



INSTRUCTIONS FOR DETERMINING THE SUSCEPTIBILITY OR RESISTANCE
 OF BODY LICE AND HEAD LICE TO INSECTICIDES¹

INDEXED

1. INTRODUCTION

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.

1.1 Establishing the base-line

Batches of lice are exposed to standard impregnated papers for different exposure periods; mortality counts are immediately recorded for organochlorines, but for organophosphates and carbamates lice are held for 24 hours before the mortality rate is determined.

Preliminary tests are carried out with a wide range of exposure periods, at logarithmic intervals. A suitable range of at least four exposure times should be chosen, some of which give partial mortalities (i.e. one of them should give 100% mortality and two from 5 to 50% mortality). Tests at these exposure times should be repeated four times with samples from the same population of lice. Controls, on paper impregnated without insecticide, should be counted after the longest exposure used for the treated papers.

1.2 Subsequent routine checks by diagnostic exposure time

In routine monitoring for resistance, it is not necessary to employ the full range of concentration/exposure times used to establish the base-line susceptibility. Use can be made of a "diagnostic exposure time" for a given standard concentration, with a high probability of killing all normally susceptible lice.

1.2.1 Choosing the diagnostic exposure time

The diagnostic 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"² the most scientific way of selecting

¹ These instructions supersede information given in WHO/VBC/75.585 Rev. 1.

² Report of the WHO Expert Committee on Resistance of Vectors and Reservoirs of Disease to Pesticides (1976) Wld Hlth Org. techn. Rep. Ser., No. 585.
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a diagnostic exposure time is to plot the base-line data on logarithmic-probability paper and find the exposure time corresponding to 99.9% mortality to a given standard concentration of insecticide. As a "rule of thumb" it is usually adequate to use an exposure double the lowest exposure that has consistently given complete kill in all tests used to establish the base-line.

1.2.2 Established diagnostic exposure time

Tentative diagnostic exposure times for lice are shown in Table 1.

These data were obtained with body lice. Such evidence as is available (e.g. Maunder, J.W., 1971, Medical Officer, 27) suggests that head lice are somewhat more susceptible. Thus, survival of head lice after such exposures would probably indicate resistance.

1.3 Condition of lice

Adult body lice of either sex can be used, provided they are not obviously starved. Large nymphs are about as susceptible as adults and can be used in the test.

Head lice are particularly difficult to collect from infested heads without damage (e.g. losing one or more legs) which may result in high control mortality. It is recommended that the specimens collected be examined carefully with a hand lens and any damaged ones discarded.

1.4 Conditions of test

Tests should be carried out in a room free from insecticidal contamination. The lice are exposed in darkness and held at a temperature between 25°C and 30°C and at a relative humidity above 50%.

2. COMPOSITION OF THE KIT

EQUIPMENT AND/OR INSECTICIDE MAY BE ORDERED SEPARATELY. FOR INSECTICIDES THE ORDER SHOULD SPECIFY BOTH THE INSECTICIDE AND THE NUMBER OF BOXES OF IMPREGNATED PAPERS. (1)

2.1 Equipment

- (a) 1 pair of forceps.
- (b) 14 halves of 9 cm Petri dishes to confine lice to the papers.
- (c) 14 clean containers (beakers, salve tins or other smooth-sided containers).
- (d) Instruction sheets, 20 report forms and 3 sheets of log-probability paper for plotting regression lines. (2)

The above items, except (d) must be provided by the investigator.

¹ Deltamethrin* 0.025%, permethrin 0.25%, propoxur 0.8%, trichlorfon 1% can be supplied on special request. These specially prepared papers are more expensive (see catalogue). The delivery time is at least two months.

² Additional report forms and log-probability papers can be ordered separately.

* Name proposed to ISO = International Standardization Organization.

2.2 Insecticides

The order should specify the insecticides and number of sets of boxes for OC and boxes of OP impregnated papers.⁽¹⁾

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|-----|----------------------|---|---------------------------------|
| (a) | 1 box ⁽²⁾ | papers impregnated with DDT (p,p'-isomer) | concentration 4% ⁽³⁾ |
| (b) | 1 box | " " " dieldrin | " 0.1% ⁽³⁾ |
| (c) | 1 box | " " " malathion | " 5% ⁽⁴⁾ |

3. PROCEDURE

3.1 Base-line

- (a) Impregnated papers to be used are laid on firm, impermeable surfaces (glass, china or metal plates are suitable).
- (b) Adult lice should be collected in a single container from one individual or, if this is not possible, from a small group of individuals. To assess the resistance level in a locality, additional samples should be taken from a sufficient number of infested individuals. The lice should be divided by random selection into lots of 20 in small containers (beakers, salve tins or other clean containers). Although it is desirable to use 20 lice in each replicate, if this is not possible the number may be reduced down to an absolute minimum of 10 per replicate.
- (c) One lot of adult lice should be placed on each treated paper and confined under half a Petri dish. Rubber bands or weights should be employed so that the dish adheres to the surface and the lice cannot escape.
- (d) At the end of the exposure period: (i) with organochlorine insecticides, the lice should be examined and the mortality recorded. Only lice capable of coordinated movements should be counted as alive; (ii) with organophosphorus or carbamate insecticides, the lice should be carefully transferred to clean sheets of paper and confined as before. Mortality counts should be made after a further 24 hours.
- (e) After a preliminary test has been performed with a wide range of exposure times tests should be carried out with the chosen series of four exposure times giving partial and complete mortality. Three replicates should be performed at each of the selected exposure times.
- (f) When the test has been repeated four times with the same population of lice, adequate data should be available for constructing a base-line of susceptibility.
- (g) Tests with control mortality in excess of 20% are unsatisfactory and should be repeated.

3.2 Diagnostic exposure times

For routine checks the same procedure is applicable except that only one exposure time to a standard concentration of insecticide, established from the base-line data or given for guidance in Table 1, is used.

¹ Papers impregnated with other organophosphorus compounds or carbamate can be supplied on request (see list of available impregnated papers in catalogue).

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

³ 1 box of CO control papers is supplied for each order of 8 boxes or less.

⁴ 1 box of OP control papers is supplied for each order of 8 boxes or less.

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 oil. To this end, the papers should be replaced in the box after use.

(b) After an impregnated paper has been removed, the package should be resealed carefully with the plastic tape provided. The package 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) To construct the time-mortality regression line the results obtained in quadruple tests at the chosen exposure times should be plotted on the logarithmic-probability paper provided. The regression line may be fitted by eye and exposure times expected to kill various percentages can be read from it. Exposure time to kill 50% and 95% are known as LT_{50} and LT_{95} . The curve can be extended to estimate the $LT_{99.9}$ (though it must be realized that this is very approximate). For accurate methods of computing various LT 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) Results obtained where control mortalities exceed 20% should be discarded.

6. INTERPRETATION OF RESULTS

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

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 (1976) Wld Hlth Org. techn. Rep. Ser., No. 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 World Health Organization, Vector Biology and Control, 1211 Geneva, Switzerland, and one copy to the appropriate WHO Regional Office.⁽¹⁾

TABLE 1. TENTATIVE DIAGNOSTIC DOSAGES FOR LICE

	Body lice	
DDT	4%	1 day
Dieldrin	0.1%	1 day
Propoxur	0.8%	10 hours
Fenchlorphos	4%	10 hours
Malathion	5%	1 day ⁽²⁾
Fenitrothion	1%	5 hours
Trichlorfon	1%	2.5 hours

¹ Addresses of WHO Regional Offices are as follows:

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

World Health Organization, Regional Office for the Eastern Mediterranean, PO 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, PO Box 2932, 12115 Manila, Philippines.

² Evaluation of malathion solutions on lice. Determination of susceptibility to malathion by M. Calade and J. Coz, VBC/EC/80.25a.

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