I2I Landscaping report: Intrinsic Toxicity Testing-Topical Bioassay

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Annabel Murphy-Fegan, Katherine Gleave

Acronym List

AI	Active ingredient
CO_2	Carbon dioxide
I2I	Innovation to Impact
LITE	Liverpool Insect Testing Establishment
LD_{50}	Lethal dose 50% mortality
LD_{90}	Lethal dose 90% mortality
µl	Microlitre
SOP	Standard operating procedure
PBO	Piperonyl butoxide
PPF	Pyriproxyfen
WHO	World Health Organization

Summary

Aim and key questions addressed	- Determining the intrinsic activity of an insecticide via topical testing. These assays are used for screening active ingredients to assess the mortality of adult mosquitoes.
Context	- Laboratory
Test item	- Mosquito colony
Mosquito population	- Laboratory reared
Number of mosquitoes per replicate	- 8-15 mosquitoes
Endpoints measured	- Knockdown, mortality
Exposure time	- N/A
Holding time	- 24, 48, 72 hours. Dependent on study protocol
Indicative of personal protection	- N/A
Suitable chemistries	- Insecticides
Appropriate controls	- Negative control: solvent used for preparation of test insecticides.

Relevant stage of production pipeline	- Development and resistance monitoring
Characterisation of output	- Endpoints well defined. Additional sub-lethal work dependent on study
Accessibility	- Equipment and set up need to be sourced, training is required
Cost	- Low cost. Relatively non-specialised equipment and straightforward method. Cost of equipment and training operator
Level of validation and characterisation of outputs	- Not identified any formal validation data
Outstanding questions, gaps and priorities	- N/A
Key references, related SOPs, guidelines and publications	- The performance of Topically Applied Bioassays LITSOP056 - Intrinsic Toxicity Testing I2I-SOP-029 - Guidelines for testing mosquito adulticides for Indoor Residual Spraying and treatment of mosquito nets (World Health Organization, 2006) - Guidelines for efficacy testing of insecticides for indoor and outdoor ground applied space spray applications, 2009. - Guidelines for efficacy testing of household insecticide products mosquito coils, vaporizer mats, liquid vaporizers, ambient emanators and aerosols.

Overview

Topical testing is used to test the efficacy of novel compounds to determine intrinsic insecticidal activity. This laboratory assay enables the precise concentration of an active ingredient (AI) to be administered via a droplet directly onto the dorsal side of the thorax of adult mosquitoes. Potential confounding effects are reduced in this method, which may arise due to insect behaviour including avoidance and grooming. This method also bypasses most barriers to uptake, controlling for the effect of any potential cuticular resistance mechanisms.

Solutions for topical applications are prepared by dissolving technical grade insecticide in acetone, a volatile solvent which has the advantage of remaining on the mosquito cuticle for a short time period. For testing, mosquitoes are anesthetised and a droplet of solution is applied to the dorsal thorax of each mosquito (see Figure 1 and 2) knockdown and mortality post exposure at 24 hours, (or longer depending on the study protocol or active ingredient (AI) tested). Topical testing bioassays can in turn be used to establish dose-response line(s) and determine the lethal dosage (LD) of an insecticide at 50% and 90% mortality (LD_{50} and LD_{90}) allowing assessment of the intrinsic activity of the insecticide against susceptible adult mosquitoes.

Figure 1: Mosquitoes set up for topical testing on damp filter paper within a petri dish taken from https://lite.lstmed.ac.uk/lite-facilities/lite-laboratories/topical-testing, date accessed 26/02/2024.

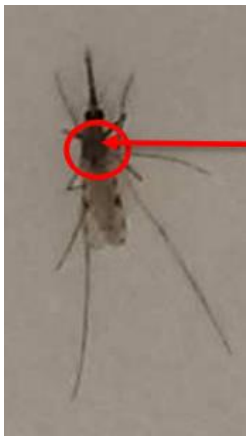

Figure 2: A single female mosquito positioned for topical application onto the dorsal thorax taken from LITSOP056.

Define Accepted Methodologies

Are there existing standard SOPs/Guidelines detailing methodologies?

An SOP has been developed by the Liverpool Insect Testing Establishment (LITE) (Liverpool, UK):

- The performance of Topically Applied Bioassays LITSOP056

An SOP has been developed by I2I:

- Intrinsic Toxicity Testing I2I-SOP-029

Guidelines developed by the World Health Organization (WHO) are:

- Guidelines for testing mosquito adulticides for Indoor Residual Spraying and treatment of mosquito nets. World Health Organization, 2006[1]

- Guidelines for efficacy testing of insecticides for indoor and outdoor ground applied space spray applications, 2009[2]

- Guidelines for efficacy testing of household insecticide products mosquito coils, vaporizer mats, liquid vaporizers, ambient emanators and aerosols, 2009[3]

Are these sufficiently detailed?

The SOPs contain a sufficient level of detail, including thorough step-by-step guidelines on the equipment required, setting up and performing the assay. The WHO provide guidelines, but these are not thorough step-by step instructions and information on the equipment required is limited to the applicator only. Information is included on how to analyse the results in the WHO guidelines that is not detailed in the SOPs.

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

Specific training is required for making up the required insecticide dilutions and carrying out the assay, though this is a standard mosquito bioassay.

Specialised equipment and training with equipment required is as follows:

- Hamilton Gastight Syringe
- Hamilton PB600-1 Repeating Dispenser
- Micro pipettes
- Use of regulated CO_2 supply
- Set up of chill table/ice bucket
- Magnifying lamp
- Soft tip paintbrush

Are there issues with the methods or their interpretation?

The suggestion for equipment in the WHO guidelines is limited to only recommending the type of applicator. The remaining equipment required is not described in detail; for example, the

guidelines state to carry out the testing on a cooled plate with no additional information. The SOPs available in LITE and I2I provide additional details, recommending that topical application is done on a petri dish lined with damp filter under a magnifying glass. It is important to specify these conditions, acetone reacts to plastics such as Petri dishes which is eliminated with the addition of filter paper.

There are variations between the WHO guidelines, I2I SOP and LITE's methods. The LITE SOP suggests testing males as well as females, with the WHO guidelines/I2I SOP suggesting females only. Males are commonly smaller in size which could impact mortality results. The LITE SOP suggests a dosage of 0.2µl in comparison to WHO guidelines/I2I SOP which suggests 0.1µl. Different volumes may also cause variations in mortality due to factors such as solvent toxicity.

Currently there is a cleaning method available for the syringes, however it is not validated. A cleaning investigation is required to ensure that there is no residual insecticide left within the syringe to ensure accurate concentrations of insecticide are being administered and there is no cross-contamination between insecticides.

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

A large number of published studies (identified by PubMed search) report results of topical testing on a range of different insecticides and compounds for multiple species of mosquitoes. Insecticides that have been documented in the published literature include permethrin, deltamethrin, malathion and tenebenal.

Additional compounds that have been documented include: veratrine, aconitine, meso-trione, nitisinone, 4-hydroxyphenylpyruvate dioxygenase, methoxyfenozide, carvone, menthone, and fenchone, piperonyl butoxide (PBO).

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

This method is frequently reported as being used in published literature however we have not identified any formal validation data.

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

Mosquito strain – characterisation of mosquito strains before testing.

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

The endpoints are clearly defined in published SOPs and WHO guidelines.

The WHO guidelines and I2I SOP instruct to record knockdown at 60 minutes and mortality at 24 hours. The LITE SOPs instructs to measure mortality at 60 minutes, 24, 48 and 72 hours. Adaptations are required however depending on the type of active ingredient used where mode of action may differ e.g. testing with chlorfenapyr, a pro-insecticide where mortality measurement beyond 24 hours is required.

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

There are the following supporting SOPs available in LITE:

- LITSOP020-Methods for performing calculations and dilutions. This SOP supports making up the insecticide dilutions required for topical testing.
- LITSOP123- Test preparation, detailing the set-up of equipment required including holding cups.
- LITSOP142-Equipment Cleaning in the LITE Laboratory Area, detailing general cleaning after testing.

There is currently no SOP available for cleaning the topical testing equipment.

Define Current Use Practices

Does everybody use the same SOP?

The level of detail given for methodology was variable between the published studies. In the studies that did report the methods used there were variations in several parameters including the volume of dose administered and the testing age range of mosquitoes. There were also variations in the equipment used e.g. using a paint brush or forceps to move the anaesthetized mosquito as well as the methods of anaesthetization, with some studies knocking the mosquitoes down on ice and others using a CO_2 supply. These inconsistencies between studies suggest that different studies follow different SOPs.

Are there differences of interpretation of the method?

There have been differences in the interpretation of the method, with different studies reporting different methodologies. WHO guidelines and the available SOPs state to use mosquitoes within an age range of 2-5 days and to administer a dose volume of 0.1µl (with the LITE SOP suggesting 0.2µl), however there have been variations in published studies on these two parameters. Additionally, the WHO guidelines and available SOPs state to anaesthetize the mosquitoes using a CO_2 supply, however studies have reported anesthetizing mosquitoes using ice.

Are the results obtained largely consistent between studies?

No published papers have used the same mosquito/insecticide combination to enable this comparison.

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

This is a standardised and robust method which has been used across multiple studies to determine the intrinsic activity of an insecticide and used routinely within industry to screen compound libraries for insecticidal activity. A validation of a cleaning method would allow the applicator used to apply insecticides to be reused whilst ensuring that there will be no contamination between insecticide.

Identify Potential Sources of Variation

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

Operator techniques are a source of variability in this method. For this method the operator is required to anesthetize the mosquitoes and manually move the mosquito to expose the dorsal thorax for application of a droplet of solution using a hand-operated micro applicator. The method requires a lot of manual handling of individual mosquitoes and variations in skill of the operator could impact results. Variations in the length of time that operators take may also cause variations in results. Delays before treatment caused by inefficient handling mean mosquitoes may begin to recover from being anaesthetized causing movement and further handling to expose the dorsal thorax, or further anesthetization may be required. Also, longer periods of time on the ice could cause the specimen to become very cold. These factors are likely to increase mortality.

Possible methods to minimise these are to ensure that adequate practice has been carried out prior to testing to ensure that all operators have a similar skill level.

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

Adaptations may be required to extend the length of time that mortality is recorded, dependent upon the type of AI used where mode of action may differ.

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

A suitable cleaning method is required. There is currently no validated cleaning method for the syringes after use. This is required to ensure there is no contamination between insecticides and that accurate doses are being administered.

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

N/A

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

N/A

References

1. *GUIDELINES FOR TESTING MOSQUITO ADULTICIDES FOR INDOOR RESIDUAL SPRAYING AND TREATMENT OF MOSQUITO NETS CONTROL OF NEGLECTED TROPICAL DISEASES WHO PESTICIDE EVALUATION SCHEME*. (2006).

2. *GUIDELINES FOR EFFICACY TESTING OF INSECTICIDES FOR INDOOR AND OUTDOOR GROUND-APPLIED SPACE SPRAY APPLICATIONS CONTROL OF NEGLECTED TROPICAL DISEASES WHO PESTICIDE EVALUATION SCHEME*. (2009).

3. *GUIDELINES FOR EFFICACY TESTING OF HOUSEHOLD INSECTICIDE PRODUCTS*. (2009).

Innovation to Impact
Pembroke Place
Liverpool L3 5QA UK

contact@innovation2impact.org
+44 151 702 9308

BILL & MELINDA GATES foundation

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