

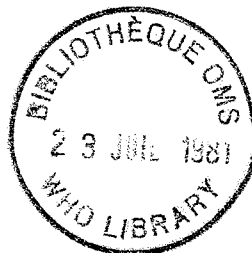


INSTRUCTIONS FOR DETERMINING THE SUSCEPTIBILITY OR RESISTANCE OF
ADULT MOSQUITOS TO ORGANOCHLORINE, ORGANOPHOSPHATE AND
CARBAMATE INSECTICIDES - DIAGNOSTIC TEST

Corrigendum

On page 4 under GENERAL REMARKS (a), the second paragraph should read:

"For organophosphates and carbamates, impregnated papers should not be left for more than 3 weeks in exposure tubes and the same precautions for wrapping and storing should be allowed."



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INDEXED

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INTRODUCTION

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

When originally investigating an insect 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 or serial time exposures to a single insecticide concentration should yield a straight-line relationship between the logarithm of the concentration or time and probit mortalities. From such data it is possible to predict by extrapolation that concentration or time will normally kill all the individuals of a susceptible population. This is the discriminating or diagnostic concentration or time.
- (ii) The frequent exposure of a population under insecticide selection pressure to this diagnostic concentration or time should serve to detect the appearance of abnormally tolerant individuals and to monitor changes in their frequency.

Established diagnostic concentration/exposure times

Tentative diagnostic concentration/exposure times for adult mosquitos are shown in 1. These data were obtained with unfed female mosquitos at 27°C. It is likely that will also apply to blood-fed specimens, though not for those entering hibernation. Since temperature affects the mortality, records of temperature should be made during the test. In general, higher temperature induces higher mortality and lower temperature results in higher survival. Therefore, the temperature/toxicity correlation should be taken into account when performing successive tests under different temperature conditions.

Tentative diagnostic concentration/exposure times are given for guidance and should be used under field conditions before final adoption.

Use of diagnostic concentration/exposure times

Tests should be made periodically with at least 75 mosquitos and preferably with 100. It is recognized, however, that it may be difficult to obtain sufficient numbers to satisfy statistical requirements, especially when the insecticide is effectively reducing numbers, or when breeding is reduced.

A warning of possible incidence of resistance is given when survivors regularly appear in a population with a correctly selected diagnostic exposure. Occasional survivors in such checks may be due to normal variation. But the regular occurrence of survivors in three successive checks constitutes a warning signal calling for further investigation.

¹ Report of the WHO Expert Committee on Resistance of Vectors and Reservoirs of Disease to Insecticides (1976) Wld Hlth Org. techn. Rep. Ser., 585.

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1.3 Condition of mosquitos

Although there is seldom a large difference in susceptibility between the sexes, female mosquitos (preferably blood-fed) should be used exclusively in field tests. This is because they survive better and show lower control mortalities.

If mosquitos are scarce, it is permissible to use a mixture of fed and unfed females provided the proportion of each is recorded. Mosquitos may be collected from sprayed and unsprayed premises in the zone, but their source should be reported on the form provided. In instances where it is not possible to collect a sufficient number of adult mosquitos for testing, specimens may sometimes be provided by collecting the immature stages and rearing them to adults. In some circumstances females without a blood meal may be used exclusively, e.g. those recently emerged from a collection of larvae.

1.4 Conditions of test

The experiments should be carried out indoors, if possible, in buildings free from insecticidal contamination and extremes of temperature, humidity, illumination and wind. Where possible, subsequent comparison tests should be made under similar conditions of temperature and humidity. Transport of insects to a base laboratory often results in mortality from causes other than the insecticide; this will show up as high mortality in the controls.

2. COMPOSITION OF THE TEST KIT

EQUIPMENT AND/OR INSECTICIDE MAY BE ORDERED SEPARATELY. FOR INSECTICIDE THE ORDER SHOULD SPECIFY BOTH THE INSECTICIDE AND THE NUMBER OF SETS OF BOXES FOR OC AND OF BOXES FOR OP AND CARBAMATE PAPERS

2.1 Equipment

(a) 8 plastic tubes, 125 mm in length and 44 mm in diameter: 2 of which (with red dot) are used to expose the mosquitos to the insecticides, 2 (with green dot) are used for the control exposure without insecticide and 4 (with green dot) are used as holding tubes for the pre-test sorting and post-exposure observation. Each tube is fitted at one end with 16-mesh screen.

(b) 4 slide units, each with a screw-cap on either side and provided with a 20 mm filling hole.

(c) 16 sheets of clean paper (12 x 15 cm) for lining the holding tubes.

(d) 8 spring wire clips to hold the papers in position against the wall of the tubes. The 6 steel clips should be used only for the holding tubes and the control exposure tubes; the 2 copper clips should be used for the insecticide exposure tubes.

(e) 2 glass aspirator tubes 12 mm internal diameter, together with 60 cm of tubing and mouthpieces.

(f) 1 roll of self-adhesive plastic tape.

(g) Instruction sheets and 20 report forms,¹ plus 3 sheets of log probability paper for plotting regression lines using variable times with one concentration.

¹ Additional report forms can be ordered separately.

Insecticides

(a)	1 box ¹ papers impregnated with DDT (p,p'-isomer) concentration	4.0% ²
	1 box papers impregnated with dieldrin concentration	0.4% ²
(b)	1 box papers impregnated with malathion concentration	5.0% ²
	1 box papers impregnated with fenitrothion concentration	1.0% ³
	1 box papers impregnated with propoxur concentration	0.1% ³

TEST PROCEDURE

- (a) Into each of the holding tubes, insert a piece of clean white paper rolled into a linder to line the wall and fasten it in position with a spring-wire clip (silver). Attach slides to the tubes.
- (b) Collect up to 100 female mosquitos with the aspirator provided (Fig. 1,A). Care resulting from careless handling of mosquitos during collecting may produce misleadingly high mortalities. Mosquitos should be collected in lots of not more than 10 (Fig. 1,B) and gently transferred to the holding tubes through the filling-hole in each side (Fig. 1,C) to 15 to 25 per tube. Any departure from these figures may impair the reliability of the tests.
- (c) A pre-test holding period may be necessary to guard against including damaged specimens in the test. For this purpose, the holding tubes are set upright, screen end up, 1 hr. At the end of this time the damaged insects are removed.
- (d) Into each of the exposure tubes introduce a sheet of impregnated paper, rolled into linder to line the wall and fastened into position with an appropriate spring-wire clip.
- (e) Introduce the mosquitos into the exposure tube by attaching it to the vacant screw-in the slide (Fig. 1,D). The slide should be pulled out to a point beyond the filling-hole so that no part of it occludes the tube openings; the mosquitos are then blown gently into the exposure tube. (If necessary, the small safety knob on the slide may be filed to facilitate this operation.) Close the slide. Detach the holding tube and set it aside.
- (f) Leave the exposure tubes standing upright with screen end up for the required exposure period (Fig. 1,E) under conditions of moderate, diffuse illumination, and adequate humidity.
- (g) At the end of the required exposure period, transfer the mosquitos to the holding tubes by reversing procedure (e). When some mosquitos have been knocked down in the course of exposure, the exposure tubes should be held horizontally and tapped to dislodge the mosquitos from the slide before the latter is withdrawn. Attach the holding tube, open the slide and gently blow the mosquitos into the holding tube; close the slide and remove the exposure tube. Then set the holding tube so that it stands on the slide and place a pad of cotton-wool on the screen (Fig. 1,F). Cardboard cartons or cups or other suitable containers may be used instead of the holding tubes, provided that they are used consistently.

¹ Each box contains 8 12 x 15 cm papers.

² One box of OC control papers is supplied for each order of 8 boxes or less.

³ One box of OP carbamate control papers is supplied for each order of 8 boxes or less. Papers impregnated with bendiocarb 0.1%; chlorphoxim 5%; deltamethrin 0.025% (name proposed by WHO = International Standardization Organization; permethrin 0.25% can be supplied, on special request, for research purposes. These specially prepared papers are more expensive than those in the catalogue). The delivery time is at least 2 months. Pirimiphos methyl impregnated papers have given variable results and should tests with this insecticide be necessary, WHO can provide technical pirimiphos methyl, filter-paper and a method to prepare the impregnated papers locally.

(h) Keep the holding tubes for 24 h in a secluded, shaded place, where the temperature does not exceed 30°C. Wherever feasible, the maximum and minimum temperature of the site of the holding tubes should be recorded. If necessary, the tubes should be protected from ants by placing them on a platform standing in a pan of water. If conditions are very hot and dry, a moist chamber may be prepared by suspending damp towelling in a container, and measuring the maximum and minimum temperature within.

(i) Mortality counts are made after 24 h. Remove the dead mosquitos by gently detaching the slide and cautiously moving the tube aside. Affected specimens that are unable to walk should be counted as dead. As an aid to counting the living specimens, they are stunned by a sharp jerk of the tube or stupified by chloroform or ether. The anaesthetics should not be allowed to come into direct contact with the plastic tube and screw-cap, which are soluble in these compounds. The results should be recorded on the forms provided. Copies of completed forms should be distributed in accordance with the instructions on page 5.

(j) Four replicate tests should be done with each of the diagnostic concentration and, preferably, 4 controls with oil-treated papers.

(k) Tests with control mortality in excess of 20%, though unsatisfactory, should be recorded. An investigation into the causes of control mortality should be made and steps taken to avoid it. A possible cause may be the collection of mosquitos from sprayed dwellings. In this case, it may be necessary to collect specimens from unsprayed ones, or to test adults reared from aquatic stages.

4. GENERAL REMARKS

(a) Each impregnated paper may be used up to 20 times, and up to 3 weeks after removal from the package, provided all possible precautions are taken against evaporation of the insecticide solution. Organochlorine papers can be left in the tubes, with the open end well wrapped, and placed in the kit box, which in turn should be kept in a cool place. No paper should be used more than 3 weeks after removal from the package.

For organophosphates and carbamates, impregnated papers should not be left for more than 3 days in exposure tubes and the same precautions for wrapping and storing should be followed.

(b) After an impregnated paper has been removed, the package should be resealed carefully with the plastic tape provided. The packages should be kept in a cool place, but not in a refrigerator, as too low a temperature may cause crystallization in the higher insecticidal concentrations. Prolonged storage at high temperatures should be avoided. Papers should not be used after the expiry date shown on the box. The expiry date is valid only if the packages are kept sealed at all times.

5. RESULTS

(a) Percentage mortality should be recorded on the report form. If the control mortality is between 5% and 20%, the percentage mortalities should be corrected by Abbott's formula:

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

(b) Results obtained where control mortalities exceed 20% should be recorded but not corrected. The accuracy of the interpretation of results depends on the reliability of the data obtained. Utilizing the maximum number of specimens per tube (25) decreases the effect of individual differences in response.

6. INTERPRETATION OF RESULTS

See Annex 1:¹ Criteria and meaning of tests for determining the susceptibility or resistance of insects to insecticides.

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 be sent to the following addresses:

For anopheline species:

1. World Health Organization, Malaria Action Programme, 1211 Geneva, Switzerland
2. The appropriate WHO Regional Office,² and
3. Project Headquarters.

The fourth copy should be retained by the investigator.

For non-anopheline species:

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

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

¹ See Annex 1: Report of WHO Expert Committee on Resistance of Vectors and Reservoirs to Pesticides (1975) WHO techn. Rep. Ser., 585.

² 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 CONCENTRATIONS AND EXPOSURE TIMES FOR ADULT MOSQUITOS

	Anopheline		<u>C. quinquefasciatus</u>	
	DDT	4%	1 hour	4%
Dieldrin	0.4%	1 hour *	4%	1 hour
Malathion	5%	1 hour	5%	1 hour
Fenitrothion	1%	2 hours	1%	2 hours
Propoxur	0.1%	1 hour	0.1%	2 hours
Chlorphoxim	4%	1 hour	-	-
Permethrin	0.25%	1 hour	0.25%	3 hours **
Deltamethrin	0.025%	1 hour	0.025%	1 hour **

* Except for An. sacharovi.

** Exposure tubes held flat so that mosquitos that are knocked down remain in contact with the paper during the entire time specified.

FIG. 1. METHOD FOR DETERMINING THE SUSCEPTIBILITY OR RESISTANCE OF ADULT MOSQUITOS TO ORGANOCHLORINE; ORGANOPHOSPHATE AND CARBAMATE INSECTICIDES

