

5. Chemical Larvicides

Larvicides are used as a source reduction method in Urban Malaria Control Programme. This method is also effective against vectors of filariasis and dengue. Evaluation is carried out both for WHOPEs passed and new insecticides. New insecticides are evaluated in 3 Phases— I, II and III, while for WHOPEs passed insecticides only Phase II and III trials are recommended.

5.1 Phase I

5.1.1. Duration

Evaluation should be carried out for 3 months.

5.1.2. Objectives

- To evaluate toxicity of the insecticide to mosquito larvae and determination of doses for treatment in Phase II evaluation
- To examine cross-resistance to other insecticides currently in use
- To evaluate toxicity to larvivorous fish *Gambusia affinis* (Gambusia) and *Poecilia reticulata* (Guppy)

5.1.3. Efficacy studies

Toxicity evaluation should be done on laboratory colonised larvae or F₁ larvae of the field collected mosquitoes of *Anopheles stephensi*, *An. culicifacies*, *Culex quinquefasciatus* and *Aedes aegypti* to the candidate insecticide and other insecticides which are currently in use in the programme. Standard WHO procedure has to be followed for bioassays (WHO 1981). Late III or early IV instar larvae in batches of 25 should be exposed to graded concentrations of the insecticide. The highest test concentration should not generally exceed 1 ppm or 1 mg/litre. Details of the test procedure are given in Box 9.

Corrected mortality for different doses should be subjected to probit analysis to determine the doses for mortality of 99.9% (LC_{99.9}). Diagnostic dose is determined by multiplying the upper fiducial limit of LC_{99.9} with a factor of 2 or 3 for routine susceptibility tests. To assess the cross-resistance to other insecticides currently in use in the programme, bioassays should be done using the diagnostic dose of the candidate and other insecticides. Data should be recorded in the format given in Table 38. For Phase II trials the doses for application in field should be in the range of 2 to 10 fold of the calculated diagnostic dose.

To determine toxicity to fish (Gambusia and Guppy) static bioassay (toxicity to fish is evaluated either by dynamic flow or static bioassay method) is to be performed using small 5 litre capacity aquaria/jars with the most effective dose (diagnostic dose as deter-

Box 9: Laboratory bioassays

- For evaluating the larvicidal activity of a compound, mosquito larvae (late III or early IV instar larvae) should be exposed to a range of test concentrations in 250 ml of chlorine free water (boiled and cooled). The highest test concentration should not exceed 1 ppm or 1 mg/litre.
- Test concentration should be prepared by adding 1 ml of standard stock solution to 249 ml of water in 500 ml capacity glass beaker or 300 ml capacity plastic bowl. Stock solution is prepared in ethanol by dissolving technical grade insecticide. A stock solution of 100 ml of ethanol containing 25 mg of insecticide and 1 ml of this stock solution when added to 249 ml of water gives a concentration of 1 ppm in the test solution. Subsequent concentrations of the test solutions should be prepared by serial dilutions of this stock solution. For control 1 ml of ethanol should be added to 249 ml of water instead of insecticide stock solution.
- After adding stock solution to water, stir with glass rod and allow the solution to stand for 20 min. Later 20–25 larvae should be introduced into the test solution. At least 5 replicates for each test concentration should be set along with 2 control replicates.
- After 24 hours, percent mortality is determined scoring the dead and moribund larvae in test replicates. Larvae, which have pupated during the test should be discarded and counted for calculation of mortality. The experiment should be repeated if more than 10% larvae pupated or when more than 20% larval mortality occurs in the controls. Corrected percent mortality should be calculated using Abbott's formula if the larval mortality in control is between 5 and 20%.

Table 38. Insecticide susceptibility test

Village/Locality Sub-centre/ward.....PHCCity/Town.....District.....
 Insecticide & Dose..... Date of test..... Test species.....

Replicate No.*	No. exposed**	No. dead + Moribund after 24 h	% Mortality	Corrected % mortality#
Replicate 1				
Replicate 2				
Replicate 3				
Replicate 4				
Control 1				
Control 2				

* : Separate row for each replicate; **: 25 larvae/replicate; #: After Abbott's formula when mortality in control replicates is between 5 and 20% (<5% no correction needed and > 20% test to be repeated).

Table 39. Toxicity to fish

Species	Replicate No.	No. exposed	No. killed	% Mortality	Corrected % mortality
1.					
2.					
3.					

mined above). Fish should be exposed for 48 h and mortality scored. During the test, food is to be provided. A total of 3 to 5 test replicates (10–15 fishes/replicate) should be used and at least 2 control replicates without insecticide should be included in the assay. Data should be recorded in the format given in Table 39.

5.2. Phase II

5.2.1. Duration

Evaluation should be carried out for 3 months.

5.2.2. Objectives

- To evaluate the candidate larvicide under different natural habitats or in simulated habitats
- To assess the persistence of the larvicide in different breeding habitats of the target vector species
- To determine the effective dose and frequency of application for Phase III trial

5.2.3. Efficacy in field

Natural breeding habitats of the target species have to be selected for the evaluation. For *Anopheles* spp. (*An. stephensi*, *An. culicifacies*) cement tanks, drums, pits, pools, water fountains and disused wells; for *Cx. quinquefasciatus* stagnant drains, pits, pools and disused wells and for *Aedes* spp. tanks, drums, discarded tyres, peri-domestic water storage containers, coolers and water fountains are best suited. Selected habitats should have a minimum density of 5 to 10 larvae per dip. A minimum of 3 replicates should be used for each type of habitat and dose. Each habitat should have at least 2 controls. Temperature, pH and water quality (polluted or clean) should be recorded.

Determine pre-treatment larval population density by dipping method using a standard dipper (300 ml capacity with 9 cm diameter) for pits, ponds, tanks, drains, drums, etc., and bucket well net (22.5 cm top diameter, 15.5 cm bottom diameter and 22.5 cm height) for wells. Number of samples to be taken from each habitat should be decided on the basis of type and size of the habitat. For small habitats such as drums, pits, pools, tanks and wells, 3 to 5 dips per site, while for stagnant drain dips at a distance of 5 m

are recommended. Larvicide formulation should be applied to the breeding habitats through a Knapsack sprayer/hand compression sprayer which should be calibrated prior to use and the rate of application be expressed per unit area.

The following formula is used to determine the application rate:

$$\text{Rate of application (ml/m}^2\text{)} = \frac{\text{Flow rate (ml/min)}}{\text{Width of swath (m) x walking speed (m/min)}}$$

The required concentration of larvicide suspension is calculated as follows:

$$\text{Concentration of larvicide (Emulsion/Suspension \%)} = \frac{\text{Dosage to be applied (g/m}^2\text{)}}{\text{Application rate (ml/m}^2\text{)}} \times 100$$

Larval sampling of treated and control habitats are made a day before the application of the insecticide for assessing the pre-treatment densities. Sampling after application is made at 24, 48 and 72 h and later at weekly intervals (minimum for 6–8 weeks) using the above mentioned methods. Larvae collected from the habitats should be categorised into I/II and III/IV instars and pupae and recorded in the format given in Table 40. The larval density per dip is calculated by dividing the total number of larvae collected by the number of dips taken. Natural predators if any, should be scored.

Percent reduction in III/IV instars larvae and pupae should be calculated using the Mulla's formula.

$$\% \text{ Reduction} = 100 - \frac{C_1 \times T_2}{C_2 \times T_1} \times 100$$

Where,

C_1 = Pre-treatment immature density in control sites

C_2 = Post-treatment immature density in control sites

T_1 = Pre-treatment immature density in treated sites

T_2 = Post-treatment immature density in treated sites

Persistence of the larvicide in different breeding habitats of the target species is determined from the post-treatment density of larvae and pupae in treated and control sites as compared to the pre-treatment density. Achievement of $\geq 80\%$ reduction in the treated habitats is considered as an effective dose for field application. Using this norm efficacious persistence period in the field can be calculated.

Table 40. Pre- and post-treatment densities of immature stages

Species.....
 Locality..... Habitat..... Location.....Surface area.....Depth of water.....
 Type of vegetation..... Application Rate..... Application Date.....
 Time of application..... Application method and comments.....

Parameters	Pre-treatment (Day 0)	Post-treatment			
		24 h	48 h	72 h	W-1 W-2 W-3 W-4
Air temperature					
Water temperature					
pH					
Polluted or non-polluted					
Density/3dips* (Total)					
Larvae (I+II)					
Larvae (III+IV)					
Pupae					
Dead adults on surface					
Non-target organisms					
Alive or dead (A or D)					
*Dipper size: Diameter 9 cm with 300 ml capacity.					
Remarks:.....					

5.2.4. Simulated field trials

These trials are conducted for the mosquito species breeding in domestic and peri-domestic habitats in clean water. Trials should be carried out in containers (drums, tanks, etc.). For *Cx. quinquefasciatus* field trials are not undertaken in simulated condition. For anophelines, cement tanks, each having a capacity of 100 litres filled with 40 to 50 litres of potable water with different concentrations of insecticides should be used. In each tank 1000 to 2000 first instar larvae of the target species should be released at weekly intervals for a period of 4 weeks.

Trials for *Ae. aegypti* should be carried out in 10 to 20 litre capacity drums with 5–10 litres of potable water. 100 to 200 larvae of *Aedes* should be introduced into the treated drums at weekly intervals for a period of 4 weeks. The tanks and drums should be covered with specially designed traps (dome shaped) to score adult emergence and prevent oviposition by other mosquito species/insects.

Water level in the tanks/drums should be maintained and finely ground larval food (yeast powder and dog biscuit mixture in a ratio of 60:40), should be provided daily until the completion of the experiment. Pre-treatment immature density should be determined by taking 3–5 dips covering the entire surface area and instar-wise data should be recorded. The live immature stages should be released back into the respective tanks/drums.

The larvicide, at three selected dosages within the recommended range of doses of application should be applied through a knapsack/ compression sprayer (delivery nozzles should be appropriate to the type of formulation and discharge rate should be determined to deliver the required dose). Each dose should be applied to a minimum of 3 tanks/drums and control replicate should be left untreated.

Larval sampling of treated and control habitats are made at 24, 48, 72 h and later at weekly intervals for 6–8 weeks using dipping method. Initial and long-term efficacy should be assessed on the basis of the observed larval and pupal density. Data should be entered in the given proforma (see section 5.2.3).

Efficacy and residual activity of the larvicide are determined from the post-treatment counts of larvae and pupae in treated and control replicates as compared to the pre-treatment counts. The criteria for determining the level of effectiveness of a candidate larvicide should be $\geq 80\%$ reduction in the pre-treatment counts (% reduction is calculated using the Mulla's formula).

5.3. Phase III

5.3.1. Duration

Evaluation should be carried out for 6 months.

5.3.2. Objectives

- To evaluate efficacy against larvae in a locality
- To assess the persistence of the larvicide
- To determine the operational dose and its frequency of use

5.3.3. Study area

A locality of one square kilometre of urban area should be selected for treatment and a locality of similar type and size for control. All the types of breeding habitats should be surveyed to ascertain the breeding of target species in order to decide the suitability of the locality for the trial. A selected locality should have a minimum of 25 percent larval habitat positivity and 5–10 larvae per dip per habitat.

5.3.4. Evaluation of larvicide

The dose and frequency of application determined in Phase II trial will be employed for Phase III trials. In order to achieve uniform distribution of the larvicide, calibration of the spray pump is essential. The larvicide application initially should cover all the habitats and subsequent applications should be undertaken either at weekly or fortnightly intervals depending on the persistence determined for the larvicide for each type of habitat in Phase II trial.

Estimation of larval density should be done at weekly intervals from different habitats on the basis of a fixed number of dips. Standard dipper should be used. Sampling should be made at a distance of 5 metre for larger habitats and 1 metre for smaller habitats. Care should be taken to cover all the sides. Data should be recorded in the format given in section 5.2.3. Thus the impact of larvicide application is monitored by sampling and calculation of efficacy of the larvicide using Mulla's formula.

In addition to estimation of larval density, adult mosquito density in the localities should be monitored fortnightly by total catch method (1.1.1.5g3). At least 8 structures randomly selected should be used for the estimation of density per structure in treated and control areas. This will provide information on the trend in the reduction of target mosquito species.

