


IRAC Susceptibility Test Methods Series

Version: 1.0 (7th February 2023)

Method No: 036

Details:

Method:	No: 036	 <p><i>Cimex lectularius</i> Photograph courtesy of BASF</p>
Status:	Approved	
Species:	<i>Cimex lectularius</i>	
Species Stage	Adults & Nymphs	
Product Class:	Carbamates (1A), Organophosphates (1B), GABA-gated chloride channel antagonists (2A, 2B) Pyrethroids (3A), nAChR agonists (4), Sodium Channel Blockers (22A, 22B)	
Comments: This method has only been approved and validated for species and ingredients indicated.		

Objectives:

Susceptibility Baseline:

Resistance Monitoring:

Description:

Materials:

- Aerated insect-proof containers
- Forceps for transferring insects
- CO₂ for anesthetizing insects
- Glass beakers/vials for test liquids
- Test insecticides
- Pipette for liquid
- Weighing balance for solid products
- Microsyringe or micropipette for topical application
- Acetone or comparable solvent for topical application
- Dental wick
- Microcentrifuge tubes-
- Petri dishes (60 x 15 mm)
- Filter paper discs (42.5 mm)
- Water bath/heat block
- Sonicator for dissolving insecticides in solvent

Methods:

- Collect enough insects from an infested location or laboratory colony and keep them in an aerated insect-proof container. Ensure that insects are not subjected to excessive stress after collection (temperature, humidity, starvation, etc.). Transfer insects to the laboratory as soon as possible. Record sample details such as collection date and location and other information that may be useful for tracking samples and interpreting susceptibility results later on.

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- After arriving in the lab, allow the insects to recover overnight prior to testing. A water source can be constructed by placing a dental wick in a microcentrifuge tube filled with water. Select uniform individuals/life stages for testing (e.g. nymphs or adults).
- Prepare appropriate test dilutions of technical grade active ingredients (AI) in 100% acetone or comparable solvent (test solvents beforehand to ensure that they are not toxic or harmful to the insects). Ensure that the product is completely dissolved. Mild heat and sonication can be used to aid dissolving AIs in solvent. Select a series of concentrations (4-6 rates) to give a range of mortality (5-100%) for a clear concentration response for the insecticide(s) being evaluated. Rates can be formulated as $\mu\text{g AI}/\mu\text{l}$ to deliver the desired concentration per insect ($\mu\text{g}/\text{insect}$).
- Anesthetize the insects with CO_2 and apply 0.5 μl to 1.0 μl drop on the dorsal thorax of each insect with a microsyringe or micropipette.
- Place 5-10 treated individuals in each Petri dish lined with filter paper. Each concentration should include at least four separate Petri dishes. Prepare at least four additional Petri dishes for solvent controls.
- All dishes should be labeled and arranged randomly and kept at $25 \pm 2^\circ\text{C}$ in an incubator with 60% relative humidity under dark conditions.
- Assess mortality between 3-7 days after treatment (test length may vary depending on the AIs being evaluated). Mortality can also be recorded daily to determine changes in mortality over time. For longer experiments, provide a water source in each dish. Insects unable to right themselves when probed should be considered dead.
- Express results as a percentage mortality. Correct for 'untreated' (control) mortalities using Abbott's formula¹ (Abbott 1925). The corrected mortality data can be subjected to a probit or logit dose response analysis to calculate an LC_{50} or LC_{90} .

² Corrected % mortality = $(\% \text{ alive control} - \% \text{ alive treated}) \times 100\% / (\% \text{ alive control})$

¹ Abbott's formula: $\text{Corrected}\% = \left(1 - \frac{nT}{nC}\right) * 100$

nT = survivors in treatment.

nC = survivors in control.

- If mortality is greater than 20% for the solvent control treatment, the study should be considered as invalid. Mortality at the highest rate must be 100%, and at least three datapoints should have mortality $>0\%$ and $<100\%$.
- For more information on validation, refer to "[IRAC Susceptibility Test Methods Series.](https://www.irc-online.org/test-methods/)"
<https://www.irc-online.org/test-methods/>

Precautions & Notes:

- a) Where glass equipment is used, it must be adequately cleaned with an appropriate organic solvent and/or lab detergents before reuse to prevent cross-contamination.
- b) Different batches of technical grade insecticide may vary in concentration of active ingredient (usually between 85-99% AI). It is recommended to use high purity AI where possible. Purity needs to be taken into account when preparing the test solutions.

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References & Acknowledgements:

1. Abbott, W.S. (1925). A method of computing the effectiveness of an insecticide. *Journal of Economic Entomology*, 18: 265-267.
2. Püntener W., 1981 *Manual for field trials in plant protection* second edition. Agricultural Division, Ciba-Geigy Limited.
3. IRAC Susceptibility Test Methods Series. Insecticide Resistance Action Committee, Test Methods. URL: <https://irac-online.org/teams/methods/documents> (accessed 1/12/23).
4. Methodology was provided by Corteva and Syngenta.