



# SOP: Wind Tunnel

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### Timeline

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1	12/07/23	Katherine Gleave	LSTM, I2I
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### Version Control<sup>1</sup>

<b>Version</b>	<b>Date</b>	<b>Updated by</b>	<b>Description of update(s)</b>
2	04/07/23	Annabel Murphy	Updated: Format and structure under sub-headings and footnotes. Added glossary of terms.

<sup>1</sup> Historical versions of SOPs can be found on the I2I website (<https://innovationtoimpact.org/>)

## Related documents

- I2I Best Practice SOP Library, 30 October 2020 (<https://innovationtoimpact.org/>)

## 1. Purpose

The aim of this study is to determine the lethal concentration (LC) of the insecticide applied as a space spray (aerosol droplets  $15 \pm 5 \mu\text{m}$ ). To enable repeatable and comparable testing, a wind tunnel system has been developed that exposes the test mosquitoes to a specified quantity of test compound under controlled environmental conditions.

## 2. Background

Mosquitoes are aspirated into a fine mesh cage, the test compound is released down a small wind tunnel, penetrating the cage, and exposing the mosquitoes to a set volume of space spray. The most important aspects of the wind tunnel design to recreate are the wind speed, the mesh of the cage, the droplet size and volume of spray material to which the test mosquitoes are exposed.

The Wind tunnel exposes adult mosquitoes to atomized insecticide, to test efficacy and effective dose of space spray formulation Knudsen *et al.*, (2018). The Wind Tunnel consists of a tube (15 cm in internal diameter metal duct) through which a column of air moves at 3 m/s. Air movement is achieved via a fan at the far end of the tube. The spray cage is a cylindrical screen cage (mesh openings  $1.2 \times 1.6 \text{ mm}$  and  $0.28 \text{ mm}$  diameter wire) made to fit the interior measurements of the wind tunnel. The cage is inserted into an opening 1 m from the wind tunnel entrance; a flexible clear plastic sheet is used to close the opening. The technical insecticide in an acetone solution (1 - 0.5 ml total volume) is atomized through a nozzle to produce droplets with a Volume Median Diameter ( $Dv_{0.5}$ ) of  $15 \pm 5 \mu\text{m}$  at the position of the cage. The  $Dv_{0.5}$  is the diameter which divides the volume of the spray into two equal halves. The nozzle is a reservoir twin fluid venturi nozzle. The orifice can be large and the pressure low because the atomization is through volatilization of the acetone carrier. This simple nozzle design allows minute quantities to be sprayed in entirety with no test compound left in a dead

space. This wind tunnel is only suitable for ULV applications and without special nozzle adjustments would not be suitable for low volume (LV) thermal fog formulations.

A total of 50 insecticide susceptible, non-blood-fed, 2–5-day-old female mosquitoes are used at each concentration, with at least five concentrations covering a range of mortality from 10% to 90%. The 50 mosquitoes are separated into duplicate cages. A minimum of three replications from separate cohorts of mosquitoes are required for each test concentration (5 doses x 1 control x 50 mosquitoes and three replicates = 900 mosquitoes). Ideally, three people should be available to work the wind tunnel. One person to insert and remove the spray cage, one person to spray the chemical and the other to transfer the dosed mosquitoes into clean holding cages. Alongside the blank control the testing of a positive control, of a standard insecticide, is encouraged. The studies are performed against well characterized laboratory reared mosquitoes, where possible all three mosquito genera (*Anopheles*, *Aedes* and *Culex*) should be tested. Finding the correct dose range can be difficult it is recommended to start with a lesser number of test mosquitoes and a large series of doses to find the appropriate range.

### 3. Materials and equipment

- Wind tunnel (extraction fan, metal duck tubing, nozzle, and nitrogen canister).
- Aspirator
- Spray cages
- Holding cups, labels, and pens
- Cottons, 10% sugar water
- Scales, dilution glass wear
- Active ingredient and diluent
- Amber vials

- CO<sub>2</sub>, a sealed box with a sealed pipe entry from a CO<sub>2</sub> canister or dry ice.<sup>2</sup>
- Gloves, lab coat, protective glasses
- Bench cover
- Biohazard bags and disposal options

## 4. Procedure

### 4.1. Preparation of test system

- Aspirate 25 mosquitoes into the spray cage labeled with an appropriate annotation for each experimental compound, dose, species, replicate and sample number. For efficiency's sake we let this label follow the mosquitoes from the spray cages to the holding cups, maintaining a precise chain of custody.
- Choose female mosquitoes that are fit, appropriately sized, and able to fly consistently. Parameters for the assessment of mosquito fitness (weight, wing length) must be defined by each test facility based on their knowledge of the species/strains of mosquito they will use for laboratory studies.
- Put spray cages into humidity and temperature-controlled room held at 27 + 2 C temperature and 80 + 10% RH until the test room and materials are prepared.
- An hour before the acclimation period, switch on the humidifier and heater in the wind tunnel room as necessary to 27 ± 2°C and 75 ± 10%.

### 4.2. Dilutions

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<sup>2</sup> A separator in the box provides a uniform distribution of CO<sub>2</sub> and protection from freezing

- Put on lab coat, gloves, and protective glasses. If possible, work in a fume cupboard, turn on air.
- Prepare the solutions and place each dose in a labeled amber glass container with a chemically resistant lid. Some compounds can be sensitive to light (amber glass) and acetone is highly volatile, so the lid reduces evaporative loss of the diluent. Only use glass measuring devices and holding jars because most pesticides adsorb to plastics.

### 4.3. Wind Tunnel Operation

- The wind tunnel is shown in Figure 1, showing the twin fluid gravity fed venturi nozzle, the baffled opening for air straightening, opening downwind from the point of atomization and at the rear an extraction fan.



*Figure 1: Wind Tunnel configuration*

- Transfer mosquitoes to the test room in the spray cages and hold for 1h acclimatization before testing. Acclimation can exceed 1 hour but should not be less than 1 hour. Remove the glucose-soaked cotton wool from the cups 1 hour before exposure.
- Place a clean bench guard on all surfaces, and fix with masking tape.

- Begin the tests with the control cage (applying acetone alone) and then with the lowest dose. Proceed with increasing concentrations, following protocol.
- Place the spray cage in the wind tunnel and close the door.
- The wind tunnel employs a twin fluid nozzle- turn the air on. Ensure that the air is flowing through the nozzle before the chemical is added to the gravity feed reservoir.
- Dispense (0.5-1.0 ml) of the test compound into the reservoir and let the chemical atomize and flow down the wind tunnel.
- Leave the spray cage for 5 seconds to ensure the all chemical has cleared the cage.
- Remove the spray cage and take to a clean bench. Lightly anaesthetize mosquitoes with CO<sub>2</sub>, place in clean holding cups, provide with 10% sugar solution on cotton wool and hold for 24 hours at 27 + 2 °C temperature and 80 + 10% RH.
- Clean the nozzle with a 1 ml spray of acetone between each series of concentrations.

#### **4.4. Post Exposure**

- Decontaminate all insecticide-contaminated material according to the specific active ingredient decontamination instructions.
- Store or dispose of the chemicals as per the instructions from the manufacturer. Dispose of bench guard paper towels and spent pipette tips in a biohazard bag. See Waste Disposal Procedure LITSOP008.

- Record knockdown 60 minutes after exposure. A mosquito is classified as knocked down if it cannot stand (e.g., has one or two legs), lies on its back moving legs and wings but is unable to take off, cannot fly in a coordinated manner or takes off briefly but falls immediately.
- Record mortality 24 hours after exposure. A mosquito is classified as dead if it is immobile, cannot stand or shows no signs of life. If control mortality is <10% the test must be repeated.

## 5. Additional data collection

Record the temperature, humidity, logger ID number, and time acclimation and time of the exposure period started on the form.

## 6. Deviations from standard protocol

## 7. Glossary of terms

LC            Lethal Concentrations

LV            Low volume

m/s          Metres per second

RH            Relative humidity



## 8. References

Knudsen, G. K., Tasin, M., Aak, A., Thöming, G. (2018). A Wind Tunnel for Odor Mediated Insect Behavioural Assays. *Journal of Visualized Experiments*, 30 (141).