

NAME \_\_\_\_\_

PERIOD \_\_\_\_\_

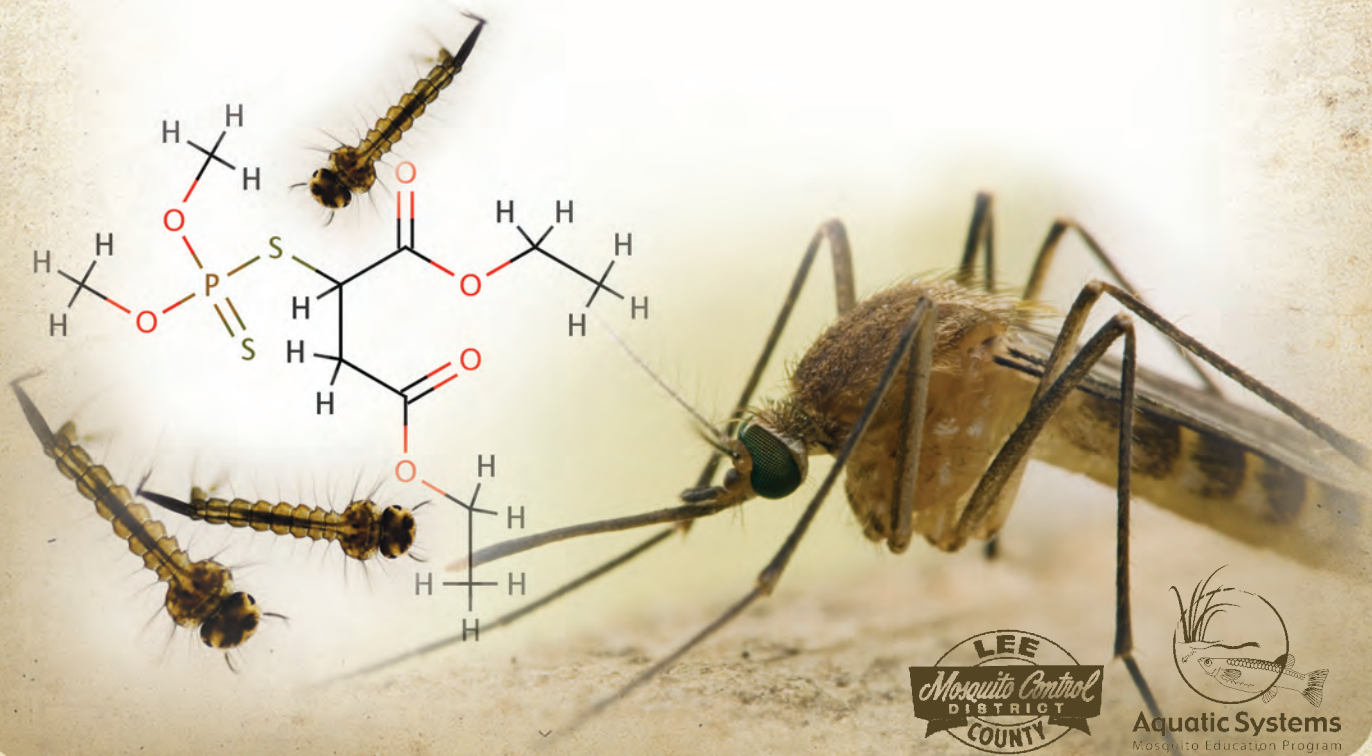
DATE \_\_\_\_\_

### Instructions for Determining the Susceptibility or Resistance of *Culex* Mosquito Larvae to Malathion

In order to detect the emergence of an insecticide-resistant strain of mosquito, it is necessary to establish base-line data for the population, either before the wide use of insecticides or with specimens from an untreated area. Where regular larvicide operations are undertaken to control mosquitoes, the normal susceptibility levels of the larvae should be determined as early as possible. To this end, several tests (a minimum of eight) should be performed at various localities to compensate for normal biological variation. Tests should then be continued at regular intervals to determine any significant reduction in susceptibility.

The history of insecticide use in the area, for both mosquito control and major agricultural uses, should be noted.

It is stressed that this test is not designed to indicate the relative effectiveness of the insecticides in the field.



Aquatic Systems  
Mosquito Education Program



# Mosquito Susceptibility

## Bioassay

### VOCABULARY

- 1. Abbott's formula** the formula used for making corrections in a bioassay when the control mortality is between 5% and 20%.
- 2. adulticide** (v) To kill adult mosquitoes; (n) a pesticide that kills adult mosquitoes.
- 3. bioassay** A test to determine the toxicity of a substance to an organism.
- 4. Diptera** The order of insects that includes flies, gnats, and mosquitoes.
- 5. entomology** The study of insects.
- 6. instar** A developmental phase of larval mosquitoes, four instars in all.
- 7. larvicide** (v) To kill mosquitoes in the larval stage; (n) a pesticide that kills larval mosquitoes.
- 8. LD<sub>50</sub>** lethal dose – 50%, the dose of an active ingredient which is expected to cause death in 50% of the test animals treated.
- 9. metamorphosis** The four stages, egg, larva, pupa and adult, in the mosquito life cycle.
- 10. molt** Shedding of the exoskeleton as part of the growth process.
- 11 moribund** dying.
- 12 organochlorines** A class of insecticides which contain carbon, chlorine, and hydrogen. Some of the more persistent insecticides are organochlorines such as DDT and Chlordane.
- 12. organophosphates** A class of insecticides which contain phosphorus. They are initially toxic but degrade quickly when exposed to air and light. Malathion and Abate are organophosphates.
- 13. resistance** the level of immunity an organism has for a particular substance.
- 14. susceptible** easily affected by diseases or poisons; unresistant.
- 15. vector** An insect capable of transmitting a disease.





# Mosquito Susceptibility Bioassay

## LAB PREPARATION

### Materials

- distilled water - approximately 2 liters
- (9) 12 oz. Styrofoam cups
- (9) 5 oz. cups
- (9) wooden stirrers
- (2) eyedroppers
- (1) 250 ml graduated cylinder
- (1) 50 ml graduated cylinder
- (1) thermometer
- Malathion at eight different concentrations \*
- diluent (methyl alcohol)
- (1) graduated eyedropper for each stock bottle of Malathion diluent
- 100 *Culex quinquefasciatus* mosquito larvae
- lab goggles - one per student
- latex gloves - one pair per group

\* The Malathion solution is in methyl alcohol at eight different concentrations. The concentrations indicated on the labels are those when 1 ml of solution is added to 249 ml of water.



Concentration #	Parts per Billion (PPB)
1	
2	
3	
4	

Concentration #	Parts per Billion (PPB)
5	
6	
7	
8	



# Mosquito Susceptibility

## Bioassay



### LAB ACTIVITY

#### Procedure

1. Label each Styrofoam cup with the pesticide name and concentration, label the last cup "Control".
2. Add 225 ml of distilled water to each of the Styrofoam cups. Be accurate!
3. Using a graduated eyedropper, add exactly 1 ml of the appropriate pesticide solution to each of the Styrofoam cups. Do not allow the dropper to touch the water in the cup. With a wooden stirrer, vigorously stir the solution for 30 seconds. Wear gloves for this step.
4. Using a graduated eyedropper, add 1 ml of diluent (methyl alcohol) to the Styrofoam cup marked "Control".
5. Measure and record the temperature of the solution in the control cup. Wash and dry the thermometer thoroughly with tap water.
6. Add 24 ml of distilled water to each 3 oz. cup.
7. Using the eyedropper, add 10 mosquito larvae to each paper cup. Be careful not to count molts as live larvae. Try to avoid adding excess water to the cup.
8. Add one 3 oz. cup of 10 mosquito larvae to each Styrofoam cup treated with pesticide.
9. Let the Styrofoam cups sit for 24 hours and read the results.

#### Results

1. Complete the Report Form on the next page as accurately as possible. Your group will fill in the column for "Replicate 1". Other groups data is used for the other replicates.
2. Count the number of dead and moribund (dying) larvae together. Dead larvae are those that cannot be induced to move when they are probed. Moribund larvae are those incapable of rising to the surface or of showing the characteristic diving reaction when the water is disturbed.
3. If the control mortality is between 5% and 20%, the percentage mortalities should be corrected by Abbott's formula.
$$\frac{\% \text{ test mortality} - \% \text{ control mortality}}{100 - \% \text{ control mortality}} \times 100$$
4. Plot, on the page provided, the percent mortality and the pesticide concentrations. Determine the LD<sub>50</sub>, the dosage causing 50% mortality. Record your observations up to 90% mortality.

## Report Form WHO Test for Insecticide-Resistance in Mosquito Larvae

Date: \_\_\_\_\_

Insecticide: DDT/ dieldrin / BHC / other <sup>1</sup> \_\_\_\_\_ Species \_\_\_\_\_

1. Investigator: \_\_\_\_\_

2. Country: \_\_\_\_\_ 3. Province: \_\_\_\_\_ 4. Locality: \_\_\_\_\_

5. History of insecticide treatment (including agriculture): \_\_\_\_\_

6. Condition of larvae: instar \_\_\_\_\_ reared / collected / other <sup>1</sup> \_\_\_\_\_

7. Results of test (abbreviations: "M" - moribund; "D" - dead)

Tests	Replicate 1		Replicate 2		Replicate 3		Replicate 4		Totals (for comparable test only)
	M & D Total (p.p.b.)	Mort. (%) corr. <sup>2</sup>	M & D Total	Mort. (%) corr. <sup>2</sup>	M & D Total	Mort. (%) corr. <sup>2</sup>	M & D Total	Mort. (%) corr. <sup>2</sup>	
Date of Test									
Temperature during Test									
Insecticide Concentration									
Control 1									

<sup>1</sup> Cross out what does not apply <sup>2</sup> Correct by applying Abbott's formula if control mortality is between 5% and 20% (see instructions)

Remarks: \_\_\_\_\_

Signature of Investigator: \_\_\_\_\_



# Mosquito Susceptibility

## Bioassay

Names

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Period  
Date

### SUSCEPTIBILITY GRAPH

