



Review article

Laboratory bioassays of volatile pyrethroid spatial repellents against medically important mosquitoes in the Asia-Pacific region: A systematic review

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Abstract

Importance of the work: Variations were identified in laboratory evaluation studies of volatile pyrethroid spatial repellents (VPSRs) in the Asia-Pacific region (APR).

Objectives: To conduct a systematic review of laboratory studies of VPSR efficacy in the APR and their adherence to World Health Organization (WHO) guidelines.

Materials and Methods: The search occurred between May 2023 until September 2024. Studies were identified by searching electronic databases and a selection process using pre-defined criteria, involving title/abstract screening and potentially full-text screening, was then applied. Strengths and weaknesses of studies were identified according to WHO guidelines and the relevant published literature. Critical comments were included on evaluation methods, design, and mosquito rearing conditions.

Results: Since 2004, VPSR studies in the APR have focused on toxic effects, as reflected by their chosen evaluation methods. Laboratory studies measured common entomological endpoints such as knockdown rate, knockdown time, and mortality. There was minimal variation in protocol due to unique study objectives. However, mosquito rearing conditions and physiological status in some studies did not adhere to WHO guidelines. This could be attributed to the unique main objectives and resources of each study. In addition, three room/chamber studies emphasized the importance of airspace volume and the capabilities of secondary devices in vaporizer formulations when conducting efficacy studies in this test arena.

Main finding: Individual VPSR studies in the APR appeared to be unique. Engagement of other APR countries would be beneficial to provide a preliminary picture of efficacy against medically important mosquitoes and to support further semi-field and field studies.

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Introduction

Spatial repellency is a term that encompasses a variety of insect responses triggered by chemical stimuli in the vapor phase. It disrupts host detection by mosquitoes (attraction inhibition) and acts as a deterrent, all without requiring direct physical or tarsal contact (Grieco et al., 2005; Achee et al., 2012; World Health Organization, 2013). Spatial repellency involves using highly volatile compounds, referred to herein as spatial repellents (SRs) that can disperse through the air, creating a mosquito-free zone. Consequently, SRs can reduce biting rates and limit contact between vectors and hosts, potentially decreasing the probability of pathogen transmission (Achee et al., 2012; Bibbs and Kaufman, 2017; Achee et al., 2018). The vapor plume generated by an SR's source establishes a safeguarding barrier that reaches a specific radius from the chemical source. Such a barrier has the capability to provide protection across an entire household (Salazar et al., 2013).

The active ingredients commonly used in SR products belong to the pyrethroid chemical class, which consists of synthetically structured compounds based on pyrethrins (Spurlock and Lee, 2008; Ogoma et al., 2012). These active ingredients are released either through passive evaporation at room temperature (passive emanators) or through active dispersion by applying heat or combustion in various forms, including mosquito coils, vaporizer mats or liquids (Ogoma et al., 2012; Tambwe et al., 2020). The optimal product would employ a passive delivery method that does not require heating to facilitate chemical dispersion, and be accessible in formats that are not only effective in disrupting mosquitoes' typical host seeking behavior, but also appealing to the consumer and reasonably priced (Achee et al., 2012). Mosquito coils only require the application of heat through ignition to volatilize their active ingredient. Coils are inexpensive and practical; however, the unpleasant smoke containing particulate matter and volatile organic compounds that can be inhaled raises health risk for its users, particularly in children and those with pre-existing conditions like asthma (Lawrance and Croft, 2004; Hill et al., 2014; Chin et al., 2017; Amelia-Yap et al., 2018). At the higher end of the market, liquid vaporizers and repellent-embedded mats used with an electric heating device offer an alternative to coils, providing a more controlled and less smoky option. However, these products may become unsuitable for some rural and urban locations in low- or middle-income nations where malaria transmission is highly endemic, since these products

require an energy source, which can raise product costs (Ogoma et al., 2012; Logan et al., 2022). On the other hand, passive emanators are designed to release volatile chemicals into the air without the requirement of an external heat source (such as electricity or fire). Passive emanators are more easily produced by impregnating active substances onto various substrates, such as paper, plastic, and cloth (Logan et al., 2022). Notably, environmental factors must be considered before implementation for both active and passive emanator formats. Environmental factors have a strong effect on the avidity of mosquitoes for biting, as well as anthropophilic and opportunistic vectors. For the medically important mosquito species, the parameters of maximum avidity are known and could be replicated in the laboratory or sought in the field such as temperature, humidity and shade (Roiz et al., 2010; Reinhold et al., 2018; Laursen et al., 2022) and the circadian clock of peak activity (Hajkazemian et al., 2022). Furthermore, the use of controls will reveal when adequate avidity, or host preference, has been achieved.

Despite lacking endorsement from the World Health Organization (WHO), spatial repellent formulations are continuously increasing in popularity, with these tools being studied extensively in different regions globally due to their versatility and availability in the public consumer market (Ogoma et al., 2012; Xue et al., 2022). The WHO requires that any new vector control product must be supported by thorough evidence demonstrating its public health value, ensuring that the intervention is both effective and cost-efficient. Serving as a foundation for all future efficacy trials, laboratory evaluations should follow and adhere to the guidelines, especially for selecting bioassays to measure the entomological endpoint of interest. As part of its normative function, the World Health Organization Pesticide Evaluation Scheme provides technical guidelines for efficacy testing of spatial repellents (World Health Organization, 2013) and household insecticide products (World Health Organization, 2009). Adoption of these guidelines will ensure less heterogeneity across studies and greater replicability of trials.

Studies were compiled for this systematic review that focused on different methodologies of laboratory evaluations of volatile pyrethroid spatial repellents (VPSRs) in the APR since 2004 to identify the strengths and weaknesses according to Luker et al. (2024) critical review and the extent of adherence to WHO guidelines. This compilation should assist researchers, decision-makers and vector control practitioners in the APR in future bioassay investigations, as well as in semi-field, and field trials in the region.

Materials and Methods

Points of interest and the population, intervention, comparison, and outcome approach

The study populations were the mosquito vectors of arboviruses, *plasmodium* and helminthic parasites sourced either from the laboratory or the wild in Asia-Pacific countries. VPSRs were considered as the intervention, including pyrethroids classified as medium to highly volatile. These pyrethroids comprised allethrin (d-allethrin, d-trans allethrin), dimefluthrin, flumethrin, meperfluthrin, metofluthrin, prallethrin and transfluthrin (Bibbs et al., 2018; Logan et al., 2022), formulated as either household insecticide products or treated materials (nets, papers, fabrics). For comparison, the control was a placebo. Outcomes were quantitative measurements of spatial repellency (complete protection time, landing inhibition, non-contact irritancy) and toxicity (pre- and post-prandial mortality).

Literature search strategy

Studies on laboratory efficacy of SR active ingredients against target mosquito species between 2004 and 2024 were searched using six database gateways: Google Scholar, PubMed, Scopus, Global Index Medicus, the Cochrane Library and the Kasetsart University Library websites. All searches included the key terms “Asia” and “Pacific”. Key terms to identify studies related to spatial repellents against mosquitoes included “malaria”, “dengue”, “volatile pyrethroid” and types of WHO-recommended assays for efficacy testing of spatial repellents (World Health Organization, 2013) and household insecticide products (World Health Organization, 2009), as shown in Table 1. Data from selected publications were reviewed and consolidated to create a summary table (Tab. 1S). Key words for literature searches are provided in Fig. 1.

- (Asia OR Pacific) AND (Malaria OR Dengue) AND “Spatial repellent” AND “Volatile pyrethroid” AND “High throughput screening system”
- (Asia OR Pacific) AND (Malaria OR Dengue) AND “Spatial repellent” AND “Volatile pyrethroid” AND “Peet-Grady chamber”
- (Asia OR Pacific) AND (Malaria OR Dengue) AND “Spatial repellent” AND “Volatile pyrethroid” AND “Y-tube olfactometer”
- (Asia OR Pacific) AND (Malaria OR Dengue) AND “Spatial repellent” AND “Volatile pyrethroid” AND “Free flight room”
- (Asia OR Pacific) AND (Malaria OR Dengue) AND “Spatial repellent” AND “Volatile pyrethroid” (Coil OR Emanator OR Vaporizer)

Fig. 1 Key search terminologies for spatial repellents (format adapted from Hii et al., 2021)

Table 1 World Health Organization guidelines for efficacy testing of spatial repellents and household insecticides (World Health Organization, 2009, World Health Organization, 2013) and their strengths and weaknesses (Luker, 2024)

Spatial repellent	Method of evaluation	Outcomes	Strengths ^c	Weaknesses ^c
Pure AI + Solvent (treated paper)	1. High-throughput screening system (HITTS) +/- Excito-repellency assay	Spatial repellency – Spatial Activity Index (SAI) <ul style="list-style-type: none"> • SAI = (Nc - Nt) / (Nc + Nt) • Nc = control; Nt = treatment 	<ul style="list-style-type: none"> • Consistent experimental design • Contact and spatial repellency testing 	<ul style="list-style-type: none"> • No mosquito attractants • Not accessible to most labs • Needs additional assays to support findings
Pure AI + Solvent (vial caps)	2. Y-tube olfactometer ^a	Host attraction inhibition (HAI) <ul style="list-style-type: none"> • HAI = (T / (T + A)) • T = towards; A = away AI 	<ul style="list-style-type: none"> • Flexibility for variations and modifications • Presence of a mosquito attractant • Tests for spatial repellency • Can test one treatment or competition between two treatments. 	<ul style="list-style-type: none"> • Variation in attractant sources • Cannot test for contact repellency • Needs control testing
Formulated Products (coils, vaporizers, emanators, etc.)	3. Free-flight rooms ^a	Protective efficacy (PE) of landing and feeding inhibition <ul style="list-style-type: none"> • %PE = ((C - T) / C) × 100 • C = control; T = treatment 	<ul style="list-style-type: none"> • Can test treatments against free-flying mosquitoes • Presence of host • Variations and modifications are possible 	<ul style="list-style-type: none"> • Needs control testing • Needs thorough decontamination • Not accessible on some labs • High probability of mosquito escapes
Pure AI + Solvent (treated paper) and Formulated products	1. Peet-Grady chamber ^b (caged bioassay)	Toxicity – Knockdown effects (50% and 95%) Mortality at 24 hr post-exposure <ul style="list-style-type: none"> • %Mortality = ((C - T) / C) × 100 • C = control; T = treatment 	<ul style="list-style-type: none"> • Ease of monitoring results • Flexibility for variations or modifications • Introduction of treatment through portals • Fitting of airflow is possible 	<ul style="list-style-type: none"> • Needs thorough decontamination • Not accessible on some labs • Absence of host or host cues • Needs control testing

a. Evaluation method retrieved from World Health Organization (2013).

b. Evaluation method retrieved from World Health Organization (2009).

c. Strengths and weaknesses retrieved from Luker (2024).

Inclusion and exclusion criteria

A set of exclusion criteria were defined and applied to filter out irrelevant studies and ensure the precision of this systematic review. The criteria were designed to eliminate studies that lacked essential details or that did not fulfill the following necessary conditions: 1) not conducted in the APR; 2) one or more active ingredients do not belong in VPSR criteria in Fig. 2; 3) conducted with other organisms (such as ticks and mites); 4) laboratory bioassays are not included in WHO guidelines (World Health Organization, 2009; World Health Organization, 2013); 5) spatial repellent product formats contain a mixture of two or more active ingredients such as other VPSR, piperonyl butoxide, insect growth regulators,

plant essential oils, or synergists; 6) not related to VPSR laboratory bioassays; and 7) unpublished full paper.

Studies were included in the systematic review if they met the criteria: 1) full-text publication written in English; 2) non-contact excito-repellency or spatial repellency effects of VPSRs in the laboratory trials against medically important mosquitoes in Asia-Pacific countries (<https://apmen.org/>); and 3) reported at least one of these defined entomological outcomes—protective efficacy, percentage of repellency or complete protection time and toxicity effects such as landing inhibition, feeding inhibition, sub-lethal incapacitation or knockdown, fecundity and pre-/post-prandial mortality. Fig. 3 provides a flow diagram of the selection criteria.

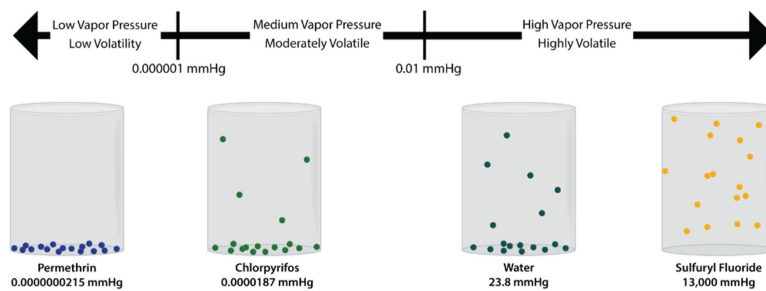


Fig. 2 Criteria of pesticide volatility (retrieved: National Pesticide Information Center, 2024)

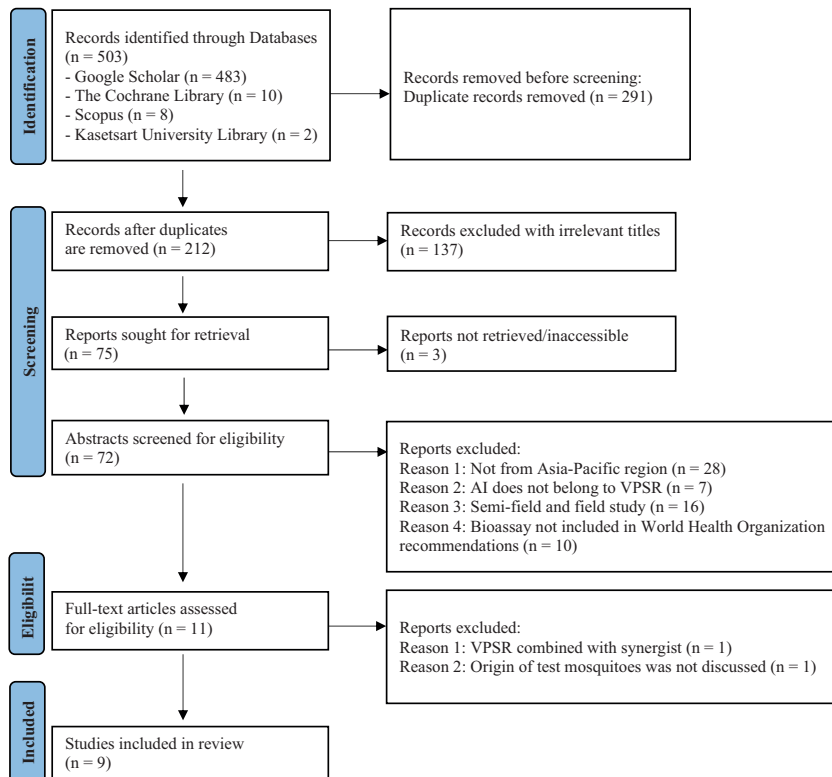


Fig. 3 Flow diagram describing the paper selection process, where VPSR = volatile pyrethroid spatial repellent and AI = active ingredient

Data extraction and characteristics of included studies

Accuracy and appraisal were improved by using a standardized data collection form for the extraction of data from the selected articles that met all the study criteria. The information collected consisted of: country, first author, year of publication, evaluation method, mosquito species, population and insecticide susceptibility, mosquito rearing condition, batch size per experiment, VPSR and its concentration, formulation and responses.

Results and Discussion

Literature search and eligibility screening

In total, 503 articles were identified based on searching electronic databases, registries and other sources (Fig. 3). These articles were screened against title and abstract eligibility, from which 12 studies were assessed for full-text eligibility, with 9 being included in this review. Studies that used mosquitoes collected from Asia-Pacific countries but conducted outside the region were excluded from this review.

Global perspective on efficacy of spatial repellents against mosquito vectors

The high volatility of VPSRs makes them an adaptable and accessible line of defense against host-seeking mosquitoes, regardless of location or financial status. In many vulnerable communities in various countries, energy access is limited (particularly for electricity), making interventions that require such infrastructure less feasible. The ability of VPSRs to passively volatilize at ambient temperatures provides a crucial advantage, allowing them to be deployed in areas without reliance on energy sources, offering protection across all socio-economic classes. However, its global efficacy is still in question due to large statistical variance in the results from different studies. According to a recently published report of the Asia-Pacific conference on mosquito vector control in 2023, a meta-analysis of VPSRs against mosquitoes revealed that metofluthrin and transfluthrin were the primary active ingredients studied, with evaluations based on human landing catch or trap density (Chareonviriyaphap et al., 2024). The overall protective efficacy was 48% (95% confidence interval, CI: 41–55%), with human landing catch yielding a higher efficacy of 74% (95% CI: 73–76%) and passive fabric

interventions showing the greatest efficacy at 64% (95% CI: 63–66%). Efficacy varied among mosquito species, with the highest observed in non-*Aedes aegypti* species and the lowest in *Anopheles funestus*. Key factors contributing to gaps in evidence include variations in product format, active ingredient, mosquito species, study type, weather and study design (Chareonviriyaphap et al., 2024). Consequently, studies in each region generally vary in methodology or protocol. Understandably, all regions of the world vary in climatic conditions, and these conditions greatly affect mosquito life table and VPSR efficacy. Therefore, it is essential (and more accurate) to investigate VPSR efficacy in each region by following a standardized guideline or protocol in order to have more consistent evidence to support the potential of VPSRs to combat medically important mosquito species adapted to their respective environments.

The World Health Organization guidelines on volatile pyrethroid spatial repellents and household insecticide testing

Mosquito behavioral responses of interest have been described by WHO in dedicated guidelines on efficacy testing of spatial repellents (World Health Organization, 2013). These responses were subdivided into laboratory, semi-field and field testing. The objectives for laboratory tests for technical materials are: 1) to establish dose-response relationships and determine the effective dosage (ED) of the active ingredient (AI) for 50% and 95% (ED50 and ED95) movement away from a chemical stimulus; and 2) to establish dose-response relationships and determine the ED50 and ED95 of the AI for host attraction-inhibition, measured using a high-throughput screening system and a Y-tube olfactometer, respectively (Table 1). For laboratory trials of formulated products, the primary outcome is the protective efficacy, specifically landing and feeding inhibition, of formulated spatial repellent products. This assessment necessitates the use of a free flight room (30 m³) by calculating the difference in inhibition of landing or feeding between treated and control groups (Table 1). In addition to the WHO guidelines for efficacy testing of spatial repellents (World Health Organization, 2013), WHO's guidelines for efficacy testing of household insecticides (World Health Organization, 2009) are necessary since VPSR products or formulations are directly intended for household use, with most AIs being incorporated into mosquito coils, liquid and mat vaporizers and ambient emanators (World Health Organization, 2009), as shown in Table 1. This latter guideline includes the use of a Peet-Grady chamber (PGC),

wind tunnel caged bioassay and indoor rooms to measure the toxicity effects such as the time it takes for the candidate VPSR to knock down 50% (KT50) and 95% (KT95) of a mosquito population, respectively, and mortality at 24 hr post-exposure.

Volatile pyrethroids used in Asia-Pacific countries

The study by van den Berg et al. (2021) measured the annual quantities of insecticides (in metric tonnes of active ingredients per insecticide class) used in spraying operations across selected countries, pooled over diseases, regions and years. The findings reported 3,042 t of organochlorines, 1,489 t of organophosphates, 611 t of carbamates, 174 t of pyrethroids, 33 t of neonicotinoids, 77 t of bacterial larvicides, 18 t of insect growth regulators and 14 t of spinosyns. In the Asia-Pacific region, pyrethroids play a crucial role in dengue control, being the primary choice for vector adulticiding, with 94.4% of their use allocated to residual spraying and space spraying. The use of pyrethroids was particularly important given the fluctuating pattern of insecticide use involving 40.4% for residual spraying, 33.2% for larviciding and 26.4% for space spraying (pooled over years). In contrast, organophosphates were mainly used for larviciding, accounting for 85.2% of their application when pooled over years (van den Berg et al., 2021).

Pyrethroids have been used in household insecticides since their discovery by Schechter and LaForge in 1949, with allethrin being the first pyrethroid discovered and mass-produced in 1953 (Matsuo, 2019). This discovery led chemists worldwide to modify pyrethroid structures, resulting in various derivatives with enhanced potency, economy and stability in subsequent years (Ujihara, 2019). These structural modifications have produced numerous synthetic pyrethroids with different vapor pressures that dictate the level of volatility or the ability to transition easily into a gaseous state (Laskowski, 2002; Matsuo 2019; Boné et al., 2020). This subset of synthetic pyrethroids is much more effectively delivered as SRs embedded in the use of vaporizers, coils and emanators than traditional adulticides (Bibbs and Kaufman, 2017). There are only a few pyrethroids that fall into the 'volatile' category, which can be further subcategorized into low, medium and high volatility, depending on their inherent vapor pressure (Fig. 2). Identified volatile pyrethroids are allethrin (0.0 ± 0.9 mmHg at 25°C), dimefluthrin (0.0 ± 0.8 mmHg at 25°C), flumethrin (0.0 ± 1.7 mmHg at 25°C), meperfluthrin (0.0 ± 0.9 mmHg at 25°C), metofluthrin (0.0 ± 0.7 mmHg at 25°C), prallethrin (0.0 ± 0.9 mmHg at 25°C), pyrethrin (0.0 ± 1.1 mmHg at 25°C) and transfluthrin (0.0 ± 0.8 mmHg

at 25°C) (Bibbs et al., 2018; Logan et al., 2022; pubchem.ncbi.nlm.nih.gov, accessed: 2024). These pyrethroids range from low to high volatility which could be a possible basis for choice of formulation or concentration. Due to their good dispersion in the air through immediate vaporization at ambient temperatures, these pyrethroids have been considered spatial repellents for mosquito control strategies (Achee et al., 2012; Salazar et al., 2013; Bibbs and Kaufman, 2017; Bibbs et al., 2018).

In this systematic review, of the VPSRs mentioned above, only transfluthrin, metofluthrin, dimefluthrin, prallethrin, allethrin, d-allethrin and d-trans-allethrin have been evaluated in various laboratory studies in the Asia-Pacific region (Table S1). This selection of AIs could have been influenced by the accessibility, preference, local policies and efficacy of the AIs formulated as household insecticides against medically important arthropods. In the Americas, the United States Environmental Protection Agency (US EPA) is reevaluating 23 pyrethroids and pyrethrins for risks to human health and the environment, with 13 of these pyrethroids approved to remain on the market and 10 still under assessment. (Erickson, 2020).

In the Asia-Pacific region, academic and research institutions commonly evaluate formulated VPSRs in their laboratories or field stations, using products purchased from consumer outlets or provided by pest control manufacturers (El-garj et al., 2015; Charlwood et al., 2016; Jung et al., 2021; Vajda et al., 2024). Other institutions procure proprietary AIs from chemical companies and create their own formulations to produce efficacy data for the intended use such as sublethal or effective concentrations (Kawada et al., 2004; Salazar et al., 2013; Yan et al., 2023, Ahebwa et al., 2024). The presence of insecticide resistance in medically important mosquito vectors in a specific geographical location also influences the choice of active ingredient for testing and evaluation (Sănu et al., 2023; Kim et al., 2023).

Laboratory studies of volatile pyrethroid spatial repellents conducted in Asia-Pacific countries

The numbers of studies published between 2004 and 2024 in the Asia-Pacific region comprise: India (1), Japan (1), South Korea (one study with a combination of PGC and room/chamber assay), Malaysia (1), Sri Lanka (1) and Thailand (4). These studies consisted of excito-repellency assay (2), high-throughput screening system (2), PGC (3) and room or chamber assay (caged/free-flying) (3). An overview of these studies is provided in the supplementary table (Tab. 1S). Evaluation of the efficacy and use of spatial

repellents requires new sets of laboratory and field assay tools, standard endpoints and detailed analyses (Achee et al., 2012). However, it is essential to have standardization or harmonization of spatial repellent bioassays to produce reliable evaluations and reproducible results. In this review, the nine laboratory studies conducted in the Asia-Pacific region for household insecticides (such as mosquito coils and emanators) were conducted in chambers, rooms or huts with different dimensions or volumes (Table 2). Air flow, temperature, humidity, and wind speed (Ritchie and Devine, 2013; Jeyalakshmi et al., 2014; Kim et al., 2024; Ahebwa et al., 2024) affect the dispersion of volatile concentrations in rooms of various sizes, resulting in variable knockdown times and delayed mortality rates. Therefore, well-controlled conditions in a standardized room size of 30 m³ (as recommended by the WHO) should provide consistent measurement of efficacy (World Health Organization, 2009; World Health Organization, 2013).

Mosquito behavioral and toxicity responses to laboratory testing of volatile pyrethroid spatial repellents

According to the WHO guidelines (World Health Organization, 2013), laboratory evaluations of spatial repellents should be conducted using approved and recommended sets of bioassays that have different points of emphasis for responses or desired outcomes. While these laboratory tests are supported by WHO guidelines, it is important to acknowledge their inherent strengths and weaknesses to ensure accurate interpretation of results and conclusions. The critical review by Luker (2024) on laboratory methods for evaluating mosquito repellents provided a basis for this review to update and assess the status of VPSRs studies in the Asia-Pacific region as shown in Table 3. One of these assays is the high-throughput screening system (HITTS) capable of evaluating toxicity and both the contact irritancy and spatial repellency behavioral responses of mosquitoes to volatile pyrethroids (Grieco et al., 2005; World Health Organization, 2013; Kim et al., 2023; Kim et al., 2024). In this review, only two VPSR studies using HITTS were carried out in the APR. Kim et al. (2023) tested transfluthrin and metofluthrin against both laboratory and field populations of *Ae. aegypti* to determine the discriminating concentration (DC) of transfluthrin. Their results indicated the successful DC determination and showed that the toxicity assay of HITTS was thus consistent and able to produce clear dose-dependent responses of *Ae. aegypti* from transfluthrin. The study of Kim et al. (2023) using HITTS was able to measure the knockdown rate after 1 hr and 24 hr post-exposure mortality.

Table 2 Strengths and weaknesses of laboratory evaluations studies of volatile pyrethroid spatial repellents (VPSRs) against medically important mosquitoes in Asia-Pacific region (APR) (World Health Organization, 2009; World Health Organization, 2013; Luker, 2024)

(Study ID)	Bioassay/evaluation method	Description	Strengths	Weaknesses
(TH1) Thailand: Kim et al. (2023)	High-throughput screening system (HITTS) (V = 11,43.97 cm ³)	Toxicity Assay of lethal and sub-lethal concentration of transfluthrin and metofluthrin against susceptible and resistant <i>Ae. aegypti</i>	<ul style="list-style-type: none"> • Replicable design • Used well-established susceptible mosquitoes • Defined mosquito generation of resistant mosquitoes to be used • Able to establish discriminating concentration of metofluthrin against <i>Ae. aegypti</i> 	<ul style="list-style-type: none"> • Lack of spatial repellency assay • Assay might not be accessible to other laboratories • Focused only on toxicity effects • Lacks host-cues
(TH2) Thailand: Kim et al. (2024)	HITTS (V = 1,143.97 cm ³)	Toxicity assay of discriminating concentration of transfluthrin treated filter papers affected by different drying times against susceptible <i>Ae. aegypti</i>	<ul style="list-style-type: none"> • Replicable design • Used well-established susceptible mosquitoes • Defined mosquito generation of resistant mosquitoes to be used • Proven weakness and inaccurate measurement of mortality elicited by discriminating concentration of transfluthrin-treated papers at standard 24 hr drying time 	<ul style="list-style-type: none"> • Lack of spatial repellency assay • Assay might not be accessible to other laboratories • Focuses only on toxicity effects • Lacks host-cues

Table 2 Continued

(Study ID) Country: Author	Bioassay/evaluation method	Description	Strengths	Weaknesses
(TH3) Thailand: Sukkanon et al. (2021)	Excito-repellency assay (ERA) (V = 8,122.5 cm ³)	Determined spatial repellency action of Transfluthrin against susceptible and resistant <i>Ae. aegypti</i> , <i>An. dirius</i> , <i>An. minimus</i>	<ul style="list-style-type: none"> • Replicable design • Used both susceptible and resistant mosquitoes • Measured both contact and non-contact irritancy (spatial repellency) response • Established lethal dose 	<ul style="list-style-type: none"> • Assay might not be accessible to other laboratories • Lacks host-cues • Assay requires constant decontamination and control testing
(TH4) Thailand: Yan et al. (2023)	ERA (V = 8,122.5 cm ³)	Established dose response of <i>An. minimus</i> against transfluthrin in ERA then impregnated into jute and cotton fabrics prior to semi-field evaluations	<ul style="list-style-type: none"> • Replicable design • Considered establishing dose response data prior to semi-field studies • Use of well-established susceptible mosquitoes 	<ul style="list-style-type: none"> • Assay might not be accessible to other laboratories • Lacks host-cues • Assay requires constant decontamination and control testing
(SL1) Sri-Lanka: Sānu et al. (2023)	Peet-Grady chamber (PGC) (V = 5.832 m ³)	Evaluation of commercially available mosquito coils and liquid vaporizers	<ul style="list-style-type: none"> • Able to evaluate a wide range of VPSRs • Use of commercially available products could give a firsthand picture of efficacy • Replicable design • Ease of result monitoring due to caged assay 	<ul style="list-style-type: none"> • Lacks host-cues • Assay requires constant decontamination and control testing • Lacks spatial repellency assay • Focused only on toxic effects • Assay might not be accessible to other laboratories
(MY1) Malaysia: Elgarj et al. (2015)	PGC (V = 5.832 m ³)	Compared spatial repellency effects of commercially available mosquito coils against laboratory population of <i>Ae. aegypti</i>	<ul style="list-style-type: none"> • Replicable design • Included standardized positive control or reference mosquito coil • Ease of result monitoring due to caged assay 	<ul style="list-style-type: none"> • Only measures two entomological endpoints • Lacks host-cues • Assay requires constant decontamination and control testing • Lacks spatial repellency assay • Focused only on toxic effects • Assay might not be accessible to other laboratories
(KR1) South Korea: Jung et al. (2021)	PGC (V = 5.832 m ³)	Compared a commercially available liquid based on prallethrin, allethrin and metofluthrin, as well as mat vaporizers against laboratory <i>Ae. Albopictus</i>	<ul style="list-style-type: none"> • Replicable design • Variation and amplification of assay by measuring spatial distribution of volatiles by using cartesian coordinates 	<ul style="list-style-type: none"> • Lacks host-cues • Assay requires constant decontamination and control testing • Only measures knockdown time • Lack of spatial repellency assay
(KR1.2) South Korea: Jung et al. (2021)	Room chamber (V = 62.4 m ³)	Compared a commercially available liquid based on prallethrin, allethrin and metofluthrin, as well as mat vaporizers against laboratory <i>Ae. albopictus</i>	<ul style="list-style-type: none"> • Flexibility in duration of exposure to VPSR 	<ul style="list-style-type: none"> • Assay requires constant decontamination and control testing • Only measures toxicity response • Lacks spatial repellency assay • Use of caged mosquitoes, not free flying in a room/chamber
(IN1) India: Jeyalakshmi et al. (2014)	Room chamber (V = 6 m ³ , 20 m ³ , 40m ³)	Evaluated the common transfluthrin concentration in commercially available transfluthrin base liquid vaporizer against <i>Cx. quinquefasciatus</i> in different room sizes	<ul style="list-style-type: none"> • Variation and flexibility of design • Room size variation reveals correlation of airspace to VPSR efficacy 	<ul style="list-style-type: none"> • Not easy to replicate since exact room size might not be accessible to some other lab
(JP1) Japan: Sugano and Ishiwatari (2011)	Room chamber (V = 28 m ³)	Evaluated wide range of VPSR mosquito coil and liquid vaporizer formulations against free flying and caged <i>Cx. quinquefasciatus</i> and <i>Cx. pipiens</i> in a room chamber	<ul style="list-style-type: none"> • Replicable design • Room volume is close to World Health Organization guideline recommendation (30 m³) • Used both laboratory and field mosquitoes 	<ul style="list-style-type: none"> • Lacks spatial repellency assay • Focused only on knockdown time effects • Assay requires constant decontamination and control testing

Ae. = *Aedes*; *An.* = *Anopheles*; *Cx.* = *Culex*.

Additionally, recovery of knocked down mosquitoes was measured using field mosquitoes. The second HITS study highlighted the importance of air-drying time for the treated papers in the toxicity assays. They found that highly volatile VPSRs, such as transfluthrin, could lose high levels of efficacy due to volatilization of molecules during a drying time of 24 hr. This outcome argues for the use of alternative assays, such as the WHO bottle bioassay, which could accommodate a highly volatile VPSR without the risk of efficacy underestimation (Kim et al., 2024)

According to the WHO guidelines, host-attraction inhibition is a desirable indicator response to VPSRs, which is evaluated using a Y-tube olfactometer to measure attraction to host odors of medically important mosquito vectors in the presence of the candidate VPSR relative to the control (World Health Organization, 2013). The current literature search using the specified key phrases revealed a lack of studies using Y-tube olfactometers for VPSR evaluation in the APR. On the other hand, compounds, such as botanicals and plant essential oils, have been evaluated for their repellency or attraction properties using Y-tube olfactometers (Uniyal et al., 2016; Islam et al., 2017). However, Kim et al. (2021) used a diffusion assay-high-throughput screening system to evaluate the attraction of synthetic lures without the need for artificial airflow in Y-tube olfactometers. The lack of studies using Y-tube olfactometers has missed the opportunity to measure the small-scale interaction between host cues and SR volatiles against field mosquitoes in the APR. This may be explained by the complexity of the equipment, which requires additional instrumentation, such as a vacuum line for airflow and activated charcoal, to filter the air, as well as the requirement for a larger airspace which could overestimate the efficacy of the SRs.

Usually, the evaluation of the protective efficacy of VPSRs in the laboratory has been performed by utilizing free-flight rooms or large chambers that can accommodate human volunteers to count mosquito landings in the presence or absence of a VPSR (World Health Organization, 2013). Three studies considered in the current review that use room chamber assays did not include human volunteers as bait, since the focus was on the toxic effects of the VPSRs. This type of room evaluation involves free-flying or caged mosquitoes, as conducted by Sugano and Ishiwatari (2012) who measured the knockdown time of both laboratory and field populations of *Culex pipiens pallens* and *Cx. quinquefasciatus*, respectively, against metofluthrin, d-allethrin, prallethrin and transfluthrin, formulated as either liquid vaporizers or mosquito coils. The lowest knockdown time (KDT) value was observed in

the field population of *Cx. quinquefasciatus* (collected from Bogor, Indonesia) against metofluthrin at a concentration of 60 mg/45 mL in a liquid vaporizer format, with a KDT value of 12 min. However, a 1 hr pre-fumigation period was instituted before mosquito introduction. The reason for the pre-fumigation period was to help with uniformity and distribution of vapors inside the room. As humans play a crucial role in vector-host-pathogen transmission, their inclusion in free-flight room SR assays provides critical endpoints such as landing inhibition, diversion and deterrence.

In South Korea, Jung et al. (2021) utilized a PGC to evaluate the movement of volatiles upon heating the liquid vaporizer device with caged mosquitoes systematically positioned in a Cartesian format. They found that the mosquitoes positioned in the upper section of the chamber exhibited faster knockdown activity than mosquitoes in other areas, indicating insecticide accumulation in the ceiling area (Jung et al., 2021). A PGC is recommended for evaluation based on the WHO guidelines for efficacy testing of household insecticides (World Health Organization, 2009) that aim to measure vapor movement from the source in the presence or absence of air movement. The PGC studies included in the current review used caged mosquitoes, as recommended by the World Health Organization (2009), to measure vapor movement, knockdown rate, knockdown time and mortality rate (El-garj et al., 2015; Jung et al., 2021; Sănu et al., 2023). In the PGC protocol, the use of caged mosquitoes ensures accurate observation of knockdown and mortality before and after exposure to SR volatiles. As stated in Jung et al. (2021), this approach facilitates the assessment of the candidate AI's toxicity by tracking the knockdown time, as a proxy indicator of SR volatility, along the gradient of the chamber airspace. This, in turn, provides insight into its initial effects on resting mosquitoes. Aside from inherent toxic properties and air movement, efficacy of VPSR usually depends on the volume of the space in which it is being deployed. Jeyalakshmi et al. (2014) used three room sizes (6 m³, 20 m³, 40 m³) for the efficacy testing of a transfluthrin vaporizer, with high and low emanation modes against *Cx. quinquefasciatus*. Their results revealed that experiments in the 6 m³ room using a high emanation mode recorded the minimum KT50 value (10.61 min), followed by experiments in the 20 m³ room using the high emanation mode (15.06 min). Furthermore, efficacy and room sizes were inversely proportional, as shown by the higher KT50 and KT95 values and the lower mortality rates after 24 hr of exposure. This emphasized the importance of the room volume on the VPSR and its format performance.

The study suggested that evaluating product efficacy in different room sizes was essential to reflect real-world conditions and to prevent household product failures in the field (Jeyalakshmi et al., 2014).

Across studies, there were procedural variations in laboratory testing of VPSRs in the APR. The mosquito-rearing conditions are important because they influence the fitness and behavior of each mosquito during exposure. The WHO recommendations for these conditions are $27 \pm 2^\circ\text{C}$, $80 \pm 10\%$ relative humidity and a light-to-darkness ration of 12 hr:12 hr. All nine studies considered in the current review had minimal variations in temperature, humidity and the light-dark cycle. In addition, each of the nine studies provided 10% sugar solution to the mosquitoes. Tests against resistant-field populations of mosquitoes revealed poor responses to VPSRs compared to susceptible-laboratory populations (Sănu et al., 2023; Kim et al., 2023) (Table S1). The mosquitoes used to determine the efficacy of d-transallethrin, metofluthrin, dimefluthrin, prallethrin and transfluthrin were derived from wild-caught populations of pyrethroid-resistant *Ae. aegypti* that were raised as colonies in the laboratory (Sănu et al., 2023), whereas Kim used both wild caught and laboratory reared mosquitoes. The results explained the poor response of the resistant field strains from Sănu et al. (2023) and Kim et al. (2023). Studies aimed at evaluating SRs against field-strain mosquitoes would benefit from using susceptible strains to establish baseline efficacy as a clear benchmark for the amount of AI needed to achieve the desired results. Once baseline efficacy has been established, complementary tests with field strains could be conducted to assess real-world applicability and resistance dynamics. In addition, there was a lack of laboratory evaluation of VPSR in the presence of a host or host-cues in the room chamber studies that were supposedly used for free-flying mosquitoes (KR1.2, IN1 and JP1, as defined in Table 2). Modifications, such as using cages, were common in the room chamber studies (Table 2). Room volume was another variable in the room chamber studies with variable room sizes being implemented. The WHO recommendation is for a 30 m³ room with smooth and light surfaces (World Health Organization, 2009). However, not all of the three reviewed studies complied with this recommendation (Table 2). In the case of Jeyalakshmi et al. (2014), the efficacy of transfluthrin was inversely proportional to room size. This finding should be valuable in considering room size for efficacy studies in both laboratory and model houses.

Conclusion

This review discussed volatile pyrethroids, such as metofluthrin, allethrin, prallethrin and transfluthrin, that had been formulated into various delivery formats, particularly mosquito coils, liquid or mat vaporizers and passive emanators in the APR. All nine laboratory studies of in the APR included in this review focused on the toxicity effects induced by the VPSRs, as indicated by the specific entomological outcomes in each assay. Although VPSRs are insecticides in nature, their main purpose is to reduce mosquito attraction and prevent mosquito entry to domestic space. The inclusion of host-cues in the form of live host or synthetic compounds could provide a solid preliminary picture on how VPSRs will perform in semi-field and field evaluations. The variations in methodology due to the unique objectives and resources across the reviewed studies for the purpose of complementing main entomological endpoints, must ensure the uniformity and replicability of results in a specific geographical location. Only 12 out of 55 countries in the APR have conducted VPSR efficacy studies that have passed the inclusion criteria, indicating limited engagement with key stakeholders and entomological laboratories in the evaluation of VPSR. Additional laboratory evaluations would be beneficial to support further semi-field and field studies to establish more concrete evidence of VPSR efficacy in the APR.

Conflict of interest

The authors declare that there are no conflicts of interest.

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