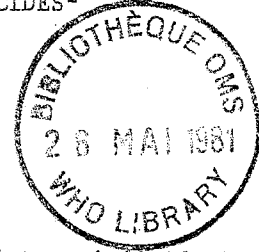




INDEXED

INSTRUCTIONS FOR DETERMINING THE SUSCEPTIBILITY OR RESISTANCE OF ADULT
MOSQUITOS TO ORGANOCHLORINE, ORGANOPHOSPHATE AND CARBAMATE INSECTICIDES¹
ESTABLISHMENT OF THE BASE-LINE



INTRODUCTION

1 Purpose and limitations of the test

The purpose of the susceptibility test is to detect the presence of resistant individuals in an insect 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 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 which 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.

Establishing the base-line

(a) For organochlorines, batches of mosquitos are exposed to different concentrations of insecticide and the mortality at each level is determined. It is suggested that a preliminary test be made at each concentration in the complete range provided, using the standard exposure time of one hour.

(b) For organophosphates and carbamates, batches of mosquitos are exposed to standard impregnated papers for different exposure periods and are subsequently held for 24 hours before mortality determination.

Depending on the class of the insecticide being tested a series of at least four concentrations for organochlorine and exposure times for organophosphate and carbamate should be chosen, some of which will give partial mortalities (i.e. at least one of them should give 5% mortality and two from 5 to 50% mortality). Tests at these concentrations or exposure times should be repeated four times with samples from the same population of mosquitos. To reveal the full range of natural variation, this set of tests should be made at several locations and at different seasons, so far as this is practicable.

¹ These instructions supersede Annex 1B (Wld Hlth Org. techn. Rep. Ser., 1970, No. 443) and WHO/VBC/75.581 and 582.

1.3 Subsequent routine checks by diagnostic concentration or exposure time

In routine monitoring for resistance it is not necessary to employ the full range of concentrations or exposure times used to establish the base-line susceptibility.

Use can be made of a diagnostic concentration or exposure time with a high probability of killing all normally susceptible mosquitos.

1.4 Choosing the diagnostic concentration of exposure time

The diagnostic concentration or exposure time is chosen on the basis of the base-line data for each insecticide.

As explained in Annex 1, "Criteria and Meaning of Tests for Determining Susceptibility or Resistance of Insects to Insecticides"¹ the most scientific way of selecting a diagnostic concentration or exposure time is to plot the base-line data on logarithmic probability paper and find the concentration or exposure time corresponding to 99.9% mortality. As a "rule of thumb" however it is usually advisable to use double this concentration or exposure time to reduce the chances of any ambiguity from exceptional susceptible survivors.

1.5 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.

1.6 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. Since temperature affects the mortality, temperatures should be recorded during the test. In general, higher temperatures induce higher mortality, and lower temperature results in higher survival. Therefore, the temperature/toxicity correlation should be taken into account when performing several tests under different temperature conditions.

2. COMPOSITION OF THE TEST KIT

EQUIPMENT AND/OR INSECTICIDES MAY BE ORDERED SEPARATELY. FOR INSECTICIDES 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) 12 plastic tubes, 125 mm in length and 44 mm in diameter; 5 of these (with red dot) are used for exposing the mosquitos to the insecticide, 2 (with green dot) are used for the control exposure without insecticide, and 5 (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.

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

- (b) 6 slide-units, each with a screw-cap on either side and provided with a 20 mm filling hole.
- (c) 40 sheets of clean paper (12 x 15 cm) for lining the holding tubes.
- (d) 12 spring wire clips to hold the papers in position against the walls of the tubes. The 7 steel clips should be used only for the holding tubes and the control exposure tubes; the 5 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 mouthpiece.
- (f) 1 roll of self-adhesive plastic tape.
- (g) Instruction sheets, 20 report forms and 3 sheets of log-probability paper for plotting regression lines,¹ and the document VBC/76.2: "Criteria and meaning of tests for determining the susceptibility of insects to insecticides."

2.2 Insecticides

- (a) 1 set of 5 boxes² of papers impregnated with DDT (p,p'-isomer) at concentrations of 0.25%, 0.5%, 1%, 2% and 4% respectively.³
- (b) 1 set of 6 boxes of papers impregnated with dieldrin at concentrations of 0.05%, 0.1%, 0.2%, 0.4%, 0.8% and 4% respectively.³
- (c) 1 box of papers impregnated with malathion at a concentration of 5%.⁴
- (d) 1 box of papers impregnated with fenitrothion at a concentration of 1%.⁴
- (e) 1 box of papers impregnated with propoxur at a concentration of 0.1%.⁴

3. TEST PROCEDURE

(a) Into each of the holding tubes, insert a piece of clean white paper rolled into a cylinder to line the wall and fasten it in position with a spring-wire clip (steel). Attach the slides to the tubes.

(b) Collect approximately 200 female mosquitos with the aspirator provided (Fig. 1,A). Damage to mosquitos from careless handling 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 give 15-25 per tube. Other methods of collection and introduction of insects into holding tubes can be used as long as they do not produce excessive mortality. Any departure from these figures may impair the reliability of the results.

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

² Each box contains 8 (12 x 15 cm) papers.

³ One box of OC control papers is supplied with each set of treated papers.

⁴ One box of OP-carbamate control papers is supplied for each order of 4 boxes or less. Papers impregnated with bendiocarb 0.1%; chlorphoxim 5%; deltamethrin 0.025% (name proposed to ISO = International Standardization Organization); and permethrin 0.25% can be supplied, on special request, for research purposes. These specially prepared papers are more expensive (see 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 paper locally.

(c) A pre-test holding period may be necessary to ensure that damaged specimens are not included in the test. For this purpose, the holding tubes are set upright, screen end up, for one hour. 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 a cylinder to line the wall, and fastened into position with a copper clip for tubes containing impregnated papers and a steel clip for those containing control papers.

(e) Introduce the mosquitos into the exposure tube by attaching it to the vacant screw-top 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 opening; the mosquitos are then blown gently down into the exposure tube. (If necessary, the small safety knob on the slide may be filed down 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 an exposure, the exposure tubes should be held horizontally and tapped to dislodge the insects 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 wet 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.

(h) Keep the holding tubes for 24 hours 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 hours. 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 stupefied 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 different concentrations/exposure times and, preferably, 4 controls with the 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 three weeks after removal from the package, provided all possible precautions are taken against evaporation of the insecticide solution. To this end, the papers should 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 three weeks after removal from the package.

(b) After an impregnated paper has been removed, the package should be resealed carefully with the plastic tape provided. For ordinary storage, the packages should be kept in a cool place, even in a warmer part of a refrigerator, remembering that 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 resealed promptly.

(c) To decide on a suitable exposure period, conduct tests with the highest concentration available until complete mortality is obtained. For 4% DDT a one hour exposure period should be used. Should this period not result in complete mortality, the exposure period should be increased to 2, 4 or 8 hours. The complete range of concentrations is then employed to determine a concentration mortality regression line at whatever exposure time is selected.

RESULTS

(a) For base-line data determinations, the concentration/mortality regression line for organochlorines or the exposure time-mortality regression line for organophosphates and carbamates should be constructed from the results obtained in quadruple tests at each chosen concentration/exposure time. For this purpose, the results should be plotted on the arithmetic probability paper provided. The regression line may be fitted by eye and concentrations/exposure times expected to kill various percentages can be read from it. The concentration to kill 50% is known as LC₅₀; that for 95% kill is LC₉₅. Similarly, exposure time to kill 50% and 95% are known as LT₅₀ and LT₉₅ for organophosphates and carbamates. The regression line can be extended to estimate the LC/LT_{99.9} (though it must be realized that this is very approximate). For accurate methods of computing various LC/LT estimates, see Swaroop.¹

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

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

(c) Results obtained where control mortalities exceed 20% should be discarded. 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. Regression lines based on similar numbers of specimens are more reliable.

INTERPRETATION OF RESULTS

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

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:

World Health Organization, Malaria Action Programme, 1211 Geneva, Switzerland.

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

² Report of WHO Expert Committee on Resistance of Vectors and Reservoirs of Disease to Pesticides (1975) Wld Hlth Org. techn. Rep. Ser., 585.

2. The appropriate 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, Division of 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.

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

