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Field Evaluation of a Spatial Repellent Emanation Vest for Personal Protection Against Outdoor Biting Mosquitoes

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Abstract

Exophilic vectors are an important contributor to residual malaria transmission. Wearable spatial repellents (SR) can potentially provide personal protection in early evening hours before people retire indoors. An SR prototype for passive delivery of transfluthrin (TFT) for protecting humans against nocturnal mosquitoes in Kanchanaburi, western Thailand, is evaluated. A plastic polyethylene terephthalate (PET) sheet (676 cm^2) treated with 55-mg TFT (TFT-PET), attached to the back of short-sleeve vest worn by human collector, was evaluated under semifield and outdoor conditions. Field-caught, nonblood-fed female *Anopheles minimus* s.l. were released in a 40 m length, semifield screened enclosure. Two collectors positioned at opposite ends conducted 12-h human-landing collections (HLC). The outdoor experiment was conducted between treatments among four collectors at four equidistant positions who performed HLC. Both trials were conducted for 30 consecutive nights. TFT-PET provided 67% greater protection ($P < 0.001$) for 12 h compared with unprotected control, a threefold reduction in the attack. In outdoor trials, TFT-PET provided only 16% protection against *An. harrisoni* Harbach & Manguin (Diptera: Culicidae) compared with unprotected collector ($P = 0.0213$). The TFT-PET vest reduced nonanophelines landing by 1.4-fold compared with the PET control with a 29% protective efficacy. These findings suggest that TFT-PET had diminished protective efficacy in an open field environment. Nonetheless, the concept of a wearable TFT emanatory device has the potential for protecting against outdoor biting mosquitoes. Further development of portable SR tools is required, active ingredient selection and dose optimization, and more suitable device design and materials for advancing product feasibility.

Key words: *Anopheles harrisoni*, *Anopheles minimus* s.l., outdoor transmission, spatial repellency, transfluthrin

Control of malaria vector mosquitoes has been mainly implemented by indoor residual-spraying (IRS) of house surfaces and distribution of insecticide-treated nets (ITNs; WHO 2015, BVBD 2018, Tananchai et al. 2019a). Collectively, these tools are designed to reduce indoor mosquito population densities by targeting indoor blood-feeding (endophagy) and resting (endophily) vector species (WHO 2015). Despite full coverage of ITNs and IRS, and the occurrence of insecticide-susceptible *Anopheles* populations (WHO 2014, Sumarnrote et al. 2017), these core intervention tools are insufficient for reducing persistent malaria transmission and outdoor vector biting (Durnez and Coosemans 2013) in malaria-endemic communities and areas beyond the village (e.g., forest) (Edwards et al. 2019). Thailand's 'malaria elimination' strategic plan intends to achieve complete interruption of all autochthonous malaria transmission in the country by 2024 (WHO 2015, BVBD 2018, Manguin and Dev 2018). To do so, one paramount concern is effectively addressing outdoor vector transmission for preventing infection (Durnez and Coosemans 2013, Hii et al. 2018, Edwards et al. 2019).

In Thailand, the biting behavior of the primary vectors (e.g., *Anopheles dirus* Peyton & Harrison, *Anopheles minimus* Theobald and *Anopheles maculatus* Theobald) and closely related sibling/group species (e.g., *Anopheles baimaii* Sallum & Peyton, *Anopheles pseudowillmori* (Theobald), *Anopheles sawadwongporni* Rattanarithikul & Green, and *Anopheles aconitus* Doenitz) show a strong tendency for blood-feeding outdoors during the early evening hours (Tainchum et al. 2015, Tananchai et al. 2019a). This exophagic behavior precludes contact with ITNs (or other indoor treated materials) and indoor insecticide-treated surfaces. Early outdoor human evening activities (e.g., bathing, social gatherings, etc.) coinciding with peak vector biting periods is an important contributor for sustained transmission and heightened infection prevalence (Durnez and Coosemans 2013). Human population movement across international borders and in rural forested habitats where vector mosquito abundance is potentially high continue to pose a higher disease risk for these more vulnerable populations (Hii and Rueda 2013, Killeen 2014, Edwards et al. 2019).

To counter host-seeking, blood-feeding, and resting behaviors, spatial repellents (SRs) can potentially provide protection outside houses during evening hours through behavioral modification (deterrence) caused by the presence of vapor phase airborne molecules (Achee et al. 2012). The delivery formats of interest, known as passive emanators, typically using fluorinated pyrethroids as active ingredients, e.g., metofluthrin (MET) and transfluthrin (TFT), impregnated on various substrates such as paper, plastic, or hessian sacking have been evaluated in different settings. As example, in Southeast Asia, MET-impregnated paper strips had a relatively high biting protection (more than 80%) against *Culex quinquefaciatus* Say (Kawada et al. 2004), whereas MET-plastic strip provided more than 11-wk protection against outdoor mosquitoes when two to four strips were hung around a shelter with no walls (Kawada et al. 2005). A slow-release emanator made of polyethylene mesh impregnated with 10% (w/w) MET also reduced outdoor landing rates of *An. minimus* and *An. maculatus* by 48–67%, but the effects of the repellent on *An. dirus*, the primary vector, were inconclusive (Charlwood et al. 2016).

TFT has high vapor phase characteristics that can result in strong spatial repellency responses in adult mosquitoes (Ogoma et al. 2012a, Sukkanon et al. 2020). In semifield system, TFT-impregnated polyester strips provided 69% protection against *Anopheles arabiensis* Patton (Andrés et al. 2015), whereas TFT-treated hessian strips reduced *An. arabiensis* attack rate on humans by more than 90% for up to 6 mo (Ogoma et al. 2012b). When used outdoors, TFT-treated hessian strips conferred more than 90% human protection against *Anopheles gambiae* Giles and *Culex* species for over 3 mo (Govella et al. 2015). Treated hessian strips also reduced outdoor biting by *An. arabiensis*, *Culex*, and *Mansonia* species for at least one year using 8.3 ml/m^2 TFT (Ogoma et al. 2017). Other TFT-treated hessian devices (e.g., decorative baskets, chairs, eave ribbons, and sandals) have also effectively reduced exposure to outdoor biting mosquito species (Masalu et al. 2017, 2020; Sangoro et al. 2020). These studies show that passive emanators (i.e., without an external energy source) are able to function at ambient temperatures through vaporization of volatile active compounds (Ogoma et al. 2012a), thus offering a potential complementary tool for protecting humans against biting mosquitoes.

Several studies have investigated the sublethal protection against mosquitoes provided by wearable spatial repellent devices containing vapor phase active ingredients (Xue et al. 2012, Rodriquez et al. 2017, Sangoro et al. 2020). The aim of this study was to evaluate the protective effects of a prototype TFT emanator vest compared with an untreated control vest in a semifield screened facility and in an open field setting against wild mosquito species. This study, therefore, addresses those situations where conventional control methods (ITNs and IRS) are either not readily accessible or specifically address outdoor biting mosquitoes. A personal repellent device may complement other methods of personal protection (e.g., topically applied repellents, insecticide-treated clothing) while exposed to mosquitoes in outside settings.

Materials and Methods

Study Site

Both semifield and outdoor trials were conducted in Pu Teuy, a small agricultural village, located in Tha Sao Subdistrict, Sai Yok District, Kanchanaburi Province, western Thailand (14°20′N; 98°59′E). The village is situated in a karst hill area (mean 420 m above sea level) and surrounded by dense primary forest and scattered small cultivated orchards and plantations. Three *Anopheles* species are common in the study locality but vary in density at different times of the year; peak activity for *An. minimus* s.l. is typically during February to April, *An. dirus* s.l. between May and September, and a relatively brief period between June and July for members of the *An. maculatus* group (Chareonviriyaphap et al. 2003, Sungvornyothin et al. 2006). The small perennial stream at the study site is heavily shaded and bordered with native vegetation, and provides the primary larval habitat of *An. minimus* s.l. (Chareonviriyaphap et al. 2003, Kengluecha et al. 2005). The Pu Teuy *An. harrisoni* has been found completely susceptible to TFT (Sukkanon et al. 2019).

Semifield Screened Enclosure

A permanent, semifield screened enclosure measuring 40 m in length (3.5-m high × 4-m width) is supported by metal frames on a concrete block foundation with corrugated iron roofing. There are various entry points, such as double doors for collectors to move. The enclosure can be modified into as many as four separate sections using screened collapsible interior partitions. For this study, the entire enclosure was opened from end to end with the placement of a collector each at either end (designated sections A and B; Fig. 1). The floors were lined with white plastic sheeting to facilitate observations and recovery of knockdown mosquitoes (Salazar et al. 2012, 2013).

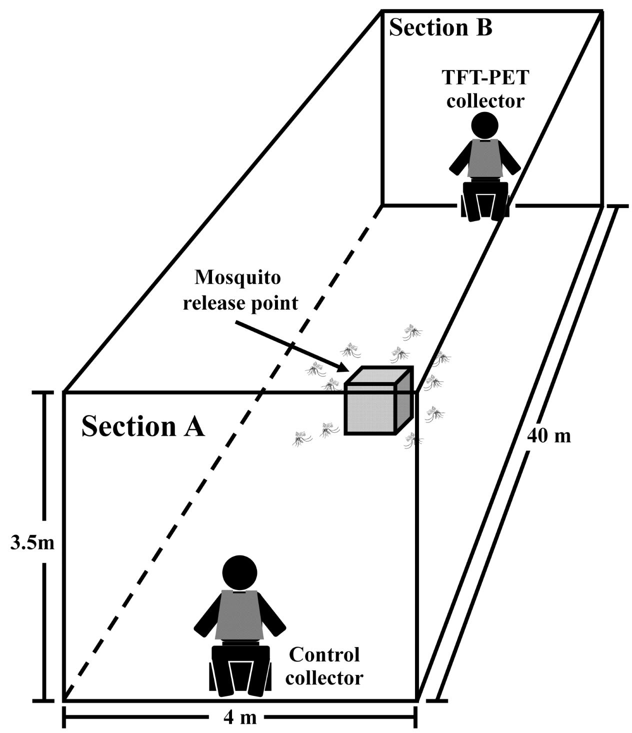

Fig. 1. Schematic of the semifield screened enclosure for baseline and spatial repellent vest evaluation.

Semifield Trials

As we aimed to simulate the 'natural' effects of TFT vest protection efficacy, wild-caught mosquitoes were used in the semifield trials. Females of *An. minimus* s.l. were collected the evening before testing using cow-bait collections (CBC) employing a double-net design containing a single cow tethered inside the inner net. During mosquito collections, the adult cow was protected from mosquito bites inside the inner net throughout the night. The cow was provided by a nearby cattle farm, and all animal care was provided by the owner. The CBC was conducted by two trained local collectors from 18:00 to 24:00 h for 15 min each hour. Unfed anopheline mosquitoes were collected resting on the inner walls of the net and placed in holding cups topped with a cotton pad soaked with 10% sugar solution. Afterward, cups were transferred to a nearby field laboratory where mosquitoes were separated by genus and anophelines (only) were morphologically identified to species (or species complex; Rattanarithikul et al. 2006). Female anophelines were deprived of sugar and provided with a water-soaked cotton pad ~12 h before testing. Approximately 6 h before the testing, mosquitoes were marked with a luminous color contact powder (BioQuip Products, Rancho Dominquez, CA) following Achee et al. (2005). Color markings facilitate the detection of knocked-down individuals and distinguish them from wild mosquitoes that may have entered the screened enclosure before, during, or after the trial period, and from those released mosquitoes from previous trials. Before release, 100 female *An. minimus* s.l. were transferred into a 1.25-liter plastic container, topped with mesh netting affixed with rubber bands. The marking powder is quickly brushed against the mesh netting of the container using a circular motion with a small 0.25 in paintbrush and repeated four times. Another 25 mosquitoes were also marked similarly (same color) and served as the paired control group. Marked mosquitoes in containers were placed in a 25-gal cooler covered with a moistened towel until the time of release at approximately 17:30 h (~30 min before beginning trial). Each replicate trial was repeated with a new set of 100 marked mosquitoes and another 25 marked mosquitoes held concurrently in the field laboratory as control. Different color markings were used each night (from a selection of 10 colors available) in sequence between adjoining trials until having to repeat color use.

Screened Enclosure Baseline

An initial trial was performed to observe the baseline behavior of released mosquitoes in the enclosure to determine whether the collector (attractant) position at opposite ends of the enclosure was a factor in natural mosquito movement (chemotaxis) given other stimuli that produced oriented movement (phototaxis, geotaxis). This baseline exercise inside the screened enclosure in the absence of an introduced repellent was to demonstrate mosquito preferences for human collectors placed at either end of the screened enclosure 40 m apart, sections A and B, respectively (Fig. 1). One hundred marked female mosquitoes from CBC were actively released by emptying the container at the center of the enclosure (20-m equidistance between enclosure ends) 30 min before, two adult male collectors wearing untreated (control) vest positioned themselves at one end of sections A and B, respectively (40 m apart). While sitting, each collector exposed both legs from ankle to knee. To ensure that mosquitoes only had access to the lower legs, work boots and a long-sleeve shirt were worn. Collectors refrained from smoking, eating, and recent alcohol consumption. Each collector was asked not to use soap for bathing at least 6 h before beginning collections.

Two collectors performed human landing collections (HLC) uninterrupted for 45 min each hour, beginning from 18:00 to 24:00 h (shift 1) followed by a second shift of two collectors conducting captures from 00:00 to 06:00 h. Collectors were allowed a 15-min break outside the enclosure before the end of each hour.

Each collector used a mouth aspirator to capture all landing mosquitoes on exposed legs. Mosquitoes were placed in holding cups marked by location (section A or B) and collection time interval. Further details on standard HLC methods are published elsewhere (Chareonviriyaphap et al. 2003). Ambient air temperature and percent relative humidity were recorded once each hour at the end of each collection interval. Re-captured mosquitoes were provided with 10% sugar solution and held at ambient temperature and humidity conditions in the field laboratory for counting. Knockdown mosquitoes were collected hourly during the 15-min break period. Collectors were rotated between sections A and B on alternate nights. Ten consecutive evening replicates were conducted.

TFT-Treated Plastic Sheet

A prototype plastic sheet made of polyethylene terephthalate (PET; 31.3 × 21.6 cm, 676 cm^2 surface area) was pretreated with 55 mg of technical grade TFT active ingredient (2,3,5,6-tetrafluorobenzyl (1*R*,3*S*)-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxy late) translating to 0.08 mg active ingredient/cm^2. Thirty minutes before beginning the trial, a single TFT-PET sheet was attached (stapled) to the back of a clean (untreated) safety mesh vest with reflective material removed (Sira Safety and Tools Co., Ltd., Thailand) to serve as the 'treatment' (Fig. 2). An identical vest was affixed with an untreated PET sheet to serve as the control. The vest was marked as treatment or control, but collectors are unaware of this marking during the entire study period. The same vest was used throughout all experiments to avoid insecticide cross-contamination. The treated and untreated TFT-PETs were replaced after each 12-h collection period.

Semifield Trials

To evaluate the protective efficiency (PE) of TFT against mosquito bites, one collector wore a TFT-PET vest ('treatment'), whereas another collector wore an untreated-PET vest only ('control'). Like the calibration procedure, marked mosquitoes were released in the middle section of the screened enclosure 30 min before (17:30 h) the HLC commencing. The treatment and control collectors were positioned at the opposite ends of the screen enclosure (sections A and B) (Fig. 1) at 18:00 h to begin collections. Four collectors, two persons per 6-h shift, performed HLC for 45 min with a 15-min break before the end of each hour. All collectors were blinded to the vest type and unaware of the vest marking during the entire study period. Ambient air temperature and relative humidity were recorded hourly at the end of each collection interval. Mosquitoes knocked down, and those not captured during the 12-h collection period were handled as described previously. Collected mosquitoes were provided with sugar solution and held at ambient temperature and humidity conditions in the field laboratory for counting. Control and treatment collectors were rotated between sections A and B each alternate night, and the control and treatment vests were also rotated between two collectors to reduce any potential bias caused by individual human factors. Thirty consecutive 12-h evening replicate trials were conducted. TFT-PET and untreated PET sheets were replaced for each replicate.

Outdoor Trials

The evaluation of PE provided by the TFT-PET was conducted against wild exophagic mosquitoes. In total, eight well-trained

Fig. 2. Transfluthrin-treated plastic sheet affixed to the back of a mesh vest. Control vest was affixed with the untreated sheet.

collectors were recruited for HLC, four collectors each per 6-h HLC shift (18:00–24:00 and 00:00–06:00). Each collector was positioned in one of four outdoor collecting stations within an open field setting at a minimum of 500 m from potential alternate sources of host attractive cues (kairomones) from other humans and livestock in the area. Study design positioning was based on an 'effective' spatial repellent activity of <1 m from active ingredient source (treatment vest) and a maximum distance of 10 m for mosquito human–host attraction (Gillies and Wilkes 1970, Moore et al. 2007). Each trial, adult male collectors wore the same treatment or control vest and were blinded to the vest type during the entire study period. Four collectors sat in designated locations, positioned 10 m from each other (Fig. 3). Two collectors each wore either a TFT treatment vest or a control vest. Routine HLC was performed for 45 min with a 15-min break each hour. Collectors changed their positions in a clockwise rotation at the beginning of every hour to minimize potential bias. Umbrellas were provided to protect the collectors from periodic rain showers and prevent possible loss of TFT from the treated sheet. All collections were terminated during periods of moderate to heavy rain and resumed immediately afterward. Collected mosquitoes were retained in holding cups labeled by the hour and collector (treatment or control vest). Mosquitoes were transferred to the field laboratory and morphologically identified soon afterward. Collected mosquitoes were provided with sugar solution and held at ambient temperature and humidity conditions in the field laboratory for counting and species identification. Thirty consecutive trial replicates were conducted. Ambient air temperature and percent relative humidity were recorded once each hour at the end of each collection interval. The control and treatment vests were rotated between collectors each alternate night. New TFT-PET and untreated PET sheets were replaced for each replicate.

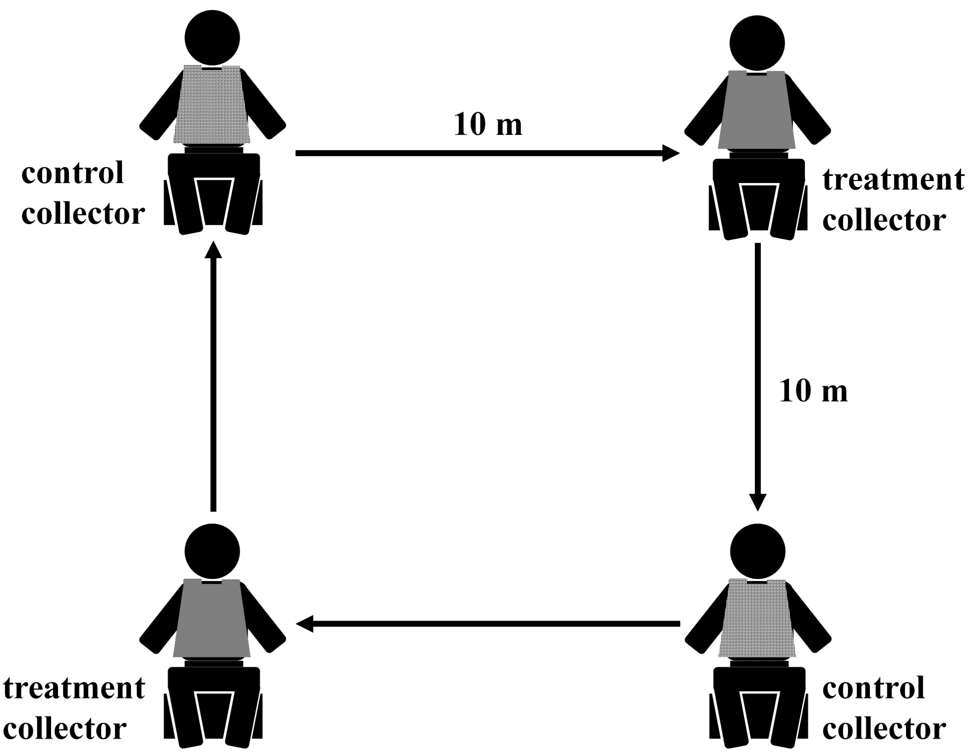

Fig. 3. Outdoor trial design consisting of two control and two treatment collectors performing evening human-landing collections during 30 consecutive nights. Two collections teams (four persons each) were divided into 6-h shifts (18:00–14:00 and 00:00–06:00 h). Collectors rotated their position in a clockwise direction every hour.

Molecular Identification of *An. minimus* sensu lato

DNA extraction and a multiplex allele-specific polymerase chain reaction (AS-PCR) procedure was performed on individual, morphologically identified *An. minimus* s.l. collected by CBC for the semifield trials and the outdoor field collections based on procedures by Sungvornyothin et al. (2006).

Data Analysis

For both semifield and outdoor data, the effect of TFT-treated vest on the risk of exposure to mosquito bites was analyzed by fitting a generalized linear mixed-effects model (GLMM) with a negative binomial distribution to account for the overdispersion of mosquito count data. This was done to include both fixed and random effects as data have more than one source of random variability. The treatment vests in the model were classified as independent variables, and the number of mosquitoes re-captured in the SFS or outdoor HLC as the dependent variable. Variations associated with fluctuations in temperature and relative humidity, date and hour of trial, and within-collector and across-collector variability were treated as random effects. The exponents of model coefficients are equal to the calculated incidence rate ratio (IRR); in this case, IRR indicates the risk of mosquito landings on the collector with respective 95% CIs. The number of mosquitoes captured by the untreated control collector in the active treatment comparison trial was compared with the two collectors using untreated vests in the SFS 'release–recapture' baseline (calibration) trials. All analyses were performed using SAS version 9 (SAS Institute, Cary, NC). All statistical significance was set at 5% ($P < 0.05$).

The protective efficacy (PE) or mean percent landing inhibition, and spatial activity index (SAI) was calculated. PE is $100 \times (C - T)/C$, where C and T are the number of mosquitoes landing on the control and treatment collector, respectively. The SAI is a measure of mosquitoes sampled at either end of the semifield screened enclosure in the calibration and TFT-PET trials (Grieco et al. 2005). SAI is calculated as $(Nc - Nt)/(Nc + Nt)$, where Nc is the number of mosquitoes collected from the untreated control and Nt the number from the treated collector. The SAI ranges from −1 to +1, with zero, negative, and positive values representing no preference to either control or treatment, a preference to treatment, or a preference to control, respectively.

Ethics Approval and Consent to Participate

Ethics approval for the use of human collectors for human-landing collections was provided by the Research Ethics Review Committee for Research Involving Human Research Participants, Health Sciences Group, Chulalongkorn University, Thailand (Certificate of approval No. 236/2016). Formal ethical clearance of study protocol and volunteer collector informed consent was obtained before commencing trials.

Results

Semifield Trials

During the 10 baseline trials, 363 female anophelines (all molecularly identified as *Anopheles harrisoni*) were re-captured out of 1,000 released mosquitoes (Table 1). No significant difference ($P = 0.5652$; IRR = 0.91) in HLC numbers was observed between opposite ends of the 40-m screened enclosure. Section A had a mean of 19.0 mosquito per night (95% CI 13.4–24.6) and B of 17.3 mosquito/night (95% CI 11.9–22.7) (Table 1). The mean temperatures (°C) and percent relative humidity (RH%) during 10 nights of baseline trials ranged from 24.6–25.7°C and 90–93% before midnight, and 23.9–24.0°C and 94–95% after midnight to dawn, respectively. Based on hourly observations, no knocked down mosquitoes were observed on the floor sheeting during the baseline (without TFT) collections. *Anopheles harrisoni,* when released at the mid-point of the screened enclosure, showed relatively equal attractiveness for either collector, each placed at either end of the enclosure and rotated positions between collection periods.

In the semifield trials, a greater number of released *An. harrisoni* ($n = 3{,}000$) landed on the control (197) compared with the treatment person (65). The mean temperatures and RH% during the 30 consecutive nights of trials ranged from 25.0–27.3°C and 70–79% before midnight, and 22.7–23.6°C and 84–87% after midnight to dawn, respectively. The treatment collector captured significantly fewer mosquitoes ($P < 0.0001$) than the control with an IRR of 0.34 (95% CI 0.25–0.44), indicating the less risk of mosquito landing on collector wearing TFT-PET vest than control collector (Table 1). Moreover, the TFT-PET vest showed a positive SAI value (>0.2) in 24 out of 30 experimental replicates (Fig. 4). No mortality was found in the control group of marked mosquitoes after the 24-h holding period, and no mosquito knockdown was observed each hour on the floor sheeting. Overall, the % protective efficacy (%PE) between TFT-PET and PET was 67% landing inhibition.

The semifield control receive significantly lower numbers of mosquitoes (6.6 mosquitoes/night) compared with baseline controls: 19.0 mosquitoes/night; $P = 0.0045$; IRR = 2.89 [95% CI 2.40–3.48] and 17.3 mosquitoes/night; $P = 0.0016$, IRR = 2.63 [95% CI 2.18–3.19], respectively. Although there was a statistically lower number of mosquitoes captured by the active trial control compared with baseline controls. However, allowing inherent biological 'variability' in mosquito and other factors (environmental conditions) due to temporal effects of comparing different testing periods, there was no evidence of strong positional or attraction bias during the treatment-control trial as collectors were rotated between locations on alternate nights. Although not part of the study design, there was no evidence that the TFT-treated vest resulted in increased mosquito landings (via behavioral diversion or other factors) to the untreated control.

Table 1. Baseline and TFT-treated vests (TFT-PET) evaluations against released *Anopheles harrisoni* in semifield screened enclosure

SFS experiment	*N*	Mean recaptured/night (95% CI)		*P*-value	*z*-score	IRR (95% CI)	%PE[a]
		Control	TFT-PET				
Baseline trial	10	19.0 (13.4–24.6)[b]	17.3 (11.9–22.7)[b]	0.5652	0.036	0.91 (0.76–1.10)	N/A
TFT-PET trial	30	6.6 (4.2–9.0)	2.2 (0.9–3.5)	<0.0001	−0.217	0.34 (0.25–0.44)	66.5

N = experimental nights (100 mosquitoes released per night).
[a]The % Protective efficacy (%PE) refers to percentage reduction in landing relative to control.
[b]Both collectors wearing untreated vest in baseline collections.

Outdoor Trials

In total, 1,798 and 1,429 mosquitoes were collected during the 30 collection nights from collectors wearing control and treatment vests, respectively (Table 2). The predominant species collected was *An. minimus* s.l. (more than 60%), followed by *Aedes* spp. (more than 15%), and *Armigeres* spp. (more than 8%). A small number of *An. dirus* s.l. (6%) and *An. maculatus* s.l. (0.3%) were recorded (Table 2). Molecular methods (AS-PCR) identified two sibling species present in the Minimus Complex, *An. minimus* s.s. (n = 41) and *An. harrisoni* (n = 1,970), the latter species representing 98% of the complex during the trials. The mean temperatures and RH% during 30 nights of outdoor trials ranged from 23.0 to 26.6°C and 71–84% before midnight, and 20.2–21.2°C and 92–96% after midnight to dawn, respectively.

In the outdoor setting, the TFT-PET vest significantly reduced landing rates for all mosquito species combined by 21% PE (P = 0.0040) and *An. harrisoni* by 16% PE (P = 0.0213) (Table 3). Although the TFT-PET vest reduced nonanopheline mosquito abundance by 29% PE, the mean landing rate was not significantly different (P = 0.0571) between control (19.8 mosquitoes/night) and treatment (14.0 mosquito/night) (Table 3). The numbers of all mosquitoes in landing collections were reduced by 20.5% compared with 15.9 and 29.2% for *An. harrisoni* and nonanopheline species, respectively (Table 3). A positive SAI (>0.10) occurred in 17 of 30 experimental nights (56.7%) indicating relatively high spatial repellent activity of TFT-PET against all mosquito species (SAI 0.16–0.61), *An. harrisoni* (SAI 0.10 to 0.71), and nonanopheline species (SAI 0.14–1.00) (Figs. 5–7).

The TFT-PET sheet had diminished spatial repellency activity in an open outdoor environment compared with the semifield enclosure results. During the studies, none of the collectors wearing the TFT-PET reported any adverse health effects such as skin irritation, respiratory symptoms, or other issues, indicating the apparent safety of using the prototype SR vest for personal protection against mosquito bites.

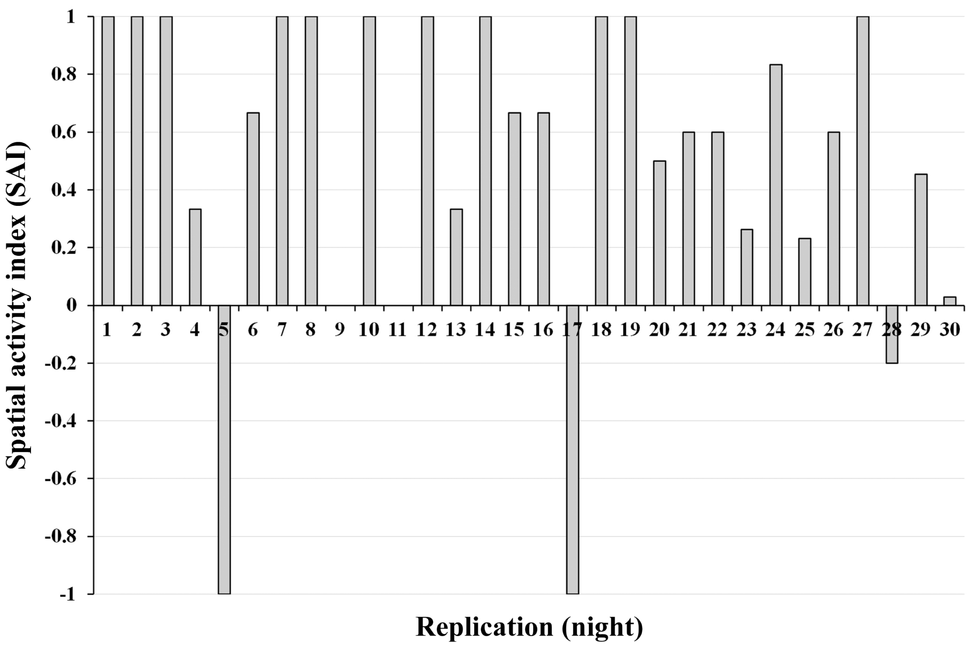

Fig. 4. The SAI for semifield trial during 30 continuous replications (nights). The SAI ranges from −1 to +1, with zero, negative, and positive values representing no preference to either control or treated collectors, a preference to active treated collectors, or preference to untreated collectors, respectively.

Table 2. Total mosquitoes from control and treatment collectors in outdoor field trial during 30 consecutive nights

Mosquito	Control		Treatment	
	Total collected (%[a])	Mean collected[b] (95% CI)	Total collected (%[a])	Mean collected[b] (95% CI)
Anopheles harrisoni	1,070 (59.7)	35.7 (25.6 to 45.7)	900 (63.0)	30.0 (18.6 to 41.4)
Anopheles minimus s.s.	22 (1.2)	0.7 (0.2 to 1.3)	19 (1.3)	0.6 (−0.1 to 1.4)
Anopheles maculatus gr	5 (0.3)	0.2 (0.03 to 0.3)	3 (0.2)	0.1 (−0.1 to 0.3)
Anopheles dirus s.l.	106 (5.9)	3.5 (1.0 to 6.0)	86 (6.0)	2.9 (1.2 to 4.5)
Aedes spp.	293 (16.3)	9.8 (4.8 to 14.7)	218 (15.3)	7.3 (3.8 to 10.7)
Culex spp.	33 (1.8)	1.1 (0.4 to 1.8)	24 (1.7)	0.8 (0.3 to 1.3)
Armigeres spp.	170 (9.5)	5.7 (1.8 to 9.5)	124 (8.7)	4.1 (1.7 to 6.5)
Mansonia spp.	99 (5.5)	3.3 (1.9 to 4.7)	55 (3.8)	1.8 (0.9 to 2.8)
Total	1,798	59.9 (41.7 to 78.2)	1,429	47.6 (30.3 to 64.9)

[a]Proportion of total mosquitoes collected.
[b]Mean number of collected mosquitoes/night.

Table 3. Outdoor field efficacy of TFT-treated vests against pooled mosquitoes, *Anopheles harrisoni* and pooled nonanopheline mosquitoes

Outdoor experiment	*N*	Mean recaptured/night (95% CI)		*P*-value	*z*-score	IRR (95% CI)	%PE[a]
		Control	TFT-PET				
All mosquitoes	30	59.9 (41.7–78.2)	47.6 (30.3–64.9)	0.0040	−0.078	0.79 (0.76–0.84)	20.5
Anopheles harrisoni	30	35.7 (25.6–45.7)	30.0 (18.6–41.4)	0.0213	−0.060	0.84 (0.78–0.91)	15.9
Non-anopheline species	30	19.8 (10.4–29.3)	14.0 (7.9–20.2)	0.0571	−0.057	0.71 (0.63–0.79)	29.2

N = number of experimental nights.
[a]% Protective efficacy is percentage reduction in landing for TFT-PET relative to untreated controls (PET).

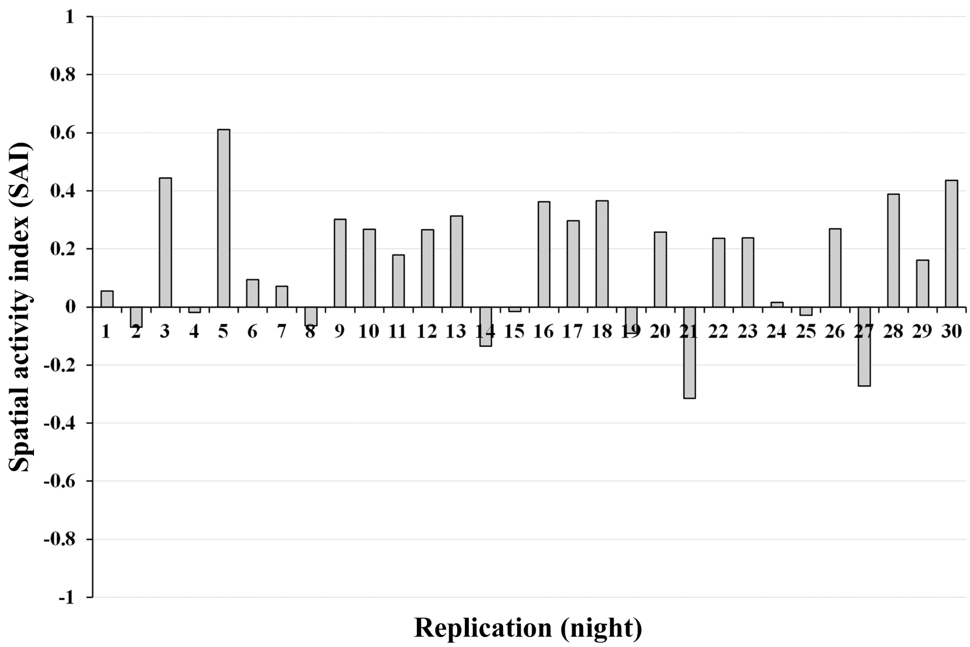

Fig. 5. The SAI for all mosquito species in outdoor trial during 30 continuous replications (nights). The SAI ranges from −1 to +1, with zero, negative, and positive values representing no preference to either control or treated collectors, a preference to active treated collectors, or preference to untreated collectors, respectively.

Discussion

A plastic PET sheet (676 cm[2]) treated with 55 mg TFT (TFT-PET), attached to the back of a vest worn by a human collector was evaluated under semifield and natural outdoor conditions. In the semifield design, the TFT-PET provided 67% protection for 12 h, with a biting pressure of 0.24 landings per person/h, a threefold reduction in attack. However, in outdoor trials, the treatment vest provided only 16–29% greater protection (landing inhibition) against outdoor mosquitoes. The TFT vest provided significant (16%) landing inhibition against *An. harrisoni* compared with the unprotected collector. The TFT-PET vest also reduced nonanophelines landing by 1.4-fold compared with the PET control with an overall 29% protective efficacy.

The differences in %PE between TFT-PET in the semifield and field trials are significant. Many factors that occurred during the two trials (time and space, for example) may have played a substantial part in the different effect outcomes; thus, direct comparisons must be made with caution. One outstanding difference between the trials was the distance between collectors. The lower %PE seen in the field setting might have been influenced by having only used 10-m distances between collectors. Unlike the SFS trial having a 40-m separation between collectors, it is possible 10-m between treated and untreated collectors was too close and may have lowered the %PE due to an area effect of volatilized TFT protecting untreated collectors as well. Further assessments of SR protective effects in the field should take into account the positioning of collectors.

The concept of TFT-treated wearable emanators such as TFT-PET sheet on clothing in this study is an innovative approach. Similarly, Sangoro et al. (2020) also demonstrated that wearable sandals fitted with hessian bands (measuring 48 cm[2]) treated with 0.15 g TFT worn by human collector reduced mosquito landings by 46 and 66% in semifield and field experiments, respectively. These wearable SR devices could potentially be a practical means of personal protection against outdoor exposure to mosquito bites, which do not require users to change their daily routines (Sangoro et al. 2020).

The semifield screen enclosure appears useful for screening candidate SR compounds and products under near-natural conditions. The objective of an SR is to disrupt host-seeking and feeding behavior via the excito-repellent actions of sublethal chemical concentrations presented in a volatile state. Whereas most laboratory assays concentrate on toxicity (killing) and related responses (i.e., knockdown), spatial repellency in either laboratory or field setting is as measured as a movement away from an offending chemical source resulting in bite suppression (Grieco et al. 2007, WHO 2013). The use of the SFS and similar semifield test systems uniquely allow for

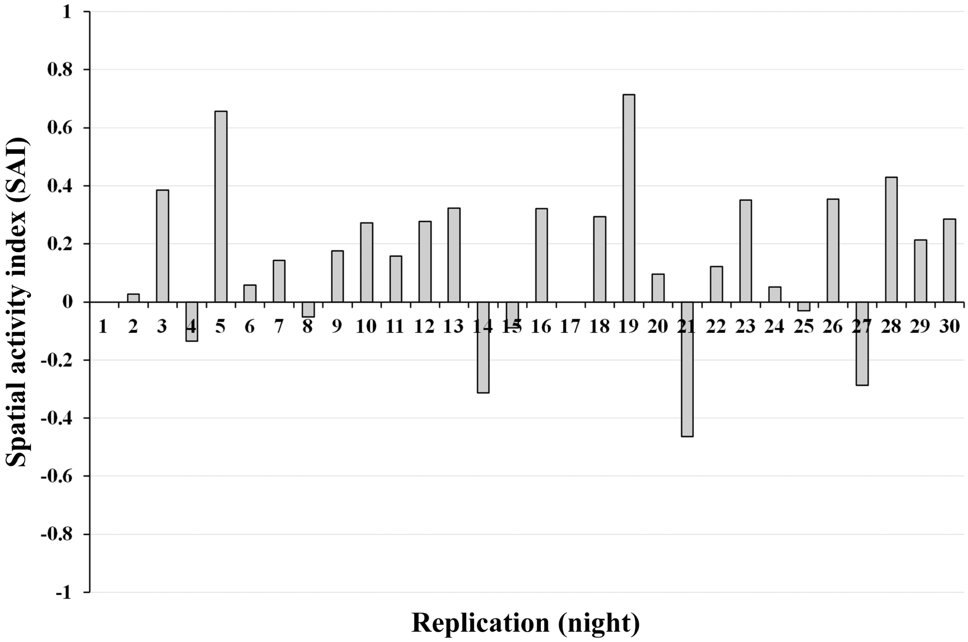

Fig. 6. The SAI for *An. harrisoni* in outdoor trial during 30 continuous replications (nights). The SAI ranges from −1 to +1, with zero, negative, and positive values representing no preference to either control or treated collectors, a preference to active treated collectors, or preference to untreated collectors, respectively.

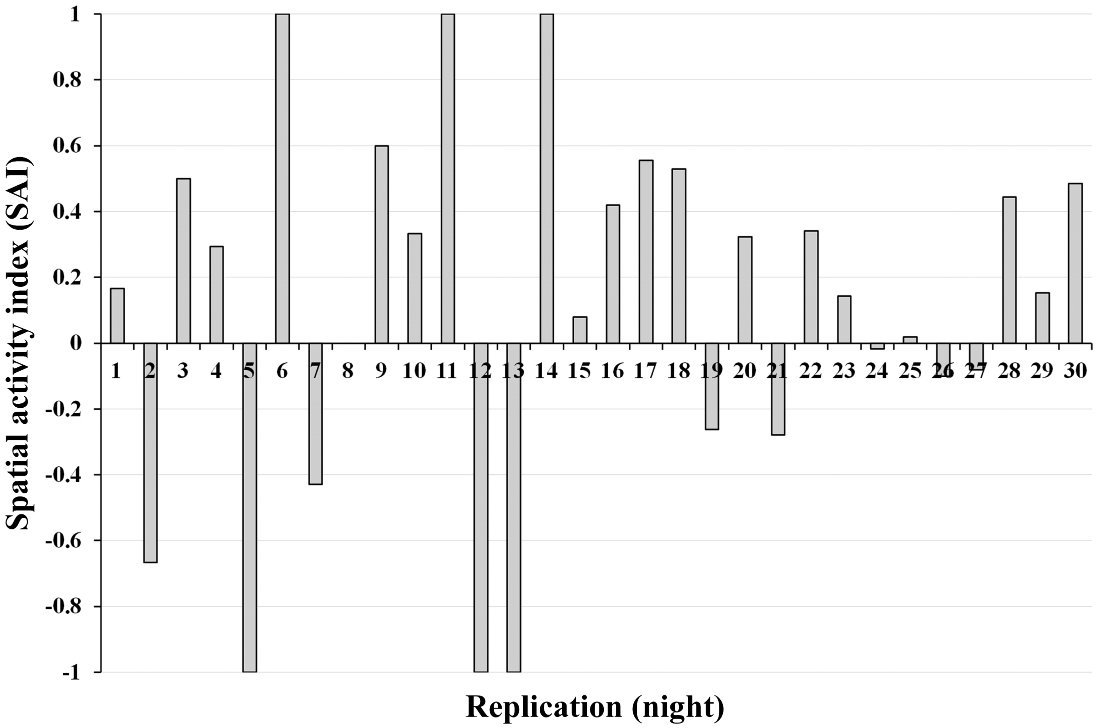

Fig. 7. The SAI for nonanopheline species in outdoor trial during 30 continuous replications (nights). The SAI ranges from −1 to +1, with zero, negative, and positive values representing no preference to either control or treated collectors, a preference to active treated collectors, or preference to untreated collectors, respectively.

these measurements within a simulated three-dimensional space. Moreover, the SFS also potentially allows assessment of mosquito diversion from the SR user to nonuser and measure protective area effects for the nonuser within the same controlled space, indicating the effective range of an SR agent. However, limitations exist when using a semifield system of mark-release-recapture of mosquitoes can depend on many variables affecting flight and orientation responses, including species, origin (colony or wild-caught), and physiological condition (age, parity) (Clements 1999). Environmental conditions (temperature, humidity, air movement) can influence adult mosquito activity and behavior as well as possible differing effects of using marked or unmarked mosquitoes (e.g., fluorescent powder). Lastly, the experimental design (e.g., method of re-capture) can impact mosquito recovery success.

Two release-recapture studies were previously conducted in the same screened enclosure using a laboratory-reared local *Ae. aegypti* (L.) field population and the BG-Sentinel mosquito trap (BGS) with an odor attractant. Salazar et al. (2012) released 100 marked (fluorescent powder) *Ae. aegypti* per day that resulted in a high re-capture rate but was dependent on the number of BGS used; 77% for one trap and up to 96% using four traps. In another experiment, moderate to high re-capture rates (58–93%) was reported in a nonexposed control group of *Ae. aegypti*, whereas BGS trap catches were reduced to 45% following exposure to TFT (5 µg a.i./cm^2) (Salazar et al. 2013). In contrast, *An. harrisoni* in the semifield trials was initially collected using a cow-baited trap the night before marking for release-recapture HLC (using human host cues). These mosquitoes were of unknown age and physiological status (e.g., parity). The natural zoophilic host preference of *An. harrisoni* compared with *Ae. aegypti*, a strong anthropophilic species, might explain the lower re-capture rate (36%) in the present study. In Thailand, Ponlawat et al. (2016) used a semifield tunnel design (50-m long) for SR experiments found laboratory colonized mosquitoes (of known age and status) collected by interception traps in the presence of a human host produced re-capture rates ranging from 91 to 69% for *Ae. aegypti* and *An. dirus*, respectively.

Temperature and wind movement can influence mosquito activity patterns, behavior, and survival (Bowen 1991). In this study, a greater number of *An. minimus* s.l. was collected during the first half of the evening (18:00 to 24:00 h) in both semifield and outdoor experiments. A decrease in mean outdoor ambient temperature from 25 to 27°C (before midnight) to 20 to 23°C (after midnight to dawn) likely resulted in the lower numbers of re-capture in both experiments. This peak distribution likely reflects normal patterns of flight and host-seeking activity. This agrees with previous studies showing the typical peak biting activity of *An. minimus* complex at Pu Teuy in the early evening immediately after sunset (Chareonviriyaphap et al. 2003, Sungvornyothin et al. 2006). *Anopheles harrisoni* was the most abundant species (93%) recorded in outdoor collections and in line with previous studies in the same location (Sungvornyothin et al. 2006). Primary malaria vectors, *An. minimus* s.l. and *An. dirus* s.l. in western Thailand show seasonal changes in densities and opportunistic feeding behavior by location (indoor and outdoor) and host (both human and animals) (Chareonviriyaphap et al. 2003; Sungvornyothin et al. 2006; Tananchai et al. 2012, 2019b; Tisgratog et al. 2012). Moreover, members of both species complexes are primarily responsible for outdoor 'residual' malaria transmission (Durnez and Coosemans 2013, Edwards et al. 2019) and the main target for control (Beier et al. 2018, Hii et al. 2018).

The protective efficacy (67% landing inhibition) produced by TFT-PET under semifield conditions is very similar to the 68.9% spatial repellent protection of TFT-treated polyester strips against *An. arabiensis* biting (Andrés et al. 2015). Additionally, the same authors showed low mosquito mortality, indicating primarily sublethal repellent effects. Compared with the TFT-PET format used in this study, other TFT emanators have shown greater reductions in preventing mosquito bites. In Tanzania, a TFT-treated hessian sacking strip provided more than 90% protective efficacy against *An. arabiensis* (laboratory strain) up to 6 mo in a semifield tunnel assay (Ogoma et al. 2012b). Comparing with our TFT-PET sheet (676-cm^2 area), the Tanzanian study used a much larger treated surface area (12,000 cm^2 using 4-m long × 30-cm wide strips), which likely explains the higher protective efficacy and longevity. Moreover, different vapor phase characteristics of active ingredients and different physical characteristics of treated materials likely contribute to active ingredient spatial availability, thus providing different efficacy and longevity profiles of volatile chemicals. For example, plastic strips treated with metofluthrin (a similar, highly volatile SR) provided longer bite protection (>11 wk) (Kawada et al. 2005) compared with treated paper strips (4 wk) (Kawada et al. 2004).

Several studies have demonstrated the efficacy of TFT emanators in outdoor field conditions. In Tanzania, TFT-treated hessian strips provided more than 92% protection against *An. gambiae* and *Culex* mosquitoes (Govella et al. 2015). Treated hessian materials modified into baskets and wall decorations placed outside reduced *An. arabiensis* and *Culex* mosquitoes entering locations up to 89 and 66%, respectively (Masalu et al. 2017). In contrast, the TFT-PET vest provided only 16% landing protection against *An. harrisoni* using a smaller plastic sheet (676-cm^2 treated area per collector). An identical TFT-PET sheet used in a cluster-randomized controlled trial in Indonesia found the primary entomological impact was 16.4% protective, but statistically inconclusive (Syafruddin et al. 2020). However, importantly, the protective efficacy in preventing malaria infection was as high as 60% between locations with houses having TFT-PET and those provided PET only. By comparison, Govella et al. (2015) demonstrated human protection using a much larger hessian strip (1.2 m^2) treatment per person, approximately an 18-fold larger treated surface area than used in Pu Teuy. The significantly larger strips presumably created a greater protection zone with vaporized TFT. The relatively low PE of the TFT-PET sheets in an outdoor setting, 4.2 times less protective compared to those used in the semifield system showing 67% landing inhibition, indicates more work is needed for developing an SR passive emanatory personal protection device. Ideally, a product should be passive (i.e., without heat or other energy requirements), cost-effective, with optimized chemistry for delivery of a greater, more prolonged spatial repellency effect. Such a product would be invaluable for providing sustained and safe protection over many weeks or longer and hopefully increase better user adherence to reduce outdoor transmission (Durnez and Coosemans 2013, Edwards et al. 2019).

As indicated previously, protective efficacy for preventing malaria infection was as high as 60% in Indonesian houses having TFT-PET compared with those without TFT (Syafruddin et al. 2020). A TFT emanator, in one form or another, could complement conventional mosquito control tools such as home improvement (e.g., mechanical screening or other barriers) and use of insecticide-treated bed nets. Numerous factors need to be considered to characterize behavioral endpoints of mosquitoes exposed to SR emanators using independent and repeatable tests. Variables include the innate behavior of mosquito species of interest, the size and configuration of laboratory test chambers or rooms, environmental factors, experimental design, active ingredient, and dosage (Ogoma et al. 2012a). Further studies are required to improve the effectiveness of TFT-PET format, including chemical dose optimization most suitable for target species, controlled release system for prolonging the protective efficacy, advances for dealing more effectively with natural physical variables and environmental conditions (e.g., temperature, wind velocity), and device design parameters for user acceptability. Additional experiments are needed to assess the impact on other outdoor-biting malaria vectors in Thailand, including members of the *An. dirus* complex and *An. maculatus* group as well as important vector aedine and culicine mosquitoes in both semifield and field conditions.

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