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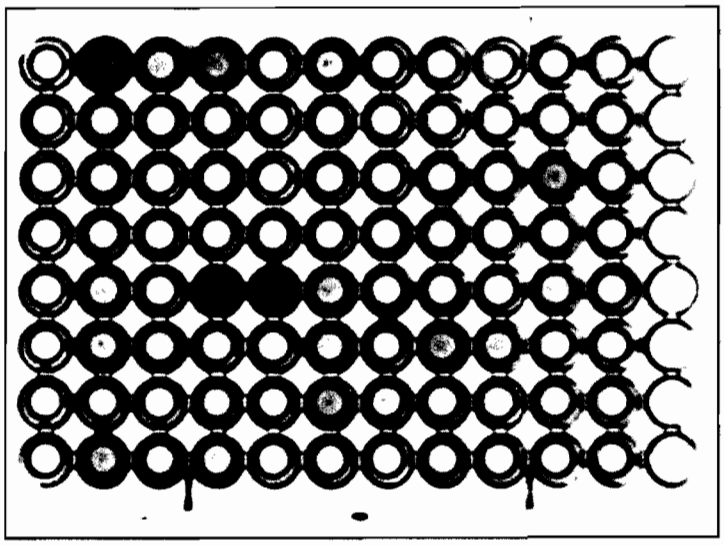
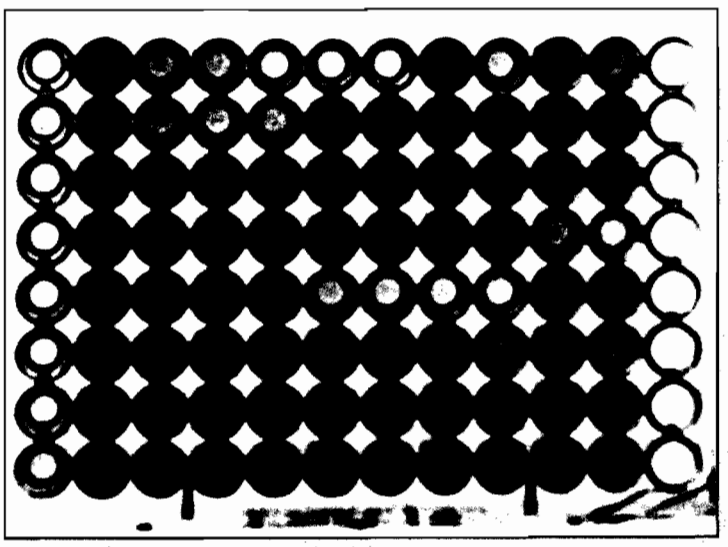
Original: English
Distr.: Limited

Techniques to detect insecticide resistance mechanisms (field and laboratory manual)





World Health Organization
Department of Disease Prevention & Control
WHO Communicable Diseases (CDS)

WORLD HEALTH ORGANIZATION

WHO/CDS/CPC/MAL/98.6
DISTR.: LIMITED
ENGLISH ONLY
DISTR.: LIMITEE

TECHNIQUES TO DETECT

INSECTICIDE RESISTANCE MECHANISMS

(Field and laboratory manual)

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1. INTRODUCTION

Insecticide resistance is an increasing problem faced by those who need insecticides to efficiently control medical, veterinary and agricultural insect pests. In many insects, the problem extends to all four major groups of insecticides. Resistance monitoring programmes should no longer rely on testing the response to one insecticide, with the intention of switching to another chemical when resistance levels rise above the threshold which affects disease control. Effective resistance management depends on early detection of the problem and rapid assimilation of information on the resistant insect population so that rational pesticide choices can be made.

The correct use of biochemical or immunological methods for resistance detection at a mechanistic level can provide a powerful tool for analyzing field and laboratory populations with the aim of improving resistance detection and management. This manual will be updated with new technologies and methodologies, as they become available. It attempts to outline the basic techniques and discusses their strengths and weaknesses. Clearly the biochemical assays provide more information about the insect population being analysed, but they also require more skill in interpretation, and those using this manual are urged to read the sections on interpretation of results carefully.

2. GENERAL OVERVIEW OF IMPORTANT RESISTANCE MECHANISMS IN ALL INSECTS

Where a mechanistic approach to resistance detection is being undertaken the investigator needs to have a basic understanding of the possible resistance mechanisms likely to be encountered. This manual currently deals only with the four major groups of insecticides, the organochlorines (with the exception of the cyclodienes), organophosphates, carbamates and pyrethroids. New compounds are clearly coming onto the market, such as insect growth regulators, but a mechanistic detection of resistance to these compounds has by necessity to be reactive rather than pro-active (i.e. we can only develop methods for resistance detection when we know the range of mechanisms selected in different insect populations). Hence monitoring for resistance to compounds outside the four well-characterised pesticide groups will still rely heavily on the standard susceptibility tests. There are four possible types of resistance mechanisms to the main insecticide groups in all insects analysed to date. These are:

- increased metabolism to non-toxic products
- decreased target site sensitivity
- decreased rates of insecticide penetration
- increased rates of insecticide excretion.

Of these four categories the first two are by far the most important. Penetration rate changes in isolation generally produce insignificant (<5-fold) levels of resistance, and only become important when found in combination with other resistance mechanisms. Increased rates of insecticide excretion are very uncommon and produce only low levels of resistance and are included in this list for completeness.

The first two categories of resistance mechanisms, which are the most common and produce the highest levels of resistance, can be sub-divided further. The enzyme groups involved in *insecticide metabolism* are:

1. esterases
2. monooxygenases
3. glutathione-*S*-transferases

The target sites involved are the sodium (Na^+) channels for the pyrethroids and DDT, and acetylcholinesterase for the organophosphates and carbamates. (The target site for the cyclodienes, such as gamma HCH, is the GABA receptor, but detection of resistance due to changes in this target site is not dealt with in this manual).

The level of resistance conferred by the different mechanisms varies depending on the insecticide and the nature of the alteration in the enzyme system involved. Exact details of this are given in the text as each mechanism is considered.

There are two major ways that the metabolic enzymes can produce resistance:

- *overproduction* of the enzyme, leading to increased metabolism or *sequestration*;
- an *alteration in the catalytic centre activity* of the enzyme, increasing the rate at which an enzyme unit metabolizes the insecticide. These two routes are not mutually exclusive and an enzyme may be both *physically changed* and *over-produced.*

When an enzyme is overproduced but the pesticide is only slowly metabolised by that enzyme, the cause of resistance may be considered to be sequestration rather than metabolism, with the increased enzyme levels acting as a means of holding the pesticide and preventing it from reaching the target site within the insect. The level of resistance conferred is then roughly proportional to the increase in the quantity of enzyme produced.

Biochemical assays/techniques may be used to establish the mechanism involved in resistance. When a population is well characterised some of the biochemical assays can be used to measure changes in resistance gene frequencies in field populations under different selection pressures. It should be stressed that at present simple field biochemical assays do not exist for all resistance mechanisms. Biochemical assays are not complete substitutes for the standard susceptibility tests which are used to measure resistance.

3. THE BIOCHEMICAL ASSAYS

Because the resistance mechanisms detected by these methods are common to all insects they are applicable across the range of insect pests. However, it is important to note that the baselines may differ between insects and where possible a known susceptible strain of the same species as the field population being tested should be analysed at the same time.

To date biochemical assays have successfully been used on mosquitoes (*Culex, Anopheles* and *Aedes*), sand flies, cockroaches, houseflies and black flies as well as some agricultural pests.

Two main variants of two of the assays are in use. To save repeating the arguments for and against each twice, these are outlined here, although both methods are later given in detail. One variant of the assays uses filter paper or another solid support media; the second variant is run in microtitre plates. The 'filter' paper or nitrocellulose membrane assays generally use one mosquito per assay and are quantified visually or using a densitometer, but provide a permanent record which can be rechecked in the future. The microtitre plate tests allow the same insect to be used for all assays and are quantified visually or with a spectrophotometer. A permanent record can be made on paper by simply using a transfer plate, but this is not an automatic result of the test.

3.1 The microtitre plate tests

Once experienced with these techniques, it is practical to run assays for altered acetylcholinesterase, elevated esterase, glutathione-*S*-transferase and protein from the same insect. It is not recommended that this is attempted initially, as it is easy for the novice to mix solutions up, and the time factor after homogenizing the insects is important, particularly if ice is not available to maintain the homogenates at a low temperature (i.e. the more rapidly the tests are run after insect homogenization, the more accurate the results will be). It is recommended that anyone attempting these assays should familiarise themselves with each assay individually, and once they are confident and competent with the techniques, start to combine them for analysis of the same insect.

3.1.1 Equipment/supplies

Equipment required for these assays are:

Microtitre plates (preferably flat-bottomed if results are to be read spectrophotometrically): if affordable, these should be disposed of, after one use. However, they can be re-used if