PAST, PRESENT AND FUTURE IN INSECTICIDE-RESISTANCE OF MOSQUITOES *

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It is now 15 years since DDT was introduced as a powerful agent for mosquito control. At half-time, that is 7 to 8 years ago, the insecticide-resistance situation was summarized by Hess, in his two review papers. The salt-marsh mosquitoes of Florida were DDT-resistant in treated areas, but still susceptible to BHC or dieldrin. The irrigation-water *Aedes* of California had become DDT-resistant in treated areas, while the *Culex* were becoming resistant to BHC and the cyclodiene group of insecticides. *Culex pipiens* in central Italy had added chlordane-resistance to DDT-resistance, while a decline in DDT-susceptibility of this species had been noted at Toledo, Ohio. There was no real DDT-resistance in *Anopheles*, limited to slight changes in *A. quadrimaculatus* in Alabama and Florida, premonitions in *A. sacharovi* in Greece, and behavioral changes in *A. albimanus* in Panama.

Now, after the lapse of a further 7 to 8 years, the resistance picture in mosquitoes has become much more serious, particularly in *Anopheles* (Table 1). No less than 21 species in this genus have now developed resistance to one or more of the chlorinated hydrocarbons used as adulticides on the walls of buildings. Of this number 7 species have developed resistance to DDT, and 20 to dieldrin. In fact, *A. sundaicus* is the only species in which DDT-resistance alone has been developed; all the others have developed dieldrin-resistance in different populations or in the DDT-resistant population itself. Dieldrin-resistance is strikingly intense, so that there is no doubt about it. For example, we all remember the question whether *A. quadrimaculatus* had developed DDT-resistance in the southeast; by the time it was decided that it was a local slight increase in tolerance, intense dieldrin-resistance had developed in Mississippi. The Greek *A. sacharovi*, whose increased DDT-tolerance took such a long time to prove, now shows strong dieldrin-resist-

* Invitation Paper on the Special Topic of Insecticide Resistance, 16th Ann. Mtg., 1960.

TABLE 1.—Resistance to DDT and dieldrin by anopheline mosquitoes.*

DDT	*A. sacharovi*	1951, Greece, Lebanon, Iran, Turkey
	A. sundaicus	1954, Java, Burma
	A. stephensi	1955, Arabia, Iraq, Iran, S. India
	A. subpictus	1955, North India, W. Pakistan
	A. albimanus	1958, Salvador, Nicaragua, Guatemala, Honduras
	A. pharoensis	1959, Egypt
	A. quadrimaculatus	1959, Georgia, Maryland, Mexico
Dieldrin	*A. sacharovi*	1952, Greece
	A. quadrimaculatus	1953, Mississippi, Maryland
	A. gambiae	1955, Nigeria, Liberia, Ivory Coast, Upper Volta, Dahomey, Cameroun, Sierra Leone
	A. subpictus	1957, Java, Ceylon, N. India
	A. constani & *A. pulcherrimus*	1957, Arabia
	A. albimanus	1958, Salvador, Guatemala, Nicaragua, Honduras, Jamaica, Ecuador, Mexico, Br. Honduras, Cuba, Dominican Rep.
	A. pseudopunctipennis	1958, Mexico, Nicaragua, Peru
	A. aquasalis	1958, Trinidad, Venezuela
	A. culicifacies	1958, E. India
	A. vagus	1958, Java, Philippines
	A. barbirostris & *A. annularis*	1958, Java
	A. sergenti	1958, Jordan
	A. fluviatilis	1958, Arabia
	A. stephensi	1959, Iran
	A. minimus flavirostris	1959, Philippines
	A. pharoensis	1959, Egypt
	A. albitarsis	1959, Colombia
	A. labranchiae	1959, Morocco

* Date given is first year of occurrence.

ance. The Mexican *A. pseudopunctipennis*, which had not lost any of its DDT-susceptibility in over 10 years of DDT spraying, went resistant to dieldrin within a few months of its introduction in the state of Morelos. The development of dieldrin-resistance in already DDT-resistant populations of *A. stephensi* around the Persian Gulf presents a most serious setback to the world-wide malaria eradication program, as does the presence of high DDT-tolerance in the dieldrin-resistant populations of *A. albimanus* in Central America. More than 5 percent of the eradication program is now confronted with resistant *Anopheles*, and this gap in the protective umbrella of insecticides is becoming larger.

In addition to physiological resistance, where populations have come to survive a lethal dose, there is behavioristic resistance, where populations have come to avoid taking up a lethal dose. Behavioristic resistance may be of two types. One is exemplified by *A. albimanus* in Panama, which derives from a hyperirritable response to DDT which causes them to leave the deposit prematurely; the other is exemplified by *A. cruzii* in southern Brazil and *A. punctimacula* in Colombia, populations of which have developed the behavior of resting outside instead of inside houses. The matter of behavioristic resistance, however, demands more exact study before it can be characterized adequately.

No less than 14 species of culicine mosquitoes have developed resistance to DDT or other insecticides (Table 2). The worst problem is the tropical house mosquito, against which the chlorinated hydrocarbons have been applied as larvicides as well as adulticides. This *Culex quinquefasciatus*, or *C. fatigans* as which it remains more generally known, is coming to rival the house fly in that there are now few parts of the world where it has not increased its natural DDT-tolerance to palpable DDT-resistance, or developed resistance to BHC, dieldrin, chlordane or toxaphene. DDT-resistance has developed

TABLE 2.—Resistance to three insecticide groups by culicine mosquitoes

	DDT group	BHC-Dieldrin gp.	Organophosphorus gp.
Culex fatigans (quinquefasciatus)	India 52, Reunion 53 Venezuela, Taiwan 56 Puerto Rico 57 S. Australia, Panama 58	Cal. 51, Malaya, India 53, E. Asia 54 S. America 56 W. Africa 57 Panama 58 Zanzibar 59	Cameroun 59
C. pipiens	Italy 47, Mass. N. J. 55, Japan, Cal. 59	Italy 50, Israel 55 France, Japan 59	
C. tarsalis	Cal. 51, Ore. 56	California 51	California 56
C. coronata	Panama 58		
C. poicilipes, C. nebulosus, C. tritaeniorhynchus		Dahomey 59	
Aedes aegypti	Trinidad, Dominican Rep. 54 Venezuela 55, Haiti 56 NE Colombia 57 Puerto Rico, Jamaica, Guadeloupe 59	Puerto Rico 59	
A. sollicitans	Fla. 47, Del. 51	Fla. 51, Del. 59	Florida 52*
A. taeniorhynchus	Florida 49	Florida 51, Ga. 59	Florida 52*
A. nigromaculis	California 49	California 51	California 58
A. dorsalis	California 51	California 51	
Psorophora confinnis, P. discolor		Mississippi 54	

* Slight increase, of 4–10 times the normal LC_{50}.

in another species of *Culex* in Panama, and dieldrin-resistance in 3 species in Dahomey. The DDT-resistance of *C. tarsalis* has spread from California to northwestern Oregon, but not to Utah. This species is increasing its dieldrin-tolerance in Weber County, Utah, and its heptachlor-tolerance at Eugene, Oregon. DDT-resistant larvae of *C. pipiens* have been discovered in testing surveys in parts of Massachusetts, New Jersey and California but evidently have not developed into a problem in Ohio or Illinois. Dieldrin-resistant larvae of this species have developed in Jerusalem, Israel, at Narbonne, France and are suspected at certain points in Massachusetts; while larvae of the variety *C. pipiens pallens* have been reported to have become resistant to chlorinated hydrocarbons at Nawasaki city, Japan.

The DDT-resistance of the salt-marsh *Aedes* larvae of Florida was very soon supplemented by resistance to the other chlorinated hydrocarbons such as BHC and dieldrin. A type of dieldrin-resistance with some cross-resistance to DDT was recently discovered in *A. taeniorhynchus* in Chatham County, Georgia. The increased tolerance of DDT that developed in *A. sollicitans* in Sussex County, Delaware, could be gradually lost if this insecticide was discontinued or substituted; however, BHC-resistance has developed in Kent County in 1959. In more northern regions the slow voltinism and high dilution factor from surrounding untreated areas evidently account for the absence of resistance, as found in a survey of *Aedes canadensis* and other species made in southern Ontario in 1953. Meanwhile, agricultural insecticides are evidently maintaining the resistance of the irrigation-water *Aedes* and *Culex* in California, since it is just as strong outside the Abatement Districts as inside them. A reduced susceptiblity to both DDT and dieldrin was detected in *A. dorsalis* in Salt Lake County, but otherwise there has been no change in the *Aedes* larvae of Utah.

A strong dieldrin-resistance, with no cross-resistance to DDT, developed in the rice-field mosquitoes *Psorophora confinnis* and *P. discolor* in Bolivar County, Mississippi, just one year after dieldrin was applied to control them. It was suggested that perhaps cotton insecticides had predisposed these populations to develop the resistance so rapidly.

In 1954 it became evident that a strong DDT-resistance had developed in certain parts of Trinidad in larvae of the yellow-fever mosquito, *Aedes aegypti*. This DDT-resistance has been subsequently found elsewhere in the Caribbean area and in the northern states of South America. Last year a most serious development was discovered; at Isla Verde, near San Juan, Puerto Rico, larvae of *A. aegypti* had become strongly resistant to dieldrin as well as to DDT.

Resistance to the organophosphorus compounds which have been substituted as mosquito larvicides is now developing in certain areas. Malathion-resistance first developed in the *Culex tarsalis* of Fresno County, California. Next, parathion-resistance was discovered in the *Aedes nigromaculis* of Kings and Tulare counties. Increased tolerances to malathion and EPN have already been reported for the salt-marsh mosquitoes in Brevard County, Florida. Recently malathion-resistance has developed in larvae of *Culex fatigans* at Duala, Cameroun.

The recognition of resistance as an ingredient of control failures became possible with the appearance of consistent test methods for susceptibility levels. Now the tests for adult mosquitoes and mosquito larvae have been standardized on a world-wide basis, and test kits are available and have been widely distributed. The application of these tests and the use of these kits in recent years has played an essential part in telling us just how far resistance has developed in vector control programmes. These tests have also proved themselves valuable tools for the more basic genetical and biochemical studies of resistance conducted in the world's laboratories. The role of WHO (The World Health Organization) in these developments is probably familiar to most of us.

In assessing the future, we must first be sure to have a correct understanding of the fundamentals of the resistance phenomenon. It does not derive from increased adaptation during the lifetime of the insect; exposure to multiple harmless doses does not increase the tolerance of that individual to the insecticide. It derives from selection of the normally more tolerant individuals in the population, and therefore requires the mortality of the more susceptible elements. In no case has it proved possible to induce resistance without mortality in the strain. Insecticide-resistance is a Darwinian phenomenon, and derives from the pre-adaptation to resistance already existing in a small segment of the population.

These pre-adaptations are due to genes. It was first found that dieldrin-resistance was inherited in *Anopheles gambiae* as if it was due to a single gene, allelic with the normal and without dominance. Similarly DDT-resistance in *Aedes aegypti* was found to be due to a single gene allele without dominance, the F_1 heterozygotes being intermediate in resistance. DDT-resistance in *Anopheles sundaicus* was found to be explicable by a single recessive gene. The allele for dieldrin-resistance of *Anopheles gambiae* was found to pre-exist in untreated areas in the interior of West Africa, where between 0.05 and 12 percent of these mosquitoes were heterozygous for it. One cycle of dieldrin spraying could transform such populations so that they comprised approximately 90 percent resistant homozygotes. The resistance hazard of a species, then, depends on whether it contains some individuals carrying the appropriate resistance gene. Attempts to evaluate this hazard by submitting laboratory colonies to insecticide pressure have been of limited value because they tested only a fraction of the gene pool of the species. It is a truism to say that a species is susceptible because it contains few or no resistant genotypes. The reason that the resistant genotypes

are normally scarce is because they have a lower survival rate than the susceptible ones in insecticide-free conditions. We have obtained a laboratory demonstration of this fact in a Malayan strain of *Aedes aegypti* which rapidly develops DDT-resistance under selection pressure but as rapidly loses it under DDT-free conditions.

Field experience and the performance of tests for cross-resistance have shown that DDT-resistance is separate from dieldrin-resistance in mosquitoes, as in house flies and many other insects. DDT-resistance extends to its analogues methoxychlor and DDD; dieldrin-resistance extends to other cylodiene derivatives such as chlordane, heptachlor and toxaphene, and also rather surprisingly to gamma-BHC. As to the mechanism of DDT-resistance in mosquitoes, it is still an open question whether it is related, as it is in the house fly, to its detoxification by dehydrochlorinase to DDE. The mechanism of dieldrin-resistance remains a mystery even in house flies; at least the associated BHC-resistance of *Anopheles gambiae* was not correlated with an increased detoxification or decreased cuticular absorption. It is however known that an increase in the fat body of adult *Anopheles maculipennis* will greatly increase their dieldrin-tolerance. With regard to malathion-resistance, we have obtained laboratory evidence with *Culex tarsalis* larvae that it is correlated with a lower level of the toxic metabolite malaoxon and a greater production of non-toxic water-soluble derivatives, much as has been found in the house fly.

There is yet another type of resistance; this is a non-specific vigor tolerance extending to insecticides in general. Characteristically it develops slowly and does not reach very high levels; genetically it derives from accumulation of multiple genes of non-specific and comparatively slight effect. It is probable that the changes formerly observed for *A. quadrimaculatus* with DDT in Alabama may have been of this nature. Another member of the *maculipennis* group, namely *A. sacharovi,* has reached a plateau of DDT-tolerance in Greece, where DDT is still moderately effective. It is extremely important to know whether any changes detected by susceptibility test indicate vigor tolerance or incipient true resistance. If the dosage-mortality lines remain parallel as they move to the right, and if similar increases in LC_{50} are shown to different insecticides, the probabilities are that it is a case of vigor tolerance. If on the other hand the d-m line becomes shallower in slope, or even more becomes flat at the higher concentrations, true resistance is indicated.

In a number of species there now exist populations which simultaneously show high dieldrin-resistance and moderate DDT-tolerance, e.g. *Anopheles albimanus* in central America, *An. sacharovi* in Greece, *An. quadrimaculatus* in Maryland, and *Aedes aegypti* in Puerto Rico. We have been attempting to separate the dieldrin-resistance from the DDT-resistance in the Puerto Rico strain by a combination of back-crossing and pressure from one or other of the insecticides; we find that the two resistances remain as a single entity, and are probably due to a single gene on chromosome 2. Microscopical examination of the chromosomes themselves is much more difficult in *Aedes* than in *Anopheles;* although certain chromosome inversion types have been associated with DDT-tolerance in *Anopheles atroparvus,* it is by no means clear whether they have anything to do with specific DDT-resistance.

The cross-resistance pattern dictates the choice of substitute insecticides. As a general rule, mosquitoes that have become DDT-resistant as a result of DDT pressure remain susceptible to dieldrin or BHC. Indeed the DDT-resistant *An. sundaicus* in northern Java have remained susceptible to dieldrin for several years. But DDT-resistance predisposes many species of mosquitoes to a rapid development of dieldrin-resistance, as was the experience with *Aedes* and *Culex* in California and Florida, with *An. sacharovi* in southern Greece and *An. stephensi* in southeastern Iran. Sometimes the dieldrin-

resistance of adult *Anopheles* forces a return from dieldrin to high doses of DDT, as in Greece and also in *An. albimanus* in El Salvador. For control of culicine larvae, certain OP compounds have been substituted. It is cold comfort to know that the OP-resistances now developed in *Culex tarsalis* and *Aedes nigromaculis* are specific to malathion and parathion respectively, without cross-resistance between the two compounds. However, we have developed by laboratory selection a malathion-resistant strain of *Aedes aegypti* which, like the house fly in similar circumstances, has a cross-tolerance to the carbamate Sevin and a very high cross-resistance to DDT.

And so we go marching on, making things more difficult for ourselves. Is there anything we can do to throw resistance into reverse? If we can find compounds whose cross-resistance is negatively correlated with DDT, we can expect that the more DDT-tolerant survivors of a DDT selection will be more susceptible than normal to this compound. Vice versa, the survivors from selection with this compound will be the more DDT-susceptible individuals, and so the compound should be able to restore DDT-susceptibility to a heterogeneous DDT-resistant strain. Such negatively-correlated compounds have cropped up from time to time for house flies or *Drosophila.* DTP, an organophosphorus preparation, was one for house flies; but attempts to repeat the results met with failure. CBA (cetyl bromoacetate) was another for house flies, but this negative correlation was not found in experiments on field strains. Apparently CFA (cetyl fluoracetate) has given better results, on *Anopheles* larvae as well as house flies. PTU (phenylthiourea) is a good one for *Drosophila,* since PTU-susceptibility is tied to the same gene as DDT-resistance and vice versa; unfortunately PTU is non-toxic to house flies. We have tested PTU, CBA and CFA in our own laboratory on various strains of *Aedes aegypti* with the following results: CFA has shown no negative correlation with DDT-resistance, while CBA and PTU are not larvicidal. Despite all these disappointments, negatively-correlated compounds offer at present our only hope of throwing resistance in reverse, and we should continue to look for them by empirical testing or by basic biochemical research.

As we peer into the future, we can only hope that such compounds will appear. Meanwhile we can hang on and make the most of what we have. The basic principle is to practice economy. Economy in extent of the area treated will reduce the resistance hazard by leaving a greater proportion of untreated population to dilute the resistant genotypes left in the treated area. Economy in choice of insecticide means avoiding a premature switch to a substitute insecticide until susceptibility tests with the standard insecticide indicate it is absolutely necessary; thus we can delay our passage from one insecticide-resistance to the next. Taking a hint from cockroach control, we can return to certain older, less effective insecticides that do not have a record of resistance, such as paris green for *Psorophora* control in Florida, and Thanite for *Culex* control in Egypt. More stress will be laid on environmental and biological means of control, even though the former will be more expensive and the latter less effective than the use of insecticides. The susceptibility levels of populations under treatment will be followed and their cross-resistances probed, while more basic research will investigate origins and mechanisms of resistance. Those responsible for management will thus be sufficiently well-informed that their anti-mosquito operations will avoid the errors of rigidity on the one hand and panic on the other. In these ways we can buy time to continue the search for definite means of counteracting resistance.