
# SOP: Cone bioassay on IRS treated surfaces in experimental huts

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| 1 | 30/10/2020 | Angus Spiers | I2I |
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**Version Control**[1]

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| 2 | June-July 2022 | Alex Wright,<br>Katherine Gleave | Related documents, purpose, materials & equipment, data collection sheet information, health and safety, glossary of terms and references added. |

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

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| | | | sheet information, glossary of terms and references added. |

**Related documents**

- I2I Best Practice SOP Library, 30 October 2020 (https://innovationtoimpact.org/)
    - SOP- Experimental Hut Spraying (I2I-SOP-014)

## 1. Purpose

This SOP details the conduct of cone bioassay on a treated surface in experimental huts. For non-pyrethroid insecticides or chemicals with a mode of action (MoA) that does not target the nervous system, a different exposure time and/or an extended observation period post-exposure may be required. In addition, other outcome measures may be included, such as the effect on fecundity post-exposure. As a guide, when testing pirimiphos-methyl, a 24-hour holding period is generally recommended. When testing products containing clothianidin, a 72hour holding period is recommended. In addition, when testing any product with a combination of AIs including a pyrethroid, pyrethroid-resistant mosquitoes are recommended to be used in the bioassay, which could include wild-caught mosquitoes as opposed to insectary-reared mosquitoes. Therefore, resistant mosquitoes should be characterized in susceptibility tests before being used, using a method such as that in I2I-SOP-016.

Always refer to the relevant study protocol for the required exposure conditions, length of exposure and post-exposure observation periods. In addition, the procedures for conducting cone assays should be closely followed so that tests are performed under the same conditions and standards, and data can be compared between different studies.

## 2. Background

For Insecticide Residual Spraying (IRS), confirmation of the insecticidal action of target dose of an insecticide applied can be carried out by spraying it on wall surfaces made of locally used

building materials and testing the bioefficacy and residual effect in World Health Organisation (WHO) cone tests. Batches of 10 non-blood-fed susceptible female mosquitoes, 2–5 days old, are put in a WHO cones and exposed for a standard exposure time (30 minutes or as stated in the study protocol) on the four walls and ceiling of each hut.

Knockdown (KD) is sub-lethal incapacitation where mosquitoes are unable to maintain normal posture and/or unable to fly immediately after exposure to a chemical although recovery may occur during the holding period. It is measured as proportion relative to the control. For the purposes of this SOP, knocked down (KD 60) mosquitoes are classified as:

- Any mosquito that cannot stand (e.g. has 1 or 2 legs)
- Any mosquito that cannot fly in a coordinated manner
- A mosquito that lies on its back, moving legs and wings but unable to take off
- A mosquito that can stand and take off briefly but falls down immediately

For the purposes of this SOP, dead mosquitoes (24-hour mortality) are classified as:

- Mosquitoes that show no sign of life or movement
- Mosquitoes that cannot stand.

## 3. Materials and equipment

### 3.1. Preparation.

- Gloves
- Labels
- Paper cups
- 10% glucose solution
- Cotton wool

### 3.2. Exposure

- Masking tape or pins
- Cones
- Cotton wool
- Aspirator with HEPA filter of battery operator aspirator
- Stopwatch
- Cool box
- Cup holding racks
- Wet towel
- 10% bleach for cleaning

## 4. Procedure

### 4.1. Preparation of test systems

4.1.1. Prepare the holding room where mosquitoes will be held pre- and post- exposure. Ensure the room is at 27 ± 2 °C and 80± 10% RH. Record temperature and humidity.

4.1.2. Request mosquitoes from the insectary, as described in protocol. The standard for WHO Phase II IRS cone assays is 2-5 days old non-blood-fed susceptible females (e.g. *An. gambiae s.s*. Kisumu or other susceptible species/strain).

4.1.3. As a guide, when testing pirimiphos-methyl, a 24-hour holding period is generally recommended. When testing products containing clothianidin, a 72-hour holding period is recommended. In addition, when testing any product with a combination active ingredient (AI) including a pyrethroid, pyrethroid-resistant mosquitoes are recommended to be used in the bioassay, which could include wild-caught mosquitoes as opposed to insectary-reared mosquitoes. Resistant mosquitoes should be characterized in susceptibility tests before being used.

4.1.4. Label the holding cups for mosquitoes with the study code, mosquito species name, date of exposure, insecticide treatment code target dose, location of cones and initial of technician.

4.1.5. Transfer 10 female mosquitoes into each paper cup. Put the cups in holding racks inside a cool box for transporting to the huts. Add cotton wool soaked with 10% glucose (or other sugar; see SOP Preparation of sugar-soaked cotton wool for feeding adult mosquitoes in holding cages or during transportation between the laboratory and field) solution to the netting on top of the cups. Place a data logger into the box and transport mosquitoes to the huts (see SOP Transportation of mosquitoes)

4.1.6. Keep cups in the cool box until the cones are attached to the hut walls.

**4.2. Exposure of mosquitoes in cones.**

4.2.1. Randomly assign the huts to treatments/trail arms according to Latin Square design. Start with the control hut, ensure you are using the correct designated aspirator for each hut (and/or each AI).

4.2.2. Put on clean nitrile gloves.

4.2.3. Attach all necessary cones to the wall with two-four strips of masking tape, or pins, on the sides. The study protocol will dictate where and how many cones to fix to the walls/ceiling/other surfaces. For each round of testing, cones are positioned in a previously unused position. Study Director uses a randomly generated list of coordinates for the position of each cone for each wall. Fix the cones to the surface in a way that the cone is securely attached, and mosquitoes will not be able to escape under the lip of the bioassay cone. Cotton wool may be needed to plug the holes on the cones.

4.2.4. Once the cones have been attached, gently aspirate 10 mosquitoes into the first cone and plug the cone with a plastic plug.

4.2.5. Start the stopwatch. Record starting time in the cone bioassay form.

4.2.6. Move to the next successive cone and aspirate in the next batch of 10 mosquitoes. Record starting time.

4.2.7. Continue to each cone in turn at two-three minute intervals until all cones contain mosquitoes.

4.2.8. After 30 minutes of exposure gently aspirate the mosquitoes from the first cone into a labelled cup, using a battery-operated aspirator or manual aspirator with HEPA filter.

4.2.9. At every two-three minute intervals remove mosquitoes from the other cones in the same order in which they were introduced, this ensuring that each batch of mosquitoes is exposed for 30 minutes.

4.2.10. Place cups containing mosquitoes back into the cool box. Where possible, use separate racks for each treatment and place into separate, labelled cool boxes to avoid contamination

4.2.11. Put on a new pair of gloves before handling sugar solution and place cotton wool moistened with 10% glucose solution onto the netting covering each of the cups in the holding racks/cool boxes. Place a wet towel on top of the cups to maintain humidity during transportation back to the holding room. Record the temperature and humidity during transit to the holding room using a data logger.

**4.3. Post-exposure period**

4.3.1. Take holding cups containing mosquitoes back to the temperature and humidity-controlled holding room and ensure glucose-soaked cotton wool is still intact. Hold mosquitoes at 27 °C ± 2°C and 80 ± 10% RH.

4.3.2. Score mosquitoes for knock down 60 minutes after the end of the test (i.e. 90 minutes after the start of exposure) using the data form.

4.3.3. Ensure that there are enough technicians to record knock down while cone tests of other experimental huts are ongoing.

4.3.4. Once all bioassays have been completed, ensure that all bioassay cones are removed from the huts and put into a plastic bin with a sealed lid for transport back to the laboratory for cleaning. Use a 10% bleach solution to soak the cones and rinse twice with tap water.

4.3.5. To reduce risk of contamination, use a new pair of gloves when moving from one experimental hut to another, and use a new/ different aspirator when conducting cone bioassays in huts with different treatments and with untreated huts.

4.3.6. At 24 hours after exposure (or as specified in the study protocol), record mortality in mosquitoes. Enter data in the data forms.

4.3.7. If required, mosquitoes should be preserved according to the relevant SOP.

**4.4. Collection and reporting of data.**

4.4.1. Ensure the following data is recorded in the data collection sheets.

- Date of exposure
- Protocol code
- Test item ID
- Test system species and strain
- Age of mosquitoes
- Abdominal status
- Exposure conditions
    - Day or night
    - Time of length of test
- Outcome measures
    - Knock-down time
    - Mortality time
        - Post-exposure holding
            - 60 minutes, 24hours (any extended holding times)
            - Data logger ID number
            - Temperature ©
            - Humidity (%)
            - Time
            - Initials of staff
        - Scoring
            - Test item code
            - Start time of exposure
            - Insecticide code
            - Replicate number

- Number of mosquitoes tested
- 60minute KD
- 24hour mortality (up to 72hours if needed)
- Study director signature and date

## 5. Health and Safety

For GLP-compliant laboratories, the following should be installed in the laboratory and field prior to Semi-Field IRS:

### 5.1. Field materials

- Spill kit for truck
- Mobile emergency shower
- Mobile emergency eye wash

### 5.2. Personal protective equipment (PPE)

- Spray suit coveralls
- Respirator mask (fit-tested for the specific individual spraying)- check AI MSDS for filter requirements
- Gloves
- Goggles and/or full-face Visor
- Over boots

## 6. Glossary of terms

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| AI | Active Ingredient |
| GLP | Good Laboratory Practice |
| HEPA | High Efficiency Particulate Air |

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| I2I | Innovation to Impact |
| IRS | Indoor Residual Spray |
| KCMUCo | Kilimanjaro Christian Medical University College |
| MSDS | Material Safety Data Sheet |
| PPE | Personal Protective Equipment |
| RH | Relative humidity |
| SOP | Standard Operating Procedure |
| WHO | World Health Organisation |
| WHOPES | World Health Organization Pesticide Evaluation Scheme |

## 7. References

WHOPES guidelines- Testing mosquito adulticides for indoor residual spray and treatment of mosquito nets


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