I2I Landscaping exercise

PEET Grady test chamber

Last updated: March 2023

Key contributors: Katherine Gleave, Fabricio Silva Martins

Acronym List

AI	Active ingredient
I2I	Innovation to Impact
KD	Knockdown
RH	Relative humidity
SOP	Standard operating procedure
WHO	World Health Organisation

Summary

Aim and key questions addressed	- Used for efficacy testing and evaluation of household insecticide products that are intended for indoor use; namely coils, vaporizer mats, liquid vaporizers, ambient emanators and aerosols
Context	- Laboratory
Test item	- Spatial repellents
Mosquito population	- Laboratory reared
Number of mosquitoes per replicate	- See relevant protocol for each spatial repellent product
Endpoints measured	- Knockdown - 24-hour mortality
Exposure time	- See relevant protocol for each spatial repellent product
Holding time	- See relevant protocol for active ingredient tested
Indicative of personal protection	- No
Suitable chemistries	- Formulations to be used as spatial repellents
Appropriate controls	- Well characterised active ingredient as a positive control
Relevant stage of production pipeline	- Product development - Efficacy assessment

Characterisation of output	- Endpoints of knockdown and mortality are well characterised, however, results can vary between studies due to differences in aerosol dispersal rates and circulating air flow.
Accessibility	- The PEET Grady chamber is a specialised piece of equipment and requires training for use. Thorough cleaning and safety methods also need to be adhered to
Cost	- Cost of PEET-Grady chamber, staff training and maintenance
Level of validation and characterisation of outputs	– Testing is not standardised, and method needs to be validated for use – These guidelines are less well developed than those available for testing contact insecticides
Outstanding questions, gaps and priorities	- There is a need to update the WHO guidelines to include more up to date methodology and input from industry on the outcomes that would be most beneficial. - Validating the updated and newly proposed method from (Martins et al., 2023) in multiple other sites is a priority
Key references, related SOPs, guidelines and publications	- Martins, W. F. S., Reid, E., Tomlinson, S., Evans, G., Gibson, J., Guy, A., … Weetman, D. (2023). Improving the efficiency of household insecticide testing against mosquitoes. Research Square, 1–18. Retrieved from https://doi.org/10.21203/rs.3.rs-2451023/v3 - World Health Organization. (2009). Guidelines for Efficacy Testing of Household Insecticide Products. World Health Organization, 3, 1–32.

Overview

As insecticide resistance continues to threaten gains made in vector-borne disease control, alternative insecticide-based tools for public health are being explored. Once such range of tools are spatial repellents which are deployed as mats, emanators, sprays, coils, and candles. Spatial repellents are generally used for personal and household protection, with limited use in large scale public health programmes. However, some studies have revealed their use is more extensive than previously thought (Loroño-Pino et al., 2014; Nalwanga & Ssempebwa, 2011), which shows a need for standardised approaches for susceptibility screening of mosquitoes against these products.

Spatial repellents aim to disrupt host-seeking and feeding behaviour of target vectors. Chemicals that have shown repellent effects include volatile pyrethroids such as metofluthrin, transfluthrin and Prallethrin, botanical compounds such as terpenoids, or volatiles found from human skin and bacteria such as 1-methylpiperazine.

The PEET Grady test chamber is recommended for efficacy testing and evaluation of household insecticide products that are intended for indoor use; namely coils, vaporizer mats, liquid vaporizers, ambient emanators and aerosols (WHO., 2009). The chamber is designed with an internal measurement of 180cm x 180cm x 180cm (Figure 1) and should be constructed using smooth internal wall panels made of either stainless steel, aluminium, glass or other suitable material to ensure easy cleaning. A tight-fitting entrance door is fixed to one of the side walls of the chamber. The chamber has a fluorescent light, as well as an exhaust fan in the ceiling to remove insecticide vapour after each test. Four hooks are fitted in the corner of the ceiling about 20cm from the side of the walls to suspend test cages. For air circulation in the chamber a 30cm diameter fan with a flat dish of 30cm attached on top of the fan rail guard is placed on the floor of the chamber facing upwards. There are two glass observation windows and four mosquito introduction and/or utility windows provided on each of the side walls of the chamber for easy introduction of mosquitoes and counting of those knocked down during the test period.

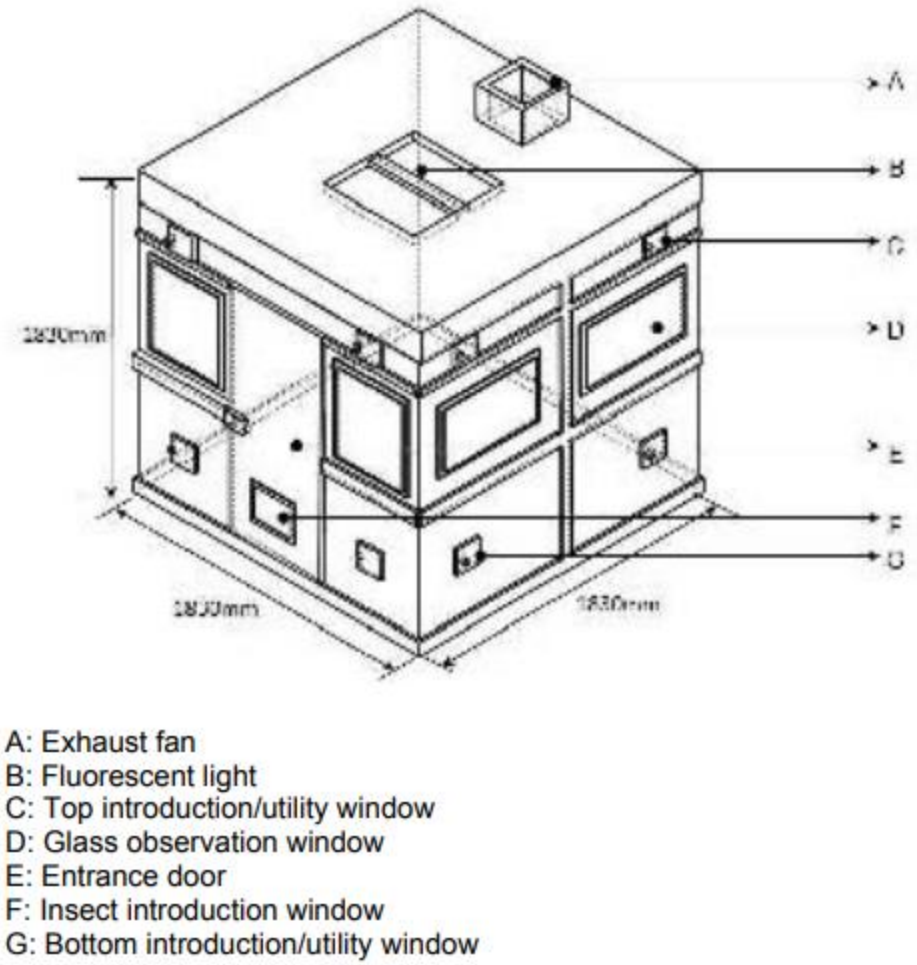

Figure 1. Diagram of the PEET-Grady test chamber (World Health Organization, 2009).

Define Accepted Methodologies

Are there existing standard SOPs/Guidelines detailing methodologies?

The WHO 2009 guidelines for testing household insecticide products are the main source of reference.

- "Guidelines for efficacy testing of household insecticide products" (WHO., 2009)
- LITSOP152 "Operation of the PEET Grady Chambers" (Liverpool Insect Testing Establishment)
- "Improving the efficiency of household insecticide testing against mosquitoes" – Supplementary Additional File 1 "Step-by-step protocol for testing aerosolized insecticides in PEET-Grady chamber" (Martins et al., 2023).

Are these sufficiently detailed?

The current method details how to perform tests, number and status of mosquitoes to use, spacing and hanging of cages, wind speed, and holding conditions post assay. Although the WHO guideline recommends the use of an automatic aerosol dispenser, specifications are not provided. These guidelines are less well developed than those available for testing contact insecticides.

Do these methods require specialised/non-standardised equipment and/or training?

The PEET Grady chamber is a specialised piece of equipment and requires training for use. Thorough cleaning and safety methods also need to be adhered to.

Are there issues with the methods or their interpretation?

As previously mentioned, these guidelines are less well developed than those available for testing contact insecticides, such as those on insecticide-treated nets (ITNs). There are also technical limitations which limit testing capacity. For instance, it is challenging to standardize exposure dose for ambient insecticides, such as volume of aerosol discharge due to cans

variable interior pressure, propellent volume and nozzle configuration. Also, the fan airflow can impact assay reproducibility and bias in results.

What AIs or combinations of AIs have the tests been used for?

Active ingredients: metofluthrin, transfluthrin, prallethrin, cypermethrin, imiprothin, d-phenothrin, tetramethrin, pyrethrum, allethein, esbiothrin, terallethrin.

Are they validated, for which AIs/entomological effects, and to what extent?

Testing is not standardised, with most studies using only one mosquito species to test formulations instead of the recommended Culex, Aedes and Anopheles.

What inputs need to be characterised? e.g., samples, mosquitoes, equipment

- Mosquito species. The WHO 2009 guidelines state tests should be done at least with *Aedes aegypti* or *Culex quinquefasciatus*
- Standardised mosquito rearing
- Inclusion of a well characterised active ingredient (AI) as a reference product or positive control
- Volume of aerosol discharge
- Standardize cage's fabrics and mesh aperture
- Specification for an automatic aerosol dispenser

Are endpoints clearly defined and appropriate? Who were they defined by?

Knock-down and mosquito mortality at 24 hours post-exposure are the endpoints set out in the 2009 guidelines. Knockdown is suggested to be scored at 'regular intervals' which leaves this open to user interpretation.

Are their supporting SOPs? e.g., cleaning SOPs, mosquito rearing SOPs required

Cleaning of the test chamber is mentioned in guidelines as: "test chambers and other instruments should be properly cleaned after completion of each test. Wash the test chamber and its internal walls thoroughly with detergent solution and water. If carried out properly, this should remove most toxic residues. Test chambers must be checked for insecticide contamination before the start of each test. The chamber shall be declared contaminated or unsatisfactory for use when the test mosquitoes held in the chamber under the same condition as test mosquitoes for 1 hour show knock-down in excess of 10%".

Define Current Use Practices

Does everybody use the same SOP?

From the limited published literature, it appears that all users of the PEET Grady for spatial repellent research follow the WHO 2009 guidelines. However, it is interesting to note that a few studies have performed bioassays in a testing room instead of a PEET-Grady chamber, using a distinct recommendation for spray discharge or air flow over the testing period. This is most likely due to the restricted availability of the PEET-Grady chamber for research purposes and so a testing room becomes the primary option for carrying out experiments.

An altered method proposed by (Martins et al., 2023) is detailed below.

Are there differences of interpretation of the method?

Bioassays carried out in testing rooms and semi-field conditions are performed in wider areas compared to the PEET-Grady chamber. Also, such studies although using cages, often do not use a source of airflow for aerosol dispersion. Furthermore, there is a lack of standardized procedure for aerosol discharge, for instance distance and direction from the cages or central point within the room as well as a volume of spray discharge per square meter.

Are there results obtained largely consistent between studies?

Issues with insecticide dispersion while running experiments can lead to inconsistencies in product efficacy between laboratories and field-based testing. The impact of air-flow circulation could also affect reproducibility and bias in results. There is a lack of published studies to compare results between due to the low availability of PEET Grady chambers for research purposes.

Is further development, refinement or validation of the method required? Based on priority, significance, and relevance of method.

(Martins et al., 2023) have refined testing methods with alterations for improved performance. A description of the newly proposed methods can be found below.

Identify Potential Sources of Variation

What are the sources of variability in the method and are their means to minimise or characterise these.

- Washing and decontamination success

- Challenges with standardizing exposure dose for ambient insecticides over testing procedures
- Absence of a commercial remote spray device with the required features for research purposes – further challenges for inferring formulations dose-response on knockdown.
- Lack of standardized aerosol density regarding testing room size
- Airflow direction, length and speed during test
- Cage's mesh aperture and fabric that restrict droplets dispersion to cage's interior

Does current method/s need to be adapted for new active ingredients/MoA/types of tool?

The guideline does not outline an approach for testing repellency effect of spatial repellents in laboratory-based conditions while using knockdown as a proxy for formulation efficacy. Nevertheless, spatial repellents are expected to primarily disrupt mosquito host-seeking behaviour and locomotion rather than killing effect. Therefore, there is a need for developing approaches to infer volatile insecticide effectiveness through behaviour disturbance.

Are new methods required? Identify areas where current method/s are not suitable or sufficient.

WHO guidelines have a very low throughput, especially when also considering the decontamination steps after each test. Several recommendations in the guidelines lack precision.

(Martins et al., 2023) have developed and validated a more standardised methodology based on the WHO guidelines but with improved reproducibility and throughput. This is a cage-based approach (Martins et al., 2023) as an alternative to free-flying bioassay and includes 3 specific innovations:

- a fast wipe-based decontamination procedure
- a dual-cage assay to increase throughput
- video cameras to collect behavioural data including mosquito knock-down

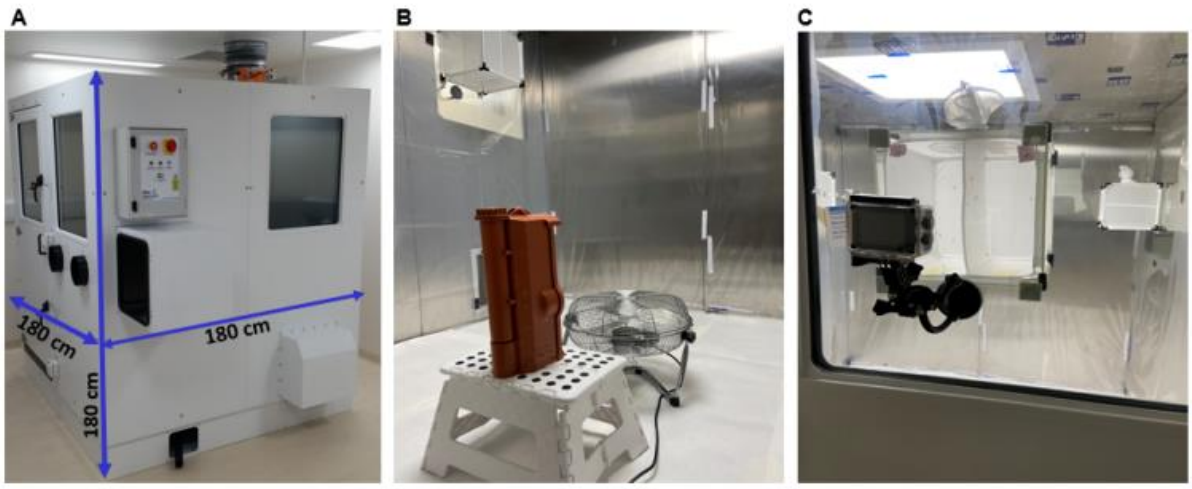

*Figure 2. PEET-Grady chamber's external and internal overview. **A** Chamber's lateral profile showing glass observation windows, electrical control panel and extract duct at the ceiling's rear. **B** Set-up of the automatic aerosol dispenser and a 30-cam diameter fan at the chamber's centre. **C** Viewing from a chamber's glass observation window with a sited action camera to assist with the scoring of mosquitoes' knockdown. (Taken from Martins et al., 2023)*

Gaps in biological or other understanding that hinder method development or validation

Due to the well-known impact of population genetic background to insecticide resistance across geographic regions, it is important to include field populations to assess effectiveness and sensitivity of new approaches.

Prioritisation – is there an issue that needs to be addressed, what specifics, how urgent is the need?

There is a need to update the WHO guidelines to include more up to date methodology and input from industry on the outcomes that would be most beneficial. Spatial repellents have previously been a small area of research compared to other vector control products and so there is not a lot of publicly available studies with data obtained from using the PEET Grady chamber.

Validating the updated and newly proposed method from (Martins et al., 2023) in multiple other sites is a priority.

References

Loroño-Pino, M. A., Chan-Dzul, Y. N., Zapata-Gil, R., Carrillo-Solís, C., Uitz-Mena, A., García-Rejón, J. E., … Eisen, L. (2014). Household use of insecticide consumer products in a dengue-endemic area in México. *Tropical Medicine and International Health*, *19*(10), 1267–1275. https://doi.org/10.1111/tmi.12364

Martins, W. F. S., Reid, E., Tomlinson, S., Evans, G., Gibson, J., Guy, A., … Weetman, D. (2023). Improving the efficiency of household insecticide testing against mosquitoes. *Research Square*, 1–18. Retrieved from https://doi.org/10.21203/rs.3.rs-2451023/v3

Nalwanga, E., & Ssempebwa, J. C. (2011). Knowledge and practices of in-home pesticide use: A community survey in uganda. *Journal of Environmental and Public Health*, *2011*. https://doi.org/10.1155/2011/230894

World Health Organization. (2009). Guidelines for Efficacy Testing of Household Insecticide Products. *World Health Organization*, *3*, 1–32.

Innovation to Impact
Pembroke Place
Liverpool L3 5QA UK

contact@innovation2impact.org
+44 151 702 9308

BILL & MELINDA GATES *foundation*

LSTM
LIVERPOOL SCHOOL OF TROPICAL MEDICINE
Since 1898