insecticide that has a distinct mode of action compared to conventional insecticides currently used in public health. There is currently no known cross-resistance to broflanilide via mechanisms of resistance to other public health insecticides. It has also shown low acute toxicity to non-target aquatic organisms, which demonstrates its high potential for use in public health and agriculture.

Before new vector control products can be introduced to the market, the optimal dose and formulation of the active ingredient must be determined. In addition, the residual efficacy of this dose must be evaluated against the target mosquitoes. It is in this context that this study aimed to determine the dose and efficacy, including the residual activity, of this new product, VECTRON™ T500, which is a wettable powder containing 50% broflanilide (w/w) as an active substance. VECTRON™ T500 was tested against susceptible and resistant strains of *Anopheles* malaria vectors in Burkina Faso. Firstly, a laboratory (Phase I) study was conducted using blocks made of different substrates, to determine the most suitable doses for field trials. Secondly, VECTRON™ T500 was tested at two application rates in an experimental hut (Phase II) trial using two different wall substrates, mud and concrete, to assess its efficacy against free flying mosquitoes following WHO guidelines.

Methods

Study area and mosquitoes

The laboratory (Phase I) study was conducted at the IRSS (Institut de Recherche en Sciences de la Santé) test facility in Burkina Faso under standard environmental conditions (27±2 °C and 75±10% relative humidity (RH)). The experimental hut (Phase II) trial was conducted at the field station in Vallée du Kou, an irrigated rice field area developed in 1970. The site is characterized by wooded savannah and covers 1,200 ha between 4°24′59″ longitude west and 11°24′ latitude and contains seven discrete villages. Mean annual rainfall is about 1,100 mm and rice is the major crop. Few insecticides are used on this crop, but they are widely used in the surrounding villages for cotton cultivation. Thanks to irrigation, the plain provides mosquitoes with permanent, sunny, and nutrient-rich breeding sites for the development of *Anopheles* larvae. Mosquitoes are found year-round, but the peak density is observed in August to September during the rainy season. *An. coluzzii* is predominant throughout the year and is highly
resistant to pyrethroids and dichlorodiphenyltrichloroethane (DDT) (kdr frequency: 0.8-0.95), with a rise in ace-1 frequency also being observed\cite{21-22}. Mechanisms of metabolic resistance, such as cytochrome P450s, esterases and also non-detoxification genes have been detected\cite{23,24}. The presence of multiple resistance genes in the mosquito populations makes this area an ideal site to evaluate the effectiveness of new insecticides against mosquitoes that are resistant to conventional insecticides.

**Laboratory (Phase I) study**

*Preparation and treatment of block substrates:* Two types of substrates were used to prepare IRS blocks for laboratory tests. Mud blocks were made by mixing 100g of mud and 25ml of water. The mud was from the experimental hut study site (Bama; 4°24’59” longitude west; 11°24’ latitude) to minimize variation between the mud used in phase I and phase II trials. Concrete blocks were made by mixing 33g cement, 66g sand and 20mL water. Blocks were shaped in Petri dishes (9 cm diameter and 1 cm thick). Mud blocks and concrete blocks were left to dry for a minimum of 1 week and for 1 month, respectively, at 27 °C ± 2 °C and 75% ± 10% relative humidity before insecticide being applied. The pH of the concrete blocks was tested on the day they were to be sprayed by scraping 5g of concrete from a block, adding 15ml distilled water, mixing thoroughly, and measuring with a pH meter (HANNA Instruments, model Hi 9813-5): blocks with a pH between 6-10 (mud and concrete) were judged suitable for use. The blocks were sprayed with the different treatments (Table 1) using a homogeneous solution of each dose. The VECTRON™ T500 product was provided by Mitsui Chemicals Agro, Inc. (MCAG). Spraying was done using a calibrated Potter Precision Laboratory Spray Tower (Burkard Manufacturing Co Ltd, Rickmansworth, UK) which is internationally recognized as the most precise method of chemical spraying in the laboratory as described in WHO testing guidelines\cite{25}. All treated blocks were stored at 30 °C ± 2 °C and 75% ± 10% RH in between bioassays. In total, five blocks of each substrate type were prepared and sprayed for each dose.

**Residual efficacy of broflanilide WP (VECTRON™ T500) in laboratory cone bioassays:** After spraying, WHO cone bioassays were performed according to WHO guidelines\cite{26} to evaluate the residual activity of insecticide on the substrates. Bioassays were performed at 1 week and then monthly up to 14 months post spraying, by attaching the cones to the treated and control blocks. For each insecticide dose and substrate type used, 100 unfed female mosquitoes aged 2 to 5 days were exposed in WHO polyvinyl chloride cones (obtained from the Vector Control Research Unit (VCRU) WHO Collaborating Centre, Universiti Sains Malaysia, Penang, Malaysia) for 30 minutes contact time with 10 mosquitoes per cone per block. Two cone bioassays were performed per block, with five blocks of each treatment. *An. gambiae* Kisumu susceptible strain and *An. coluzzii* VK laboratory resistant strain, reared at the IRSS insectary under standard controlled conditions (27±2°C and 75±10% relative humidity), were used. After removal from cones, mosquitoes were transferred into holding cups, provided access to 10% sucrose soaked cotton wool, and held under the same conditions described earlier. Mortality was recorded at 24 hours, 48 hours and 72 hours post exposure in cones.

**Experimental hut (Phase II) trial**

*Design of huts:* The experimental huts used were of the West African design\cite{27}. An experimental hut is a simulated house in which all entering, exiting (exophily), dead and blood fed mosquitoes can be recorded. It is made of local material and is characterized by the presence of a gutter or moat around the hut to protect against ants which would eat dead mosquitoes. It is also characterized by the presence of veranda traps to catch mosquitoes which may exit during the night due to either behavioural or insecticidal effects. Mosquitoes can enter through four window slits constructed from pieces of metal,

| Treatments       | Application rates of treatments | Substrates | Number of blocks |
|------------------|---------------------------------|------------|-----------------|
| VECTRON™ T500    | 50 mg a.i./m²                    | Concrete   | 05              |
| VECTRON™ T500    | 100 mg a.i./m²                   | Concrete   | 05              |
| VECTRON™ T500    | 200 mg a.i./m²                   | Concrete   | 05              |
| Negative control | Distilled water                  | Concrete   | 05              |
| VECTRON™ T500    | 50 mg a.i./m²                    | Mud        | 05              |
| VECTRON™ T500    | 100 mg a.i./m²                   | Mud        | 05              |
| VECTRON™ T500    | 200 mg a.i./m²                   | Mud        | 05              |
| Negative control | Distilled water                  | Mud        | 05              |
fixed at an angle to create a funnel with a 1 cm wide gap. The ceiling of the huts was made of plastic. For interior wall surfaces, two types of material were used: concrete and mud.

**Treatments**: VECTRON™ T500 was evaluated at two application rates (100 mg a.i./m² and 150 mg a.i./m²) on concrete and mud walls in the experimental huts. The reference product was Actellic® 300CS (Syngenta), which contains the organophosphate insecticide pirimiphos-methyl as the active ingredient. It was used at the recommended dose of 1000 mg a.i./m². A negative control, sprayed only with distilled water, was also included. Table 2 below summarises the different treatment arms and substrates that were tested.

**Insecticide application**: The IRS treatments were applied at the specified dosages (Table 2) to the internal walls of experimental huts and the hut ceiling using a MICRON CS-10 10L compression sprayer, fitted with a red 4.2 bar CFV and a T-Jet 8002E flat fan nozzle. The target volume ejected was 560 mL/min. All sprayers were calibrated with water prior to treatment of huts. All sprayers were equipped with pressure gauges, and initial pressure settings were conducted at 60 psi for consistency. The huts were prepared before spraying by marking swaths on the walls and ceiling, each swath being 75 cm in width and with a 5 cm overlap with the next swath. The safety precautions, mixing, handling, spray techniques and spray tank washing were all done according to standard procedures as outlined in the WHO manual for IRS. Prior to spraying, the spray operator practiced several times on blank walls using a tank filled with water to ensure that a constant flow rate was obtained before treatment started. A digital metronome (freeware from Metronome Beats v. 2.3.3, Stonekick 2013) synchronized with a digital stopwatch was used to enhance the consistency of applications (6 seconds per spray swath). The use of the digital metronome provided an audible guide to spray operators. An orientation pole was used to maintain the correct distance of the sprayer nozzle from the walls. It was attached to the handle of the sprayer during spraying. Five labelled filter papers (Whatman™ No. 1 10cm x 10cm) were fixed onto the four walls and ceiling of the huts.

The filter papers were removed after spraying, dried, grouped by hut and treatment, and carefully packed in aluminium foil for subsequent High performance Liquid Chromatography (HPLC) analysis at the Liverpool School for Tropical Medicine (LSTM), to provide a measure of the quality of the treatment applications. Insecticides were mixed homogeneously in the spray tank. Spraying was done alternately from roof to floor and then from floor to roof to treat each hut. After spraying the wall, the tarpaulin ceiling previously arranged on a plastic support was also sprayed. The sprayer tank was shaken frequently to ensure proper mixing. After spraying of each treatment, the solution remaining in the pump was removed and the volume measured. This measurement made it possible to determine the actual quantity of treatment solution applied per hut.

**Trial procedure**: Evaluation of free flying mosquitoes started five days after applying the treatments inside the huts. Cows were used as bait for mosquito attraction in place of human volunteers as the local *An. coluzzii* population is relatively zoophilic. In total, 14 cows, male and female, aged between 2 and 3 years, were purchased locally. A veterinarian was recruited to follow their health. They were used in this study according to his requirements to ensure their good health. Cows were divided in two groups: one group was used during one week and the second during the following week. The cows of each group were randomized on the first day of use and placed inside the huts in containment crates made of wood. Cows were rotated between huts each night according to a Latin square design to control for any variation in the attractiveness of individual cows to the mosquitoes. Thus, every cow spent one night in each hut during the round of 6 nights. They were placed inside huts at dusk (7:00 pm) and remained inside until dawn.

Each morning, volunteers, recruited from the village around the huts station and trained in mosquito collection, entered the huts to collect the mosquitoes that had entered overnight. Dead and live mosquitoes were collected from the floor, the walls and the ceiling of the hut and from the veranda trap, and placed into collection tubes. Mosquitoes were put in different...
bags for each collection compartment and transferred to the laboratory. Species identifications were made using the appropriate taxonomic keys. Mosquitoes were scored by location as dead or alive and as fed or unfed. Live mosquitoes were placed in cups covered with clean netting and provided with a 10% glucose solution for assessment of delayed mortality up to 72 hours after collection.

The main outcomes measured were:
- Deterrence: reduction in treated hut mosquito entry rates relative to the negative control hut;
- Induced exophily: proportion of mosquitoes that exit early and are found in exit traps;
- Blood-feeding inhibition: the reduction in blood feeding of mosquitoes compared with those in the negative control huts;
- Immediate and delayed mortality: proportion of mosquitoes that are found killed early morning and after 72 hours of holding.

**Evaluation of insecticide residual activity using cone tests:**
Residual activity of the different treatments was assessed at 1 week and then monthly after spraying up to 9 months for the VECTRON™ T500 100 mg a.i./m² and Actellic® 300CS treatments and up to 12 months for the VECTRON™ T500 150 mg a.i./m² treatment. Females of the susceptible strain *An. gambiae* Kisumu strain and the resistant strain *An. coluzzii* VK strain were tested using WHO standard cone bioassays on the treated walls and ceiling⁷. Two cones were attached with masking tape on each of the hut inner walls (the four walls and the ceiling) to obtain ten (10) cones per hut. Ten (10) females were exposed in each cone by plugging the cone with cotton wool after the introduction of mosquitoes. After 30 minutes of contact, mosquitoes were removed and placed in 150-ml plastic cups with access to the glucose solution (10%) provided via cotton wool. Mosquitoes were quickly transferred to the holding room in Bobo-Dioulasso (30 min drive) and maintained at a temperature of 27°C ± 2 °C and 75% ± 10% RH. Knockdown was recorded 60 minutes after exposure, and mortality was recorded 24, 48 and 72 hours after exposure in cones.

**Chemical residue analysis**
The five labelled filter papers (10 cm x 10 cm) fixed to the walls and ceiling of each hut before spraying were removed after spraying, packed in aluminium foil separately, and put in labelled bags. The packed samples were stored in a refrigerator at +4°C temperature and were then shipped to LSTM/LITE in Liverpool for HPLC analysis.

Broflanilide content was determined by reversed-phase high-performance liquid chromatography (HPLC) using UV detection at 226 nm and dicyclohexyl phthalate (DCP) as an internal standard. Briefly, a hole punch (0.635cm radius) was used to cut 12 circles from each filter paper. The pieces of each filter were placed into a glass tube and 5 ml of extraction solution was added, consisting of 100 µg/ml of DCP in methanol. The glass tubes were capped with tin foil and a screw cap and placed into a water bath sonicator. Samples were sonicated for 60 minutes at room temperature. Once sonication was completed, a syringe and PTFE filter (0.2 µm) was used to transfer 1 ml of each solution to a 1.5 ml Eppendorf tube. Using a 200µl micropipette, a 100µl aliquot of each sample was pipetted into a labelled HPLC vial. The HPLC was equipped with a detector suitable for operation at 226 nm, a constant temperature column compartment and an injector capable of delivering 20 µl injection volume. A Thermo Scientific Hypersil Gold column (Thermo Fisher Scientific, U.K.; Particle size: 5µm) was attached to the HPLC equipment. The mobile phase was acetonitrile and water mixed in a 7:3 ratio (v/v) with a flow rate of 1 ml/min and detection at 226 nm. The column temperature was between 23°C and 25°C.

The aim of this chemical analysis was to assess the quality of the spraying by comparing the doses of insecticides on the papers with the target doses. The difference of the two doses in percentage should be in the range of ±50% of the target dose according to WHO recommendation⁷.

**Supplementary tests: molecular and WHO tube test**
A sub-sample of mosquitoes (610 individual mosquitoes) collected in treated huts were submitted for polymerase chain reaction (PCR) analysis to determine species and the presence of kdr resistance by genotyping. The cycling conditions were 10' [30",30",60"] 35°C @ 54°C for Sine and 3' [30", 30", 10"] 35°C @ 55°C for Kdr. The reagents and kits (details in supplementary file 11, Extended data⁷) used are Pool Master Mix, Primers, sterile water, Trizma base, boric acid, EDTA, Agarose Multi, Purpose Agarose, Hexadecyltrimethylammonium bromide, sodium chloride, Trizma hydrochloride solution Ph 8.0, 1M and Ethylenediaminetetraacetic acid solution, 0,5 M aq. Soln, pH 8.0 Liquid. The primers were: S200X 6.1F: TCG-CCT-TAG-ACC-TTG-CTA, S200X 6.1R: CTC-TTC-AAG-AAT-TCG-AGA-TAC, Kdr_w D1: ATA-GAT-TCC-CGG-ACC-ATG, Kdr_w D2: AGA-GAA-GGA-TGA-TGA-ACC, Kdr_w D3: AAT-TTG-CAT-TAC-TTA-CCA-CA and Kdr_w D4: CTG-TAG-TGA-TGA-ATT-TA. The main equipments were composed by thermocyclers (Eppendorf, Biorad, Applied Biosystems), transiluminator, migration cuve (Fluorabnder, Apelx), vortex, centrifuges, Eppendorf pipettes, Electrophoresis Power Supply (E 815 CNSort and E 844 CNSort) (details in supplementary file 11, Extended data⁷). The species identification to identify *An. gambiae* complex species used the standard protocol⁸ and the presence/absence of kdr mutation L1014F (kdr-w) was determined using the protocol described by Martinez-Torres et al.⁹. Mosquitoes were identified as *An. arabiensis*, *An. gambiae* sensu stricto or *An. coluzzii*. For the resistance assays, mosquitoes were classified as SS, RS or RR i.e., homozygous susceptible, heterozygous, or homozygous resistant for the kdr mutation L1014F.

Phenotypical resistance was evaluated by using the WHO tube test method⁹. Larvae were collected from the field site.
and reared in the insectary to adults 2 to 5 days old. Papers impregnated with pyrethroids, carbamate, organophosphate insecticides and the synergist piperonyl butoxide (PBO) from the WHO laboratory in Malaysia were used.

Data management and statistical analysis
WHO cone bioassay and experimental hut trial data were entered using EpiData v 3.1 software (RRID:SCR_008485). Mortality was calculated from the total number of mosquitoes tested per period. If the mortality of the negative control in WHO cone bioassay was between 5% and 20%; mortality of treated mosquitoes was corrected using Abbott’s formula.

**Abbott’s formula:**
\[
\text{Corrected mortality} = \frac{\% \text{ treated mortality} - \% \text{ negative control mortality}}{100 - \% \text{ negative control mortality}} \times 100
\]

If negative control mortality was above 20% at 24 hours after exposure, the test data for that day was discarded, and the cone bioassays repeated. In the experimental hut trial, the free flying mosquito data such as the number of mosquitoes that entered, exited, or were dead inside the hut. The number that succeeded in blood feeding on the cows were calculated for each treatment by compiling the data collected over 12 weeks. The main analyses were performed using R statistical software (RRID:SCR_001905) version 4.1.0 with a significance level of 0.05 for rejecting the null hypothesis following a predefined analysis plan. Mixed effect logistic regression model analysis was conducted using the lme4 package, to compare proportional data by taking mosquitoes exited, blood fed and dead (total mortality) as dependent variables and treatment as categorical covariates (fixed effect), sleepers (cows) and months of the trial (random effect). For overall comparison, the negative control (untreated hut) was kept as a reference category. The primary criteria in the evaluation were blood feeding inhibition and 72 hours mortality. All graphs were produced using Excel 2016.

Ethical considerations
Institutional ethical approval for the study was obtained on October 2016 from the Institutional (Institut de Recherche en Sciences de la Santé) Ethics Committee for Health Sciences Research (N/Réf. 023- 2016/CEIRES). The cows used in experimental huts to attract mosquitoes were maintained according to the institution’s recommendations. Care was taken that the cows were not traumatized. A veterinarian was recruited to monitor their hygiene and health. All sick cows were replaced and treated appropriately. During the day, the cows were allowed to graze freely in an open field. The study was performed according to relevant international animal use guidelines\(^1\). This manuscript is reported in line with the ARRIVE (Animal Research: Reporting of In Vivo Experiments) guidelines\(^1\).

**Results**

**Laboratory (Phase I) study: Residual efficacy of VECTRON™ T500 on blocks**

The residual efficacy of VECTRON™ T500 was investigated at application rates of 50, 100 and 200 mg a.i./m² on concrete and mud blocks under laboratory conditions. The substrates of each type of block treated at these different rates were tested in WHO cone bioassays at 1 week post spraying and at monthly or two months intervals up to 14 months using the insecticide susceptible *An. gambiae s.s.* Kisumu strain and the pyrethroid-resistant *An. coluzzii* VK strain sampled from Vallée du Kou, Burkina Faso. On concrete blocks, the 50 mg a.i./m² and 100 mg a.i./m² application rates of VECTRON™ T500 resulted in 100% mortality for up to 12 months before falling below 80% during the last two months, while the 200 mg a.i./m² dose of VECTRON™ T500 gave complete mortality of susceptible *An. gambiae s.s.* Kisumu strain for up to 14 months (Figure 1a)\(^2\). VECTRON™ T500 on mud blocks showed a longer residual efficacy than on concrete blocks. Indeed, as shown in figure 1b, all applications to mud blocks induced 100% mortality for up to 14 months after spraying with the *An. gambiae s.s.* Kisumu strain (Figure 1b).

The residual efficacy of VECTRON™ T500 against the pyrethroid resistant VK strain of *Anopheles coluzzii* is shown in Figure 2. The results of mortality following exposure to treated concrete blocks showed that this was dose dependent. Indeed, for the 50 mg a.i./m² application rate, the mortality was around 80% at 5 months after block treatment before decreasing below 60% during the last six months. However, at the highest doses, the mortality rate was still high at 10 months after spraying, reaching 89.81% and 100% for the 100 mg a.i./m² and 200 mg a.i./m² doses, respectively, before decreasing below 80% during the last three months (Figure 2a). As with the susceptible *An. gambiae s.s.* Kisumu strain, VECTRON™ T500 showed better residual efficacy on mud blocks compared with concrete blocks against the resistant VK strain of *Anopheles coluzzii*. Indeed, 100% mortality was recorded up to 14 months for the 200 mg a.i./m² application rate. The lowest application rate (50 mg a.i./m²) demonstrated good residual efficacy up to 8 months after spraying with mortality up to 98.16% during that period (Figure 2b). The 100 mg a.i./m² application rate induced mortality up to 92% 14 months after spraying, although there was some variation in mortality between months 10 and 13 (Figure 2b).

**Efficacy of VECTRON™ T500 in an experimental hut (Phase II) trial**

**Efficacy of VECTRON™ T500 against free flying mosquitoes**

Residual efficacy of VECTRON™ T500 against wild free flying pyrethroid resistant malaria vectors was investigated in an experimental hut trial at the IRSS field station in Vallée du Kou (Bama, Burkina Faso). Experimental huts simulate the conditions in domestic dwellings and are, therefore, used to assess the efficacy of indoor vector control interventions in terms of mosquito entry rates, induce early exit of vector mosquitoes, prevention of mosquito feeding and induced mosquito mortality. Cows were used in place of human volunteers for mosquito attraction in this study as the toxicity, and potential risk of the VECTRON™ T500 product had not been fully assessed at the time of study initiation. In addition, previous studies have shown that the local vector mosquitoes are highly attracted to blood-feed on cows. Results of the different outcome measures are presented in Figure 3 and Table 3. In total 19,552
Figure 1. Monthly mortality of *Anopheles gambiae* s.s. Kisumu strain exposed on treated concrete (a) and mud (b) blocks substrates in World Health Organization (WHO) cones bioassay. Approximately 100 mosquitoes 2-5 days old were exposed for 30min to each treatment and mortality recorded 72 hours after exposure. Overall, 10 cones per dose and 10 mosquitoes per cone were used at each time point. Each histogram represents the mean mortality rate and error bars represent ± 95% confidence interval (CI). The dotted line represents WHO threshold.

Figure 2. Monthly mortality of *Anopheles coluzzii* pyrethroids resistant strain exposed on treated concrete (a) and mud (b) blocks substrates in World Health Organization (WHO) cones bioassays. Approximately 100 mosquitoes 2-5 days old were exposed for 30min to each treatment and mortality recorded 72 hours after exposure. Overall, 10 cones per dose and 10 mosquitoes per cone were used at each time point. Each histogram represents the mean mortality rate and error bars represent ± 95% confidence interval (CI). The dotted line represents WHO threshold.

Figure 3. Overall mortality per month of wild free-flying pyrethroid-resistant *Anopheles gambiae* s.l. collected daily inside treated huts for 4 months evaluation. Each histogram represents monthly mean mortality rate of mosquitoes collected inside each hut during the month and error bars represent ± 95% confidence interval (CI).
Table 3. Overall mortality 72h after collection, deterrence, blood-feeding rates and exophily induced by treatments on free flying *Anopheles gambiae* s.l. collected in treated huts during 12 consecutive weeks evaluation. Values bearing the same letter superscript along a row are not significantly different at the 5% level (P>0.05). CI=confidence interval.

| Type of wall | Concrete | VECTRON™ T500 100 mg a.i./m² | VECTRON™ T500 150 mg a.i./m² | Actellic® 300CS mg a.i./m² | VECTRON™ T500 100 mg a.i./m² | VECTRON™ T500 150 mg a.i./m² |
|--------------|----------|-----------------------------|-----------------------------|---------------------------|-----------------------------|-----------------------------|
| Treatments   | Control  | VECTRON™ T500              | VECTRON™ T500               | Actellic® 300CS           | VECTRON™ T500              | VECTRON™ T500               |
| Total caught | 3453     | 3546                        | 4330                        | 1535                      | 3498                        | 3190                        |
| % Deterrence | -        | 0                           | 0                           | 55.54                     | 0                           | 7.61                        |
| Number dead  | 192      | 2142                        | 3033                        | 1535                      | 1942                        | 2336                        |
| Global 72h % mortality | 5.56* | 60.40*                      | 70.04*                      | 100*                      | 55.51*                      | 73.22*                      |
| 95% CI       | (4.84-6.37) | (58.78-62.00)          | (68.66-71.39)     | -                        | (53.95-57.25)          | (71.76-74.73)               |
| Blood-fed caught | 3214      | 3277                        | 4006                        | 1457                      | 3109                        | 3038                        |
| % Blood-feeding | 93.07a     | 92.41b                     | 92.51b                      | 94.91e                     | 88.87a                      | 95.23e                      |
| 95% CI       | (92.18-93.87) | (91.49-93.24)          | (91.69-93.26)     | (93.70-95.90)       | (87.95-90.02)          | (94.43-95.92)               |
| % Blood-feeding inhibition | -       | 0.71                       | 0.60                        | 0                         | 4.51                        | 0                           |
| Total exit in veranda | 1139     | 679                        | 1705                        | 337                       | 511                         | 917                         |
| % Exophily   | 32.98a   | 19.14b                     | 39.37c                      | 21.95d                     | 14.60e                      | 28.74f                      |
| 95% CI       | (31.43-34.57) | (17.88-20.47)          | (37.93-40.84)     | (19.95-24.09)        | (13.50-15.84)          | (27.20-30.34)               |

*An. gambiae* s.l. mosquitoes were collected between August and October 2018. Mortality of free flying mosquitoes was recorded up to 72 hours after collection from huts, due to the delayed mortality effect of brofanilide, the active ingredient of VECTRON™ T500. Mortality rates of free flying *An. gambiae* s.l. indicate that the 150 mg a.i./m² dose of broflanilide was the most effective in killing mosquitoes. Indeed, this dose induced the highest overall mosquito mortality rates on concrete (70.04%) and on mud (73.22%) during the four months of mosquito collection post spraying. Statistically, the 150 mg a.i./m² dose performed significantly better (P<0.001) on mud than on concrete. During this period, mortality with the 150 mg a.i./m² dose of VECTRON™ T500 ranged from 59.74% to 79.06% on concrete walls and from 55.33% to 79.33% on mud walls. Mortality of mosquitoes collected in the huts treated with 100 mg a.i./m² VECTRON™ T500 ranged from 54.57% to 72.87% on concrete walls and from 40.31% to 63.54% on mud walls with 60.40% and 55.51% as global mortality, respectively. In contrast, 100 mg a.i./m² dose of VECTRON™ T500 performed significantly better (P<0.0001) on concrete than mud. On the concrete and mud substrates, there was a significant difference (respectively) between the 150 mg a.i./m² dose and the 100 mg a.i./m² dose in terms of mortality (P<0.0001; P<0.0001). The positive reference product, Actellic® 300CS, induced 100% mortality during the four months of evaluation. Deterrence, blood-feeding inhibition and exophily obtained with VECTRON™ T500 treated huts compared to negative control were very low (Table 3). The Actellic® 300CS treatment showed 55% deterrence. As expected of IRS treatments, blood-feeding rates of mosquitoes were very high in all huts (>90%). There was a significant difference in blood-feeding rates between the two application rates of VECTRON™ T500 (100 mg a.i./m² and 150 mg a.i./m²) for both concrete and mud substrates (P<0.05, Table 3). The natural exophily rate in the control hut was high. Due to this natural exophily, it was not possible to determine the insecticide-induced exophily.

**Residual efficacy of insecticide applied in experimental huts using cone tests**

Cone bioassays were performed monthly in experimental huts up to 9 months for the 100 mg a.i./m² application rate of VECTRON™ T500 and for the Actellic® 300CS treatment. The 150 mg a.i. /m² application rate of VECTRON™ T500
was evaluated up to 12 months after spraying, to assess the residual efficacy on the different hut wall substrates (mud and concrete). Unfed adult females (3-5 days old) of the susceptible *An. gambiae* Kisumu and resistant *An. coluzzii* (reared from larvae collected at the experimental field site) were used. The residual efficacy in cone bioassays with the susceptible Kisumu strain is presented in Figure 4. The 100 mg/m² and 150 mg/m² doses of VECTRON™ T500 induced 100% mortality on both concrete and mud walls up to 9 months after spraying. The three extended monthly test performed with 150 mg/m² showed better efficacy up to 12 months on mud walls (100%) than concrete walls (<80%). The Actellic® 300CS reference product applied to concrete showed variable mortality from 4 months post-treatment onwards, and mortality at 6 and 7 months was below 80%. Residual efficacy against the pyrethroid resistant VK strain *An. coluzzii* is shown in Figure 5. The 100 mg a.i./m² and 150 mg a.i./m² doses of VECTRON™ T500 performed better on mud walls (mortality over 80%) 9 months after spraying than on concrete walls (mortality below 80%) at the same time. The Actellic® 300CS showed variable residual efficacy from 4 months post-treatment onwards, but mortality was below 80% at 8 and 9 months.

**Insecticide application quality**

The results of the HPLC analysis of filter papers treated during the application of treatments to experimental hut walls are shown in Table 4. The percentage difference between the target dose and the actual dose sprayed onto filter papers was within the range of ±50% of the target doses for all treatments confirming that the spraying met WHO statement for spray quality.

**Phenotypical and genotypical resistance**

To determine the prevalence of phenotypic resistance, larvae were collected from breeding sites near to the experimental hut station and reared in the insectary to adults 2-5 days old. WHO susceptibility bioassays were performed using insecticide impregnated papers obtained from the Vector Control Research Unit (VCRU) WHO Collaborating Centre, University Sains Malaysia, Penang, Malaysia in Malaysia. The insecticide susceptible Kisumu strain of *An. gambiae* s.s. was also tested for data quality control purposes. The results summarized below (Table 5) showed full susceptibility (100% mortality) of the Kisumu strain and a very high level of resistance to pyrethroids (<2% mortality) in the field population of *An. gambiae* s.l. The increase in mortality (39%) with deltamethrin following exposure to the cytochrome P450 synergist piperonyl butoxide (PBO), indicates the role of a P450 metabolic mechanism of pyrethroid resistance in this population. Resistance was also observed in the field population to the carbamate bendiocarb (84%), but it was fully susceptible to the organophosphorus insecticide pirimiphos-methyl.

Mosquitoes sampled from treated huts (610 individual mosquitoes) were used in PCR tests to determine species and to detect kdr resistance mutations. Of these mosquitoes, 8 mosquitoes did not amplify. A high proportion of the mosquitoes collected from huts were *An. coluzzii* (98%), and these had a high frequency of the kdr (L1014F) mutation (0.65). The proportion of heterozygote, homozgyote susceptible was respectively 42.6%, 44.5% and 12.8% (Table 6). The kdr mutations confer cross-resistance between pyrethroids and DDT in these mosquitoes.  

![Figure 4](image-url)  
**Figure 4.** Mortality of *Anopheles gambiae* Kisumu strain exposed to treated huts surfaces in cone bioassays. Approximately 100 mosquitoes 2-5 days old were exposed for 30min to the hut walls and ceiling, and mortality recorded 72 hours after exposure. Overall, 10 cones per hut, two per side and 10 mosquitoes per cone were used at each time point. Each histogram represents the mean mortality rate of tested mosquitoes at each time point and error bars represent ± 95% confidence interval (CI). The dotted line represents World Health Organization (WHO) threshold.
Figure 5. Mortality of *Anopheles coluzzii* pyrethroids resistant strain exposed to treated huts surfaces in cone bioassays. Approximately 100 mosquitoes 2-5 days old were exposed for 30min contact to the treated hut walls and ceiling, and mortality recorded 72 hours after exposure. Overall, 10 cones per hut, two per side and 10 mosquitoes per cone were used at each time point. Each histogram represents the mean mortality rate of tested mosquitoes at each time point and error bars represent ± 95% confidence interval (CI). The dotted line represents World Health Organization (WHO) threshold.

Table 4. Analysis results of filter papers treated during the huts spraying to determine accuracy of indoor residual spraying (IRS) applications.

| Walls | Concrete | Mud |
|-------|----------|-----|
| Treatments | VECTRON™ T500 | VECTRON™ T500 | Actellic 300CS | VECTRON™ T500 | VECTRON™ T500 |
| Target doses (mg/m²) | 100 | 150 | 1000 | 100 | 150 |
| Filter paper doses (mg/m²) | 109.17 | 188.84 | 800.55 | 121.14 | 150.90 |
| Deviation from target doses (%) | 9.17 | 25.89 | -19.94 | 21.14 | 0.60 |

Table 5. Knock down and mortality of mosquitoes tested in World Health Organization (WHO) tube using impregnated papers to evaluate phenotypic resistance. %: Pourcentage, KD: Knock down, PY: Pyrethroids, OP: Organophosphate, PBO: piperonyl butoxide.

| Strains | Susceptible *An. gambiae* Kisumu strain | *An. gambiae* s.l. from field larval collections |
|---------|------------------------------------------|-------------------------------------------------|
| treatments | number tested | % (KD) | % mortality 24h | number tested | % (KD) | % mortality 24h |
| Control PY | 54 | 0 | 4 | 54 | 0 | 0 |
| Permethrin_0.75% | 103 | 100 | 100 | 100 | 0 | 0 |
| Alpha-cypermethrin_0.05% | 100 | 100 | 100 | 102 | 0 | 0 |
| Deltamethrin_0.05% | 102 | 100 | 100 | 105 | 0.93 | 1.87 |
| Control/OP | 54 | 0 | 0 | 52 | 0 | 1.96 |
| Pirimiphos_methyl_0.25% | 105 | 100 | 100 | 109 | 12 | 99 |
| Bendiocarb_0.1% | 101 | 100 | 100 | 99 | 93088 | 84.69 |
| Control/PBO (4%) | - | - | - | 53 | 0 | 0 |
| PBO (4%) + Deltamethrin_0.05% | - | - | - | 99 | 52.53 | 39.39 |
Discussion

Control of malaria vectors is dominated by use of insecticides on LLINs and applied by IRS. Unfortunately, the major malaria vector species have become resistant to many of the classes of insecticides currently recommended by WHO for use in public health\(^1\). Managing insecticide resistance is a major challenge for malaria control and elimination\(^2\) and implementation of insecticide resistance management strategies is a key method for the continued control of malaria. Such strategies require new insecticides, with modes of action effective against resistant strains of mosquito.

Insecticide susceptibility assays showed high resistance of An. gambiae s.l. to all pyrethroids tested (deltamethrin, permethrin and alphacypermethrin) and moderate resistance to carbamate (bendiocarb) in the malaria vector population in Vallée du Kou, Burkina Faso. The molecular diagnostic (PCR) testing detected a high frequency of the kdr (L1014F) mutation in the mosquito population. Recent studies of this population have shown a high resistance to the three main classes of insecticides (DDT, carbamate and pyrethroids) used in vector control throughout the country\(^3\). Pre-exposure to the synergist PBO in bioassays with deltamethrin increased mortality, suggesting the presence of a cytochrome P450-based mechanism of resistance in this mosquito population. However, pre-exposure to PBO did not fully restore the susceptibility of the mosquitoes to deltamethrin, which indicates the presence of other resistance mechanisms\(^4,5\). To address the urgent need for new insecticides with novel modes of action to control malaria vectors, we investigated the bioefficacy of broflanilide insecticide to give high levels of mortality (>80%) in pyrethroid resistance mosquito strains indicates that VECTRON™ T500 for field use and its efficacy against resistant mosquitoes in comparison to a WHO listed IRS product. Mortality following exposure to VECTRON™ T500 treated surfaces was assessed at 72 hours post-exposure due to the slower mode of action of broflanilide on the mosquitoes\(^6\). Clothianidin and chlorfenapyr, which also give delayed mortality with mosquitoes, are prequalified by WHO-PQT for use as an IRS products and an insecticide treated net for malaria vector control\(^7,8,9\).

The results of the laboratory and experimental hut trials clearly demonstrate the ability of broflanilide insecticide to give high levels of mortality (>80%) in pyrethroid resistance mosquitoes up to 6 months post-spraying. The lowest dose tested in the experimental hut study, 100 mg a.i./m\(^2\), gave more than 80% mortality 6 months after application to mud or concrete in laboratory cone bioassays and in situ cone tests carried out in the experimental huts. Similar results were reported in other studies performed in Benin and Tanzania with An. gambiae s.l. and An. Arabiensis, respectively\(^10,11\). The residual activity through in situ cone bioassays on treated experimental huts walls with susceptible and pyrethroid-resistant vector mosquito strains indicates that VECTRON™ T500 performed as well as Actellic® 300CS, a WHO listed IRS product, during 4 to 9 months post-spraying. This demonstrates the potential of VECTRON™ T500 to provide prolonged vector control in many malaria-endemic African villages where the interiors of houses are largely plastered with mud only. High levels of mortality were seen with free flying mosquitoes entering huts treated with VECTRON™ T500 or Actellic® 300CS during the four months of evaluation. Other studies have shown residual efficacy of Actellic® 300CS up to six months\(^12,13\). Our data corroborate these studies. Both 100 mg a.i./m\(^2\) and 150 mg a.i./m\(^2\) applications of VECTRON™ T500 gave extended residual efficacy in experimental huts, residual efficacy being longer on mud

### Table 6. Species and kdr genotyping by polymerase chain reaction (PCR).

| Species            | Number | Genotypes of the kdr-w | f(L1014F) |
|--------------------|--------|------------------------|-----------|
|                    |        | 1014L 1014L            | 1014L 1014LF | 1014F 1014LF |         |
| An. coluzzii       | 591    (98%) | 76(12.8%) | 252(42.6%) | 263(44.5%) | 0.65    |
| An. gambiae        | 10(1.6%) | 0        | 0          | 10(100%)  | 1       |
| An. arabiensis     | 1(0.1%) | 0        | 0          | 1(100%)   | 1       |
| total              | 602    | 76(12.6%) | 252(41.8) | 274(45.5) | 0.66    |
substrate than on concrete. With its novel mode of action and efficacy for IRS against pyrethroid-resistant malaria vectors, VECTRON™ T500 shows potential for use with other IRS insecticide formulations in an IRS rotation strategy to help manage insecticide resistance and extend the effective lives of the insecticides used in IRS.

**Conclusion**

IRS products should have an effective duration of action of at least 6 months to cover the period of malaria transmission following a single application. The laboratory and experimental hut trials reported here have demonstrated the extended residual efficacy of VECTRON™ T500, a wettable powder formulation of broflanilide, against both susceptible and pyrethroid-resistant mosquito strains for 6 months or more, post-spraying onto mud and concrete substrates. These trials have also helped define the dose of VECTRON™ T500 that should be applied for use in the community. For future studies, including community trials, VECTRON™ T500 can be sprayed at a target concentration of 100 mg a.i./m².

**Data availability**

Underlying data

Zenodo: koamabayili/VECTRON: Laboratory and experimental hut trial evaluation of VECTRON™ T500 for indoor residual spraying (IRS) against insecticide resistant malaria vectors in Burkina Faso. https://doi.org/10.5281/zenodo.64698367.

This project contains the following underlying data:
- file 1-laboratory cone test raw data.xlsx
- file 2-free flying raw data.xlsx
- file 3-residual efficacy inside huts raw data.xlsx
- file 4-filter papers analysis raw data.xlsx
- file 5-PCR raw data.xlsx
- file 9-PCR revelation for especies.pdf
- file 10-PCR revelation for kdr.pdf

**Extended data**

Zenodo: koamabayili/VECTRON: Laboratory and experimental hut trial evaluation of VECTRON™ T500 for indoor residual spraying (IRS) against insecticide resistant malaria vectors in Burkina Faso. https://doi.org/10.5281/zenodo.64698367.

This project contains the following extended data:
- file 6-script of laboratory data.R
- file 7-script of free flying data.R
- file 8-script of residual efficacy data.R
- file 11-PCR reagents and equipments details.docx

**Reporting guidelines**

Zenodo: ARRIVE checklist for ‘Laboratory and experimental hut trial evaluation of VECTRON™ T500 for indoor residual spraying (IRS) against insecticide resistant malaria vectors in Burkina Faso’.

https://doi.org/10.5281/zenodo.64698172.

Data are available under the terms of the Creative Commons Zero “No rights reserved” data waiver (CC0 1.0 Public domain dedication).

**Acknowledgements**

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Reviewer Report 16 June 2022

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Rosemary S. Lees
Department of Vector Biology, Liverpool School of Tropical Medicine, Liverpool, UK

Katherine Gleave
Department of Vector Biology, Liverpool School of Tropical Medicine, Liverpool, UK

Giorgio Praulins
Department of Vector Biology, Liverpool School of Tropical Medicine, Liverpool, UK

George Parsons
Department of Vector Biology, Liverpool School of Tropical Medicine, Liverpool, UK

Koama Bayili et al. have conducted an important piece of evaluation work for the VECTRON T500 IRS product, which follows on in a logical fashion from previously published laboratory evaluation of the active ingredient Tenebenal (Lees et al. 2020). Standard laboratory and semi-field methods have been applied by an experienced team to demonstrate the residual activity of the IRS formulation applied to mud and concrete blocks in controlled laboratory conditions, and to the walls of mud and concrete experimental huts, against laboratory colonies and free-flying wild mosquitoes, respectively. Experiments were conducted according to the WHO guidelines, with a good level of quality control and additional checks, for example, the information about training, HPLC analysis, and genotyping as well as phenotyping of free flying mosquitoes.

There are some small points of clarification and suggestions for improvement below. The main limitation of the manuscript is that some of the methodological detail is not very well described, and so the experimental design is confusing in some places. Although the study type will be familiar to many readers, to those who are less familiar with this form of testing it would be hard to replicate the experiments.

General:

- There is some confusion over how the product under evaluation is referred to - it is sometimes called VECTRON T500, sometimes just VECTRON, and in Figures 4 and 5, for example, as Tenebenal 50WP. It seems like the same product was used throughout, but it would be helpful to either explain the different names in the Introduction or ideally be
consistent in the naming.

- There are references to two versions of the WHO guidelines for evaluation of IRS products, which do not differ in the methods they present but it would be better to cite them consistently.

- There is some repetition between the Methods section and Introduction.

- There are some typos and misspellings, for example, the use of capital letters for species names and reference to ‘resistance mosquitoes’ rather than ‘resistant’.

**Introduction:**

- It would be useful to have a sentence to explain the need for the 72h observation of mortality, which is not standard but required for this chemistry which is slower acting than pyrethroids. This could be included in the section of the Introduction which describes Tenebenal, and which would be usefully expanded to introduce the new chemistry to readers who are not familiar with it.

- The Introduction uses WHOPES terminology to refer to Phase I and Phase II testing, which could be updated to reflect current WHO PQT/VCP terminology.

**Methods:**

- I would like to see some more detail in the Methods section, for example, details on the batch number and quality control of the product being used in this study, and how the Potter Tower was calibrated. If HPLC or other analysis was done to confirm the rate of application by the Potter Tower I would like to see this data. In particular, I found it difficult to understand the experimental hut trial procedure – how many rounds of 6 nights of testing were completed over the 12 weeks of testing, how the huts and cows were rotated, etc. A schematic or diagram of some sort would be helpful for readers not familiar with this type of study.

- It would be nice to see a justification for why the concentrations of IRS changed between the laboratory and experimental hut phase of testing.

- It is helpful that the authors shared their R scripts, though they should also be cited appropriately in the text. It would also be helpful if they shared the code for the mixed effects model, to allow results to be reproduced.

**Results/Discussion:**

- The very low blood feeding inhibition, deterrence, and exophily in the experimental hut experiment is interesting, alongside good levels of mortality. I would like to see the authors discuss the implications of this for the expected performance of the product against pyrethroid resistant populations of mosquitoes.

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Is the work clearly and accurately presented and does it cite the current literature?
Yes

Is the study design appropriate and is the work technically sound?
Yes

Are sufficient details of methods and analysis provided to allow replication by others?
Partly

If applicable, is the statistical analysis and its interpretation appropriate?
I cannot comment. A qualified statistician is required.

Are all the source data underlying the results available to ensure full reproducibility?
Yes

Are the conclusions drawn adequately supported by the results?
Yes

Competing Interests: One author on this paper, Graham Small, is employed by IVCC, which is part of the LSTM group. HPLC analysis of samples was performed at LSTM. Myself and my co-reviewers are employed by LSTM, but have not been involved in any way in this study, and do not work directly with any of the authors. I do not believe this has affected our ability to provide a impartial and unbiased review for this article.

Reviewer Expertise: Medical entomology, development and evaluation of vector control tools, insecticide resistance

We confirm that we have read this submission and believe that we have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Author Response 22 Jun 2022
koama bayili, Institut de Recherche en Sciences de la Santé, Bobo-dioulasso, Burkina Faso

Dear Rosemary S. Lees et al.

We thank you and your colleagues for your constructive comments and suggestions to improve the manuscript. The manuscript will be revised accordingly. All changes or additions in the text will be done in the revised version before submitting it. All the changes will be in blue in the revised version.

I tried to respond to your below comments:

There is some confusion over how the product under evaluation is referred to - it is sometimes called VECTRON T500, sometimes just VECTRON, and in Figures 4 and 5, for
example, as Tenebenal 50WP. It seems like the same product was used throughout, but it would be helpful to either explain the different names in the Introduction or ideally be consistent in the naming.

**Response: I corrected the word and the right name is VECTRON T500**

There are references to two versions of the WHO guidelines for evaluation of IRS products, which do not differ in the methods they present but it would be better to cite them consistently.

**Response: Yes**

There is some repetition between the Methods section and Introduction.

**Response: Yes, we just want some time to remind you about some right information.**

There are some typos and misspellings, for example, the use of capital letters for species names and reference to ‘resistance mosquitoes’ rather than ‘resistant’.

**Response: Yes, corrected**

Introduction:

- It would be useful to have a sentence to explain the need for the 72h observation of mortality, which is not standard but required for this chemistry which is slower acting than pyrethroids. This could be included in the section of the Introduction which describes Tenebenal, and which would be usefully expanded to introduce the new chemistry to readers who are not familiar with it.

**Response: Yes included**

- The Introduction uses WHOPES terminology to refer to Phase I and Phase II testing, which could be updated to reflect current WHO PQT/VCP terminology.

**Response: Yes, corrected**

Methods:

- I would like to see some more detail in the Methods section, for example, details on the batch number and quality control of the product being used in this study, and how the Potter Tower was calibrated. If HPLC or other analysis was done to confirm the rate of application by the Potter Tower I would like to see this data. In particular, I found it difficult to understand the experimental hut trial procedure – how many rounds of 6 nights of testing were completed over the 12 weeks of testing, how the huts and cows were rotated, etc. A schematic or diagram of some sort would be helpful for readers not familiar with this type of study.

**Response: The batch number of products is included. Unfortunately, we missed the potter Tower calibration results that would be filter papers analysis. We did...**
12 rounds with 6 nights each. The huts were not rotated only the cows were rotated as in the example below:

| Hut Number | 1 | 2 | 3 | 4 | 5 | 6 |
|------------|---|---|---|---|---|---|
| Day 1      | C1| C2| C3| C4| C5| C6|
| Day 2      | C2| C3| C4| C5| C6| C1|
| Day 3      | C3| C4| C5| C6| C1| C2|
| Day 4      | C4| C5| C6| C1| C2| C3|
| Day 5      | C5| C6| C1| C2| C3| C4|
| Day 6      | C6| C1| C2| C3| C4| C5|

C1 to C6 refers to cows

- It would be nice to see a justification for why the concentrations of IRS changed between the laboratory and experimental hut phase of testing.

**Response:** As in the lab 100mg/m² was performed as 200mg/m² and the product production cost is very expensive, we judged that it will be good to reduce the concentration in the field.

- It is helpful that the authors shared their R scripts, though they should also be cited appropriately in the text. It would also be helpful if they shared the code for the mixed-effects model, to allow results to be reproduced.

**Response:** Please see in the data availability section R scripts are in files 6, 7, and 8.

Results/Discussion:
- The very low blood feeding inhibition, deterrence, and exophily in the experimental hut experiment is interesting, alongside good levels of mortality. I would like to see the authors discuss the implications of this for the expected performance of the product against pyrethroid resistant populations of mosquitoes.

**Response:** I tried to explain it in the results section by the lowest action of insecticide on the mosquitoes.

Thank you very much again for your time in reviewing this manuscript

**Competing Interests:** No competing interests
General comment on the paper: The authors provide background to why it is important to develop and evaluate new indoor residual spray formulations following the increase and wide spread of mosquito resistance against currently used vector interventions mainly long-lasting insecticidal nets (LLINs) and indoor residual spray (IRS). The authors introduce VECRTONTM T500, a novel insecticide formulation that may have potential as a new IRS vector control product.

The objectives of the study are clearly stated as 1) to determine the dose required for the application of VECRTONTM T500 and its residual efficacy in the lab and in the wild mosquito population. The study and the experiment were conducted in Latin-square design. Although the study was a Latin-square, it is not stated why the positive control Actellic 300CS was not tested in mud which would provide a 7 x 7 treatment arms.

Here are minor comments that I suggest the author correct/clarify before indexing:

1. Currently, WHO PQT does not use Phase I or Phase studies. In stated laboratory study & Experimental hut should be used. I suggest correcting this throughout the manuscript.

2. In the lab study, the author used 50, 100, and 200 mg/m2 but in the experimental hut study, the author decided to use 150 mg/m2. No statement about this decision

3. On page 5, the author mentions that cows were used because wild mosquitoes are zoophilic. On page 7, the author mentions that cow was used because the safety profile of VECRTONTM T500 is not established. This is confusing. In addition, it raises serious safety concerns and really suggests the author needs to clarify or correct this.

4. There is inconsistency in figure keys, labels, and legends.

5. On page 9, the authors included numbers which I failed to find in the table of results. I suggest clarifying which figure or table they are linked to. Or if it is not shown, it should be stated.

6. In the discussion section, the author is reintroducing the subject using several sentences, which I think are not necessary and makes the discussion unnecessarily long.

7. All my comments are in the manuscript I reviewed. I suggest the author have a look and address them whenever necessary. (Bayili et al feedback.pdf)

8. It is odd that the author did not include a section for study limitations. I noted several limitations. 1) It would be good to use WHO papers with 5x discriminating concentration as this gives important info on the resistance intensity. The author did not do a second 7x7 latin square to look at effects up to 8 months post spray. This is important when the Vectron looks better than Actellic in the cone test.
Is the work clearly and accurately presented and does it cite the current literature?
Yes

Is the study design appropriate and is the work technically sound?
Partly

Are sufficient details of methods and analysis provided to allow replication by others?
Yes

If applicable, is the statistical analysis and its interpretation appropriate?
Yes

Are all the source data underlying the results available to ensure full reproducibility?
Yes

Are the conclusions drawn adequately supported by the results?
Yes

**Competing Interests:** No competing interests were disclosed.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Author Response 22 Jun 2022
koama bayili, Institut de Recherche en Sciences de la Santé, Bobo-dioulasso, Burkina Faso

Dear Emmanuel Mbuba

We thank you for your constructive comments and suggestions to improve the manuscript. The manuscript will be revised accordingly.

As your comments and suggestion are below, I tried to respond to them in the revised manuscript. Please see my comments in bold.

1. Currently, WHO PQT does not use Phase I or Phase studies. Instead laboratory study & Experimental hut should be used. I suggest correcting this throughout the manuscript.

   **Response:** All corrections are done accordingly

2. In the lab study, the author used 50, 100, and 200 mg/m² but in the experimental hut study, the author decided to use 150 mg/m². No statement about this decision

   **Response:** You are right. In the lab, 100mg/m² was performed as 200mg/m² and we don't want to use much product due to its production cost, which is why we
reduced the dose.

3. On page 5, the author mentions that cows were used because wild mosquitoes are zoophilic. On page 7, the author mentions that cow was used because the safety profile of VECRTON™ T500 is not established. This is confusing. In addition, it raises serious safety concerns and really suggests the author needs to clarify or correct this.

Response: I tried to correct and clarify this in the manuscript.

4. There is inconsistency in figure keys, labels, and legends.

Response: All corrections are done accordingly.

5. On page 9, the authors included numbers which I failed to find in the table of results. I suggest clarifying which figure or table they are linked to. Or if it is not shown, it should be stated.

Response: All corrections are done accordingly reference was done to Figure 3 and Table 3.

6. In the discussion section, the author is reintroducing the subject using several sentences, which I think are not necessary and makes the discussion unnecessarily long.

Response: All corrections are done and discussion is reduced.

7. All my comments are in the manuscript I reviewed. I suggest the author have a look and address them whenever necessary. (Bayili et al feedback.pdf)

Response: Yes I got them and took them in count on the revised manuscript.

8. It is odd that the author did not include a section for study limitations. I noted several limitations. 1) It would be good to use WHO papers with 5x discriminating concentration as this gives important info on the resistance intensity. The author did not do a second 7x7 latin square to look at effects up to 8 months post spray. This is important when the Vectron looks better than Actellic in the cone test.

Response: Yes these are the limitation of our study and we will consider these suggestions in other studies that will be run in the same area.

Thank you very much again for your time in reviewing this manuscript.

Competing Interests: No competing interests
Johnson J. Matowo
Department of Parasitology and Entomology, Faculty of Medicine, Kilimanjaro Christian Medical University College, Moshi, Tanzania

Reviewer’s report on the manuscript titled Laboratory and experimental hut trial evaluation of VECTRON™ T500 for indoor residual spraying (IRS) against insecticide resistant malaria vectors in Burkina Faso

Reviewer: Dr. Johnson Matowo (PhD)

The manuscript by Bayili et al. investigated the optimum effective dose and efficacy of VECTRON™ T500 against susceptible and resistant strains of Anopheles in Burkina Faso

Title:
Seems to be okay

Abstract:
World Health Organization recommended insecticides for vector control are limited in number

To be rephrased as follows:

The World Health Organization (WHO) recommended insecticides for vector control are limited in number

Background
Okay

Methods
1. The title Chemical residue analysis is to be changed to Insecticide application quality

2. Supplementary tests: To be changed from Phenotypical and genotypical resistance to Polymerase Chain Reactions (PCR) and WHO resistance assay

3. Page 6-7

The following text

*Phenotypical resistance was evaluated by using the WHO tube test method30. Larvae were collected from the field site and reared in the insectary to adults 2 to 5 days old. Papers impregnated with pyrethroids, carbamate, organophosphate insecticides and the synergist piperonyl butoxide (PBO) from the WHO laboratory in Malaysia were used.*
Could be rephrased as follows:

Phenotypic resistance was evaluated using 2 to 5-day old adult female mosquitoes according to the standard WHO susceptibility test method\(^\text{30}\). The insecticide and PBO treated papers (impregnated papers) were obtained from WHO laboratory in Malaysia.

4. **Page 7**

The following sentence

*In the experimental hut trial, the free flying mosquito data such as the number of mosquitoes that entered, exited, or were dead inside the hut…….;* seems to be incomplete. This should be addressed.

5. **Page 5**: The local *An. coluzzii* population is relatively zoophilic. Please provide a citation for this

**Results**

1. The title Phenotypical and genotypical resistance is to be deleted. Instead, the text could be placed under two headings

   WHO susceptibility assays

   Phenotypic resistance data (%mortality and %knockdown data)

2. Species identification and kdr genotyping

   Species composition and *kdr* mutations data

3. The first paragraph of each subsection of the Results section is part of methodology section. This is to be shifted to the methodology section.

4. **Figures**

   Figure legends

   The second sentence for figure legends figures 1,2,4, and 5 is part of the methodology section and should be deleted. For example *figure 1: Approximately 100 mosquitoes 2-5 days old were exposed for 30min to each treatment and mortality was recorded 72 hours after exposure. Overall, 10 cones per dose and 10 mosquitoes per cone were used at each of tested mosquitoes at time point*

5. **Tables**

   Headings for tables 4,5, and 6 are to be rephrased as follows

   - Table 4: HPLC analysis or Insecticide application quality
   - Table 5: WHO susceptibility assay
   - Table 6: Species identification and kdr genotyping
The following should appear as footnotes, below the tables, not on the heading

- Table 3: Values bearing the same letter superscript along a row are not significantly different at the 5% level (P>0.05). CI=confidence interval
- Table 5: %: Percentage, KD: Knock down, PY: Pyrethroids, OP: Organophosphate, PBO: piperonyl butoxide
- Table 6: f(1014F): frequency of the 1014F resistant kdr allele

The format of ALL tables could be formatted. Instead of highlights, the tables to have few horizontal lines (column headings and base of tables) with no vertical lines

For table 5, two cells for strains and treatments to be merged and the word strains to be deleted, the cell to be for treatments.

Discussion
The authors are not focused. There is unnecessary background information on background and methodology.

1. The first paragraph could be followed by the following paragraph

To address the urgent need for new insecticides with novel modes of action to control malaria vectors, we investigated the bioefficacy of broflanilide in a wettable powder formulation, VECTRON™ T500, for use in IRS, through the conduct of laboratory (Phase I) and experimental hut (Phase II) studies. Insecticide susceptibility assays showed high resistance of An. gambiae s.l. to all pyrethroids tested (deltamethrin, permethrin and alphacypermethrin) and moderate resistance to carbamate (bendiocarb) in the malaria vector population in Vallée du Kou, Burkina Faso. The molecular diagnostic (PCR) testing detected a high frequency of the kdr (L1014F) mutation in the mosquito population. Recent studies of this population have shown a high resistance to the three main classes of insecticides (DDT, carbamate and pyrethroids) used in vector control throughout the country10. Pre-exposure to the synergist PBO in bioassays with deltamethrin increased mortality, suggesting the presence of a cytochrome P450-based mechanism of resistance in this mosquito population. However, pre-exposure to PBO did not fully restore the susceptibility of the mosquitoes to deltamethrin, which indicates the presence of other resistance mechanisms8,10

2. Then the following text

Broflanilide, discovered by Mitsui Chemicals Agro, Inc., has an unique chemical structure characterized as a meta-diamide and shows strong activity against various pests15. This insecticide, with a new mode of action for malaria vector control, shows no cross-resistance to existing pyrethroid resistance mechanisms34. This is an important consideration for future use in IRS product rotations for insecticide resistance management purposes. VECTRON™ T500 was tested as an IRS against pyrethroid susceptible and pyrethroid-resistant strains of malaria vectors following application to mud and concrete, the two principal substrates used in Bama village, Vallée du Kou, inside the houses. Residual efficacy was assessed via cone bioassays and by assessing mortality of free flying mosquitoes entering experimental huts. The purpose of these investigations was to determine the appropriate application rate of VECTRON™ T500 for field use and its efficacy against resist[1]ant mosquitoes in comparison to a WHO listed IRS product. Mortality following exposure to VECTRON™ T500 treated surfaces was assessed at 72 hours post-
exposure due to the slower mode of action of broflanilide on the mosquitoes. Clothianidin and chlorfenapyr, which also give delayed mortality with mosquitoes, are prequalified by WHO-PQT for use as an IRS products and an insecticide treated net for malaria vector control could be deleted.

3. The following text

Other studies have shown residual efficacy of Actellic® 300CS up to six months. Our data corroborate these studies.

To be rephrased as follows:
Residual efficacy of Actellic® 300CS is in the present study is similar to the findings of a previous study.

Conclusion
1. The first sentence to be deleted

2. The third and fourth sentences to be combined as follows:

The present study has defined the dose for VECTRON™ T500 to be used in Community trials i.e. 100 mg a.i./m2

Grammatical and typing/spelling errors

There are few typing errors. For example:
  ○ Pourcentage instead of Percentage (table 3)
  ○ Resistance instead of resistant (page 12)
  ○ resistant instead of resistance (abstract)

I find the manuscript as the one that requires minor corrections before it can be considered for indexing in Gates Open Research

Sincerely,

Dr. Johnson Matowo (Ph.D)

Is the work clearly and accurately presented and does it cite the current literature?
Yes

Is the study design appropriate and is the work technically sound?
Yes

Are sufficient details of methods and analysis provided to allow replication by others?
Yes

If applicable, is the statistical analysis and its interpretation appropriate?
Yes

Are all the source data underlying the results available to ensure full reproducibility?
Yes

**Are the conclusions drawn adequately supported by the results?**
Yes

**Competing Interests:** No competing interests were disclosed.

**Reviewer Expertise:** Medical entomology, with focus on understanding of the Biochemical and Molecular Basis of Insecticide Resistance in Malaria Vectors.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Author Response 18 Jun 2022

koama bayili, Institut de Recherche en Sciences de la Santé, Bobo-dioulasso, Burkina Faso

Dear Dr. Johnson Matowo

We thank Dr. Johnson Matowo for his constructive comments and suggestions to improve the manuscript. The manuscript will be revised accordingly. All changes or additions in the text will be done in the revised version before submit it. All the changes will be in yellow in the revised version.

Thank you very much again for your time in reviewing this manuscript.

**Competing Interests:** No competing interest