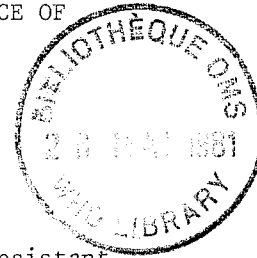


WORLD HEALTH ORGANIZATION
ORGANISATION MONDIALE DE LA SANTÉ

INSTRUCTIONS FOR DETERMINING THE SUSCEPTIBILITY OR RESISTANCE OF
MOSQUITO LARVAE TO INSECTICIDES¹



INTRODUCTION

1.1 Purpose and limitations of the test

The purpose of the susceptibility test is to detect the presence of resistant individuals in a mosquito larval population as soon as possible so that alternative control plans can be made in time to deal with the situation when the insecticide in question is no longer having the desired effect.

When originally investigating larval population two approaches are necessary:

(i) The establishment of the base-line susceptibility of a normal population. By "normal" is meant a population never subjected to insecticidal pressure and in which resistant individuals are rare. Exposure of such a population to serial concentrations of insecticide should yield a straight-line relationship between the logarithm of the concentration and probit mortalities. From such data it is possible to predict by extrapolation that concentration which will normally kill all the individuals of a susceptible population. Double this concentration is taken to be the discriminating or diagnostic concentration or that concentration normally producing a complete kill of populations lacking resistant genes.

(ii) The frequent exposure of a population under insecticide selection pressure to this diagnostic concentration should serve to detect the appearance of abnormally tolerant individuals and to monitor changes in their frequency.

1.2 Establishing the base-line

Batches of mosquito larvae are exposed to different concentrations of insecticides and the mortality at each level is determined. It is suggested that a preliminary test be made on a wide range of concentrations using the standard exposure of 24 hours. This will indicate the general level of susceptibility; further tests should then be made with at least 4 concentrations, some of which will give partial mortality (i.e. at least one of them should give 100% mortality and two from 5 to 50% mortality). Four replicate tests at each concentration should then be made and from the results a log-probit regression line constructed on the logarithmic-probability paper provided. The line should be straight if the population is homogeneous and, if so, the concentration expected to produce 99.9% mortality can be extrapolated from it.

1.3 Subsequent routine checks by diagnostic concentrations

In routine monitoring for resistance, it is not necessary to employ the full range of concentrations used to establish the baseline of susceptibility. Only the diagnostic concentration should be used. Tentative diagnostic concentrations for mosquito larvae are shown in Table 1.

¹ These instructions supersede WHO/VBC/75.583

These data were obtained with IV instar larvae at 23°C. It is possible that they will not correspond to those for wild-caught larvae under field conditions and are only given for guidance; they should be checked under various field conditions before use.

Tests at the diagnostic concentration should be repeated periodically with at least four replicates of 25 larvae. The occurrence of survivors at this concentration will rarely be due to normal variability provided that the physiological age and condition of the larvae and the experimental conditions are the same as when establishing the base-line data. If survivors are repeatedly found, resistance is confirmed.

2. COMPOSITION OF THE KIT

EQUIPMENT AND INSECTICIDE SHOULD BE ORDERED SEPARATELY. FOR INSECTICIDE THE ORDER SHOULD SPECIFY THE INSECTICIDE AND THE NUMBER OF EACH STANDARD SOLUTION.

2.1 Equipment

- (a) 4 1-ml pipettes for insecticides and 1 for ethanol and 5 rubber suction bulbs.
- (b) 3 droppers with rubber suction bulbs.
- (c) The following materials for use in making a strainer: 2 wire loops, 1 piece of nylon netting (30cm²) and 1 tube of cement. It is suggested that 2 pieces of netting be cut and cemented to opposite sides of the large end of the wire loop. More cement should then be applied around the outside of the loops to join the 2 pieces of netting. When dry, the netting may be trimmed with scissors. The kit contains sufficient netting for replacement purposes.
- (d) Instruction sheets, 20 report forms and 3 log-probability papers for plotting regression lines.¹

The user is expected to provide his/her own collecting and test vessels. Disposable plastic cups which hold 250 ml to a rim, may be used when available.

2.2 Insecticides

Standard solutions:

Malathion	781.25 mg/l, ²	156.25 mg/l,	31.25 mg/l,	6.25 mg/l
Temephos	156.25 mg/l,	31.25 mg/l,	6.25 mg/l,	1.25 mg/l
Bromophos	31.25 mg/l,	6.25 mg/l,	1.25 mg/l,	0.25 mg/l
Fenitrothion	"	"	"	"
Fenthion	"	"	"	"
Chlorpyrifos	6.25 mg/l,	1.25 mg/l,	0.25 mg/l,	0.05 mg/l

All these alcoholic solutions are supplied in 50 ml bottles. One 50 ml bottle of alcohol for control is supplied for each order of 4 standard solutions or less.

Caution: alcohol used for solutions and control has been denatured by addition of 2% butanone.

¹ Additional report forms and log-probability papers can be ordered separately.

² mg/l unit in accordance with the International system of Units = ppm.

3. PROCEDURE

3.1 Base-line

(a) For a complete test with one insecticide, sufficient larvae should be collected from the field in order that about 300 individuals of the same species may be selected; they should be in their third or early fourth instar and should be retained in the water in which they were collected until selected for testing. Any larvae showing abnormalities, for example a fuzzy appearance due to the presence of parasites on the body surface, should be discarded. Lots of 20-25 larvae are distributed in each of 12 small beakers, each containing 25 ml of water. Their transfer is effected either by means of the strainer, or by means of a dropper; during the process they should be rinsed lightly in clean water.

(b) Into each of 12 glass vessels, approximately 7.5 - 10 cm in diameter, (jars, bowls or 500 ml beakers)¹ place 225 ml of water. The vessels should be such that the depth of water is between 2.5 and 7.5 cm. Distilled water, rain water or tap water² may be used, or even water obtained from a well or stream, but it should be as free as possible from chlorine or organic contaminants. It should be noted that distilled water obtained commercially may contain traces of poisonous heavy metals and this will give high mortalities in the controls. Certain species such as salt-marsh or tree-hole mosquitos may suffer upon transfer to relatively pure water, an effect that will also be reflected in high control mortalities; in this case water from the breeding site should be used provided that it is free from insecticides and filtered to exclude most of the organic matter. The average temperature of the water should be recorded and should be approximately 25°C; it must not be below 20°C or above 30°C.

(c) Prepare the test concentrations by pipetting 1 ml of the appropriate standard insecticide solution just above the surface of the water in each of the glass vessels and stirring vigorously for 30 seconds with a glass rod. In preparing a series of concentrations, the most diluted should be prepared first. There should be 2 replicates at each concentration, and 2 control replicates. The 2 controls should be prepared by the addition of 1 ml of the alcohol to the water in each container. To obtain intermediate concentrations, pipette 0.5 ml of any standard solution instead of 1 ml.

(d) Within 15-30 minutes of the preparation of the test concentrations, add the mosquito larvae to them by tipping the contents of the small beakers into the vessels.

(e) After a period of 24 hours, make mortality counts. In recording the percentage mortalities for each concentration, the moribund and dead larvae in both replicates should be combined. Dead larvae are those that cannot be induced to move when they are probed with a needle in the siphon or the cervical region. Moribund larvae are those incapable of rising to the surface (within a reasonable period of time) or of showing the characteristic diving reaction when the water is disturbed; they may also show discolouration, unnatural positions, tremors, incoordination or rigour.

¹ Containers other than glass, plastic or enamel can interfere with the action of the pesticide.

² Hard or chlorinated tap water should not be used with organophosphorus insecticides.

(f) Discard the larvae that have pupated during the test. If more than 10% of the control larvae pupate in the course of the experiment, the test should be discarded. Tests with a control mortality of 20% or more are unsatisfactory and should be repeated.

(g) When 4 replicates have been performed with the same population of mosquito larvae, adequate data should be available for constructing a base-line susceptibility. The results should be recorded on the forms provided. Completed forms should be distributed in accordance with the instructions on page 5.

3.2 Diagnostic concentrations

For routine checks, the same procedure is applicable except that larvae are exposed to only one concentration, established from the base-line data or given for guidance in Table 1.

4. GENERAL REMARKS

(a) The accuracy of the concentrations provided will be affected if the alcohol is allowed to evaporate from the standard solutions. The bottles should therefore be tightly stoppered after use. The contents should no longer be used when they have decreased below 5 ml; fresh standard solutions should then be prepared from the stock solutions.

(b) Test vessels should be carefully cleaned after use to remove traces of insecticide. They should be thoroughly rinsed, scrubbed with detergent and water (or cleaned with potassium dichromate and sulfuric acid), and rinsed again. Pipettes should be thoroughly cleaned with acetone or alcohol.

5. RESULTS

(a) To construct the dosage-mortality regression line the results obtained should be plotted on the logarithmic-probability paper provided. The regression line may be fitted by eye and the concentrations expected to kill various percentages can be read from it. The concentration to kill 50% is known as LC₅₀; that for 95% kill as LC₉₅, etc. The curve can be extended to estimate the LC_{99.9} (though it must be realized that this is very approximate). For accurate methods of computing various LC estimates, see Swaroop.¹

(b) If the control mortality is between 5% and 20%, the percentage mortalities should be corrected by Abbott's formula:

$$\frac{\% \text{ test mortality} - \% \text{ control mortality}}{100 - \% \text{ control mortality}} \times 100$$

(c) The accuracy of the interpretation of results depends on the reliability of the data obtained. Utilizing the maximum number of specimens per test (25) decreases the effect of individual differences in response. Regression lines based on similar numbers of specimens offer greater reliability.

6. INTERPRETATION OF RESULTS

See Annex 1: "Criteria and Meanings of Tests for Determining the Susceptibility or Resistance of Insects to Insecticides."²

¹ Swaroop, S. (1966) Statistical methods in malaria eradication, Geneva, World Health Organization (Monograph Series, No. 51).

² Report of the WHO Expert Committee on Resistance of Vectors and Reservoirs to Pesticides (1976). Wld Hlth Org. techn. Rep. Ser., 585.

7. DISTRIBUTION OF REPORTS

It is of considerable importance that WHO should receive copies of results obtained from the use of this test kit. It is therefore requested that copies of all reports be sent to the following addresses:

For anopheline species

1. World Health Organization, Division of Malaria Eradication, 1211 Geneva, Switzerland
2. The WHO Regional Office;¹ and
3. Project Headquarters.

The fourth copy should be retained by the investigator.

For non-anopheline species

1. World Health Organization, Vector Biology and Control, 1211 Geneva, Switzerland; and
2. The appropriate WHO Regional Office.¹

The third and fourth copies should be retained by the investigator.

¹ Addresses of WHO regional offices are as follows:

World Health Organization, Regional Office for Africa, P.O. Box No. 6, Brazzaville, Congo.

World Health Organization, Regional Office for the Eastern Mediterranean, P.O. Box 1517, Alexandria, Egypt.

World Health Organization, Regional Office for South-East Asia, World Health House, Indraprastha Estate, Mahatma Gandhi Road, New Delhi - 110002, India.

World Health Organization, Regional Office for the Americas/Pan American Sanitary Bureau, 525, 23rd Street, N. W., Washington, D. C., 20037, United States of America.

World Health Organization, Regional Office for Europe, 8 Scherfigsvej, DK-2100 Copenhagen Ø, Denmark.

World Health Organization, Regional Office for the Western Pacific, P.O. Box 2932, 12115 Manila, Philippines.

TABLE 1. TENTATIVE DIAGNOSTIC DOSAGES FOR LARVAL MOSQUITOS
(mg/litre)

	Anophelines	<u>Culex quinquefasciatus</u>	<u>Aedes aegypti</u>
Malathion	3.125	1.00	1.0
Fenitrothion	0.125	0.125	0.06
Fenthion	0.05	0.05	0.05
Temephos	0.25	0.02	0.02
Chlorpyrifos	0.025	0.01	0.01

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