PRACTICAL IMPLICATIONS OF INSECTICIDE RESISTANCE IN CULICINE MOSQUITOES

by

R. PAL*

1 - INTRODUCTION

The control/eradication of culicines is receiving increasing attention, since these mosquitoes are not only carriers of important diseases, such as filariasis, yellow fever, haemorrhagic fever, but have also been incriminated as important carriers of other arbo viruses of man and animals. Furthermore, these mosquitoes are a great nuisance since they feed on man with great avidity. Control of culicine mosquitoes is presenting a problem since the common species are ubiquitous with high reproductive potential. With increasing urbanization general sanitation is lagging behind in most of the developing countries, with the result that some of the culicine species, especially the *Culex pipiens* complex, are breeding in large numbers. The current methods for control of these species are based largely on the use of insecticides. The adult mosquitoes, particularly in the *Culex pipiens* complex, are naturally refractory to chlorinated hydrocarbon insecticides and develop resistance fairly rapidly to these compounds; in addition, recent reports indicate that the species are developing resistance to other groups of insecticides, such as organophosphorus compounds. Larvicides can be used in certain situations, but there are increasing cases of resistance to insecticides even in the larval stage. The present status of resistance in culicine mosquitoes is briefly summarized as follows :

2 - AEDES

Aedes aegypti is normally very susceptible to DDT and house spraying with this insecticide has virtually eliminated the species from a number of areas. An *Aedes aegypti* eradication campaign was started in the Americas in 1947 and towards the end of 1963 the eradication of the mosquito from 17 countries and territories had been certified, with Argentina about to be added to the list as the eighteenth country (KERR, 1964). Success has been achieved in Mexico, all of Central America and all of South America except the Caribbean where *Aedes aegypti* has developed resistance to DDT and in some places both to DDT and dieldrin *(vide infra)*. Several territories from which *Aedes aegypti* has been eradicated have been reinfested by resistant strains. The present status of the *Aedes aegypti* eradication campaign in the Americas is given in Figure 1. A programme for the eradication of *Aedes aegypti* has also been started in the United States of America. With the recent outbreaks of haemorrhagic fever in South-East Asia, plans are afoot to start *Aedes aegypti* control/eradication campaigns in some of the countries in that region.

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* Division of Environmental Health, World Health Organization, Geneva.

GENERAL STATUS OF THE AEDES AEGYPTI ERADICATION CAMPAIGN IN THE AMERICAS AS OF DECEMBER 1963

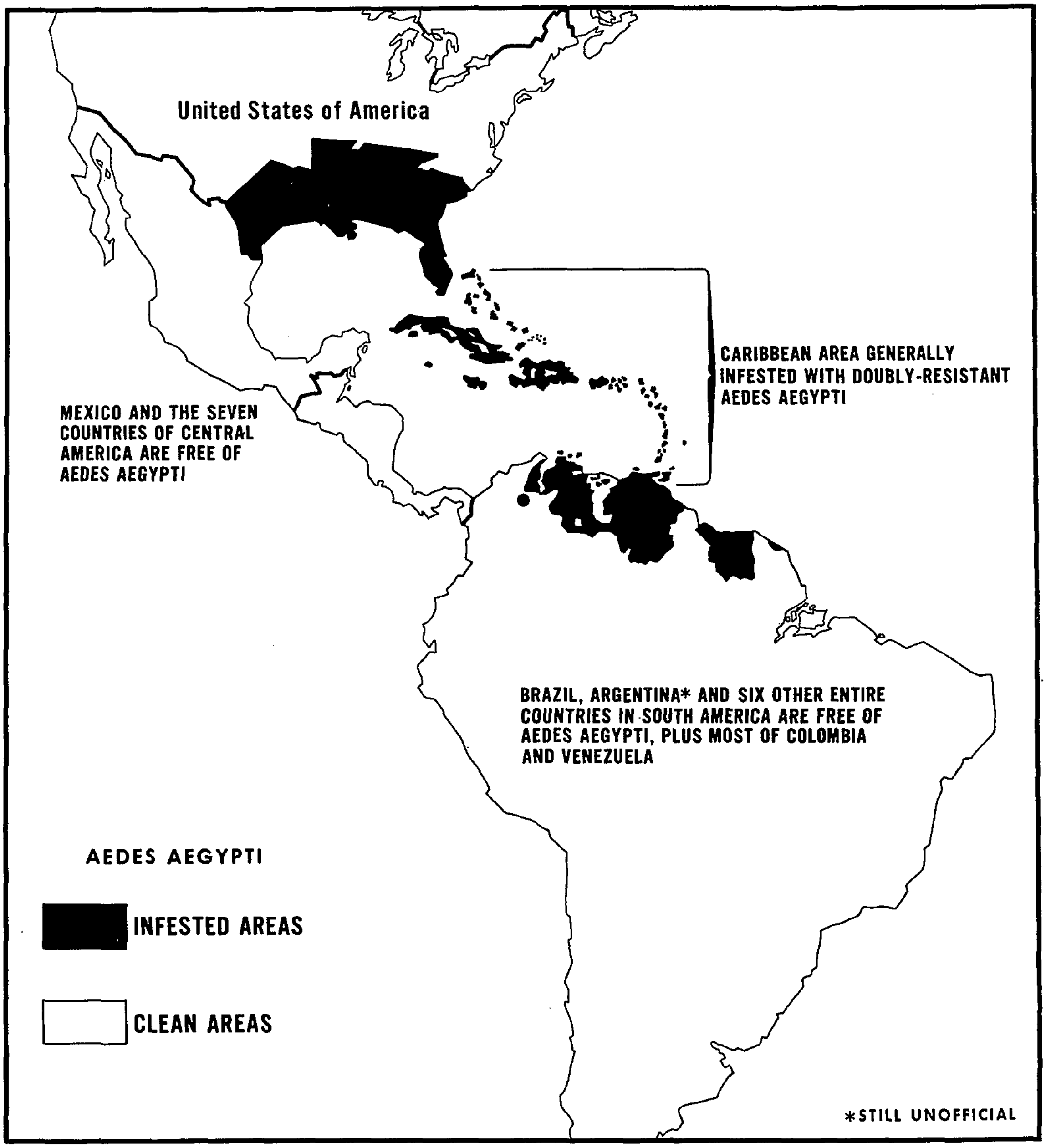

Figure 1

The present status of resistance in *Aedes* mosquitoes is briefly summarized below :

In 1953, larvae of *Aedes aegypti* were reported to be resistant to DDT in Surinam; the following year, DDT-resistance appeared in the Dominican Republic. In 1955, resistance was present in Trinidad and the three eastern-most states of Venezuela. In 1956, resistance was found in Cucuta, in north-eastern Colombia and in Haiti. By 1961, resistance had been reported in Guadeloupe, Puerto-Rico and Jamaica. Many of the Caribbean strains of *Aedes aegypti* became so DDT-resistant that the LC_{50} in laboratory determinations approximated 5 p.p.m. (ABEDI & BROWN, 1961).

Increased DDT-tolerance has also been observed in the field in South Vietnam, Japan and Fiji, and very recently in Thailand (NEELY, 1964).

Double resistance to both DDT and dieldrin has been observed in Isle Verde and Puerto Rico, and has now been confirmed in a number of Caribbean countries, i.e. Jamaica, Haiti, the Dominican Republic, Puerto Rico, Curacao and the Grenadines. This double resistance is also common in *Aedes aegypti* populations of Surinam. A strain of *Aedes aegypti* from French Guiana has been reported very recently to be resistant to DDT and dieldrin and tolerant to malathion and fenthion (ABEDI, 1964. Communication to WHO).

Increased tolerance has been developed by laboratory selection in strains from Nigeria and Malaya. By the same method it has been shown that strains of *Aedes aegypti* from New Orleans and Key West, Florida, are capable of developing high DDT-resistance in the larvae, the results paralleling those obtained from a strain from Cucuta and Colombia. Thus the DDT-resistance hazard in populations in Southern USA appears to be just as high as in the Caribbean region, and perhaps this may also be true of other *Aedes aegypti* populations elsewhere. In some of the territories in the Americas resistance to dieldrin has been found where this compound had not previously been used. This makes it very doubtful that dieldrin can be used to any great extent to replace DDT in those areas in which resistance to the latter has become pronounced (CAMARGO, 1964).

The salt marsh mosquitoes *Aedes sollicitans* and *Aedes taeniorhynchus* were also found to be resistant to DDT and dieldrin in Florida. The malathion-resistance found in the species in the year 1952 was transitory and of no great significance (BARR *et al*, 1962). Both *Aedes dorsalis* and *Aedes nigromaculis* were found to be resistant to chlorinated hydrocarbon insecticides in California. A malathion-resistant population of *Aedes nigromaculis* was discovered in Fresno County with an LC_{50} of 0.1 p.p.m. instead of the normal 0.01 p.p.m. Resistance to parathion in the species has spread in different parts of the USA. BROWN *et al* (1963) recently observed that the parathion-resistance in *Aedes nigromaculis* also extended to other organophosphorus compounds, e.g. malathion and fenthion. Thus resistance in this case was rather unspecific and appeared to be due to multiple factors. Of all the species of culicines tested in Canada, *Aedes cantator* was found to be resistant to DDT and dieldrin, the LC_{50} of both the insecticides having risen more than 2.5. p.p.m. DDT-resistance was indicated in a population of *A. vexans* in Kamloops, British Columbia, Canada, which had been under spraying with DDT for 16 seasons. *Aedes pseudoscutellaris* in Fiji was found to be susceptible to DDT, dieldrin and gamma-BHC, but certain populations of the species were heterogenous as regards response to DDT. On selection, a highly resistant strain of the species was found (BURNETT & ASH, 1961). Apart from these species, DDT-resistance was noted in *Aedes detritus* in Southern France and *Aedes cantans* in Germany.

The present status of resistance in *Aedes* mosquitoes is briefly summarized in Table I.

3 - CULEX

The number of insecticide-resistant populations of culicine mosquitoes in steadily increasing. *Culex pipiens fatigans* larvae with normal LC_{50} levels of 0.023-0.061 p.p.m. DDT have shown LC_{50} levels from 0.6-15.0 p.p.m. in differents parts of the world. In addition resistance to dieldrin and gamma-BHC is widespread in this species. The pre-existing normal DDT-tolerance of the adult stage is particularly conspicuous. DDT- and dieldrin-resistant strains are generally susceptible to 0-P compounds; however, MOUCHET et al (1960) reported that high resistance to chlorinated hydrocarbon insecticides was accompanied by a significant tolerance to 0-P compounds. Malathion-resistant strains have been observed to revert to normal susceptibility when insecticide pressure is removed (MOUCHET, 1964).

It will be recalled that is was in *Culex pipiens molestus* that DDT-resistance in mosquitoes was first discovered by MOSNA in 1947, only one year after the commencement of house spraying in Latina (Italy). So far this species has been reported to be DDT-resistant in Italy, USA, Israel and Japan. More recently DDT-resistance has been found in *Culex pipiens* in several localities in New York and New Jersey and a few localities in Massachusetts, Ithaca and Utah. Dieldrin-resistance has been observed in Italy, Israel, France and Japan. Resistance to organophosphorus compounds has not been reported so far.

The present status of *Culex pipiens* species resistance may be summarized as follows*:

DDT : Australia, Brazil, British Guiana, Cameroons, Ceylon, Colombia, Congo, Ecuador, Fiji, France, French Guiana, Greece, India, Israel, Italy, Hawaii, Japan, Kenya, Malaya, Okinawa, Panama, Philippines, Puerto Rico, Reunion, Taiwan, Tanganyika, Turkey, USA, Venezuela, Trinidad.

BHC dieldrin : Brazil, British Guiana, Cameroons, Ceylon, Fiji, France, French Guiana, Hong Kong, India, Israel, Japan, Liberia, Malaya, New Caledonia, Okinawa, Panama, Puerto Rico, Reunion, Tanganyika, USA, Upper Volta, Venezuela, Trinidad, Zanzibar.

O-P : Cameroons, USA, Sierra Leone.

Culex tarsalis has been found to be DDT-resistant and dieldrin resistant in California. Heptachlor and dieldrin, which were introduced for the control of this species in Oregon, became ineffective by the development of dieldrin-resistance in the species. Resistance to malathion is also widespread, an LC_{50} as high as 3.0. p.p.m. having been observed. The specific malathion-resistance (LEWALLEN, 1961) discovered in a population in Fresno County, California, in 1956 was found to have completely reverted by 1960.

Culex tritaeniorhynchus : The presence of DDT- and dieldrin-resistance has been indicated in this species in Okinawa. It was also found to be resistant to dieldrin in Dahomey (HOLSTEIN 1959).

Certain species of *Culex,* however, remain susceptible to DDT and dieldrin.

Culex peus remains susceptible to DDT in California, but was reported to be resistant in Oakridge, Oregon, USA (BARR et al, 1962). The presence of dieldrin-resistance in *Culex restuans* was indicated at Camp Drum, New York. The survival of a few mosquitoes was observed at even as high a dosage as 0.5 p.p.m., whereas the LC_{100} for a susceptible strain was 0.02 p.p.m. By using a different technique, FRENCH & KITZMILLER -1963) noted the presence of individuals resistant to the dieldrin group of insecticides at Illinois. An indication of DDT-resistance in the adults of *Culex erythrothorax* in Fort Conkhite, California, was also obtained. A DDT-resistant population of *Culex coronator* has been found in Panama.

4 - PRACTICAL IMPLICATIONS

The *Aedes aegypti* eradication programme in the Americas has been in operation for a number of years. At first, DDT water dispersible powder was used as a larvicide, and later dieldrin water dispersible powder was substituted. Both insecticides were applied in such a manner that, in addition to their larvicidal effect on water, there was a residual effect on surfaces

* The instances cited are those for which correct testing has been done. It is more than probable that the incidence of resistance is even more widespread than reports indicate.

in close proximity to the breeding places where adult mosquitoes, on emergence, are likely to rest. This "perifocal"spraying of insecticides was a great success at the beginning, but recently the development of resistance of *Aedes aegypti* to DDT and dieldrin has rendered the use of these insecticides ineffective, so much so that the eradication programme is at a standstill with interruption of activities in the British Virgin Islands and Antigua. This is also true in Jamaica where, in spite of the occurrence of a severe outbreak of dengue, the suspended campaign was not re-started, owing to the lack of effective substitute insecticides. On theoretical grounds, perhaps these developments could have been expected, since perifocal spraying exerted intensive selection pressure on both larvae and adults, thereby precipitating early resistance.

In most countries in South-East Asia and the Western Pacific regions, *Aedes aegypti* control is at present carried out only around and in the near vicinity of airports, with a view to keeping the *Aedes aegypti* index as low as is required under the International Sanitary Regulations. Both anti-larval and anti-adult measures are carried out, mostly by using chlorinated hydrocarbon insecticides. Except for the information on development of resistance in South Vietnam, Japan and Fiji, no other data are available from this region. However, it is likely that with the intensification of control procedures resistance will develop in *Aedes aegypti.*

Two important points should be considered :

1 - baseline data on the susceptibility of *Aedes aegypti* in these regions should be obtained as early as possible, at any rate before any intensive control operations are started,

2 - as far as possible "perifocal" spraying of insecticides should be avoided since it precipitates resistance much earlier than if the insecticide is used either as a larvicide or as an adulticide.

Although at the beginning of spraying perhaps DDT or other chlorinated hydrocarbon insecticides would be used, it will be necessary to find substitute insecticides to combat any development of resistance. Promising alternatives (OMS-187, 236, 315, 495, 658, 711 and 800 - see Annex 1 -) have been recommended by the Directors of Laboratories collaborating in the WHO scheme for the evaluation and testing of new insecticides, some of which have a low mammalian toxicity and can even be used in drinking water.

WHO has been developing a pattern for the control of major vectors of disease by considering the application of a number of insecticides in sequence so that if resistance develop to one another can be substituted; in this way effective control might be obtained for at least 10-15 years. These studies include the speed of development of resistance to various groups of insecticides, e.g. starting with chlorinated hydrocarbons, followed by organophosphorus compounds, and carbamates, if necessary. In the case of *Aedes aegypti,* particular attention will have to be paid to the toxic hazards of these insecticides to man, since in most of the above-mentioned countries *Aedes aegypti* breeds in containers used either for drinking water or for storage purposes. The most common breeding places are cisterns, water pitchers, ornamental pools, disused cans and tyres, etc.

The control procedures are therefore based on anti-larval measures, although theoretically there is no doubt that anti-adult measures should succeed, though *Aedes aegypti* bites and rests indifferently both indoors and outdoors.

The control of *Culex fatigans* likewise presents a difficult problem since it is not only refractory to insecticides but resistance emerges fairly rapidly. Attempts to control filariasis by both anti-larval and anti-adult measures directed against *Culex fatigans* in India and elsewhere have been disappointing, so much so that this method of control has been replaced by the application of oil on breeding places. The high organic contamination of polluted water in which *Culex fatigans* breeds has made it difficult to obtain prolonged residual effectiveness of larvicides. Either these do not penetrate the surface or they rapidly break down and are absorbed by the mud bottom complex. The World Health Organization is at present testing a number of new insecticides against the species (OMS-2, 236, 315, 437, 648, 658, 659, 711 and 800 - see Annex 1 -) and it is hoped that satisfactory control will be achieved over a number of years using a number of compounds on the lines referred to above. Dichlorvos dispensers in storm-sewers, cess-pits, etc., have proved effective for the control of *Culex fatigans* and further tests are planned.

Examples of *Aedes aegypti* and *Culex fatigans* have been taken to illustrate the practical implications of insecticide resistance in culicine mosquitoes since these are the two most important culicine species : the others, some of which are of local importance only, have not been discussed in detail. However, it is becoming increasingly obvious that some *Aedes* and *Culex* may be responsible for transmission of virus-borne diseases, and constant vigilance will have to be maintained with regard to development of resistance in these species.

5 - CONCLUSION

It is evident that the development of resistance in both *Aedes* and culicine mosquitoes has created a serious problem and its repercussions on disease transmission are even more serious, as is evident from the recrudescence of yellow fever in the Americas and haemorrhagic fever in the Orient. However, although resistance has caused setbacks in many eradication programmes, there are still several promising insecticides available for the control of larvae. It seems that it would not be possible to carry out effective control of adult mosquitoes due to natural refractory type behaviour and lack of persistence of insecticides when applied as residual deposits on porous surfaces. Nevertheless, larvicidal control to achieve an effective reduction in mosquito populations could be instituted with new larvicides followed by appropriate sanitation measures to control breeding. In the case of the *Culex pipiens* complex, toxic larvicides could be used in polluted, stagnant water, since such water cannot be used for drinking or other purposes. In the case of *Aedes* mosquitoes, larvicides with low mammalian toxicity could be used in drinking waters without any health hazard. It is obvious that control of culicine mosquitoes is more difficult than that of most other mosquitoes and presents a challenge to the inginuity of vector control specialists.

6 - ACKNOWLEDGEMENTS

The author is greatly indebted to Dr. J. AUSTIN KERR of the Pan American Sanitary Bureau, Washington, for permitting the use of Figure 1, and to Professor A.W.A. BROWN, University of Western Ontario, London, for his valuable comments on the paper.

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TABLE I

PRESENT STATUS OF RESISTANCE OF *AEDES* MOSQUITOES TO INSECTICIDES *

Species - Country	Insecticide	Year of report
Aedes aegypti		
Colombia	DDT and dieldrin	1957 and 1960
Dominican Republic	DDT	1958
Fiji	DDT	1960
Guiana (French)	DDT	1959
Guadeloupe	DDT	1959
Haiti	DDT	1957
Japan	DDT and lindane	1958
Malaya	DDT	1958
Puerto Rico	DDT, diazinon, dieldrin Dipterex, lindane, malathion	1960
Surinam	DDT	1953-54
USA	DDT	1961
Venezuela	DDT	1957
Vietnam	DDT	1958-59
Antigua	DDT	1957
Jamaica	DDT	1959
Trinidad	DDT	1957
Thailand	DDT	1964
Aedes cantans		
Germany	DDT	1958
Aedes cantator		
Canada	DDT and dieldrin	1961
Aedes detritus		
France	DDT	1959
Aedes dorsalis		
USA	BHC, DDT, dieldrin and Toxaphene	1960, 1950, 1951 and 1958 respectively

* As given in US Army Environmental Health Agency publication "Insecticide resistance of medically important arthropods" (1962) Rev. 3 + subsequent additional reports of resistance.

Aedes fijiensis		
Fiji	DDT	1960
Aedes nigromaculis		
USA	Aldrin	1955
	Chlordane	1953
	DDT	1950
	Dieldrin	1955
	Heptachlor	1955
	Lindane	1953
	Malathion	1960
	Parathion	1958
	Ronnel	1958
	Trithion	1958
Aedes poicilius		
Philippines	DDT	1957
Aedes sollicitans		
USA	Chlordane	1949
	DDT	1950
	Dieldrin	1961
	EPN	1953
	Lindane	1956
	Malathion	1953
	TDE	1950
Aedes taeniorhynchus		
USA	Chlordane	1949
	DDT	1950
	Dieldrin	1961
	EPN	1953
	Lindane	1956
	Malathion	1953
	TDE	1950
Aedes vexans		
USA	DDT	1960

ANNEX 1

CHEMICAL NAMES OF THE PROMISING INSECTICIDES FOR USE AS MOSQUITO LARVICIDES

OMS-2	0,0-dimethyl 0-(4-methylthio-m-tolyl) phosphorothioate (fenthion)
OMS-187	2,4-dimethylbenzyl chrysanthemumate (dimethrin)
OMS-236	0,0-dimethyl 0-(4-methylthio-3,5-xylyl) phosphorothioate
OMS-315	0,0-dimethyl S-(p-chlorophenyl) phosphorothioate
OMS-437	toluene-α α -dithiol bis (0,0-dimethyl phosphorodithioate)
OMS-658	0-(4-bromo-2,5-dichlorophenyl) 0,0-dimethyl phosphorothioate
OMS-659	0-(4-bromo-2,5-dichlorophenyl) 0,0-diethyl phosphorothioate
OMS-800	dimethyl 1-hydroxy-2,2,2-trichloroethylphosphonate (Dipterex)