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INSECTICIDE RESISTANCE IN MOSQUITOES: A PRAGMATIC REVIEW

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ABSTRACT. Descriptions of the World Health Organization standard methods of assessing susceptibility or resistance in larval and in adult mosquitoes are presented, and the evaluation of their results are discussed. Other susceptibility test methods are also mentioned, including those based on esterase zymograms. Recent work on the biochemical mechanisms of resistance and cross-resistance are reviewed, along with possible countermeasures for the problem of mosquito resistance, now known in 113 species of culicines and anophelines.

INTRODUCTION

This review is an update of a section originally written for the revision of AMCA Bulletin No. 2 (Ground Equipment and Insecticides for Mosquito Control) in 1976, and subsequently brought up to date twice. Finally, the abandonment of a general revision for Bulletin No. 2 resulted in certain of its sections, this being among them, being chosen for publication in the *Journal of the American Mosquito Control Association.*

The original purpose having been to introduce and explain the test methods used to detect and measure insecticide resistance in mosquitoes, the present review also discusses the recent research on the biochemistry and genetics of resistance and the ideas for remedial action where resistance occurs. The coverage of the literature includes that subsequent to a previous detailed review which extended up to 1980 (Brown 1983).

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[3] Resistance of vectors and reservoirs of disease to pesticides: 10th report of the Expert Committee on Vector Biology and Control. W.H.O. Tech. Rep. Series (1986).

NATURE AND EXTENT OF INSECTICIDE RESISTANCE

The development of resistance by mosquitoes to the compounds used against them as larvicides and adulticides was first observed in 1947, when the salt-marsh mosquitoes *Aedes taeniorhynchus* and *Ae. sollicitans* began to show resistance to DDT in Florida. Since then, populations which have developed resistance to organochlorines (DDT and/or dieldrin) are known in 109 mosquito species throughout the world; 58 species have developed resistance to organophosphorus (OP) insecticides, of which 4 had not been recorded as organochlorine-resistant. Also among these species, 17 have now shown adult resistance to the carbamates propoxur or bendiocarb, and 10 have shown either resistance or cross-resistance to certain pyrethroids. Multiple resistance to all 4 of the above-mentioned chemical groups in the same population of a mosquito species has been developed in certain areas by *Ae. aegypti, Culex pipiens, Cx. quinquefasciatus, Anopheles albimanus, An. culicifacies, An. pseudopunctipennis, An. sacharovi* and *An. stephensi.*

The development of resistant populations is observed in the field as a progressive decrease in the control obtained by the dosage recommended on the basis of its effectiveness when the insecticide was first introduced. To obtain proof that the control failure observed is due to resistance in the target mosquitoes themselves, and not to such factors as deficiency of the formulation, inefficient application, or unfavorable meteorological conditions, it is necessary to submit a sample of the target population to a set test of its susceptibility to the insecticide. Methods for such susceptibility-resistance tests, of international validity, have been stan-

dardized by the World Health Organization (WHO) for both adult and larval mosquitoes.

Such susceptibility/resistance tests should be regularly made as an integral adjunct of pest management, so that the progressive development of resistance to the insecticide in use may be detected before it reaches the point of a control failure. For example, the normal LC_{50}[4] levels to malathion for *Aedes* larvae range between 0.01 and 0.04 ppm; instances of control failure where the target population shows an LC_{50} in excess of 0.25 ppm may be concluded to be true cases of resistance; moreover the survival of any larvae at all at this test dosage indicates that the population sampled is at least on its way to becoming resistant.

MECHANISMS OF RESISTANCE. The characteristic of insecticide resistance is inherited, and in most cases it has proved to be due to unitary genetic factors (gene alleles) for resistance. The resistance allele may be either recessive (as in certain DDT-resistances), or dominant (as in OP-resistance), or codominant, the resistant-susceptible hybrids being intermediate (as in dieldrin-resistance). In a mosquito population, resistance is induced by a process of selection which increases the proportion of resistant genotypes by killing off, generation after generation, the individuals with the normal susceptible alleles. Laboratory strains are known which are genetically pure for resistance, all the individuals being homozygous for the resistance allele, but resistant field populations almost invariably contain some heterozygotes and the susceptible alleles are always infiltrating back from surrounding untreated areas (Georghiou 1980a).

Resistance is not general but is usually specific to the insecticide which induced it, with greater or less cross-resistance to those other insecticides which are in the same molecular group. Among the organochlorine insecticides, two different types of resistance occur: DDT-resistance does not extend to dieldrin and its relatives, nor to OP compounds: dieldrin-resistance extends to gamma-HCH but not to DDT, nor to the OP insecticides. OP-resistance may be subdivided to the extent that malathion often induces a resistance that does not include other OP insecticides; however, selection with any OP compound usually induces cross-resistance to the others in greater or less degree.

Resistance is usually due to a detoxication of the insecticide due to mutant enzymes (isozymes) engendered by the resistance gene alleles, but some resistance may also be conferred by a reduced uptake of the toxicant. In DDT-resistant mosquitoes (including *An. gambiae*), the resistance is mainly due to an increase in the enzyme DDT-dehydrochlorinase, a type of glutathione S-transferase (Clark and Shamaan 1984) which detoxifies DDT to DDE. Another mechanism of DDT-resistance is nerve insensitivity, due to a knockdown-resistance (*kdr*) gene which also confers pyrethroid-resistance; it involves a difference in nerve ultrastructure in that there are probably fewer target site receptors for DDT and pyrethroids. Dieldrin-resistance is evidently (from studies in the German roach) associated with a deficiency in those tertian receptors in nerves which are blocked by picrotoxinin as well as cyclodienes; a decrease in the number of these receptors results in the dieldrin-R types having less binding affinity for the cyclodiene compounds or gamma-HCH so that the target sites have less chance of being blocked by them (Kadous et al. 1983).

In *Culex p. pipiens, Cx. quinquefasciatus* and other culicids, OP-resistance is due to esterase isozymes which can break down OP compounds by phosphatase-type hydrolysis (in the case of malathion by carboxylic-ester hydrolysis as well), and individual mosquitoes may be tested by electrophoretic chromatography for these detoxifying esterases (Pasteur and Georghiou 1980). In *Anopheles albimanus* strains in El Salvador, however, malathion-resistance is due to their acetylcholinesterase (AChE) being an isozyme which is insensitive to inhibition by malathion and malaoxon; it is also insensitive to propoxur, a carbamate which is an anticholinesterase like the OP insecticides (Ayad and Georghiou 1975). The OP-insensitive AChE was 5 times more slowly inhibited by malaoxon or fenitroxon than a normal strain (Hemingway and Georghiou 1983). Whereas carbamate-resistance in *An. albimanus* is partly due to the AChE being insensitive to propoxur also, in *Cx. quinquefasciatus* it proved to be due to increased detoxication (e.g., of propoxur) by oxidative enzymes (Shrivastava et al. 1971).

ORGANOCHLORINE-RESISTANCE. In this review a minimum of attention is paid to the organochlorine compounds in the DDT and cyclodiene groups, since they are no longer employed in the USA. However, DDT is still widely used as a residual adulticide for malaria control and eradication in the developing world, and among the anophelines 56 species have developed DDT-resistance (Table 1). The total number of the various species known to have developed dieldrin-resistance (a resistance which extends to gamma-HCH also) stands at 50 in this table, but dieldrin is no longer used

[4] The concentration at which 50% of the specimens are killed.

Table 1. Occurrence of resistance in anophelines to residual organochlorine adulticides (some countries omitted where insufficient space).

Species	Resistance to DDT	Resistance to dieldrin/HCH
**Anopheles aconitus*	Nepal, Bangladesh, Indonesia, Thailand	Indonesia
**albimanus*	Mexico, Central America, Caribbean, Colombia	Mexico, Central America, Caribbean, Ecuador
albitarsis	Colombia, Brazil	Venezuela
**annularis*	Pakistan, Nepal, India, Burma, Thailand	Pakistan, Nepal, India, Indonesia
apicimacula	Panama	—
aquasalis	—	Trinidad, Venezuela, Brazil
**arabiensis*	Senegal, Sudan, Swaziland, Mauritius	Mauritania, Ethiopia, Zimbabwe, Madagascar
atroparvus	UK, Portugal, Spain, Romania, USSR	Spain, Romania, Bulgaria
balabacensis	Bangladesh, Burma, Malaysia, Thailand	—
barbirostris	India, Indonesia, Sri Lanka	Indonesia, Thailand
coustani	Egypt	Egypt, Saudi Arabia
crucians	Mexico	South Carolina, Dominican Rep.
**culicifacies*	Iran, Pakistan, India, Sri Lanka, Burma	Oman, Afghanistan, Pakistan, India, Nepal
darlingi	Colombia	—
donaldi	Malaysia	Malaysia
flavirostris	Malaysia	Philippines
fluviatilis	Pakistan, India, Nepal	Pakistan, India, Saudi Arabia
funestus	Mali	Mali, Ghana, Benin, Nigeria, Cameroon, Kenya
**gambiae*	Liberia, Niger, Togo, Cameroon, Zaire, R.S.A.	Mauritania, Mali, Zaire, Kenya, Madagascar
hyrcanus	USSR, Turkey, Afghanistan	Turkey, Afghanistan, India, Sri Lanka
jamesi	Burma	Sri Lanka
kochi	India	—
koliensis	Indonesia	—
labranchiae	Morocco, Algeria, Tunisia	Morocco, Algeria, Tunisia
littoralis	Malaysia	—
maculatus	Pakistan, India, Burma, Thailand	—
**maculipennis*	Romania, USSR, Greece, Turkey, Iran	Greece, Turkey
martinius	USSR	—
melanoon	Turkey	Turkey
messeae	Romania, Bulgaria, USSR	Romania, Bulgaria
minimus	Thailand	Indonesia, Thailand
multicolor	Saudi Arabia	Egypt, Saudi Arabia
nigerrimus	Pakistan, India, Burma, Indonesia, Thailand	Pakistan, India, Sri Lanka, Burma
nivipes	Thailand	—
pallidus	India, Sri Lanka	Sri Lanka
peditaeniatus	Indonesia, Vietnam	—
**pharoensis*	Egypt, Sudan, Ethiopia, Angola	Israel, Egypt, Sudan
philippinensis	Bengal, Burma, Thailand	Sabah
**pseudopunctipennis*	Mexico, Guatemala, Honduras, Panama, Peru	Mexico, Guatemala, Honduras, Nicaragua, Venezuela
pulcherrimus	USSR, Iraq, Afghanistan, Pakistan	Syria, Saudi Arabia, Pakistan
punctimacula	Panama, Colombia, Ecuador	—
punctulatus	Indonesia	—
quadrimaculatus	Maryland, Georgia, Mexico	Mississippi, Georgia, Mexico
**sacharovi*	USSR, Greece, Turkey, Syria, Iraq, Iran	Greece, Turkey, Lebanon, Syria, Iraq
**sergenti*	Egypt	Jordan
**sinensis*	Vietnam, China, Japan	S. Korea
splendidus	India	Pakistan
**stephensi*	Sudan, Arabia, Iran, Iraq, Pakistan, India	Arabia, Oman, Iran, Iraq, Afghanistan, India
strodei	—	Venezuela
subpictus	Afgh., Pakistan, India, Indonesia, Vietnam	Afghan., India, Bangladesh, Sri Lanka, Indonesia
sundaicus	Indonesia, Malaysia, Thailand	Indonesia, Malaysia
superpictus	USSR, Afghanistan	—
tessellatus	India, Nepal, Sri Lanka, Indonesia	India, Sri Lanka
triannulatus	Bolivia	Colombia, Venezuela
turkhudi	Afghanistan	—
vagus	Bangalesh, Malaysia, Thailand, Vietnam	Nepal, Indonesia, Vietnam, Philippines
varuna	India, Nepal, Sri Lanka	Sri Lanka
vestitipennis	Mexico, Guatemala	—

In addition, records of the following species resistant to dieldrin/HCH only: *d'thali* (Iran), *farauti* (Solomons), *filipinae* (Philippines), *neomaculipalpis* (Trinidad, Colombia), *nili* (Ghana), *rangeli* (Venezuela), *rufipes* (Mali).

* Important malaria vectors in which insecticide resistance has had a serious impact.

for domiciliary application. The countries in which these resistances have developed, for all but the most recent records, are shown in detail in Annex 1 of the 22nd report of the WHO Expert Committee on Insecticides (World Health Organization 1980). Among culicine mosquitoes, DDT-resistance is known in 39 species, and dieldrin-resistance in 31 species, (Table 2), also shown in more detail in Annex 1 of the 22nd report. Neither DDT nor dieldrin are acceptable for the larviciding and area adulticiding that culicine control usually requires.

ORGANOPHOSPHORUS-RESISTANCE. It is the organophosphorus (OP) group of insecticides which demands attention, since it constitutes almost all of the present-day larvicides, and their use is increasing as residual adulticides also. The carbamate compounds have not as yet proved effective as larvicides, but they have

Table 2. Occurrence of resistance in culicines to organochlorine larvicides or adulticides.

Species	Resistance to DDT	Resistance to dieldrin/HCH
Aedes		
aegypti	Almost every infested country except African	Almost every infested country except African
albopictus	India, Malaysia, SE Asia, Philippines, Japan	India, Malaysia, SE Asia, Philippines, Japan
atroparvus	Oklahoma	—
cantans	W. Germany, Czechoslovakia	Czechoslovakia
cantator	New Brunswick	New Brunswick
caspius	Kuwait, Sudan	Kuwait
detritus	S. France	S. France
fijiensis	Fiji	—
melanimon	California	California
nigromaculis	California, Utah	California, Utah
polynesiensis	French Polynesia, Fiji	—
pseudoscutellaris	Fiji	—
sierrensis	California	—
sollicitans	Florida, Delaware	Florida, Delaware
taeniorhynchus	Florida, Georgia, Cayman	Florida, Georgia, Cayman
togoi	S. Korea	—
vexans	British Columbia	—
Armigeres		
subalbatus	Sri Lanka, Malaysia, Japan	Sri Lanka, Japan
Culex		
andersoni	Ethiopia	—
antennatus	Egypt	Egypt
coronator	Panama	—
erythrothorax	California	—
fuscocephalus	Taiwan	Taiwan
gelidus	India, Bangladesh, Thailand	India, Thailand
nebulosus	—	Benin
nigripalpus	Florida	—
peus	California	California
pipiens pipiens	USA, N. Africa, Europe, Middle East	USA, N. Africa, Europe, Middle East
pipiens pallens	China, Japan, Korea	China, Japan, Korea
quinquefasciatus	Tropics, subtropics & adjacent temperates	Tropics, subtropics & adjacent temperates
poicilipes	Benin	—
pusillus	Egypt	Egypt
restuans	Illinois, New York	Illinois, New York
salinarius	New Jersey	Texas
tarsalis	California, Oregon, Washington, Utah	California, Oregon
theileri	—	Egypt
tritaeniorhynchus	Benin, Nigeria, Bangladesh, China, Japan, Korea	Benin, Nigeria, China, Japan, Korea
univittatus	Egypt	Egypt
vishnui	Taiwan	Taiwan
Culiseta		
inornata	N. California	N. California
Mansonia		
annulifera	Thailand	Thailand
indiana	Thailand	—
uniformis	—	Thailand
Psorophora		
confinnis	—	Mississippi
discolor	—	Mississippi

been employed as residual adulticides and in adulticidal mists. OP-resistance is now known in 59 species of mosquitoes (see below); carbamate-resistance has been found in 13 species of anophelines and in *Culex pipiens*. The most severe problems have developed in agricultural areas, where large volumes of OP insecticides such as parathion-methyl and fenthion, and certain carbamates such as carbaryl and propoxur, are applied to crops, e.g., in the Adana region of Turkey, El Salvador and western Nicaragua in Central America, and the San

Joaquin valley of California. In southern Spain, the resistance level of the *An. atroparvus* in the Cadiz region to temephos, fenitrothion and malathion was highest in those areas that had been most heavily treated with agricultural insecticides, a situation analogous to the OP-resistance of *An. albimanus* in El Salvador (A. Encinas Grandes and E. Astudillo Sagrado, J. Med. Entomol., in press).

Culicines. OP-resistance is known to have developed in 28 species of culicine mosquitoes (Table 3), a more complete locality list being

Table 3. Occurrence of resistance in culicines to organophosphorus (OP) adulticides or larvicides.*

Species	Adulticide or larvicide				
	Malathion	Fenthion	Fenitrothion	Chlorpyrifos	Temephos
Aedes					
aegypti	Carib., SE Asia	Carib., Malaysia	Caribbean		Carib., Malaysia
albopictus	Sing., Vietnam	Malaysia	Madagascar		
canadensis				N. Central USA	
caspius	Kuwait				France, Spain
detritus					France
dorsalis	Utah	Utah, N.M.			
melanimon	California	California			
nigromaculis	California	Calif., Utah	California	California	California
sollicitans	N.J., Virginia				SE USA
taeniorhynchus	Florida				
togoi		S. Korea		S. Korea	
vexans	Utah	Utah			Spain
Armigeres					
subalbatus	Sri Lanka, Japan				
Culex					
antennatus	Egypt		Egypt		
annulirostris	Australia				
fuscocephalus	Taiwan	Taiwan	Taiwan	Taiwan	Taiwan
gelidus	Sri Lanka				
peus	California	California		California	California
pipiens pipiens	USA, W. Eur., Mid-E.	France, Eg., Isr.	Egypt, Kuwait	France, Eg., Isr.	USA, Fr., Eg., Isr.
p. pallens	China, Jap. Korea	China	China, Japan	Japan	China, Japan
quinquefasciatus	General	General	General	General	General
restuans	USA				
tarsalis	California	California	California	California	California
theileri	Spain		Spain		Spain
tritaeniorhynchus	Japan, Korea	Japan, Korea	China, Jap., Korea	Japan, Korea	China, Jap., Korea
vishnui	Taiwan				Taiwan
Culiseta					
inornata	N. California	N. California		N. California	N. California

* Also resistance to bromophos in *Cx. pusillus* (Egypt), and to pirimiphos-methyl in *Cx. pipiens pallens* (China) and *Cx. quinquefasciatus* (Bangladesh).

given in Table 2 (Annex 1) of the WHO publication mentioned above. The history of the OP-resistance of *Aedes nigromaculis* in Kings and Tulare counties of California, where parathion larvicide was introduced as early as 1952 because of the high organochlorine-resistance, epitomizes the problems faced today in the chemical control of mosquitoes. Parathion-resistance reached 70-fold[5] by 1961, and parathion-methyl was substituted since its LC_{50} had by then become one-fifteenth that of parathion. However, by 1963 the *Ae. nigromaculis* had developed a 200-fold resistance to parathion-methyl. Fenthion was introduced in 1962, being then effective although its LC_{50} had already been increased about 20-fold by cross-resistance from the two parathions. Control failures with fenthion started to occur in 1968. Chlorpyrifos and temephos were introduced in 1966, the cross-resistance to them being at that time only 7 and 12 times the normal respectively, but control failures with them began to appear in 1969. This pasture mosquito had by 1975 become resistant to all these OP larvicides throughout the San Joaquin valley and most of the Sacramento valley (Gutierrez et al. 1976). *Aedes nigromaculius* was also found to have developed resistance to fenthion and parathion in 'Davis county, Utah (Hart and Womeldorf 1976).

In California, *Ae. melanimon* first became resistant to parathion in Tulare County in the south and Yuba County in the north (Gillies et al. 1971), and subsequently showed resistance to malathion and fenthion (Guttierez et al. 1976). In *Ae. dorsalis,* fenthion-resistance was first observed in Santa Fe, N.M. (Harmston, *in* Brown and Pal 1971); subsequently this resistance was found in Utah southwards from Weber County, where *Ae. vexans* had also devel-

[5] i.e., the LC_{50} of some samples was 70 times the base-line LC_{50}.

oped resistance to fenthion and malathion (Merrell and Wagstaff 1977).

In Florida, where *Ae. taeniorhynchus* had been combatted by aerosols and airsprays of malathion since 1955, control failures due to malathion-resistance appeared in Lee County in 1965; by 1978 this resistance had become prevalent along most of its Atlantic coast and the southern half of its Gulf coast, being highest on the Florida keys (offshore islands), where the adult resistance ranged up to 40-fold, but with no resistance to fenthion and naled (Boike et al. 1978). Malathion-resistance had developed in *Ae. sollicitans* on Langley Air Force Base, Virginia, in 1968 after 10 years of malathion aerosol treatments (Mount et al. 1969). In certain areas of New Jersey, subpopulations of *Ae. sollicitans* now require a 10-fold increase in malathion dosage for ULV kill of adults (Sutherland et al. 1983). Laboratory selection with malathion for 13–14 generations further doubled this level of adult malathion-resistance (Sutherland and Khoo 1984).

In the Caribbean area, malathion and fenthion had been employed in domestic perifocal treatments against *Ae. aegypti* because of the development of DDT-resistance in 1955 and soon after. By 1974 malathion-resistance was present on 6 of the islands, and fenthion-resistance on 8 of them, while on the Virgin Islands and Bahamas resistance was developing to the temephos which had been substituted (Pan American Health Organization, *in* Pal 1976). OP-resistance was also found in persisting populations of *Ae. aegypti* in Colombia, Venezuela and French Guiana. Meanwhile, *Ae. albopictus*, an indigenous vector of the dengue viruses in the eastern hemisphere, developed resistance to malathion in Vietnam and Malaysia, and to fenitrothion in Madagascar (World Health Organization 1980).

In *Culex tarsalis,* vector of western equine encephalitis virus, resistance to malathion appeared near Fresno, California in 1956, in an area which had been treated with malathion granules for the preceding 5 years. This particular resistance, specific to malathion alone among the OP compounds and due exclusively to increased detoxication by carboxyesterase activity, disappeared by 1959. However, by 1969 populations of *Cx. tarsalis* in the San Joaquin valley had developed a generalized type of OP-resistance, which in Kings County included chlorpyrifos and temephos (Georghiou et al. 1969). By 1975, this generalized OP-resistance extended almost continuously throughout the San Joaquin and Sacramento valleys (Gutierrez et al. 1976).

In the *Culex pipiens* complex, resistance to fenthion, fenitrothion and malathion appeared in *Cx. quinquefasciatus (fatigans)* in certain spots in tropical Africa, the Far East and California. Sometimes the loss of susceptibility is very slow (as in Rangoon, Burma) or the resistance may revert to susceptibility (as at Douala, Cameroon); in California the resistance includes chlorpyrifos and temephos as well, occurring principally in the Kings, Delano, Turlock and San Mateo areas. The use of chlorpyrifos for *Cx. quinquefasciatus* control in several urban areas of Tanzania was followed by resistance to chlorpyrifos, malathion, fenthion and other OP insecticides (Curtis and Pasteur 1981). This generalized type of OP-resistance has occurred in *Cx. p. pipiens* in Egypt, Israel and France, and in *Cx. pipiens pallens* in Japan, China and South Korea. OP-resistance is also developing in *Cx. tritaeniorhynchus* and two other *Culex* vectors of Japanese B encephalitis virus in these western Pacific areas (World Health Organization 1980).

Anophelines. In the world-wide campaign against malaria, OP-resistance is now known in 31 species of *Anopheles* (Table 4). Resistance to malathion was first found in *An. albimanus* in El Salvador and western Nicaragua (Georghiou et al. 1972), 4 years after it had been introduced in 1965 because of the ineffectiveness of organochlorine residual sprays; this OP-resistance had evidently been induced by agricultural applications. The El Salvador strain was resistant to most OP compounds except fenthion, and also resisted the carbamate propoxur (Herath and Davidson 1981c). When malathion had been introduced against organochlorine-resistant *An. culicifacies* in two states of western India in 1968, malathion-resistance was discovered in 1973 (Rajagopal 1977) and had become common by 1975; it was accompanied by resistance to fenitrothion, although this OP had not been used against malarial vectors (Herath et al. 1981). Subsequently, the introduction of malathion in Iran induced malathion-resistance in *An. stephensi* both on the coast (Manouchehri et al. 1976) and in the interior (Eshghy 1978), and in Iraq it induced malathion-resistance in Basrah province (Manouchehri et al. 1980). This resistance was later reported to extend to fenitrothion and to be present in *An. stephensi* in India (World Health Organization 1980).

Malathion has also been employed since 1968 against *An. sacharovi* in the Adana coastal area of southern Turkey, but by 1976 it was failing to control malaria transmission. Adult susceptibility tests revealed that the resistance was weak to malathion and pirimiphos-methyl but strong to fenthion and fenitrothion (Ramsdale et al. 1980), thus implicating agricultural insecticides such as parathion-methyl and fenthion as being the main causative agents. A similar resistance

Table 4. Occurrence of resistance in anophelines to organophosphorus (OP) adulticides or larvicides.

Species	Adulticide or larvicide				
	Malathion	Fenthion	Fenitrothion	Chlorpyrifos	Temephos
An. albimanus	Mex., Cent. Am.		Mex., Cent. Am.	El Salvador	
annularis	Sri Lanka		Sri Lanka		
arabiensis	Sudan				
atroparvus	Spain	Portug., Spain			Spain
balabacensis			Malaysia		
barbirostris	Sri Lanka		Sri Lanka		
coustani	Egypt		Egypt		
culicifacies	Pak., India		Pak., India	Un. Arab. E.	
d'thali					Jordan
fluviatilis	Pakistan		Pakistan		
funestus	Mali		Mali		
hyrcanus	Sri Lanka	Turkey	Turkey		
jamesi	Sri Lanka				
karwari	Sri Lanka				
labranchiae	Italy				
maculipennis		Romania	Rom., Greece, Turk.		
messeae	Romania	Romania			
multicolor	Egypt	Egypt		Egypt	Jordan
nigerrimus	Sri Lanka	Pak., Sri Lanka	Sri Lanka		
pallidus	Sri Lanka		Sri Lanka		
pharoensis	Egypt	Egypt	Egypt	Egypt	
pseudopunctipennis	Honduras		Guatemala		
rhodesiensis				Djibouti	
sacharovi	Turkey	Turkey	Bulg., Greece, Turk.	Turkey	
sergenti					Jordan
sinensis	China, Japan	Japan, Korea	China		
stephensi	Iran, Iraq, Pak.	Pak., India	Iran, Iraq, Pak.		India
subpictus	Sri Lanka		Sri Lanka		
tessellatus	Sri Lanka				
vagus	Sri Lanka		Sri Lanka		
varuna	Sri Lanka		Sri Lanka		

was found in this species in central Greece, in the Akkar agricultural district of coastal Lebanon, and in Syria; moreover, resistance to fenitrothion was present in *An. hyrcanus* in Turkey, and in *An. maculipennis* in Greece and Romania.

Malathion-resistance has been recently found in *An. messeae* in Romania and *An. labranchiae* in Italy, as well as *An. atroparvus* in Spain and *An. arabiensis* in Sudan (Hemingway et al. 1980). Whereas in *An. arabiensis* the resistance was restricted to malathion, in *An. messeae* it extended to fenthion and in *An. atroparvus* it included fenitrothion, fenthion and chlorphoxim (Hemingway 1982a) and temephos (Encinas Grandes and Astudillo Sagrada, in press). Resistance to fenitrothion has been reported for *An. funestus* in Mali (Touré 1982).

Among the 31 species records of OP-resistance in anophelines (Table 4), 26 have involved malathion, 20 fenitrothion, 10 fenthion, 6 chlorpyrifos and 5 temephos. Resistance to other OP residual insecticides has been found in the following species:

Chlorphoxim: *albimanus* (Salvador), *hyrcanus* (Turkey), *nigerrimus* (Sri Lanka), *sacharovi* (Turkey), *stephensi* (Iran), *vagus* (Sri Lanka).

Pirimiphos-methyl: *albimanus* (Salvador), *labranchiae* (Morocco), *stephensi* (Iran, Iraq).

Bromophos: *multicolor* (Egypt)

The areas left blank in Table 4 do not necessarily indicate non-resistance, but are simply the limitations of the reports available.

RESISTANCE TO OTHER INSECTICIDE GROUPS

Carbamate-resistance. The following 14 species of anophelines have developed field populations resistant to carbamates, often as a direct result of the use of carbamates in agriculture;

albimanus (El Salvador)
atroparvus (Portugal, Romania)
culicifacies (Oman, India)
hyrcanus (Turkey, USSR)

labranchiae (Morocco)
maculipennis (Greece, Romania)
multicolor (Egypt)
nigerrimus (Sri Lanka)
pharoensis (Egypt)
pseudopunctipennis (Honduras)
sacharovi (Bulgaria, USSR)
stephensi (Iran, Pakistan, India)
subpictus (Sri Lanka)
vagus (Sri Lanka)

In fact, the resistance mechanism of less-sensitive AChE is stronger against carbamates than OP compounds, and has been found not only in *An. albimanus* but also *An. atroparvus, An. nigerrimus* and *An. sacharovi,* as well as *Culex pipiens, Cx. quinquefasciatus* and *Cx. tritaeniorhynchus.* Resistance to the carbamate propoxur (Baygon) was shown by the *An. albimanus* in Central America (Georghiou 1972) and the *An. sacharovi* in southern Turkey (Ramsdale et al. 1980). Both instances were probably induced by the applications of carbaryl in agriculture, intensified in the case of *An. albimanus* by the use of propoxur in anti-malaria programs since 1968. The OP-resistance in the Spanish strain of *An. atroparvus* was accompanied by propoxur-resistance, multifactorial genetically, which also extended to bendiocarb (Hemingway 1982a). Among culicines in the USA, incipient resistance to propoxur applied in adulticidal mists was discovered to exist in adult *Cx. quinquefasciatus* in the Pixley area, Tulare County (Georghiou 1977), and erratic results had been obtained with propoxur mists against *Ae. nigromaculis* in some districts of California (Womeldorf et al. 1971). In various parts of southern California, none of the highly OP-resistant larval populations of *Cx. quinquefasciatus* showed any resistance to propoxur (El-Khatib and Georghiou 1985).

Pyrethroid-resistance. The following 10 species of anophelines and culicines have developed populations resistant or cross resistant to pyrethroids:

An. albimanus (El Salvador)
arabiensis (Sudan)
culicifacies (India, Sri Lanka)
multicolor (Egypt)
nigerrimus (Sri Lanka)
An. pseudopunctipennis (Guatemala)
sacharovi (Turkey)
stephensi (India)
Ae. aegypti (Malaysia, Thailand, Guyana, USA)
Cx. quinquefasciatus (India, USA)

These discoveries in field populations had been preceded by the induction of pyrethroid-resistance in laboratory selection experiments. For example, trans-permethrin induced a strong larval resistance in *Cx. quinquefasciatus* in the laboratory (Priester and Georghiou 1978). Permethrin-resistance has also been induced in a DDT- and pyrethroid-tolerant strain of *Aedes aegypti* from Bangkok by trans-permethrin selection, where it was associated with increased DDT-dehydrochlorinase, and an ability to tolerate the permethrin absorbed into the adult, characteristic of the *kdr* type of resistance as originally known in house flies (Brealey et al. 1984). Larval cross-resistance to pyrethroids has been shown by DDT-resistant populations of *Ae. aegypti* on the Demerara coast, Guyana (Prasittisuk and Busvine 1977) and at Bangkok, Thailand (Chadwick et al. 1977). Adult pyrethroid-resistance was found present in the malathion-resistant *An. arabiensis* from the Gezira, Sudan (Davidson and Curtis 1979), and larval pyrethroid-resistance resulted from the DDT selection of a strain of *An. stephensi* from Kasur, Pakistan (Omer et al. 1980). In the Pakistan *An. stephensi,* larval neuromuscular site-insensitivity was indicated, the body absorption of the permethrin being always much more in the resistant than in the susceptible larvae (Priester and Georghiou 1980).

Other resistances. In laboratory selection experiments with Insect Development Inhibitors (or IGRs—Insect Growth Regulators), the juvenile-hormone mimic methoprene (Altosid) has induced a 100-fold larval resistance in a normal strain of *Cx. p. pipiens* (Brown et al. 1978) and a multiresistant strain of *Cx. tarsalis* (Georghiou et al. 1974). The chitin-synthetase inhibitor diflubenzuron (Dimilin) did not induce resistance in either of these laboratory-cultured species of *Culex.* In the field, resistance to juvenoids has been found in *Culex p. pipiens* in Iraq, and in *Cx. quinquefasciatus* in the USA and Tanzania. Resistance to diflubenzuron has also been detected in *Cx. quinquefasciatus* in Tanzania.

TEST METHODS TO DETERMINE SUSCEPTIBILITY OR RESISTANCE

The function of susceptibility-resistance tests is not only to provide confirmation where resistance was responsible for the failure of control; their most important use is to detect the decay in susceptibility level and the emergence of resistance sufficient to cause a control failure. Target populations of mosquito species should be tested from year to year in order to ascertain whether their LC_{50} or LC_{95} levels, as measures of susceptibility, are increasing over the original level. This baseline will have been already determined from field populations or laboratory colonies which have not yet been exposed to the insecticide involved in the control operation. A later and less laborious test is to detect the presence of individual resistant lar-

vae by exposing the sample to a diagnostic concentration of the insecticide which assuredly kills all but the resistant individuals.

The original larval test (World Health Organization 1970) has been revised successively by mimeographed documents WHO/VBC/75.583 and WHO/VCB/81.807.[6] A tentative test for resistance to insect development inhibitors was originally printed (World Health Organization 1976), and has later been revised (document WHO/VBC/81.812).

LARVAL TESTS. Resistance tests performed on larvae are applicable to culicine control, where larvicides are used against urban and suburban *Culex*, or when source control is applied against *Aedes* or *Culex*.

In the WHO standard test, mosquito larvae in lots of 20–25 are exposed for 24 hours to the test insecticide in water at 4 different concentrations. About 300 larvae in the 3rd or early 4th instar are required for the complete test. The WHO test kit (Fig. 1) provides standard solutions of the principal larvicides in ethanol, so designed that aliquots of 1 ml of the standard added to 250 ml water give the following test concentrations in mg/liter (ppm):

Malathion	3.125	0.625	0.125	0.025			
Temephos		0.625	0.125	0.025	0.005		
Fenthion			0.125	0.025	0.005	0.001	
Fenitrothion			0.125	0.025	0.005	0.001	
Chlorpyrifos				0.025	0.005	0.001	0.0002

Bromophos is also available, but subsequent to 1980 parathion, DDT, dieldrin and gamma-HCH have been discontinued as test larvicides. The concentrations in the standard solutions are 250 times those in the test resulting from their use, e.g., a standard of 156.25 mg/liter gives a test concentration of 0.625 mg/liter (ppm). It will also be noted that each concentration for which a standard is provided is 5 times higher than the preceding one, i.e., a 5-fold concentration interval. This wide-mesh net serves to provide sufficient range to obtain test mortalities above 0% and less than 100% for any population sample of larvae, whatever its susceptibility level. Intermediate concentrations may be intercalated into the test, to give a concentration interval of the square root of 5, i.e., 2.24. Thus if "partial" mortalities (between 0 and 100%) were obtained at 0.005 and 0.25 ppm, intermediate concentrations of 0.0112 ppm, as well as 0.00224 and 0.0558 ppm, may be added to the test; thus a series of concentrations is achieved which come at regular intervals on a logarithmic scale. The dosage-mortality figures obtained are plotted on graph paper relating the dosage on a logarithmic scale to the percentage mortality on a probability (probit) scale, and a regression line is drawn to fit these points. From this line the susceptibility level may be read off in terms of the LC_{50}, that concentration in mg/liter (ppm) which is expected to cause 50% mortality; figures in a higher mortality range, e.g., the LC_{90}, may also be read off.

The results obtained with the population tested are then compared with the base-line figures for that species, obtained from populations at such a time and place that their previous insecticide history had been sufficiently negligible that they can be considered as being of normal susceptibility (e.g., Table 5). Utilizing these base-line figures, routine surveillance and the monitoring for resistance can be made by tests at a single concentration which is just sufficient to ensure complete kill (or rather 99.9% mortality on the log-probit graph paper) as indicated by the regression line for the normal population of base-line susceptibility. To serve as a guide, the World Health Organization (1980) has proposed, on the basis of experience of many years, the following tentative diagnostic dosages (in mg/liter):

	Malathion	Temephos	Fenthion	Fenitrothion	Chlorpyrifos
Aedes aegypti	1.0	0.02	0.05	0.06	0.01
Culex pipiens complex	1.0	0.02	0.05	0.125	0.01
Anopheles spp.	3.125	0.25	0.05	0.125	0.025

Survival of any larvae from exposure to the diagnostic dosage would indicate the possibility of resistance among the population tested, and the necessity of performing the full multiple-concentration test to validate the results.

For insect development inhibitor (IDI) compounds (also known as juvenoids or IGRs), a special WHO test method and kit is available, in which 4th-stage larvae, shortly before they are due to pupate, are exposed to the test concentrations for 6 hours; those that pupated during the test are discarded from consideration. In scoring, the percentage determined as affected consists of those that subsequently failed to pupate or to emerge free of the pupal case. Standard solutions of methoprene and diflubenzuron are provided for the following test concentrations in ppm:

0.002 0.004 0.001 0.05 0.25

[6] Instructions for determining the susceptibility or resistance of mosquito larvae to insecticides 6 pp. mimeo. Obtainable from Vector Biology and Control, World Health Organization, 1211 Geneva 27, Switzerland, along with a catalogue of test insecticides available and price list for test kits. Enquiries may also be made to the Pan American Health Organization, 525 Twenty-third Street N.W., Washington D.C. 20037, USA.

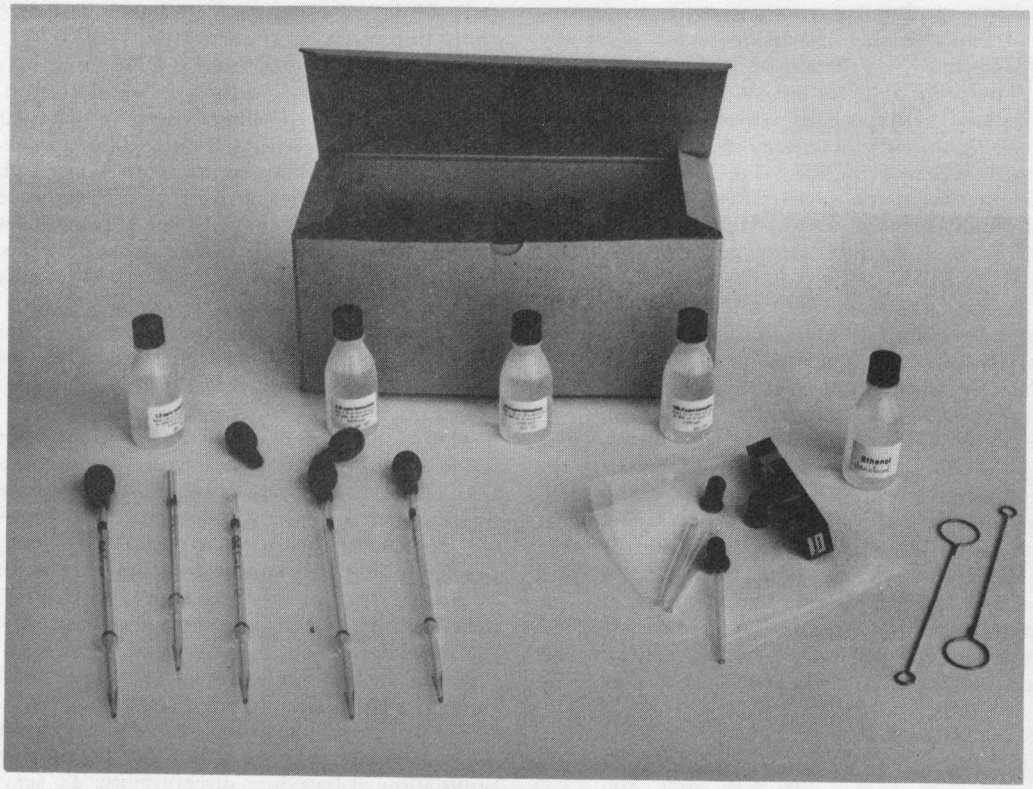

Fig. 1. Test kit for determining susceptibility of mosquito larvae to insecticides. (Photo furnished by World Health Organization, Geneva, Switzerland).

Larvae that pupated during the exposure period of the test, along with those that had not yet pupated 30 hours later, are discarded and disregarded in the denominator for the percent-effect results obtained. An EC_{50} figure is derived from the dosage-effect regression line.

ADULT TESTS. Resistance tests performed on adults are applicable to anopheline control by means of intradomiciliary residual adulticide applications. Tests with such residual deposits have been standardized by WHO and are designed for field use by means of portable test kits containing insecticide-impregnated papers. First printed 16 years ago (World Health Organization 1970), they have been revised in 1975 (mimeographed documents WHO/VBC/75.581 and 582), and again in 1981 (documents WHO/VBC/81.805[7] and 806[7]). Especially useful in the world malaria control and eradication campaign, they have also been employed to test *Cx. quinquefasciatus* in Texas (Micks and Rougeau 1977).

Where culicines are submitted to adult control by means of fine sprays (ULV or LV), tests are performed with fine droplets of the insecticide either directly applied topically to the mosquito, or made available in laboratory mist applications. *Aedes taeniorhynchus* and other culicines in Florida have been tested in wind tunnels with either thermal aerosols or non-thermal spray mists, a method originally designed to test different mosquito populations for their susceptibility level. Lots of 25 caged female mosquitoes are exposed either to 0.5 ml of graded insecticide dilutions moving at 3 mph in a 6-inch cylinder (Rathburn 1969, Boike and Rathburn 1975), or to 0.25 ml moving at 4 mph in a 4-inch cylinder (Mount et al. 1970). Adult mosquitoes have also been tested individually by topical application of a micro-droplet of graded insecticide dilutions by means of a hypodermic syringe and needle. Adult *Ae. sollicitans* in New Jersey are tested by topical application of 0.5 μl of an acetone solution of malathion to the thoracic pleuron by means of a syringe needle bent by 90° and ground flat (Khoo and Sutherland 1983). The method was

[7] Instructions for determining the susceptibility or resistance of adult mosquitoes to organochlorine, organophosphate and carbamate insecticides—a) Establishment of the base-line, 7 pp., document 805; b) Diagnostic tests, 7 pp., document 806.

Table 5. Base-line LC_{50} levels in mg/liter (ppm) to OP larvicides obtained for some important mosquito species by 24-hr exposure test.

Species	Larvicide					Reference
	Malathion	Fenthion	Fenitrothion	Chlorpyrifos	Temephos	
Aedes nigromaculis	0.011	0.0011	0.002	0.0007		Brown et al. (1963) Schaefer and Wilder (1970)
Ae. taeniorhynchus	0.017	0.00094			0.00073	Boike and Rathburn (1969)
Ae. aegypti	0.029	0.0026	0.0081	0.0007	0.00056	W.H.O. (1970)
Cx. pipiens complex	0.035	0.0023	0.0041	0.003	0.00039	W.H.O. (1970)
Anopheles albimanus	0.085	0.023	0.025	0.0063	0.005	Georghiou (1972)

originally used for *An. quadrimaculatus*, a smaller mosquito, with 0.25 μl of an ethanol solution of chlorinated hydrocarbon insecticides (Ludvik 1953).

In the WHO standard test, adult female mosquitoes in lots of not more than 10 are exposed for 1 hour to the test insecticide impregnated into filter paper, and are transferred to clean paper for a subsequent 24-hour holding period. In the test kit (Fig. 2), impregnated papers are provided for the organochlorines DDT and dieldrin, for the organophosphates malathion and fenitrothion, and for the carbamate propoxur. DDT is still widely used as a residual adulticide in malaria control and eradication; dieldrin papers were included to test for resistance to gamma-HCH, occasionally so used. For the two organochlorines, tests are made with a uniform exposure period (usually 1 hour) and with the multiple concentrations (in percent of

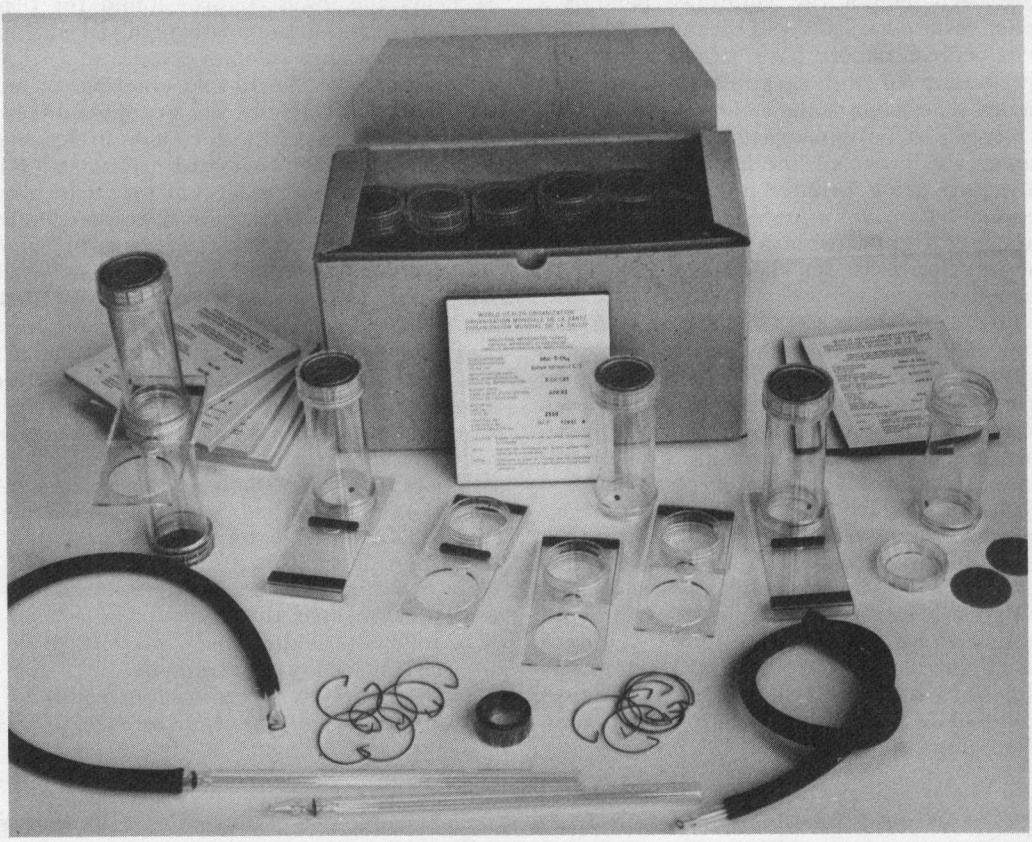

Fig. 2. Test kit for determining susceptibility of adult mosquitoes to insecticides. (Photo furnished by World Health Organization, Geneva, Switzerland).

the impregnant solution) provided by the following papers:

DDT			0.25	0.05	1.0	2.0	4.0
Dieldrin	0.05	0.1	0.2	0.4	0.8	1.6	4.0

The dosage-mortality when plotted yields a regression line and the LC_{50}. On the other hand, tests with the two organophosphates and the carbamate are made with a single concentration and multiple exposure periods. The concentrations provided (in percent) are as follows:

Malathion	Fenitrothion	Propoxur
5.0	1.0	0.1

and the series of exposure periods employed is usually 0.5, 1, 2 and 4 hours. The exposure-mortality figures when plotted yield a regression line and the LT_{50} (the exposure time at which 50% mortality is expected). Prior to 1980, papers impregnated at one-tenth of these concentrations had been also available, but these have been discontinued, along with fenthion papers at 2 concentrations. Papers impregnated with chlorphoxim, bendiocarb, permethrin and deltamethrin have been available on special request for research purposes. Bendiocarb papers have been temporarily withdrawn pending a better surface availability of the insecticide, and deltamethrin papers have lacked a uniform distribution of the pyrethroid. Pirimiphos-methyl is available as the technical-grade product itself, to be impregnated into the filter-papers also provided, the instructions for the procedure being included in the kit. For these residual adulticides as used in malaria control, the investigator should request WHO's catalogue/price list for choosing the materials needed.

Since such scalar tests require at least 200 adult mosquitoes, which are difficult to obtain in anti-malaria operations, they are designed primarily to determine the base-line level. Routine surveillance and monitoring for resistance are performed by utilizing a diagnostic dosage, which is set at the concentration or exposure time which is just sufficient to ensure complete kill (or rather 99.9% mortality on the log-probit graph paper) of a normal population. In such tests with anophelines, blood-fed females are always employed, and old specimens are avoided since they may give a false impression of susceptibility. The tentative diagnostics re concentration and exposure time proposed by the World Health Organization (1980) are as follows:

	DDT	Dieldrin	Malathion	Fenitrothion	Propoxur
Anopheles spp.	4% 1 hr	0.4% 1 hr	5% 1 hr	1% 2 hr	0.1% 1 hr
Cx. quinquefasciatus	4% 4 hr	4% 1 hr	5% 1 hr	1% 2 hr	0.1% 2 hr

The diagnostic dosages for chlorphoxim, permethrin and deltamethrin are respectively 4%, 0.25% and 0.025% for 1-hour exposure of adult anophelines. When and where survivors eventually make their appearance, the complete multiple-exposure scale test is performed to confirm the loss of susceptibility and validate the onset of resistance.

EVALUATION OF TEST RESULTS. Although larviciding induces more larval resistance than adult resistance, and adulticiding may produce more adult resistance than larval, resistance is not restricted to one or the other stage. Whereas mosquito control procedures may be confined to one stage, the insecticides employed in agricultural areas are not so restricted in their effect. The larval test by its very nature is more sensitive than the adult test in detecting changes in susceptibility level: roughly, a 2-fold increase in adult LC_{50} is accompanied by a 10-fold increase in larval LC_{50}, and a 4-fold adult by a 100-fold increase in larval LC_{50}. Again roughly, a population may be termed resistant when its larval LC_{50} has increased by 10 times (Knipling 1950). Base-line LC_{50} levels for several important species are shown below (Table 5), being the lowest figures found for field populations and in some cases being taken from pristine laboratory colonies.

Application of the 10-fold criterion, or any other criterion derived from susceptibility tests alone, should be used with caution in deciding on the use of the buzzword "resistant." For example, highly potent larvicides such as chlorpyrifos and temephos, with a recommended application rate (0.05 lb/a) fully one-fifth that of the recommended rate for malathion (0.025 lb/a), have LC_{50} levels less than one-fiftieth that of malathion, and thus there is room for a considerable increase in a target population's LC_{50} for chlorpyrifos or temephos before the recommended application rate is confronted with a control failure. It was the experience in California that successful control of pest *Aedes* with parathion or fenthion became problematic when the larval LC_{50} had risen above 0.005 ppm and the LC_{90} was at least twice that figure (Hart and Womeldorf 1976).

For exposure of adults to diagnostic dosages, as with anophelines, the evaluation criteria may be as follows (Davidson and Zahar 1973):

98% mortality = susceptible,
80–98% mortality = verification required,
80% mortality = resistant individuals present.

The verificatory test is to take the survivors (blood-fed and probably already fertilized when collected in the field) and confine them over water in vials or tubes; their larval offspring are then reared and the F_1 females are tested (blood feeding not necessary). If they show less mortality than the P generation, it may be concluded that resistance is present.

Susceptibility test results are of the greatest value in anticipating or confirming resistance, but it is the combination of field observations of control failure with the test results which add up to what can be called a true case for resistance, i.e., to the recommended application rate of the insecticide.

TESTS FOR RESISTANCE MECHANISMS AND CROSS-RESISTANCE

The results of multiple-concentration tests on mosquito larvae leading to an LC_{50} are easily reconciled with what might happen with larvicides in the field, the two situations being analogous. With adult tests, and particularly for anophelines, the paucity of available test material puts a premium on single diagnostic dosages, although multiple-time tests can lead to an LT_{50}. For the assessment of OP-resistance in anophelines, biochemical tests to identify resistant phenotypes on the basis of their esterase content will simultaneously shed light on the resistance mechanisms involved and on the cross-resistance spectrum.

A simple test whereby homogenates of single adults, or alternatively 4th-instar larvae, are spotted on filter paper which is then immersed in a buffered solution of alpha-naphthyl acetate and a chromogen (Fast Garnett GBC or Fast Red TR) will reveal those individuals having an OP-detoxifying esterase that releases alpha-naphthol. The method has been described in detail by Pasteur and Georghiou (1981). In OP-resistant *Culex quinquefasciatus* the principal esterase is Est-2, determined by the principal OP-resistance gene Est-B (Georghiou and Pasteur 1980), and in *Aedes aegypti* it is esterase-6 (Rees et al. 1985). Such spot tests thus rapidly reveal those individuals, among those sampled, that are heterozygous or homozygous for an OP-resistance gene.

In the *Culex pipiens* s.l. complex, OP-resistance in the field may develop only against the selecting agent, with little or no cross-resistance. An example was the development of resistance to malathion but not to fenitrothion in *Cx. p. pipiens* breeding in irrigated truck gardens near Naples, Italy which had been treated mainly with malathion for crop pest control (Breeden et al. 1984). Even when a more generalized type of OP-resistance has developed, it tends to be the most intense against the selecting agent. In *Cx. p. quinquefasciatus*, the OP-resistance developed by chlorpyrifos treatments in Tanzania was the highest to chlorpyrifos, while the use of fenthion in Sri Lanka gave a resistance which was highest to fenthion. Since the Est-2 bands were equally intense in both of these strains, they evidently were specialized for detoxifying the selecting compound the most strongly (Villani et al. 1983).

The biochemical mechanisms and the cross-resistance spectra of malathion-resistant anophelines have been studied in detail. In *An. arabiensis* from the Sudan the malathion-resistance was purely due to an increase in carboxyesterase enzyme, the cross-resistance extending to no other OP insecticide except phenthoate, a dithioate ester like malathion (Hemingway 1983). The malathion-resistant *An. culicifacies* from Maharashtra state, India, on the other hand, were also resistant to fenitrothion (Herath et al. 1981) and other OP compounds such as chlorphoxim and pirimiphos-methyl (Herath and Davidson 1981a); this type of resistance was not only due to increased carboxylesterase but also a mixed-function-oxidase (MFO) system (Herath and Davidson 1981d). Malathion-resistance in *An. stephensi* from southern Iran (Herath and Davidson 1981b) and Pakistan (Hemingway 1982b) was also proved due to carboxylesterase because it was counteracted by the classical carboxylesterase inhibitor TPP (triphenyl phosphate), and it also extended to fenitrothion (World Health Organization 1980). Malathion-resistant *An. atroparvus* in southern Spain, resistant not only to other OP compounds such as fenitrothion and chlorphoxim but also to the OP fenthion and the carbamate bendiocarb, sufficiently resemble the OP-carbamate-resistant *An. albimanus* of El Salvador that it is probable that their monogenic OP-resistance is due to an insensitive AChE, while the carbamate-resistance determined by the OP-resistance gene plus at least one other gene is due to increased oxidative enzymes as well (Hemingway 1982a).

A direct approach to discover the resistance mechanisms involved in a given sample of mosquitoes has been proposed in the form of a test kit containing the chromogenic means of testing for carboxylesterase, MFOs and glutathione S-transferase, and of differentiating between esterases by means of electrophoresis zymograms. A less-sensitive AChE target enzyme is another resistance mechanism which may be found in any species of anopheline or culicine; indeed, the three genotypes for the *Ace* gene which determines this resistance mechanism may be distinguished in *Cx. p. pipiens* by a single-mosquito test (Raymond et al. 1985). The

use of microtiter plate wells instead of filter paper allows the density of the enzyme to be determined spectrophometrically, and is adaptable to AChE and other enzymes as well as esterases (Brogdon 1984). Synergists may also be used as diganostic tools, piperonyl butoxide (PB) to reveal MFOs, DEF to reveal esterases, and F-DMC to reveal glutathione S-transferase.

COUNTERMEASURES FOR RESISTANCE

RESISTANCE HAZARD. Ever since resistance was first suspected in field populations, the possible fate of a given insecticide against a given species has been probed by selecting laboratory strains for about a dozen generations. Recently there have been some comparisons made between mosquito larvicides to assess the effect of selection pressure with them. From studies with larvicides of different modes of action applied against a laboratory strain of *Culex quinquefasciatus,* it was found by Georghiou et al. (1983) that diflubenzuron induced essentially no resistance, temephos a resistance that rapidly reverted on relaxation of pressure, permethrin a resistance that more gradually reverted, and propoxur a resistance that was relatively stable. Selection with the toxin of *B.t.i.* [*Bacillus thuringiensis* var. *israelensis* (H-14)] induced only a 70% increase in tolerance in 15 generations, rising eventually to a 12- to 17-fold resistance during 45 succeeding generations of selection (Vazquez-Garcia and Georghiou, unpublished data).

When temephos was compared with chlorpyrifos for its resistance potential in larval selection experiments with *Aedes aegypti* and *Cx. quinquefasciatus,* it was found to be the faster of the two in inducing resistance to itself, and to give a higher cross-resistance to chlorpyrifos; whereas selection with chlorpyrifos induced resistance to itself more slowly, giving a lower cross-resistance to temephos (Ziv et al. 1969). These results, of course, are limited to the laboratory strains employed. Experiments on field populations would compensate for restricted gene pools, but are open to immigration of susceptible phenotypes. The collation of insecticide histories in MAD operations with the test results for susceptibility levels and cross-resistance spectra could provide more relevant information on the fate of target populations in the field.

CHOICE OF SUBSTITUTE INSECTICIDES. The obvious countermeasure for a case of resistance is to switch to another insecticide, if not to an entirely different method of control (e.g. biological, environmental). For example, resmethrin synergized with piperonyl butoxide 1:3 was effective for quick adult kill of malathion-resistant *Ae. sollicitans* in New Jersey (Sutherland et al. 1983). In *Cx. quinquefasciatus,* OP-resistant strains due to esterase were fully susceptible to 26 different pyrethroids, whereas *Cx. quinquefasciatus* resistant to DDT and propoxur, and *An. albimanus* resistant to DDT, OPs and carbamates, were somewhat tolerant to some of the pyrethroids (Priester et al. 1981). However, addition of resmethrin to malathion aerosol sprays did not increase the kill of malathion-resistant *Aedes taeniorhynchus* in Florida (Rathburn and Boike 1981).

The policy considered most prudent has been to continue with one insecticide until the susceptibility test results indicate that resistance, in terms of a control failure, is imminent (Metcalf 1983). The weapon in reserve may be in an entirely new chemical group (e.g., pyrethroids, oils, amines, IDIs), but the most common situation is the necessity to switch from one OP insecticide to another. The basic question becomes the order of succession in which the compounds are introduced; in other words, which insecticide should be used first and which should be held in reserve. The best strategy is to use first the compound which gives the least cross-resistance, reserving those which induce a generalized OP-resistance until the very last; in the house fly, for example, scientific knowledge of the biochemical genetics involved has made it clear that malathion should be the first and dimethoate the last (Keiding 1977). In mosquitoes, the use of malathion usually induces a type of resistance restricted to malathion only, being due to detoxication by a carboxyesterase enzyme. The other OP compounds, and particularly the larvicides chlorpyrifos and temephos, induce a more general OP-resistance, usually due to phosphatase detoxication. The type of generalized resistance due to insensitive AChE is the most dangerous of all, since it negates both the OP and the carbamate anticholinesterase insecticides. It is probable that this AChE isozyme mechanism results more readily from selection with carbamates than from the OP compounds. For residual insecticides in anopheline control against malaria, it is best to start with malathion and hold fenitrothion or pirimiphos-methyl in reserve.

RESISTANCE MANAGEMENT. General principles to minimize the resistance problem (Metcalf 1983) include: (1) avoiding insecticides that select for resistance to other insecticides also; (2) as a general rule, avoiding mixtures of insecticides, thus inducing more than one type of resistance at the same time; and (3) avoiding the use of the same insecticide treatment against adults as that used against larvae.

Bearing in mind the resistance mechanisms involved, as well as the genetic, biological and operational influences on resistance development, the concept of resistance management has been developed by Georghiou (1980b). Thus attention could be paid to the types of usage practice on a broad basis rather than simply finding remedial insecticides for individual appearances of resistance. These types of resistance management strategies (Table 6) have been tabulated by Georghiou (1983); types B and C, which call for more positive action than type A, include the use of synergists, mixtures or rotations.

Synergists. Two esterase inhibitors have proved effective in reducing OP-resistance in *Cx. quinquefasciatus* in the laboratory. The esterase inhibitor DEF, when added to temephos, progressively reduced the temephos-R types in the strain and finally eliminated them. Unfortunately DEF, which is S,S,S-tributyl phosphorotrithioate, is a defoliant (Ramasinghe and Georghiou 1979). IBP, which is S-benzyl-0,0-diisopropylophosphorothioate (the fungicide Kitazin P), when added to malathion eliminated the malathion-resistant gene as fast as a period of relaxation of malathion selection, although it did not have such an effect on temephos-resistance (Hemingway and Georghiou 1984). A third compound, TPP (triphenyl phosphate), resembled DEF and IBP in being a direct synergist reducing the naphthyl esterase activity of OP-resistant *Cx. quinquefasciatus* larvae down to that of a normal susceptible larva (Georghiou 1984a). IBP was also highly synergistic with malathion against the malathion-resistant Pakistan strain of *An. stephensi,* but curiously enough was even more synergistic in a malathion-susceptible strain (Hemingway 1984).

Table 6. Chemical strategies of resistance management (from Georghiou 1983).

A.	Management by moderation
	Low dosages, sparing a proportion of susceptible genotypes
	Less frequent applications
	Chemicals of short environmental persistence
	Avoidance of slow-release formulations
	Selection directed mainly against adults
	Localized rather than area-wide applications
	Certain generations or population segments left untreated
	Preservation of "refugia"
	Higher pest population threshold for insecticide application
B.	Management by saturation
	Rendering R gene "functionally" recessive by higher dosages on target
	Suppression of detoxication mechanisms by synergists
C.	Management by multiple attack
	Mixtures of chemicals
	Alteration or rotation of chemicals

Mixtures. Combinations of two or more different insecticides are seldom considered, since they tend to produce more than one resistance simultaneously. A notable exception was found in a susceptible compounded California strain of *Cx. quinquefasciatus,* in which selection with a permethrin-temephos larvicide mixture failed to induce any resistance; moreover, the temephos-resistance induced by temephos selection was abolished by subsequent permethrin selection, and vice versa (Georghiou et al. 1980). This is the first example among mosquitoes of a pair of compounds negatively correlated for cross-resistance, long sought since the discovery in 1958 that DDT and phenylthiourea were negatively correlated for *Drosophila melanogaster* (vide Ogita, *in* Brown and Pal 1971). Mixtures of 2 bactericides to which resistance is shown only by bacilli carrying the 2 resistance genes simultaneously have succeeded in delaying the onset of bactericide-resistance in tuberculosis therapy.

Alterations or rotations. In experiments to delay the onset of insecticide resistances in *Culex quinquefasciatus,* some success has been obtained with an arsenal of 5 entirely different insecticides, namely propoxur, temephos, permethrin, diflubenzuron and BTI, when the changes were rung every 5–9 generations or so (Georghiou et al. 1983). Long-term sequential selection, where the change of insecticide is arbitrarily made several generations after its introduction, has an advantage over the present practice of waiting until resistance develops before making a switch; making the change before that happens has the effect of denying the target population the opportunity of developing fitness alleles to counteract the reduction of fitness which characterizes the incipient stage of resistance development. In laboratory experiments, this long-term sequential selection of *Cx. quinquefasciatus* did achieve delays in the general resistance picture, in contrast to short-term sequential selection (i.e., alternation among two or rotation among 3 in each generation). In such short-term rotations the *kdr*-resistance induced by permethrin continued to be induced by the other alternating compounds (loc. cit.). Initial results with a field trial in the area around Long Beach, Cal., indicated that rotating *B.t.i. (Bacillus thuringiensis* var. *israelensis*) with chlorpyrifos did delay the development of resistance to chlorpyrifos by *Cx. quinquefasciatus* (Mellon and Georghiou 1985).

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