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PROTOCOL FOR LARVICIDE BIOASSAYS [↗](#)

PLOS One

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ABSTRACT

This protocol is designed for the evaluation of the larvicidal activity of perspective insecticides on *Anopheles* larvae. The tests are performed according to the World Health Organization methodology (WHO, 2005).

EXTERNAL LINK

<https://doi.org/10.1371/journal.pone.0227811>

THIS PROTOCOL ACCOMPANIES THE FOLLOWING PUBLICATION

Mesquita RdS, Kyrylchuk A, Grafova I, Kliukovskyi D, Bezudnny A, Rozhenko A, Tadei WP, Leskelä M, Grafov A (2020) Synthesis, molecular docking studies, and larvicidal activity evaluation of new fluorinated neonicotinoids against *Anopheles darlingi* larvae. PLoS ONE 15(2): e0227811. doi: [10.1371/journal.pone.0227811](https://doi.org/10.1371/journal.pone.0227811)

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Preparation of the stock solutions

Dissolve accurately weighed quantity of the test substance in an appropriate volume of dimethylsulfoxide (DMSO) and/or distilled water (depends on the sample solubility) to obtain the concentration of **[M]2000 Parts per Million (PPM) (pg/mL)**

Final volume suggestion: **25 ml** to **50 ml**, depending on the amount of the material available for the assay.

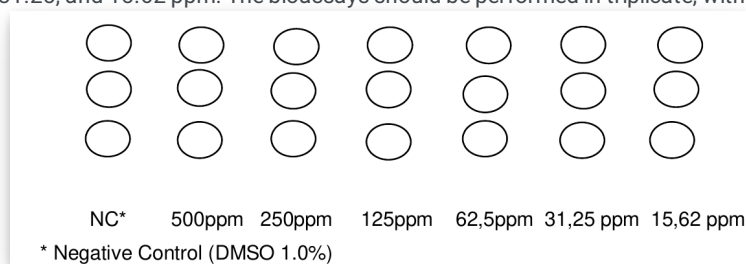
Negative Control: Prepare **[M]1.0 Mass Percent** aqueous solution DMSO (the solution concentration should not exceed **[M]5.0 Mass Percent**).

The solutions should be stored in a refrigerator in dark glass bottles, preferably wrapped in aluminium foil to prevent material degradation.

Selective Bioassays

Purpose of Selective Bioassays: To screen the toxicity of substances exposed to larvae at different reading intervals (24, 48 and 72 h). Those substances showing mortality above 50 % will be considered active.

Concentrations to be evaluated: In the case of substances with unknown larvicidal activity, the assays should start at the maximum concentration of 500 ppm. Other concentrations should be obtained by serial dilution as follows: 500, 250, 125, 62.5, 31.25, and 15.62 ppm. The bioassays should be performed in triplicate, with 3 disposable cups for each concentration:



Each sequence of 3 cups corresponds to a concentration, where each cup should contain:

- **10 ml** of distilled water;
- Ten 3rd instar larvae (be careful when handling the larvae as they are sensitive to sudden movements);
- Some powdered food (porosity of the food suitable for Aedes or Anopheles);
- Test substance in the prescribed concentration (volume to be withdrawn from the stock solution varies with the concentration to be tested);

Negative Control Composition:

- **10 ml** of distilled water or **1.0 Mass Percent** aqueous solution of the DMSO (if the test substance was solubilized in DMSO);
- Ten 3rd instar larvae (be careful when handling the larvae as they are sensitive to sudden movements);
- Some powdered food (porosity of the food suitable for Aedes or Anopheles);

Important: The experiment is performed only once. Thus, verify larval mortality after 24 and 48 hours of exposure by counting the number of larvae alive.

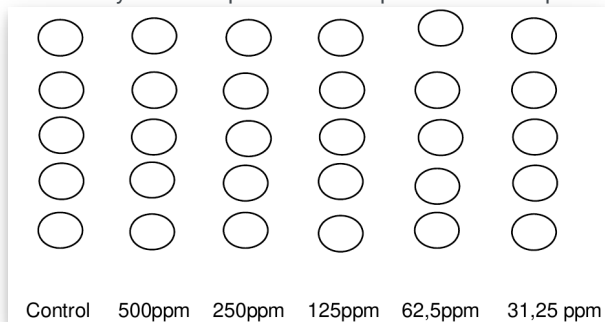
Dose Bioassay

Purpose of Dose Bioassay: The concentrations showing larval mortality above 50% in the selective bioassays will be selected for the dose bioassays and subsequent calculation of LC50 and LC90.

Concentrations to be evaluated: Larvicidal bioassays should contain at least five different concentrations (chosen from the selective bioassays) and the negative control, whereas mortality in the control group should not exceed 10% if repeated assays should be performed.

Assembly of the experiments:

The bioassays must be performed in triplicate with 5 disposable cups for each concentration:



Each sequence of 5 cups corresponds to a concentration, where each cup should contain:

- **50 ml** of distilled water;
- Twenty 3rd instar anopheline larvae (be careful when handling the larvae, as they are sensitive to sudden movements);
- Powdered food (porosity of food suitable for Aedes or Anopheles);
- Concentration to be tested (volume to be withdrawn from the stock solution varies with the concentration to be tested);

Negative Control Composition:

- **10 ml** of distilled water or **1.0 Mass Percent** aqueous solution of the DMSO (if the test substance was solubilized in DMSO);
- Ten 3rd instar larvae (be careful when handling the larvae as they are sensitive to sudden movements);
- Some powdered food (porosity of food suitable for Aedes or Anopheles);

Important: In the dose bioassays, it is necessary to repeat the experiment at three different times. Thus, verify larval mortality after 24 and 48 hours of exposure by counting the number of larvae alive.

Statistical analysis

To obtain the LC50, the mortality data are treated by the Polo plus software (Robertson, 2003) with a 95% confidence interval and $p < 0.05$ value considered statistically significant (Finney, 1971).

In cases when mortality in the control group exceeds 10%, the larval mortality has to be corrected by Abbott's formula (Abbott, 1925).

$$(\text{mortality \% in the assay} - \text{mortality \% in the control}) / (100\% - \text{mortality \% in the control})$$

References

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