RESEARCH ARTICLE

Efficacy of targeted indoor residual spraying with the pyrrole insecticide chlorfenapyr against pyrethroid-resistant Aedes aegypti

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Abstract

Background

There is an increased need to mitigate the emergence of insecticide resistance and incorporate new formulations and modes of application to control the urban vector Aedes aegypti. Most research and development of insecticide formulations for the control of Ae. aegypti has focused on their peridomestic use as truck-mounted ULV-sprays or thermal fogs despite the widespread knowledge that most resting Ae. aegypti are found indoors. A recent modification of indoor residual spraying (IRS), termed targeted IRS (TIRS) works by restricting applications to 1.5 m down to the floor and on key Ae. aegypti resting sites (under furniture). TIRS also opens the possibility of evaluating novel residual insecticide formulations currently being developed for malaria IRS.

Methods

We evaluated the residual efficacy of chlorfenapyr, formulated as Sylando 240SC, for 12 months on free-flying field-derived pyrethroid-resistant Ae. aegypti using a novel experimental house design in Merida, Mexico. On a monthly basis, 600 female Ae. aegypti were released into the houses and left indoors with access to sugar solution for 24 hours. After the exposure period, dead and alive mosquitoes were counted in houses treated with chlorfenapyr as well as untreated control houses to calculate 24-h mortality. An evaluation for these exposed cohorts of surviving mosquitoes was extended up to seven days under laboratory conditions to quantify “delayed mortality”.

Results

Mean acute (24-h) mortality of pyrethroid-resistant Ae. aegypti ranged 80–97% over 5 months, dropping below 30% after 7 months post-TIRS. If delayed mortality was considered (quantifying mosquito mortality up to 7 days after exposure), residual efficacy was above
90% for up to 7 months post-TIRS application. Generalized Additive Mixed Models quantified a residual efficacy of chlorfenapyr of 225 days (ca. 7.5 months).

Conclusions

Chlorfenapyr represents a new option for TIRS control of *Ae. aegypti* in urban areas, providing a highly-effective time of protection against indoor *Ae. aegypti* females of up to 7 months.

Author summary

Vector control (VC) for managing *Aedes aegypti* and reducing transmission of *Aedes*-borne diseases has largely focused on peridomestic insecticide applications. However, the indoor resting behavior of *Ae. aegypti* and the acceleration of insecticide resistance owed to reduced modes of action have diminished the effectiveness of many VC tools. A targeted Indoor residual spraying (TIRS) modality in experimental housing units was employed to investigate the potential of chlorfenapyr, a pyrrole-class insecticide with known effectiveness to resistant mosquito species. This was the first investigation for chlorfenapyr use against locally resistant *Ae. aegypti* (Merida, Mexico) with this approach. Two treatment arms were investigated in the present study: TIRS and a control house where only water was sprayed. A comparison of entomological efficacy for TIRS applied to interior perimeter walls below 1.5 m with chlorfenapyr (formulated as Sylando 240SC) at 250 mg/m² over 12 months was assessed. TIRS chlorfenapyr treatments were highly efficacious and led to acute mortalities (after 24 exposure) above 80% up to 5 months; delayed mortalities (to *Ae. aegypti*) were monitored over seven days post-exposures vs untreated controls. When delayed mortality was considered, residual efficacy of chlorfenapyr extended to 7 months. These data provide evidence that TIRS chlorfenapyr is an effective *Aedes* management tool that surpassed efficacy profiles for other TIRS insecticides that have been previously reported with this method. Further, Chlorfenapyr emerges as a novel addition to *Ae. aegypti* VC, and future studies should focus on its effectiveness and residual power as part of Phase II-III TIRS trials.

Introduction

Controlling the anthropophilic disease vector *Aedes aegypti* has long been conducted by peridomestic application of truck-mounted ultra-low volume spraying, thermal fogging and larviciding [1,2]. Adult female *Ae. aegypti* are typically found indoors in urban settings, where they feed frequently and almost exclusively on human blood [3–5] and rest on surfaces that are unreachable with the routinely used insecticide methods. Peridomestic mosquito control tactics, therefore, lead to poorly-efficient and in the best case, transient control of the epidemiologically important biting female mosquitoes (e.g., *Ae. aegypti*) and thus, with limited impact in preventing arboviral disease transmission [6].

A novel application technique, which exploits *Aedes aegypti* resting behavior, termed targeted indoor residual spraying (TIRS), focuses the selective application of residual insecticides in lower walls (<1.5m) and other primary *Ae. aegypti* resting locations (under beds and furniture), reducing insecticide volumes and treatment time [7,8]. The development of TIRS was rooted on prior success in controlling *Ae. aegypti* using perifocal spraying of DDT [6,9,10],
and recent evaluations in a novel experimental house setting in Merida, Mexico [7]. Effectiveness of TIRS implementation has been confirmed in Cairns, Australia, where coverages of 60% or more led to reductions in dengue virus incidence of >86% [8]. Furthermore, modeling studies indicate that the highest effectiveness of TIRS occurs when the method is deployed preventively prior to the regular transmission season, instead of reactively to cases [11–13]. Preventive TIRS, while considered an approach that can overcome the limitations of IRS and increase insecticide application effectiveness, is dependent on having insecticide molecules to which Ae. aegypti is susceptible and insecticide formulations that can provide sustained control for 5 months or more [12].

Recent advancements in new and repurposed chemistry to mitigate mosquito-borne diseases have been seen from the development of non-pyrethroid IRS formulations to control malaria vectors [14–18]. Some of the innovation in new molecules stands from their unique toxicity mechanisms, which rely more on mosquito physiology than on “usual” neurological or simple detoxification pathways. Chlorfenapyr (commercially available as Phantom Termite-Cide -Insecticide in the United States, BASF for urban pest control and Sylando 240SC, BASF for public health use) is a new insecticide class (pyrrole) that acts as a physiological toxin, requiring activation as a pro-insecticide [19,20] to exert mosquito mortality [19]. Chlorfenapyr is a halogenated pyrrole that uncouples oxidative phosphorylation processes in mitochondria [20]; in other words, affects insect’s ability to produce energy in their mitochondria which consequently affects crucial and vital functions until eventual death. The mode of action of chlorfenapyr on an insect’s metabolism is particularly relevant for the control of vectors harboring metabolic insecticide resistance mechanisms (e.g., cytochrome P450, glutathione S-transferases), as increased metabolic activity increases the activation of the toxin and increase mosquito mortality [19]. Furthermore, as these new physiological insecticides depend on the mosquito metabolism to act, they generally present delayed toxicity (in the order of 1–5 days) when insects are inactive or constrained to a cage, making their evaluation using conventional neuro-toxic tests (e.g., WHO cone bioassay) challenging [21]. While chlorfenapyr has recently been evaluated against Anopheles sp. Vectors, no rigorous evaluation of its efficacy on Ae. aegypti has been published.

TIRS evaluation of the carbamate Bendiocarb on a novel experimental house setting established in Merida, Mexico (i.e., typical residential houses rented long-term and double-screened to allow for free-flying mosquitoes to be exposed to diverse insecticide treatments), led to a 4-month residual efficacy against pyrethroid-resistant Ae. aegypti [22]. Such experimental setup provides a unique opportunity to evaluate new insecticide formulations for TIRS against Ae. aegypti, as it saves the cost of running experiments in the open field or in expensive lab enclosures. Here, we evaluated the residual efficacy of chlorfenapyr (Sylando 240SC; BASF) against a locally-derived, pyrethroid-resistant, strain of Ae. aegypti.

Methods

Ethics statement

This was an experimental study, and because mosquitoes were released into uninhabited houses rented on long-term contracts, we did not require an Institutional Review Board evaluation.

Experimental house layout

We conducted this evaluation within two experimental houses located in Ciudad Caucel, a neighborhood of the subtropical city of Mérida, México [22]. The houses are rented long-term by the Universidad Autónoma de Yucatán (UADY) after explaining the purpose and extent of
The study to their owners. Distance between experimental houses was 0.3 km. The houses were similar in floor plan and design; all were concrete, single-story and had a living-dining room, two bedrooms, one bathroom and one kitchen (Fig 1). Houses were on average $57.8 \pm 2.8 \text{ m}^2$ (mean $\pm$ SEM) and uniformly had walls $2.5 \text{ m}$ in height. Construction characteristics were that of subsidized middle to low-income housing in Mérida, typical of areas with high ABD transmission [23].

To prevent any mosquitoes used in the experiments from escaping from the houses, all windows and doors (inside & outside), including furniture were sealed. A double screened entrance was also installed. Simulated furniture was standardized in each house. Buckets of water with cloth piece and oscillating fans were installed to keep optimal humidity and temperature as showed in the figure.

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To prevent any mosquitoes used in the experiments from escaping from the houses, all windows and doors were screened on both the outside and inside of each house before the study began. Additionally, a double screened-door vestibule was built into the main entrance of each house to allow personnel to enter and exit while preventing mosquitoes from escaping (Fig 1). Sinks, drains and toilets were also sealed with window screening. Existing furniture within houses was removed, and where furniture could not be removed (e.g., built-in kitchen or closet cabinets) it was sealed with window screening. Houses were then refurnished with standardized furniture and clothing that represented typical elements found within houses (Fig 1). Furniture within the living room included two black plastic tables and four plastic chairs. Within each bedroom was a bed made out of PVC tubing and black cloth, a black plastic nightstand and six articles of clothing (3 black and 3 white) hung within the closet. Four plastic buckets (1 L) were half filled with water and a dark cloth and placed throughout each house to provide
moisture into the environment and reduce mosquito mortality due to desiccation. Ant baits (Antex Gel, Allister de México with 0.05% abamectin) were placed next to each door or any other location where ants were observed to enter the experimental houses. The house layout was carefully designed to mirror elements and surface materials found in most homes but ensuring standardization in a way that allowed replication and comparability between replicates.

**Insecticide application**

Insecticide and untreated controls (water) were applied within experimental houses on 18 March 2019. Manual compression sprayers, IK-Vector Control Super (Goizper Group, Antzuola, Spain) were fitted with 8002EVP nozzle and a Goizper Low Pressure Control Flow Valve (output pressure 1.5 bar) to administer sprays to houses at a flow rate of 580 mL/min (±5%), according to following preparations: Sylando 240SC Target dose 250 mg/m² and 286 mL diluted in 7.5L water as recommended by the manufacturer in the proposed label and detailed in prior IRS trials conducted in Africa and India by WHOPES [24]. All applications were performed by the same applicator. TIRS application was conducted as described in Dunbar et al. [22]. Briefly, insecticide (or water, for the control) was applied to walls below 1.5 m and under furniture or to the undersides of furniture. Furniture was not removed from experimental houses during the insecticide application and insecticide was not applied to clothing or the plastic buckets with water.

**Mosquito strain**

To test the residual efficacy of each IRS application method, groups of 100 Ae. aegypti females three to seven days old from F4 generation were released within each experimental house. The strain used (Juan Pablo strain, JP) was locally derived, had a high level of resistance to pyrethroids but full susceptibility to carbamates [25,26]. The JP strain was reared and maintained at the insectaries of the Unidad Colaborativa para Bioensayos Entomológicos (UCBE), UADY, Mérida, México, at constant laboratory conditions (27°C and 60% RH). Resistance is maintained by periodic mixing of the colony with recently hatched larvae from field-collected eggs and monitored using the CDC bottle bioassay [27] and genotyped using standard PCR methods [25,26] to detect two of the most common single nucleotide polymorphisms of the voltage gated sodium channel gene (i.e., at positions 1,016 and 1,534) as described elsewhere [25,26]. Mosquitoes released into houses had only been provided sugar solution and were non-bloodfed.

**Intervention evaluation**

Post-insecticide application, mosquitoes were released into the experimental houses (both in houses treated with chlorfenapyr as well as untreated control houses) eleven times over a 12-month period; 1) +1 day, 2) +14 days, 3) +1 month, 4) +2 months, 5) +5 months, 6) +7 months, 7) +8 months, 8) +9 months, 9) +10 months, 10) + 11 months, and 11) +12 months (see Table 1). Replication of this design occurred by conducting three independent releases, on three consecutive days, for each period (Fig 2). To facilitate mosquito detection, all experimental houses were vacuumed and swept clean of any debris on the floor one day prior to mosquito release. After a 24 hr exposure in the houses, a team of four field technicians entered each house and searched for live mosquitoes using a Prokopack aspirator [28] and searched by hand for dead mosquitoes. This 24-h exposure period allowed quantification of acute mortality. Searching for Ae. aegypti ceased when either 100 mosquitoes were collected or > 20 minutes elapsed after the last mosquito was collected (circa 30–40 min / house). Sampling dates are provided in Fig 2 for release of cohorts into experimental houses. Acute (24-h) mortality
was calculated from the number of dead/live *Ae. aegypti* found at the end of the exposure period in the houses. Exposed mosquitoes were held at the UCBE insectary inside bugdorm cages (30x30x30 cm) for 7 days at 26 ± 2°C and 75 ± 5%RH and monitored daily for signs of intoxication to quantify “delayed mortality” because uncoupling of oxidative phosphorylation and the necessary requirement for mosquitoes to enzymatically convert parent chlorfenapyr (CL303630) to its n-dealkylated metabolite (CL303268) delay the appearance of toxicity effects in mosquitoes [19,29]. On each house, we placed three unsprayed control cups (250 mL) containing 10 JP strain females each during the 24-h exposure period to have an independent measure of mosquito mortality due to the temperature and humidity conditions of the experimental houses. This measure was estimated at the +5, +7, +8–12 months post-application evaluations, which coincided with the warmest periods of the year in Merida.

**Statistical analyses**

For each sampling period, acute and delayed mortalities were calculated per house by dividing the number of dead individuals by the number of individuals released. Missing individuals were assumed to be dead. Due to the mortalities in the control group (when they were observed) which ranged from 2–23%, the mortality calculation was corrected according to the

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**Table 1. Sampling dates for Release of Mosquitoes into experimental houses in Ciudad Caucel neighborhood of Merida, MX.** For each of the two experimental houses, three consecutive releasing events were implemented in each period of time to evaluate, using days as replicates (also see Fig 2). The mosquito strain (Juan Pablo) was pyrethroid-resistant; resistance was maintained by periodic reseeding of populations with field-collected eggs (see methods).

| Post-application releasing | Days post application | Releasing dates  | Number of Mosquitoes |
|----------------------------|----------------------|------------------|----------------------|
| 1 day                      | 1                    | 19–21 March, 2019| n = 600              |
| 2 weeks                    | 14                   | 2–4 April, 2019  | n = 600              |
| 1 month                    | 30                   | 16–18 April, 2019| n = 600              |
| 2 months                   | 60                   | 27–29 May, 2019  | n = 600              |
| 5 months                   | 150                  | 11–13 August, 2019| n = 600             |
| 7 months                   | 210                  | 21–23 October, 2019| n = 600           |
| 8 months                   | 240                  | 20–22 November, 2019| n = 600        |
| 9 months                   | 270                  | 15–17 December, 2019| n = 600         |
| 10 months                  | 300                  | 19–21 January, 2020| n = 600         |
| 11 months                  | 330                  | 16–18 February, 2020| n = 600         |
| 12 months                  | 360                  | 17–19 March, 2020 | n = 600            |

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formula of Abbott (1925). On each evaluation date, corrected acute mortalities were compared to the 80% threshold set by WHO as the cutoff for effective insecticidal effect of indoor residual spraying [30]. Further, both acute and delayed mortalities were compared between treatment and control using binomial generalized linear mixed models (GLMM) in R 4.0.5 statistical software (https://www.r-project.org/) using package lme4. For each date, treatment was classified as fixed effect and experimental replicate was classified as a random effect.

A Generalized Additive Mixed Model (GAMM) determined the association between acute and delayed mortality and the time (in days) since TIRS application. Time to intervention was calculated by estimating the number of days that elapsed between TIRS and the entomological evaluation. The full model had the form: Mortality = $\alpha + f(\text{Days}) + Z(\text{Replicate}) + \epsilon$. Where $Z(\text{Replicate})$, represents a random effects term associated with observations from the same time point, $\alpha$ the model constant and $\epsilon$ the error term. We fitted $f(\text{Days})$, the non-linear term of mortality and days since TIRS, by applying a penalized cubic spline function to the data and a Gaussian link function to fit the model. The parameter $f(\text{Days})$ was fitted separately to the control and chlorfenapyr data. Exploration of fitted $f(\text{Days})$ allowed assessing the temporal trend in Ae. aegypti mortality after TIRS. Specifically, since $f(\text{Days})$ describes the non-linear fit of the time since TIRS application to the mortality data, we used the parameter’s 95% credible interval (95%CI) to quantify: 1) if the 95%CI of $f(\text{Days})$ differed significantly between control and chlorfenapyr treatments; and 2) at what time point the predicted non-linear fit for chlorfenapyr (with 95% CI) went from positive to negative, indicating a loss of impact of the insecticide on mosquito mortality. The package mgcv was used to fit and plot the results of the GAMM.

Results

TIRS was implemented according to standard protocol (spraying walls below 1.5 m and under furniture) on March 18, 2019. A total of 7,200 Ae. aegypti females were released within the experimental houses throughout the trial. Recapture of released mosquitoes (dead and alive) averaged 97.5 ± 5.3% (Mean ± SEM; n = 66 releases). Based on prior studies applying TIRS, we attribute high recovery to pre-cleaning the floors of experimental houses the day before mosquitoes were released and to effective management of ants using baits. Mortality within cups left inside houses to monitor natural mortality averaged 3.2 ± 1.1, 4.8 ± 0.8, 2.3 ± 1.5, 1.9 ± 0.6, 4.4 ± 1.3%, 1.5 ± 0.7% and 5.0 ± 1.7% (Mean ± SEM) for evaluations from +5, +7, +8, +9, +10, +11 and +12 months post-application, respectively, indicating negligible effect of high summer temperatures on mortality. Before the first release, recently emerged female Ae. aegypti mosquitoes were tested for susceptibility to permethrin, deltamethrin and chlorpyrifos (100 females per insecticide). At the diagnostic time for each insecticide, 72%, 94% and 100% female mosquitoes were dead in the permethrin, deltamethrin, and chlorpyrifos groups, respectively. After 6 months, and to maintain genetic diversity and resistance mechanisms, the laboratory strain was mixed with a batch of 1,000 recently emerged larvae from field-collected eggs. Emerging adults from the mixed colony experienced mortalities at the diagnostic time of 62% for permethrin, 92% for deltamethrin and 100% for chlorpyrifos. A subsample of 141 female Ae. aegypti from the mixed colony was genotyped for the presence of the two most common kdr mutations. For the 1,016 mutation, 27.7% mosquitoes were homozygous susceptible, whereas 26.2% were homozygous resistant and 46% were heterozygous. For the 1,534 mutation, only 10.6% were homozygous susceptible, whereas 66.0% were homozygous resistant and 23.4% heterozygous. This information is indicative of pyrethroid resistance in the population.

Acute mortality of female Ae. aegypti released into the houses was significantly higher and sustained in houses sprayed with chlorfenapyr compared to control houses up to 11 months.
post spraying (Fig 3, Table 2). Abbott-corrected average mortalities (including their standard error) were equal or higher than the 80% mortality threshold up to 5 months post-TIRS. A remarkable reduction on the mortality (15–16%) was observed at 8 to 11 months, whereas no mortality was observed at 12 months (Fig 3, Table 2).

Delayed mortality was recorded during 7 days post-exposure for most collection periods (Fig 4). Total delayed mortality (100%) was observed after 48 hr of exposure at 1 and 2 months post-TIRS application. At 5 and 7 months post TIRS application, delayed mortality was 96.6% and 99.3% after 2–7 days of observation, respectively. At 8 and 9 months the delayed mortality reached 75% and 64% after 7 days of observation respectively. At 10 & 11 months the maximum mortality reached after 7 days of observation was 41% and 36% respectively. At 12 months no delayed mortality was observed (0% after 7 days of observation). Similar levels of statistical significance as described for acute mortality when comparing chlorfenapyr and control data were observed for delayed mortality (S1 Table).

Fig 5 shows the plot of f(Days), obtained after fitting a GAMM to the mortality data of the control and chlorfenapyr houses. The y-axis can be interpreted as the effect of time since TIRS on mosquito mortality. When the predicted value and its 95% credible interval are negative, it means that there is a significant reduction in mortality. Chlorfenapyr led to a significant reduction in mortality up to 225 days (ca. 7.5 months, vertical line on right panel of Fig 5A and 5B) post-TIRS application.
This study provides information about a new insecticide chemistry for the urban control of pyrethroid-resistant *Ae. aegypti* using a novel experimental house system that incorporates typical living conditions in urban areas of an endemic area for ABVs. Results from this study show that a single TIRS application of chlorfenapyr (Sylando, 240SC Target dose 250 mg/m²) led to mosquito mortalities above 80% for up to 5 months and to delayed mortalities above the 80% threshold for up to 7 months. Operationally, results suggest that a single application of chlorfenapyr can provide a new highly-effective and sustainable alternative for TIRS application for ministry of health institutional programs to control *Ae. aegypti* in urban areas.

Studies both in the laboratory and field environments have shown the ability of many insect species to rest on surfaces treated with chlorfenapyr for extended periods of time [31–33]. The non-repellent nature of chlorfenapyr, described in other studies on mosquitoes [21,34–36], may have led to greater resting times and insecticide uptake compared to pyrethroids, contributing to observed mortalities in experimental houses. The physiological effect of chlorfenapyr on free-flying mosquitoes may have also contributed to the extended and significant direct and delayed mortality effects observed. The enzymatic transformation of parent chlorfenapyr (CL303630) to its pro-insecticidal metabolite (CL303268) can be slow and quite variable, but generally unidirectional once conversion has started [29]. The uncoupling of oxidative phosphorylation can be influenced by many exogenous and endogenous factors: temperature, cuticular penetrations, physical movement of challenged insects, host-seeking behaviors,

### Table 2. Average (min-max) raw acute (24-h) mortality data and Abbott-corrected mortality throughout the 11 sample periods (24 hours, 2 weeks, 1, 2, 5, 7, 8, 9, 10, 11 and 12 months) and results from a Generalized Linear Mixed Model (GLMM) quantifying the significance in mortality between control and treatment measures (control used as baseline).

| Days post TIRS | Treatments | Recapture after 24-h | Mortality | Corrected Mortality | Coefficient (std. error) | P-value |
|---------------|------------|---------------------|-----------|--------------------|--------------------------|---------|
| 1             | Chlorfenapyr 87.3 (82–94) | 93.4 (87–100) | 92.7 (86–100) | 0.792 (0.05) | 0.0001 |
|               | Control 92 (88–94) | 13.5 (6.7–19.1) | — | — | — |
| 14            | Chlorfenapyr 92 (86–96) | 93.9 (87.8–98) | 93.6 (87.2–97.8) | 0.868 (0.04) | <0.0001 |
|               | Control 96.7 (94–98) | 6.8 (4–10.2) | — | — | — |
| 30            | Chlorfenapyr | 100 | 97.6 (95.2–100) | 97.5 (95.2–100) | 0.949 (0.02) | <0.0001 |
|               | Control 100 | 2.7 (0–4) | — | — | — |
| 60            | Chlorfenapyr 96.7 (94–100) | 93.1 (87.2–97.3) | 82 (77.3–88.7) | 0.677 (0.07) | 0.0007 |
|               | Control 92.7 (78–100) | 14.4 (2–23.1) | — | — | — |
| 150           | Chlorfenapyr 93.3 (90–96) | 90.4 (88.2–93.1) | 88.6 (86–91.8) | 0.734 (0.02) | <0.0001 |
|               | Control 100 | 15.3 (14–16) | — | — | — |
| 210           | Chlorfenapyr 100 | 72.9 (56.5–83.3) | 72.9 (56.5–83.3) | 0.730 (0.008) | 0.0009 |
|               | Control 100 | 0 | — | — | — |
| 240           | Chlorfenapyr 100 | 17.3 (12–28) | 16.3 (12–24.9) | 0.149 (0.05) | 0.0298 |
|               | Control 98.7 (96–100) | 1.4 (0–4.2) | — | — | — |
| 270           | Chlorfenapyr 100 | 15.3 (8–22) | 15.3 (8–22) | 0.153 (0.05) | 0.0194 |
|               | Control 100 | 0 (0–0) | — | — | — |
| 300           | Chlorfenapyr 100 | 25.3 (18–38) | 25.3 (18–38) | 0.253 (0.06) | 0.0164 |
|               | Control 100 | 0 (0–0) | — | — | — |
| 330           | Chlorfenapyr 100 | 16.7 (14–18) | 16.7 (14–18) | 0.166 (0.01) | 0.0002 |
|               | Control 100 | 0 (0–0) | — | — | — |
| 360           | Chlorfenapyr 100 | 0 | 0 | N/A | 1 |
|               | Control 100 | 0 (0–0) | — | — | — |

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blood-feeding status of mosquitoes, concentrations of chlorfenapyr challenged to insects from different substrates, degree of metabolic activity already within target pests and antagonisms by known metabolic inhibitors or competing resistant mechanisms (e.g., Glutathione S-Transferases or GSTs are not known to favor similar intoxication routes as cytochrome P450s) [21,29,37,38]. Ultimately, as chlorfenapyr is a physiological toxin, normal mosquito behaviors during their circadian rhythms will favor intoxication [19,20] and its evaluation in small cages may yield different (poorer) results compared to experimental houses.

Novel chemistries are challenging the original ‘neurotoxic thinking’ of the mode of action of insecticides and are pushing testing procedures to move beyond quantification of acute mortality to account for delayed mortality and other physiological and behavioral effects. Delayed mortality has been reported for novel chemistries currently being used or evaluated for malaria IRS, clothianidin [39,40], broflanilide [17,41] and chlorfenapyr [21,34]. Delayed intoxication has also been shown for pyriproxyfen, which reduced life-span and female *Anopheles* sp. fecundity when exposed to new generation nets [42]. Our study shows for the first time the delayed mortality effect of chlorfenapyr on exposed *Ae. aegypti*. Not considering delayed mortalities may lead to considering the molecule’s efficacy to be shorter than it actually is (in our case, 5 months instead of 7). This aspect was noted in an IRS WHOPES phase III trial.
Fig 5. Generalized Additive Linear Mixed Model (GAMM) fitted to the association between mortality [s(Mortality)] and days since TIRS application [f(Days)] for the control and chlorfenapyr houses. The gray vertical line on the right panel shows the threshold of change from positive to negative impact of chlorfenapyr on mosquito mortality.

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Fig 6. Comparison of acute mortality after TIRS application of bendiocarb (Dunbar et al. [7], blue line) and chlorfenapyr (orange, present study) in experimental houses from Merida, Mexico.

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conducted in the Gambia where researchers observed that although the threshold for standard mortality metrics were observed to be declining, there was indeed a broader epidemiological impact of chlorfenapyr IRS compared to DDT [24]. Our delayed mortalities >80% of up to 7 months were comparable to WHOPES phase II hut trials against Anopheles sp mosquitoes, which showed 8 months efficacy after accounting for delayed mortality [24].

Some studies have demonstrated that detoxifying enzymes (P450s) in mosquitoes that are responsible for converting parent chlorfenapyr (CL303630) to its pro-insecticide metabolite (CL303268) can be inhibited with known inhibitors like PBO in measurable ways both in vivo and in vitro [29,43,44]. To less experienced researchers with this mode of action, the tendency to assume resistance rather than poor conversion (from parent to pro-insecticidal metabolite) requires consideration of laboratory or field-testing conditions which might interfere with chlorfenapyr’s mode of action [29,38] as influenced by numerous endogenous and exogenous elements [21]. Other studies point to induction routes which favor pre-exposures to neurotoxic chemistries (e.g., alpha-cypermethrin or others) which may actually enhance the conversion rates of the more toxic form of chlorfenapyr to mosquitoes as do more metabolically resistant mosquito strains (68,74). The lack of cross-resistance [43,45] and general trends for intoxication to various metabolic resistant dipterans [46] makes chlorfenapyr relevant for insecticide resistance management.

Having demonstrated utility and regional acceptance [47], the TIRS application method may provide important public health benefits when applied preventively before the transmission season [12]. Such benefit relies on the availability of long-lasting residual insecticides. Mathematical modeling showed that effectiveness of TIRS can be increased up to 90% compared to not conducting TIRS when residual efficacy of the insecticide lasts 5 months [12]. An ongoing Phase III two-arm clinical trial is evaluating the epidemiological impact of preventive TIRS on Aedes-borne viruses [9] using insecticides to which Ae. aegypti is susceptible. In urban tropical environments, pyrethroids such as deltamethrin have residual efficacies of up to 3–6 months but are severely challenged by the presence of resistance in the mosquito population [26]. Alternative chemistries (to which Ae. aegypti is susceptible) exist, and the carbamate bendiocarb has provided not only to control pyrethroid-resistant Ae. aegypti [26] but also to exert mortalities >80% for up to 4 months in experimental houses [7]. Our study shows that, in experimental houses, chlorfenapyr can extend TIRS residual efficacy against Ae. aegypti up to seven months (Fig 6). Future studies should evaluate the entomological impact of chlorfenapyr TIRS against Ae. aegypti in field randomized trials, providing evidence of the value of this new chemistry for the management of pyrethroid resistance and the prevention of Aedes-borne viruses.

Supporting information

S1 Table. Results from a Generalized Linear Mixed Model (GLMM) quantifying the significance in delayed mortality between control and treatment measures (control used as baseline).

(DOCX)

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References

1. Achee NL, Gould F, Perkins TA, Reiner RC Jr., Morrison AC, Ritchie SA, et al. A critical assessment of vector control for dengue prevention. PLoS neglected tropical diseases. 2015; 9(5):e0003655. https://doi.org/10.1371/journal.pntd.0003655 PMID: 25951103; PubMed Central PMCID: PMC4423954.

2. Ritchie SA, Devine GJ, Vazquez-Prokopec G, Lenhart A, Manrique-Saide P, Scott TW. Insecticide-based approaches for dengue vector control. Innovative strategies for vector control. 6. Wageningen, ND: Wageningen Academic Publishers; 2021. p. 59–89.

3. Dzul-Manzanilla F, Ibarra-Lopez J, Bibiano Marin W, Martini-Jaimes A, Leyva JT, Correa-Morales F, et al. Indoor Resting Behavior of Aedes aegypti (Diptera: Culicidae) in Acapulco, Mexico. Journal of medical entomology. 2017; 54(2):501–4. https://doi.org/10.1093/jme/tjw203 PMID: 28011725.

4. Chadee DD. Resting behaviour of Aedes aegypti in Trinidad: with evidence for the re-introduction of indoor residual spraying (IRS) for dengue control. Parasites & vectors. 2013; 6(1):255. https://doi.org/10.1186/1756-3305-6-255 PMID: 24004641; PubMed Central PMCID: PMC3847653.

5. Perich MJ, Davila G, Turner A, Garcia A, Nelson M. Behavior of resting Aedes aegypti (Culicidae: Diptera) and its relation to ultra-low volume adulticide efficacy in Panama City, Panama. Journal of medical entomology. 2000; 37(4):541–6. https://doi.org/10.1603/0022-2585-37.4.541 PMID: 10916294.

6. Bowman LR, Donegan S, McCall PJ. Is Dengue Vector Control Deficient in Effectiveness or Evidence?: Systematic Review and Meta-analysis. PLoS neglected tropical diseases. 2016; 10(3):e0004551. https://doi.org/10.1371/journal.pntd.0004551 PMID: 26986468; PubMed Central PMCID: PMC4795802.

7. Dunbar MW, Correa-Morales F, Dzul-Manzanilla F, Medina-Barreiro A, Bibiano-Marin W, Morales-Rios E, et al. Efficacy of novel indoor residual spraying methods targeting pyrethroid-resistant Aedes aegypti within experimental houses. PLoS Negl Trop Dis. 2019; 13(2):e0007203. Epub 2019/03/01. https://doi.org/10.1371/journal.pntd.0007203 PMID: 30817759; PubMed Central PMCID: PMC6394901.

8. Vazquez-Prokopec GM, Montgomery BL, Horne P, Clennon JA, Ritchie SA. Combining contact tracing with targeted indoor residual spraying significantly reduces dengue transmission. Science advances. 2017; 3(2):e1602024. https://doi.org/10.1126/sciadv.1602024 PMID: 28232955; PubMed Central PMCID: PMC5315446.

9. Manrique-Saide P, Dean NE, Halloran ME, Longini IM, Collins MH, Waller LA, et al. The TIRS trial: protocol for a cluster randomized controlled trial assessing the efficacy of preventive targeted indoor residual spraying to reduce Aedes-borne viral illnesses in Merida, Mexico. Trials. 2020; 21(1):839. Epub 2020/10/10. https://doi.org/10.1186/s13063-020-04780-7 PMID: 33032661; PubMed Central PMCID: PMC7542575.
10. Samuel M, Maoz D, Manrique P, Ward T, Runge-Ranzinger S, Toledo J, et al. Community effectiveness of indoor spraying as a dengue vector control method: A systematic review. PLoS Neglected tropical diseases. 2017; 11(8):e0005837. https://doi.org/10.1371/journal.pntd.0005837 PMID: 28859087; PubMed Central PMCID: PMC5578493.

11. Cavany SM, Espana G, Lloyd AL, Waller LA, Kitron U, Astete H, et al. Optimizing the deployment of ultra-low volume and indoor residual spraying for dengue outbreak response. PLoS Comput Biol. 2020; 16(4):e1007743. Epub 2020/04/21. https://doi.org/10.1371/journal.pcbi.1007743 PMID: 32310958.

12. Hladish TJ, Pearson CAB, Patricia Rojas D, Gomez-Dantes H, Halloran ME, Vazquez-Prokopec GM, et al. Forecasting the effectiveness of indoor residual spraying for reducing dengue burden. PLoS neglected tropical diseases. 2018; 12(6):e0006570. Epub 2018/06/26. https://doi.org/10.1371/journal.pntd.0006570 PMID: 2939983; PubMed Central PMCID: PMC6042783 providing quality, unbiased scientific consulting for hire; it sells no other product or service. All authors declare that no competing interests exist.

13. Hladish TJ, Pearson CAB, Toh KB, Rojas DP, Manrique-Saide P, Vazquez-Prokopec GM, et al. Designing effective control of dengue with combined interventions. Proc Natl Acad Sci U S A. 2020; 117(6):3319–25. Epub 2020/01/25. https://doi.org/10.1073/pnas.1903496117 PMID: 31974303; PubMed Central PMCID: PMC7022216.

14. Ngufor C, Critchley J, Fagbohon J, N’Guessan R, Tadjinou D, Rowland M. Chlorfenapyr (a Pyrrole Insecticide) Applied Alone or as a Mixture with Alpha-Cypermethrin for Indoor Residual Spraying against Pyrethroid Resistant Anopheles gambiae s.l: An Experimental Hut Study in Cove, Benin. PLoS One. 2016; 11(9):e0162210. Epub 2016/09/03. https://doi.org/10.1371/journal.pone.0162210 PMID: 27588945; PubMed Central PMCID: PMC5010291.

15. Ngufor C, Fongnikin A, Hobbs N, Gbegbo M, Kiki L, Odjo A, et al. Indoor spraying with chlorfenapyr (a pyrrole insecticide) provides residual control of pyrethroid-resistant malaria vectors in southern Benin. Malar J. 2020; 19(1):249. Epub 2020/07/15. https://doi.org/10.1186/s12936-020-03325-2 PMID: 32660479; PubMed Central PMCID: PMC7359555.

16. Ngufor C, Fongnikin A, Rowland M, N’Guessan R. Indoor residual spraying with a mixture of clothianidin (a neonicotinoid insecticide) and deltamethrin provides improved control and long residual activity against pyrethroid resistant Anopheles gambiae s.l in Southern Benin. PLoS One. 2017; 12(12): e0189575. Epub 2017/12/19. https://doi.org/10.1371/journal.pone.0189575 PMID: 29252986; PubMed Central PMCID: PMC5734732.

17. Ngufor C, Goveetchan R, Fongnikin A, Vigninou E, Syme T, Akogbeto M, et al. Efficacy of broflanilide (VECTRON T500), a new meta-diame insecticide, for indoor residual spraying against pyrethroid-resistant malaria vectors. Sci Rep. 2021; 11(1):7976. Epub 2021/04/14. https://doi.org/10.1038/s41598-021-86935-3 PMID: 3486394; PubMed Central PMCID: PMC8042056.

18. Ngufor C, N’Guessan R, Boko P, Odjo A, Vigninou E, Asidi A, et al. Combining indoor residual spraying with chlorfenapyr and long-lasting insecticidal bed nets for improved control of pyrethroid-resistant Anopheles gambiae: an experimental hut trial in Benin. Malar J. 2011; 10:343. Epub 2011/11/18. https://doi.org/10.1186/1475-2875-10-343 PMID: 22037506; PubMed Central PMCID: PMC3229591.

19. Hunt DA, Treacy MF. Pyrrole Insecticides: A New Class of Agriculturally Important Insecticides Functioning as Uncouplers of Oxidative Phosphorylation. In: Ishaya I, Degheele D, editors. Insecticides with Novel Modes of Action: Mechanisms and Application. Berlin, Heidelberg: Springer Berlin Heidelberg; 1998. p. 138–51.

20. Hollingworth R, Gadelhak G, Kuhr R, Motoyama N, editors. Mechanisms of action and toxicity of new pesticides that disrupt oxidative phosphorylation1998.

21. Oxborough RM, N’Guessan R, Jones R, Kitau J, Ngufor C, Malone D, et al. The activity of the pyrrole insecticide chlorfenapyr in mosquito bioassay: towards a more rational testing and screening of non-neurotoxic insecticides for malaria vector control. Malar J. 2015; 14:124. Epub 2015/04/17. https://doi.org/10.1186/s12936-015-0639-x PMID: 25879231; PubMed Central PMCID: PMC4390098.

22. Dunbar MW, Correa-Morales F, Dzul-Manzanilla F, Medina-Barreiro A, Bibiano-Marin W, Morales-Rios E, et al. Efficacy of Novel Indoor Residual Spraying Methods Targeting Pyrethroid-Resistant Aedes aegypti. PLoS neglected tropical diseases. 2019; 13(2):e0007203. https://doi.org/10.1371/journal.pntd.0007203 PMID: 30817759

23. Bisanzio D, Dzul-Manzanilla F, Gomez-Dantes H, Pavia-Ruz N, Hladieh TJ, Lenhart A, et al. Spatio-temporal coherence of dengue, chikungunya and Zika outbreaks in Merida, Mexico. PLoS Neglected tropical diseases. 2018; 12(3):e0006298. https://doi.org/10.1371/journal.pntd.0006298 PMID: 29543910; PubMed Central PMCID: PMC5870998.

24. World Health Organization. Report of the sixteenth WHOPES working group meeting: WHO/HQ, Geneva, 22–30 July 2013: review of Pirimiphos-methyl 300 CS, Chlorfenapyr 240 SC, Deltamethrin 62.5 SC-PE, Durenat LN, Netprotect LN, Yae LN, Spinosad 83.3 Monolayer DT, Spinosad 25 Extended release GR.2013.
25. Grossman MK, Uc-Puc V, Rodriguez J, Cutler DJ, Moran LT, Manrique-Saide P, et al. Restoration of pyrethroid susceptibility in a highly resistant Aedes aegypti population. Biol Lett. 2018; 14(6). Epub 2018/06/15. https://doi.org/10.1098/rsbl.2018.0022 PMID: 29899128; PubMed Central PMCID: PMC6030600.

26. Vazquez-Prokopec GM, Medina-Barreiro A, Che-Mendoza A, Dzul-Manzanilla F, Correa-Morales F, Guillermo-May G, et al. Deltamethrin resistance in Aedes aegypti results in treatment failure in Merida, Mexico. PLoS neglected tropical diseases. 2017; 11(6):e0005656. https://doi.org/10.1371/journal.pntd.0005656 PMID: 28646468; PubMed Central PMCID: PMC5481028.

27. Deming R, Manrique-Saide P, Medina Barreiro A, Cardena EU, Che-Mendoza A, Jones B, et al. Spatial variation of insecticide resistance in the dengue vector Aedes aegypti presents unique vector control challenges. Parasites & vectors. 2016; 9:67. Epub 2016/02/06. https://doi.org/10.1186/s13071-016-1346-3 PMID: 26846468; PubMed Central PMCID: PMC4743324.

28. Vazquez-Prokopec GM, Galvin WA, Kelly R, Kitron U. A new, cost-effective, battery-powered aspirator for adult mosquito collections. Journal of medical entomology. 2009; 46(6):1256–9. Epub 2009/12/08. https://doi.org/10.1603/033.046.0602 PMID: 19960668; PubMed Central PMCID: PMC2800949.

29. Black B, Hollingworth K, Ahammadshahib C, Kuvel S, Donovan S. Insecticidal Action and Mitochondrial Uncoupling Activity of AC-303,630 and Related Halogenated Pyrroles. Pesticide Biochemistry and Physiology. 1994; 50(2):115–28.

30. World Health Organization. Guidelines for testing mosquito adulticides for indoor residual spraying and treatment of mosquito nets. Zaim/WHOES DR, editor. Geneva: WHO; 2006.

31. Agnew JL, Romero A. Behavioral Responses of the Common Bed Bug, Cimex lectularius, to Insecticide TREATMENT. J Econ Entomol. 2005; 98(2):485–92. Epub 2005/05/14. https://doi.org/10.1603/0022-0493-98.2.485 PMID: 15889742.

32. Buczowski G, Scharf ME, Ratliff CR, Bennett GW. Efficacy of simulated barrier treatments against laboratory colonies of Pharaoh ant. J Econ Entomol. 2005; 98(2):485–92. Epub 2005/05/14. https://doi.org/10.1603/0022-0493-98.2.485 PMID: 15889742.

33. Rust MK, Saran RK. Toxicity, repellency, and transfer of chlorfenapyr against western subterranean termites (Isoptera: Rhinotermitidae). J Econ Entomol. 2006; 99(3):864–72. Epub 2006/07/04. https://doi.org/10.1603/033.046.0602 PMID: 16819853.

34. Mosha FW, Lyimo IN, Oxborough RM, Malima R, Tenu F, Matowo J, et al. Experimental hut evaluation of the pyrrole insecticide chlorfenapyr on bed nets for the control of Anopheles arabiensis and Culex quinquefasciatus. Trop Med Int Health. 2008; 13(5):644–52. Epub 2008/04/19. https://doi.org/10.1111/j.1365-3156.2008.02059.x PMID: 18419583.

35. Bayili K, N’do S, Namountougo M, Sanou R, Ouattara A, Dabire RK, et al. Evaluation of efficacy of Insecticide-Resistant Anopheles gambiae and Culex quinquefasciatus mosquitoes chlorfenapyr and alpha-cypermethrin against pyrethroid-resistant. Trop Med Int Health. 2017; 11(1):293. Epub 2018/05/12. https://doi.org/10.1186/s13071-018-2869-6 PMID: 29747684; PubMed Central PMCID: PMC5422893.

36. N’Guessan R, Boko P, Odjo A, Knols B, Akogbeto M, Rowland M. Control of pyrethroid-resistant Anopheles gambiae and Culex quinquefasciatus mosquitoes with chlorfenapyr in Benin. Trop Med Int Health. 2009; 14(4):389–95. Epub 2009/02/21. https://doi.org/10.1111/j.1365-3156.2009.02245.x PMID: 19228349.

37. Massue DJ, Kisinza WN, Malongo BB, Mgaya CS, Bradley J, Moore JD, et al. Restoration of pyrethroid susceptibility in a highly resistant Aedes aegypti population. Biolog Lett. 2018; 14(6). Epub 2018/06/15. https://doi.org/10.1098/rsbl.2018.0022 PMID: 29899128; PubMed Central PMCID: PMC6030600.

38. Vazquez-Prokopec GM, Medina-Barreiro A, Che-Mendoza A, Dzul-Manzanilla F, Correa-Morales F, Guillermo-May G, et al. Deltamethrin resistance in Aedes aegypti results in treatment failure in Merida, Mexico. PLoS neglected tropical diseases. 2017; 11(6):e0005656. https://doi.org/10.1371/journal.pntd.0005656 PMID: 28646468; PubMed Central PMCID: PMC5481028.

39. Deming R, Manrique-Saide P, Medina Barreiro A, Cardena EU, Che-Mendoza A, Jones B, et al. Spatial variation of insecticide resistance in the dengue vector Aedes aegypti presents unique vector control challenges. Parasites & vectors. 2016; 9:67. Epub 2016/02/06. https://doi.org/10.1186/s13071-016-1346-3 PMID: 26846468; PubMed Central PMCID: PMC4743324.

40. Vazquez-Prokopec GM, Galvin WA, Kelly R, Kitron U. A new, cost-effective, battery-powered aspirator for adult mosquito collections. Journal of medical entomology. 2009; 46(6):1256–9. Epub 2009/12/08. https://doi.org/10.1603/033.046.0602 PMID: 19960668; PubMed Central PMCID: PMC2800949.
42. Ngufor C, N’Guessan R, Fagbohoun J, Odjo A, Malone D, Akogbe M, et al. Olyset Duo(R) (a pyriproxifen and permethrin mixture net): an experimental hut trial against pyrethroid resistant Anopheles gambiae and Culex quinquefasciatus in Southern Benin. PLoS One. 2014; 9(4):e93603. Epub 2014/04/05. https://doi.org/10.1371/journal.pone.0093603 PMID: 24699827; PubMed Central PMCID: PMC3974762.

43. Raghavendra K, Barik TK, Sharma P, Bhatt RM, Srivastava HC, Sreehari U, et al. Chlorfenapyr: a new insecticide with novel mode of action can control pyrethroid resistant malaria vectors. Malar J. 2011; 10:16. Epub 2011/01/27. https://doi.org/10.1186/1475-2875-10-16 PMID: 21266037; PubMed Central PMCID: PMC3039634.

44. Raghavendra K, Barik TK, Bhatt RM, Srivastava HC, Sreehari U, Dash AP. Evaluation of the pyrrole insecticide chlorfenapyr for the control of Culex quinquefasciatus Say. Acta Trop. 2011; 118(1):50–5. Epub 2011/02/15. https://doi.org/10.1016/j.actatropica.2011.02.001 PMID: 21315680.

45. Dagg K, Irish S, Wiegand RE, Shillu J, Yewhalaw D, Messenger LA. Evaluation of toxicity of clothianidin (neonicotinoid) and chlorfenapyr (pyrrole) insecticides and cross-resistance to other public health insecticides in Anopheles arabiensis from Ethiopia. Malar J. 2019; 18(1):49. Epub 2019/02/24. https://doi.org/10.1186/s12936-019-2685-2 PMID: 30795788; PubMed Central PMCID: PMC6387473.

46. Sheppard CD, Joyce JA. Increased Susceptibility of Pyrethroid-Resistant Horn Flies (Diptera: Muscidae) to Chlorfenapyr. Journal of Economic Entomology. 1998; 91(2):398–400. https://doi.org/10.1093/jee/91.2.398

47. Pan American Health Organization. Manual for Indoor Residual Spraying in Urban Areas for Aedes aegypti Control. PAHO, editor. Pan American Health Organization; 2019.