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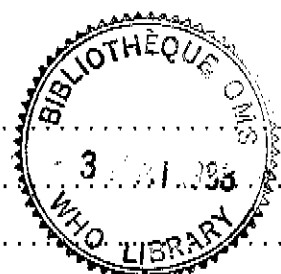
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INFORMAL CONSULTATION ON DETECTION
OF INSECTICIDE RESISTANCE

Geneva, 10-12 June 1987

CONTENTS

	<u>Page</u>
1. Introduction	2
2. Mechanisms of insecticide resistance	3
3. Tests for detecting resistance	4
3.1 High esterase tests	4
3.1.1 Filter-paper test (FP/Est.test)	4
3.1.2 Nitrocellulose membrane test (NC/Est.test)	4
3.1.3 Microtitre plate assay (MT/Est.test)	4
3.2 Immunological test	5
4. Field studies on esterase tests	5
4.1 Filter-paper test (FP/Est.test)	5
4.2 Microtitre plate assay (MT/Est.test)	5
4.3 Carboxylesterase	6
4.4 Glutathione S-transferase	6
4.5 Multi-function oxidase (mfo) assay	6
4.6 Insensitive acetylcholinesterase (AChE)	7
4.7 Target acetylcholinesterase microplate assay	8
5. Conclusions	8
6. Recommendations	9
References	11
Annex I - List of participants	12
Annex II - Table	13



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1. INTRODUCTION

The Informal Consultation on Detection of Insecticide Resistance was held in Geneva from 10 to 12 June 1987. Dr R. Slooff, Director of the Division of Vector Biology and Control, opened the meeting. He emphasized the need to develop new methods and techniques to further facilitate the early detection of vector resistance to pesticides and the mechanisms involved that could help in the monitoring and management of the resistance problem.

Since the late 1950s WHO has been actively engaged in promoting measures to counter resistance to insecticides in vectors of disease. One early and important action was the development of standard test methods for detecting and measuring resistance. Accordingly, a number of test kits based on bioassay methods were designed.

In the early years, it was sufficient to test only two insecticides (DDT and dieldrin) for the detection of resistance to chlorinated hydrocarbons used in public health. Later on, a number of organophosphates, carbamates and pyrethroids were introduced in vector and pest control programmes. This complicated the procedure of providing a representative number of standard solutions and impregnated papers for testing larval and adult stages, respectively, since no one organophosphate, carbamate or pyrethroid allowed a unequivocal detection of resistance to other members of the insecticide class concerned. The test kits, therefore, had to include impregnated papers and solutions of all new insecticides.

The characteristic of the standard methods as expressed at the time were simplicity, validity, reliability and comparability. The equipment involved had to be simple, cheap, easily available, and compact for easy use and transportation to the field. The methods of application were to be simple and easy enough to be understood and implemented by mid-level field workers. Preliminary methods and kits were subjected to field testing and critical evaluation before submitting them to expert committees on resistance of vectors to pesticides for standardization. At present WHO has standard methods and test kits for almost all insects of medical importance.

Results of tests performed with these test kits throughout the world were collected by WHO, computerized and periodic summaries issued either in the Expert Committee report or by individual reviewers. Results were also provided to research and field workers as well as countries and WHO regions.

The simplification of the procedure in test kits had some negative effects in that unqualified staff who started using them made a number of procedural mistakes, of which some could have significantly affected the interpretation of results. In some instances a hasty switch to other insecticides was made which was not justified. It was recommended that the detection of resistance by these methods should not be taken as conclusive evidence for a control failure and that supporting entomological and epidemiological data on the failure to control the disease should be obtained before a decision on the change of insecticide was made.

In 1970, the Expert Committee on Insecticide Resistance and Vector Control recommended basic research on the genetics and biochemistry of resistance. These aspects of insecticide resistance were studied in depth by some laboratories, which led to a better understanding of the mechanisms of resistance in certain vectors and the development of prototypes of new biochemical methods of detecting vector resistance. These test methods have the advantage of detecting resistance in individual insects and identifying the type of resistance mechanism involved.

An informal consultation was convened in London in 1985 to further stimulate research on the new leads and define how close various laboratories were to being able to produce simple biochemical tests for the major resistance mechanisms and to examine the possibility of introducing a prototype kit for these tests in the field. A number of recommendations on the exchange of information, on cost of the future kits, on field testing of available methods, on development of field methods for testing those mechanisms not yet evaluated under field conditions, and on the possibility of using immunological methods, were made by the group.

The present informal consultation aimed at (a) considering the progress made since the last meeting and (b) formulating recommendations concerning the use of biochemical methods for field determination of resistance, their application, their use to back up existing methods, and their limitations.

It should be borne in mind that these test methods will sometimes have to be used in remote areas where highly trained personnel may not be available.

2. MECHANISMS OF INSECTICIDE RESISTANCE

The vast amount of literature on insecticide resistance mechanisms gathered over the last two to three decades indicates that there are only a limited number of ways in which any insect species develops resistance to pesticides. These can be summarized briefly as:

- (i) an increase in the rate of pesticide detoxification;
- (ii) a reduction in the sensitivity of the insecticide target site within the insect;
- (iii) a decrease in penetration or increase in excretion of the pesticide from the intact insect.

The first two categories are by far the most important. These can be further subdivided as follows:

- (i) (a) esterase;
- (b) oxidase;
- (c) glutathione S-transferase;
- (ii) (a) kdr;
- (b) insensitive acetylcholinesterase (AChE).

The enzymes involved in resistance can either be qualitatively and/or quantitatively changed. The former involves a change in the active site of the enzyme, allowing it for example to metabolize the insecticide more rapidly; the latter involves production of a larger amount of the same enzyme, again resulting either in faster conversion of the insecticide or, perhaps in some cases, sequestration of the insecticide by the enzyme.

A complete susceptibility test system should ideally enable the identification of resistance mechanisms, indicate the level of resistance, and suggest the spectrum of cross-resistance conferred by each mechanism.

The biochemical assays with the exception of the target acetylcholine microassay described below do not in isolation give information on the occurrence or level of resistance. A causal correlation between these mechanisms and resistance should be verified.

The susceptibility tests developed so far are based on enzymatic and immunological reactions and are mainly concerned in the detection of enzymes involved in organophosphate and carbamate resistance.

3. TESTS FOR DETECTING RESISTANCE

3.1 High esterase tests

Testing for high esterase activity as a means of detecting resistance presumes that the relationship between esterase activity and resistance in the population in question has been established through appropriate genetic tests.

There are two approaches to test for high activity of esterases against naphthyl esters: one is based on the measurement of metabolic activity, the other on immunology.

3.1.1 Filter-paper test (FP/Est.test)

A small drop of single mosquito homogenate is deposited on filter-paper, incubated in a solution of naphthyl acetate, and stained for the product of esterase metabolism. The level of esterase activity is revealed by the development of a stain on each spot (Pasteur & Georghiou, 1981). The test has been optimized with respect to the type of paper used, homogenization of the mosquito, enhanced recovery of esterase, and length of incubation and staining periods.

The insects can be visually classified qualitatively as possessing high or low esterase activity. The FP/Est.test holds promise in the determination of the degree of resistance. This is accomplished through quantification of the esterase in the homogenate and estimation of the amount of α -naphthol produced by referring to a standard curve for α -naphthol vs. colour intensity. The intensity of colour produced can be determined using a densitometer or by reference to a chart.

The advantage of the filter-paper test is its simplicity and speed with which it can be performed, even by non-professional individuals. Another important aspect is that the results of the assay (the test papers) can be permanently preserved and may thus be re-evaluated at a later time, for quantification or other purposes, or may serve as baselines for comparisons with subsequently obtained data. The test materials are relatively inexpensive. A further simplification of this test would be the use of naphthyl acetate pre-impregnated papers on which mosquitos could be directly squashed.

3.1.2 Nitrocellulose membrane test (NC/Est.test)

This method for the detection of non-specific esterases is a variant of the filter-paper test and is based on the use of nitrocellulose membranes instead of filter-papers. It has the advantage that nitrocellulose binds the esterase molecules more firmly, and thus the stain has no tendency to diffuse on the membrane as may occasionally be the case with filter-papers. Despite this advantage, the test holds little promise for routine application due to the high cost of nitrocellulose membranes.

3.1.3 Microtitre plate assay (MI/Est.test)

This method involves the addition of a known quantity of naphthyl acetate substrate solution to a microtitre plate well containing a known quantity of homogenate from a single insect. This is followed by an incubation period after which a dye solution is added and results are then either determined visually or spectrophotometrically

(Hemingway & Georgioui, 1984). Individuals with high esterase activity give a much stronger colour than those with low esterase activity. Quantification of the esterase activity can be obtained by preparing serial dilutions of the mosquito homogenate and comparing this to that of a reference curve of naphthol.

Precise quantification of esterase activity may require that the amount of naphthol produced be expressed on the basis of the quantity of protein contained in the homogenate. This may be done using the microtitration protein assay (Brogdon, 1984a,b).

A permanent record may be obtained by transferring samples of assay well contents to filter-paper using a transfer plate.

3.2 Immunological test

An immunological test has been used in combination with the high esterase activity tests in order to introduce a higher degree of specificity. When a positive high esterase result is obtained the same individual¹ is "challenged" with an antiserum prepared against a native β -naphthyl acetate specific esterase from African Culex quinquefasciatus using an ELISA test. Earlier laboratory results have indicated that this antiserum cross-reacts with a number of different Culex β -naphthyl acetate specific high esterases but not with the α -naphthyl specific esterases. Similar results were obtained with an antiserum prepared against a SDS-denatured β -naphthyl esterase B1 of C. quinquefasciatus from California.

This β -naphthyl acetate antiserum mentioned above has been used to detect and quantify β -naphthyl acetate esterases by a dot blot method in which homogenates of single insects are deposited on a nitrocellulose filter rather than a microplate as in ELISA. Results on USA field populations were consistent with bioassay results as well as with results obtained with filter-paper and microplate assays for esterases (Pasteur, personal communication). High esterase activity in Anopheles has not, to date, shown any cross-reaction with antiserum against natural β -naphthyl acetate esterase (Hemingway et al., 1986a). However, a cross-reaction was observed with Aedes aegypti and Musca domestica using the antiserum prepared against SDS-denatured esterase B1.

4. FIELD STUDIES OF ESTERASE TESTS

4.1 Filter-paper test (FP/Est.test)

The validity of the filter-paper test for detection of non-specific esterases and resistance to organophosphates was tested in 1986 on 15 collections of C. quinquefasciatus and C. pipiens from various localities in the USA. These populations were evaluated against four insecticides (chlorpyrifos, temephos, fenthion and malathion), using the filter-paper test, electrophoresis, discriminating doses and complete bioassays. The proportion of mosquitos lacking high esterase activity as revealed by electrophoresis was significantly correlated with the presence of susceptible individuals estimated by discriminating doses and by complete bioassays with each of the four insecticides. This same study also showed that the proportion of mosquitos in which optical density of the esterase spot exceeded an established threshold level was correlated with the LC_{95} observed for chlorpyrifos, temephos and fenthion.

4.2 Microtitre plate assay (MT/Est.test)

A combination of the MT/Est.test and protein assay on the same field strains from California indicate that the MT/Est.test holds a high resolution power for quantification of resistance. A high degree of similarity was observed in the shapes of the curves obtained by bioassay with several organophosphate insecticides and of curves of esterase activity distribution in each population.

¹ Homogenate from one single insect.

In Haiti, bioassays and MT/Est. tests were used in conjunction to detect fenitrothion resistance due to high esterase in A. albimanus. A significant correlation was established between bioassay and the MT/Est. assay. Similarly, high esterase activity in a number of Sri Lankan Culex species (see table) was well correlated with organophosphate resistance.

Field work on Sri Lankan Anopheles confirmed the need to verify that the phenomenon of high esterase activity is directly equated with resistance. Here, of three species showing high esterase activity in the MT/Est. assay, only one gave evidence of resistance dependent on esterase metabolism after bioassay, synergist, and laboratory metabolism studies were conducted. In Guatemala, high esterase activity associated with resistance to fenitrothion did not produce cross-resistance to malathion at the same frequency.

4.3 Carboxylesterase

The resistance mechanism based on carboxylesterase cannot be differentiated from the non-specific esterase mechanism by a change in activity against naphthyl acetates. A specific reliable field assay has yet to be developed; however, it can be detected, though not specifically identified, using the target acetylcholinesterase microplate method (see page 8). A specific test would be useful as this mechanism confers resistance to malathion, one of the major insecticides used in public health vector control at present.

4.4 Glutathione S-transferase

Glutathione S-transferase is involved in resistance to DDT and organophosphate insecticides in a number of species.

It has not yet been established whether resistance mechanisms involving this enzyme are determined by quantitative or qualitative changes in the enzyme. However, there is some evidence in two species of Anopheles that qualitative changes are involved, whereas data from Musca domestica show a quantitative change.

At present there are two laboratory methods for detecting this type of resistance mechanism and which can be adapted for use in microtitre plates (Booth et al., 1961; Clark et al., 1984). Neither of the reactions involved induces a colour change in the visible range. Microtitre plate tests can then only be read in a specialized microtitre plate reader with a 340-nm filter and a UV source light. Although they are useful laboratory methods, in their present form they are of little use for field studies.

4.5 Multi-function oxidase (mfo) assay

The principle of a prototype method explored so far on A. subpictus is based on the observed faster in vitro conversion of fenitrothion to its oxon analogue in resistant mosquitos than in susceptible individuals. It is assumed that the faster conversion of thion to oxon is accompanied by faster detoxication of the oxon in vivo. Fenitrothion was chosen for this assay because at a concentration which yields sufficient oxon to give high-level acetylcholinesterase (AChE) inhibition, it does not itself inhibit the AChE enzyme and remains in solution. Hence, compared to the controls, this insecticide has little or no effect on absorbance readings in test wells in the absence of the mfo resistance mechanism.

The fenitrothion and mosquito homogenate are pre-incubated for one hour before addition of the normal AChE substrate, acetylthiocholine iodide. This pre-incubation allows production of sufficient quantities of the oxon to give a high level of AChE inhibition in the susceptible strain compared to the resistant strain, without allowing the normal uninhibited AChE activity to decline in vitro to a level too low to yield a good comparison between test and control wells.

At present the assay has not been tested against any other species whose resistance mechanism is based on mfo since no other mfo-based resistant material was available when the prototype test was being developed.

This assay was field-tested in Sri Lanka using the F1 generation of A. subpictus. It was already known from earlier bioassay, synergist, enzyme-kinetic, and metabolism studies that in Sri Lanka the resistance mechanism based on mfo is highly frequent in this species (Hemingway et al., 1987). Individual families were "genotyped": half the progeny from each family (one-day-old adults) underwent bioassays after a two-hour exposure to 1% fenitrothion-impregnated papers; the remaining progeny were subjected to the prototype mfo assay. Results obtained by the two methods indicated good agreement on the resistance status of the family evaluated.

It is possible, but not yet demonstrated, that this resistance mechanism may be detected using the target acetylcholinesterase microplate assay (see page 8) and specifically identified using piperonyl butoxide as a synergist.

4.6 Insensitive acetylcholinesterase (AChE)

These tests exploit the differences in inhibition characteristics that exist between insensitive and normal AChE when they are incubated in the presence of a carbamate or an organophosphate insecticide.

The tests developed in the different laboratories use either nitrocellulose membranes as a support for the homogenates (Dary & Georghiou, personal communication) or are performed in microtitre plates (Hemingway et al., 1986b; Raymond et al., 1985; Brogdon & Dickinson, 1983; Brogdon & Barber, personal communication). In the former test AChE activity is revealed using the method of Karnovsky & Roots (1964), in the latter by the method of Ellman et al. (1961). Acetylthiocholine is used in both methods as a substrate for AChE since it yields thiocholine after a chemical reaction.

In all tests, insensitive AChE is identified by comparing visually and photometrically the quantity of thiocholine iodide produced by AChE activity in the presence and absence of insecticide. In the case of C. pipiens, A. nigerrimus and A. albimanus, homozygous susceptible and resistant as well as heterozygous insects are readily distinguished using propoxur as the inhibitor.

The AChE test using the method of Hemingway et al. (1986b) has now been used in the field in Sri Lanka for over two years. Data collected for 22 species of Anopheles and a number of Culex species, comprising many tests, suggest that this test is reliable under field conditions. However, approximately 2% of the mosquitos exhibited very low (uninhibited) AChE activity and could not be classified. It is not clear whether they represent homozygous resistant individuals.

Likewise, tests using 2000 mosquitos and following the method of Raymond et al. (1985) gave an equally good performance in a survey of insensitive AChE distribution in C. pipiens from southern France (Magnin & Pasteur, personal communication).

Up to now, the nitrocellulose test has been used only on C. pipiens and C. quinquefasciatus populations from the USA and, as expected, showed the absence of the insensitive AChE in these species (Dary & Georghiou, personal communication).

Insensitive AChE (together with high esterase) microplate assay methods were incorporated into field studies of the geographic distribution of organophosphate- and carbamate-resistant A. albimanus and A. pseudopunctipennis in Guatemala (Brogdon et al., personal communication). Areas of complete susceptibility to organophosphates and carbamates were observed along with others where resistance phenotypes represented up to 98% of the population. The high esterase activity and insensitive AChE mechanisms were independently associated, the high esterase activity mechanism being the predominant resistance mechanism.

In these studies (Brogdon et al., personal communication), resistance data from bioassay and microplate assay methods were collected and correlated. Resistance thresholds were established for microplate assay and bioassay methods of resistance detection. Correlations were established between insensitive AChE microplate assay data and data from bioassays. In tests for detection of resistance to propoxur an absorbance at 410 nm of greater than 0.3 in microplate assay was correlated with survival at 60 min in bioassays.

4.7 Target acetylcholinesterase microplate assay

A prototype assay method has been developed for detection of resistance to organophosphates and carbamates, based upon the measurement of AChE activity of mosquitos pre-exposed to organophosphates. This in vivo exposure to organophosphate insecticides allows the activated insecticide to reach the AChE target enzyme rapidly. Residual AChE activity is assayed using the method of Ellman et al. (1961).

For A. albimanus, A. arabiensis, and A. stephensi bioassay exposure thresholds of 2 h (5% malathion) or 3 h (1% fenitrothion) were established. To determine resistance to carbamates, the existing method of insensitive AChE microplate assay is used on the same homogenate since the rapid decarbamylation step prevents use of in vivo exposures; also, carbamates (e.g., propoxur) work well in vitro (Ellman et al., 1961). A drawback of this approach is that resistance mechanisms based on metabolism, specifically those conferring resistance to carbamates but not organophosphates, will be missed. Such mechanisms are generally rare, but they do exist. In these instances, bioassays using carbamates will need to be incorporated into the testing scheme.

The prototype test method features advantages of both the bioassay and microplate assays. The method may be augmented, as necessary, through the use of specific assays (e.g., esterase, AChE) to detect other resistance mechanisms, by using portions of the same homogenate used in the Ellman assay. As a microplate assay, in its augmented form, it shows the resistance mechanism involved and, in certain instances, reveals the phenotype of the individual from small portions of single mosquitos. This test method has allowed the detection of resistance to malathion due to carboxylesterase in A. arabiensis and A. stephensi, as well as multi-resistance due to high esterase activity and insensitive AChE in Guatemalan A. albimanus.

The method has also the advantage of allowing collection of mortality data on the same mosquitos used for microplate assay and may be more effective in the identification of cross-resistance patterns than the specific biochemical assays described earlier.

5. CONCLUSIONS

1. In developing new biochemical tests, all assays should be verified by running the new tests in combination with bioassays and, when necessary, with more complex laboratory methods to verify that there is causal correlation between results obtained with biochemical tests and resistance.
2. Esterase and acetylcholinesterase biochemical assays have been laboratory- and field-tested, and have been shown to correlate well with bioassay data.
3. A tentative test for the mfo resistance mechanism was presented. This was developed and field-tested on one species showing this resistance mechanism. Although there was a good agreement between bioassay data and the mfo test, this method needs further testing against resistance and susceptible populations of a much wider range of species.
4. Two laboratory tests for glutathione S-transferase were identified. The reaction in these tests does not give visually detectable colour changes, hence they may be useful laboratory tools but are unsuitable for field use.

5. No specific tests are yet available for carboxylesterase or kdr-type resistance mechanisms.

6. A novel approach, the target acetylcholinesterase microplate assay, was proposed for detection of resistance to organophosphates and/or carbamates. This test combines elements of the bioassay with microplate assay. It has been laboratory- and field-tested, and the results are encouraging. The method has allowed to detect organophosphate resistance mechanisms based on high esterase, carboxylesterase and insensitive AChE.

7. Immunological tests have been used for detection of esterases. The tests are more specific than the high esterase tests with regard to the types of enzymes involved in the reaction. However, introduction of this type of test in the field is unlikely in the immediate future due to a number of limitations.

6. RECOMMENDATIONS

The following recommendations were made by the participants in the informal consultation:

6.1 General

6.1.1 Bioassay methods must continue to play their role in the detection and monitoring of resistance. Further research should be carried out with existing compounds to improve the accuracy of the tests and modifications sought to extend their applicability to new chemical compounds being introduced in the market.

6.1.2 Resistance assessment should be a field activity. However, the training for a better understanding of the phenomenon of resistance and for conducting resistance tests should be promoted.

6.1.3 When results of bioassay tests are inconclusive regarding resistance status (e.g., when few insect specimens survive the discriminating concentrations), biochemical methods such as the target enzyme tests may be used on survivors to confirm resistance instead of the time-consuming procedure of rearing survivors and testing progeny. When approved, description of the appropriate test methods should be annexed to the instructions for determining the susceptibility/resistance of insect vectors.

6.1.4 Biochemical methods, at their present stage, may help in the identification of the mechanism involved in resistance and the assessment of changes occurring in a field population of insects. Their introduction should provide additional data to supplement information obtained from existing bioassays.

6.1.5 Work on the introduction and development of biochemical tests should be pursued at both the laboratory and field levels.

6.1.6 Promising test methods should be described in detail and appropriate test kits developed. After further review, arrangements should be made for their testing in carefully selected areas.

6.1.7 Training in the use of biochemical methods should be promoted as soon as such methods become operationally available.

6.1.8 Multidisciplinary research should be continued with the objective of providing information essential for the development of biochemical tests.

6.2 Specific

6.2.1 Further efforts should be devoted to refining the test methods already developed and producing prototype field methods for those resistance mechanisms for which biochemical methods are not yet available (e.g., general resistance to pyrethroids/DDT, resistance due to specific carboxylesterase and resistance due to glutathione S-transferase).

6.2.2 Test instructions should take into account extremes of environmental conditions (humidity, temperature, light) likely to be encountered in the field.

6.2.3 New methods should be tested against the widest possible variety of species and populations.

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ANNEX I

PARTICIPANTS

Temporary advisers

Dr W. Brogdon, Research Entomologist, Malaria Branch, Parasitic Diseases Division, Centers for Disease Control, Department of Health and Human Services, Atlanta, Georgia, USA

Professor G. P. Georghiou, Professor of Entomology, Department of Entomology, University of California, Riverside, California, USA (Chairman)

Dr Janet Hemingway, Royal Society Research Fellow, Department of Entomology, London School of Hygiene and Tropical Medicine, Keppel Street, London, United Kingdom (Rapporteur)

Dr Nicole Pasteur, Directeur de Recherche au CNRS (UA 327), Institut des Sciences de l'Evolution, Université de Montpellier II, Place Eugène Bataillon, Montpellier, France

Secretariat

Dr J. F. Copplestone, Chief, Pesticide Development and Safe Use Unit, Division of Vector and Biology Control

Ms F. Lebtahi, Ecology and Control of Vectors, Division of Vector Biology and Control

Dr C. Pant, Chief, Ecology and Control of Vectors, Division of Vector Biology and Control

Dr G. Quelemec, Pesticide Development and Safe Use Unit, Division of Vector Biology and Control (Secretary)

Mr G. Shidrawi, Ecology and Control of Vectors, Division of Vector Biology and Control

Dr R. Slooff, Director, Division of Vector Biology and Control

SPECIES ON WHICH WHO BIOASSAYS AND NEW BIOCHEMICAL METHODS HAVE BEEN RUN IN COMBINATION,
AND THE CONCLUSIONS WITH REGARD TO INSECTICIDE RESISTANCE MECHANISMS

Species	Bioassays*					Synergist	Biochemical tests			Enzyme activity		Metabolism			Comments	Location		
	F	M	D	P	Pn		E	Est	Mfo	AChE	Target	Gst	Mfo	Fenit			DDT	Mal
<u>A. culicifacies</u> (B)	S	R	R	S	S		S	S	S		R	S		✓	✓	A carboxylesterase involvement in Mal-R, GST involvement in DDT-R.	Sri Lanka	
<u>A. subpictus</u>	R	R	R	(R)	S	R	R	S			R	R		✓	✓	✓	Esterase involved in carb-R in larvae. Oxidase involved in OP-R GST in DDT (plus in OP-R?).	Sri Lanka
<u>A. nigerrimus</u>	R	R	R	R	S	R	S	S	R		S	S		✓	✓	✓	AChE giving broad spectrum OP+carbamate resistance; DDE major metabolite in DDT metabolism but no evidence of increased GST activity.	Sri Lanka
<u>A. jamezi</u>	S	S	R	S	S	S	S	S	S		S	S						Sri Lanka
<u>A. annularis</u>	S	S	R	S	S	S	S	S	S									
<u>A. aconitus</u>	S	S	S	S	S	S	S	S	S									
<u>A. subpictus</u> (B)	S	S	R	S	S	S	S	S	S									
<u>A. kawasi</u>	S	S	S	S	S	S	S	S	S									
<u>A. barbirostris</u>	S	S	R	S	S	S	S	S	S									
<u>A. stephensi</u>	S	R	R	S	S	S	S	S	S	R	S	S			✓	Carboxylesterase involvement Mal-R.	Pakistan Delhi	
	S	S	S	S	S	S	S	S	S	S								
<u>A. arabiensis</u>	S	R	R	S	S	S	S	S	R		R	S			✓	✓	Carboxylesterase involvement GST DDT-R.	Sudan Zaire
	S	R	S	S	S	S	S	S	S									
<u>A. albimanus</u>	R	R	R	R	S	R	S	S	R			S					AChE giving broad spectrum OP/carb-R elevated esterase insensitive AChE elevated esterase.	El Salvador Guatemala Panama Haiti
	R	R	R	R	R	R	R	R	R									
	S	S	S	S	S	S	S	S	S									
	R	S	S	S	S	S	R	S	S									
<u>A. gambiae</u>	S	S	R	S	S	S	S	S	S		R	S					GST → DDT R.	Tanzania
<u>C. pipiens</u>	R	R	R	R	S	R	R	S	R			S			✓	Altered AChE + high esterase → OP + carbamate R.	Italy	
<u>C. quinquefasciatus</u>	R	R	R	R	S	R	R	S	S			S		✓	✓	High esterase → OP + carb-R.	Liberia	
<u>C. quinquefasciatus/pipiens</u>	R	R	R	R	S	R	R	S	S			S		✓	✓	High esterase → OP + carb-R.	Israel	
<u>C. tritaeniorhynchus</u>	R	R	R	R	S	R	R	S	S			S		✓	✓	High esterase + AChE → OP + carb-R.	Sri Lanka	

Annex II

SPECIES ON WHICH WHO BIOASSAYS AND NEW BIOCHEMICAL METHODS HAVE BEEN RUN IN COMBINATION,
AND THE CONCLUSIONS WITH REGARD TO INSECTICIDE RESISTANCE MECHANISMS (continued)

Species	Bioassays*					Synergist	Biochemical tests				Enzyme activity		Metabolism			Comments	Location	
	F	M	D	P	Fn		B	Est	Mfo	AChE	Target	Gat	Mfo	Fenit	DDT			Mal
<i>C. vishnui</i>	R	R						R	S								High ester. OP-R.	Sri Lanka
<i>C. pseudovishnui</i>	R	R						R	S								High ester. OP-R.	Sri Lanka
<i>C. helioides</i>	R	R						R	S								High ester. OP-R.	Sri Lanka
<i>C. quinquefasciatus</i>	R	R	R	R	S			R	S								High ester. OP-R.	Sri Lanka
<i>A. albopictus</i>	S	S	S	S	R	S		S	S	S		S	S				Electrophysiology indicates kdr-type pyrethroid-R.	
<i>Ae. aegypti</i>	S	R	R	R	S			R									Esterase based OP resistance.	Caribbean
<i>C. quinquefasciatus</i>	R	R						R	S			-	✓	✓			Esterase based OP-R oxidase based carb-R kdr and oxidase based pyrethroid-R.	USA USA Lab. USA Lab.
				R	R							R	R					
					R													
<i>C. nipiensis</i>				R	R			R	R			✓	✓				OP+carb-R high est. + insensitive AChE.	France
<i>C. quinquefasciatus</i>		R	S	R	S			R	S								OP-R → high esterase DDT + pyrethroid-R → kdr.	Côte d'Ivoire

Key

Bioassays: F = fenitrothion
M = malathion
D = DDT
P = propoxur
Fn = permethrin
B = bendiocarb
* = bioassays conducted on larvae, adults or both

Biochemical test: Est = high esterase
Mfo = multi-function oxidase
AChE = acetylcholinesterase

Enzyme activity: GST = glutathione S-transferase
Mfo = multi-function oxidase

Synergist: DEF = defoliant (S,S,S-triethyl-phosphorothioate)
TPP = triphenyl phosphate
p.b. = piperonyl butoxide

Resistance/susceptibility: R = resistant
S = susceptible

() = indicate results in larvae only.

NOTE: Much of information provided in this table is unpublished or tentative. It may be modified as further research is conducted. List is not complete.