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Resistance of *Aëdes aegypti* to Certain Chlorinated Hydrocarbon and Organophosphorus Insecticides in Puerto Rico*

IRVING FOX[1]

Failure to control Aëdes aegypti *in Puerto Rico has suggested resistance to the insecticides, particularly DDT. A laboratory colony (named Isla Verde strain) was established from material obtained near the International Airport. This strain proved highly resistant to DDT and dieldrin as well as various other insecticides. The scientific and practical significance of this is far-reaching. Heretofore, it was thought that populations of* Aëdes aegypti *could be resistant to the DDT group of insecticides or to the dieldrin group but not to both, and that one type of resistance involved biochemical, toxicological and genetic characteristics different from the other. This theory must now be modified or even abandoned. From the practical point of view, it is clear that the continued use of DDT or dieldrin for* Aëdes aegypti *control in Puerto Rico is questionable, for it is not wise to attempt to overcome high resistance by increasing the concentration or rate of application. Further, the situation demonstrated for Puerto Rico may also obtain in other islands of the Caribbean where* Aëdes aegypti *is known to be DDT-resistant.*

INTRODUCTION

The outbreaks of yellow fever in Trinidad and the northward advance of the disease in Central America in recent years have alarmed the local and federal public health authorities of America. Puerto Rico, in the West Indies but a part of the United States of America, just as all the port cities, land entry points, and airports where *Aëdes aegypti* occurs in the southern USA, has been designated a yellow fever receptive area (Hughes & Porter, 1958; Tinker & Hayes, 1959).

The Department of Health of Puerto Rico has always been aware of the danger from yellow fever and in 1950 instituted a programme of DDT residual spraying and house-to-house inspections. This programme, although it did not eradicate *Aëdes aegypti*, was successful in keeping the population in the area of San Juan, the capital city, at a relatively low level. However, in 1956 a recrudescence in the population of *A. aegypti* seemed to have occurred and the species was found to be rather common in the vicinity of the International Airport, Isla Verde, not far from San Juan (Fox, 1958). This suggested resistance to DDT, inasmuch as resistant strains have been reported from various near-by countries of the Caribbean region, such as Haiti (Sautet & Vuillet, 1956; Sautet et al., 1958), Trinidad, Venezuela, Surinam, Dominican Republic, and Colombia (Brown, 1958a).

THE LABORATORY COLONY

The experiments with the Puerto Rico strain of *Aëdes aegypti* reported upon here were accomplished using specimens obtained from a recently established laboratory colony. It must be pointed out, however, that the World Health Organization Expert Committee on Insecticides (1960) recommends that insecticide resistance tests be made on lots of larvae obtained from various localities and at different seasons. This is undoubtedly the more desirable procedure but it is exceedingly expensive, requiring

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[1] Associate Professor of Entomology, School of Medicine–School of Tropical Medicine of the University of Puerto Rico, San Juan, Puerto Rico.

personnel, time, and transport facilities to obtain from the field the large number of healthy specimens needed for resistance tests, particularly in places where control efforts have been in progress for some time. It is not likely, in my opinion, that a laboratory colony established from specimens collected in an area subject to control operations and replenished from time to time differs greatly from the population in nature when resistance is shown by experiments. Moreover, it has been pointed out that the laboratory-reared specimens may be more uniform in reactions to insecticides than those collected in the field, which vary in nutrition and exposure to chemicals and suffer from the effects of transportation (Lewallen & Nicholson, 1959). It seems to be a good principle as regards the question of laboratory colony *versus* field-collected specimens that where resistance is shown the results should be considered valid but where susceptibility is demonstrated confirmation should be sought from specimens taken in the field.

In San Juan, Puerto Rico, room temperature varies from 25°C to 30°C in the spring and summer months and conditions of humidity and light are favourable for small colonies of *Aëdes aegypti*. The colony used in the experiments described here was begun in November 1958 from specimens obtained as adults and larvae at Boca de Cangrejos, Isla Verde, Puerto Rico, not far from the International Airport.

SOURCES OF INSECTICIDES

The composition and sources of the insecticides used in these experiments were as follows.

1. *DDT* (purified *p,p'*-isomer). World Health Organization Test Kit L-37 for surveys on susceptibility of mosquito larvae to insecticides, received from the Pan American Sanitary Bureau, Washington, D.C. This kit includes standard solutions in ethyl alcohol which, when added to water, give concentrations of 0.004, 0.02, 0.10, 0.50 and 2.50 parts per million (p.p.m.).

2. *Lindane* (BHC, pure γ-isomer). The same as above.

3. *Dieldrin* (purified HEOD). The same as above.

4. *Chlordane* (technical chlordane, 74.00%; aliphatic hydrocarbons, 16.40%; emulsifying agent (anhydrous), 9.60%). Velsicol Corporation, New York, N.Y., USA; received from the Puerto Rico Department of Health, San Juan, Puerto Rico.

TABLE 1

PERCENTAGE MORTALITY OF *AËDES AEGYPTI* LARVAE (AVERAGE OF 2 REPLICATES, DEAD LARVAE ONLY) AFTER 24 HOURS' EXPOSURE TO INSECTICIDES [a]

Insecticide	Concentration (p.p.m.)								
	0.004	0.02	0.10	0.5	1.0	2.5	5.0	7.5	10
DDT	0	0	5.5	10.0	8.0	55.0	75.0	91.5	100
Lindane	0	1.5	5.0	44.0	34.0	56.5	95.0	97.5	100
Dieldrin	0	0	5.5	14.0	21.5	70.0	92.0	100	
Chlordane	0	0	0	2.5	3.0	22.5	23.0	32.5	14.5
Bayer 21/199	0	5	33.5	96.5	100				
Dipterex	8.0	3.5	0	72.0	67.5	100			
Malathion	5.0	33.0	10.0	85.0	95.0	100			
Diazinon	5.5	14.5	12.5	74.5	92.0	100			

[a] Control mortalities for the experiments reported in Tables 1-4 were 0; for those in Table 5 they were mostly 0 and never more than 4%. According to the instructions with WHO test kits, if control mortalities are 5% or less, the mortalities observed may be used without further calculation.

5. *Bayer 21/199* (30% wettable powder, also called Asuntol). Geo. F. Novey, Inc., Panama; received from the US Navy, San Juan, Puerto Rico.

6. *MGK Dipterex soluble powder* (0,0-dimethyl-1-hydroxy-2,2,2-trichloroethyl phosphonate, 80%; inert ingredients, 20%). Developed by Farbenfabriken Bayer and licensed to Chemagro Corporation, New York, N.Y., USA; manufactured for McLaughlin Gormley King Co., Minneapolis, Minn., USA; received from Rodriguez US Army Hospital, San Juan, Puerto Rico.

7. *Malathion* (57% liquid concentrate, emulsifiable). Cyanamid Inter-American Corp., Division Agropecuaria, New York, N.Y., USA; received from the Puerto Rico Department of Health, San Juan, Puerto Rico.

8. *Diazinon* (25% liquid, emulsifiable). Geigy Agricultural Chemicals, Ardsley, N.Y., USA; received from the Puerto Rico Department of Health, San Juan, Puerto Rico.

METHODS [1]

The first group of experiments was carried out from 13 March through 24 July 1959, at temperatures varying from 25°C to 29°C. In the beginning the tests were made using 30 fourth-stage larvae in 1000 ml of distilled water; most of the experiments with DDT, dieldrin, and lindane were done in that way. Later, however, in keeping with recent changes in instructions for WHO test kits, 20 larvae in 200 ml of distilled water in disposable paper cups were used (J. A. Kerr—personal communication, 1959). Appropriate controls were always used. In general two replicates were made, but in cases of inconsistent results more were done. When more than two replicates were made the two lowest percentages of mortality were considered to be the most significant. In calculating percentage mortality, many authorities recommend that moribund and dead larvae be combined (Brown, 1958b); but I found it exceedingly difficult to distinguish between moribund and living larvae, while there was no doubt concerning the dead larvae.

RESULTS

Table 1 gives the average of the two lowest percentages of dead larvae after 24 hours' exposure to the insecticides in the concentrations of 0.004, 0.02, 0.10, 0.5, 1.0, 2.5, 5.0, 7.5, and 10 p.p.m. for this group of experiments. In Table 2 the data for DDT, lindane, and dieldrin at 0.5, 1.0, and 2.5 p.p.m. in six replications are given, with the dead larvae and the dead plus the moribund larvae treated separately. This table shows the wide variation in mortality that occurred; thus DDT at 0.5 p.p.m. gave 0 to 85% mortality after 24 hours' exposure in six replications. It also demonstrates

TABLE 2

PERCENTAGE MORTALITY OF *AËDES AEGYPTI* AFTER 24 HOURS' EXPOSURE TO DDT, LINDANE OR DIELDRIN [a]

Replicates	Concentration (p.p.m.)					
	0.5		1.0		2.5	
	D	D+M	D	D+M	D	D+M
DDT						
1	33	33	5	16	80	86
2	39	39	11	11	74	74
3	75	75	80	95	55	93
4	85	85	60	60	55	79
5	0	5	35	50	73	87
6	15	15	40	40	65	79
Average	41.2	42.0	38.5	45.3	67.0	83.0
Lindane						
1	48	70	33	44	97	97
2	77	83	38	50	97	100
3	80	90	50	50	58	58
4	40	50	35	40	55	63
5	68	68	94	94	100	100
6	78	78	84	84	100	100
Average	65.2	73.2	55.7	60.3	84.5	86.3
Dieldrin						
1	18	79	30	30	89	100
2	70	70	11	11	95	100
3	40	40	40	40	85	90
4	55	65	55	55	84	89
5	25	25	40	40	80	80
6	10	20	20	20	60	60
Average	36.3	49.8	32.6	32.6	82.2	86.5

[a] See footnote to Table 1. D = dead larvae. D + M = dead and moribund larvae.

[1] Readers who desire detailed information on how concentrations were obtained are referred to the previous article by the present author (Fox, 1960).

TABLE 3

PERCENTAGE MORTALITY AFTER 24 HOURS OF *AËDES AEGYPTI* ADULT FED FEMALES EXPOSED FOR ONE HOUR TO DDT OR DIELDRIN [a]

Replicates	DDT (4%)		Dieldrin (1.6%)	
	No.	% mortality	No.	% mortality
1	9	11	6	16
2	10	30	10	0
3	11	45	7	0
4	20	35	20	15
5	22	63	22	22

[a] See footnote to Table 1.

that interpretation as to what constitutes living and moribund larvae may make a difference in the results; thus DDT at 2.5 p.p.m. when only dead larvae are counted gives a mortality of 55%-80% but if dead plus moribund larvae are counted the mortality is 74%-93%. Sometimes a higher concentration gave less mortality than a lower one; thus DDT, lindane, dieldrin and Dipterex gave a greater percentage mortality at 0.5 p.p.m. than at 1.0 p.p.m. in some replicates (Tables 1 and 2). Erratic results were obtained with chlordane, which in six replications gave 100%, 100%, 0, 0, 100% and 100% mortality at 0.1 p.p.m., and gave a lower mortality at 10 p.p.m. than at 2.5, 5.0, or 7.5 p.p.m. WHO test kit No. 276 was used to determine the susceptibility of adult females to DDT and dieldrin in the first group of experiments accomplished in May. A total of 72 specimens tested at 4% DDT and 1.6% dieldrin (the highest concentrations in the test kit) showed a range of mortality for DDT from 11% to 63% and for dieldrin from 0 to 22% (Table 3), confirming the high resistance to these insecticides demonstrated by the larval tests.

Two to five months after the experiments the results of which are shown in Tables 1, 2, and 3 —that is, in September and October 1959, or after about 10-25 further generations of continuous inbreeding in the colony—another group of experiments was made using fourth-stage larvae and a new test kit (No. 259). DDT, lindane, and dieldrin at 2.5 p.p.m. and Bayer 21/199 at 0.5 p.p.m. gave the results shown in Table 4. DDT at 10 p.p.m. gave the following results (average of two replications): dead, 96%; dead plus moribund, 100%.

TABLE 4

PERCENTAGE MORTALITY OF *AËDES AEGYPTI* FOURTH-INSTAR LARVAE AFTER 24 HOURS' EXPOSURE TO DDT LINDANE, DIELDRIN OR BAYER 21/199 2-5 MONTHS AFTER EXPERIMENTS IN TABLE 2 [a]

Replicates	Bayer 21/199 (0.5 p.p.m.)		DDT (2.5 p.p.m.)		Lindane (2.5 p.p.m.)		Dieldrin (2.5 p.p.m.)	
	D	D+M	D	D+M	D	D+M	D	D+M
1	100	100	50	61	74	74	13	20
2	84	100	40	40	70	75	18	25
3	91	100	48	48	78	78	15	15
4	90	100	75	75	96	96	11	11
5	100	100	73	73	93	93	13	26
6	100	100	68	76	86	96	7	27
Average	94	100	59	62	83	85	13	21

[a] See footnote to Table 1. D = dead larvae. D + M = dead and moribund larvae.

Lindane at 10 p.p.m. gave 77.5% dead and 100% dead plus moribund. Dieldrin at 10 p.p.m. gave 66% dead and 80% dead plus moribund. From these data it is clear that the colony became even more resistant to the three insecticides, more particularly to dieldrin. However, the degree of susceptibility to Bayer 21/199 remained about the same. Confirmatory experiments were also made with adults, using new test papers with DDT at 4% and dieldrin

TABLE 5

PERCENTAGE MORTALITY AFTER 24 HOURS OF *AËDES AEGYPTI* ADULT FEMALES EXPOSED FOR ONE HOUR TO DDT OR DIELDRIN 4-5 MONTHS OF INBREEDING AFTER EXPERIMENTS IN TABLE 3 [a]

Replicates	DDT (4%)		Dieldrin (1.6%)	
	No.	% mortality	No.	% mortality
1	19	35	21	23
2	20	35	20	5
3	20	65	21	14
4	21	42	21	9
5	22	18	22	10
6	22	27	20	15

[a] See footnote to Table 1.

at 1.6%, kindly provided by Dr J. Austin Kerr. These experiments were done in September and October 1959, or four to five months after the experiments shown in Table 3, which were done in May. The results are shown in Table 5. To confirm the results for dieldrin and to use higher concentrations, since 1.6% is the highest concentration in the WHO test kit, a formulation labelled "Dieldrin (Ortho) 15%, Calif. Spray Co., 1.5 lbs. per gal.", which was kindly supplied by the US Public Health Service, San Juan, Puerto Rico, was tested against adults. Test papers similar to those of the WHO test kit were made by spraying WHO blank holding-tube paper sheets (12 cm × 15 cm) at the rate of 3.6 mg of solution per cm^2. Desired concentrations were obtained by diluting with water. After the solution was weighed, four parts of water were added to obtain an even spread on the paper, which was dried overnight before use. The 24-hour percentage mortalities after one hour's exposure to various percentages of dieldrin were as follows (average of two replications): for 1.87% dieldrin, 33% mortality; for 3.75% dieldrin, 34.5% mortality; for 7.5% dieldrin, 50.5% mortality; and for 15% dieldrin, 57.5% mortality.

CONCLUSIONS

It is clear that the Isla Verde, Puerto Rico, strain of *Aëdes aegypti* is highly resistant to DDT, lindane, dieldrin and chlordane (Tables 1-5). Calculation of the LC_{50} under these circumstances is useless. According to the World Health Organization, 100% mortality results when non-resistant larvae are exposed to 0.02 p.p.m. of DDT or dieldrin; the Puerto Rico strain requires about 10 p.p.m. to effect a 100% kill with these insecticides, indicating that this strain is about 500 times as DDT-resistant as susceptible strains. Comparison with the results obtained by Dr R. W. Fay using a strain from Trinidad (Brown, 1958a; Fay, 1956) shows that the Puerto Rico strain is less resistant to DDT but more resistant to lindane and dieldrin. The Puerto Rico strain also appears to be about 10 times more resistant to malathion than susceptible strains and five times more resistant than the Trinidad strain. Dipterex and diazinon were similar in effectiveness to malathion. Of all the insecticides tested Bayer 21/199 was clearly superior, and the 100% 24-hour mortality at 1.0 p.p.m. shown in Table 1 was obtained in six replicates. However, further experimentation, as well as practical trials, will be necessary before it can be decided whether Bayer 21/199 is the answer to the problem of resistant strains of *Aëdes aegypti* in the Caribbean region. Of particular interest is the remarkable resistance to dieldrin which developed in the colony after continuous inbreeding for a number of months (Table 4). This appears to be the first report of a strain which exhibits a high degree of resistance to both DDT and dieldrin. Heretofore, resistance to one insecticide or the other but not to both has been reported. It is therefore apparent that dieldrin is not likely to be successful if used to overcome DDT-resistance in Puerto Rico, and this may also apply to other places in the Caribbean region where DDT-resistance has been encountered.

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RÉSUMÉ

En raison de la présence d'*Aëdes aegypti* sur son territoire et de la proximité de zones où des cas de fièvre jaune se sont produits au cours des dernières années, Porto Rico est considérée comme zone de réceptivité amarile. Dès 1950, le Département de la Santé a mis sur pied un programme de pulvérisations par le DDT et d'inspection de maison par maison qui eut pour effet, sinon de supprimer le moustique vecteur, du moins de limiter fortement sa densité dans la zone de San Juan, la capitale. En 1956 cependant, cette densité parut augmenter, en particulier autour de l'aéroport de Isla Verde. On soupçonna une résistance au DDT, d'autant plus plausible qu'elle était signalée dans des régions avoisinantes des Antilles et de l'Amérique du Sud.

Les essais de sensibilité aux insecticides portèrent sur une colonie maintenue en laboratoire, provenant de moustiques récoltés à Isla Verde. Ces moustiques montrèrent une résistance très élevée au DDT — 500 fois supérieure

à celle des souches sensibles —, ainsi qu'au lindane et à la dieldrine (il fallait 7,5 p.p.m. de cette substance pour obtenir 100 % de mortalité chez les larves après 24 heures d'exposition à l'insecticide). Dans une autre série d'essais, après quelques mois de développement en colonie, 10 p.p.m. de DDT ou de lindane n'assuraient pas une mortalité de 100 %, et la résistance à la dieldrine s'accentua au point que 10 p.p.m. ne donnaient plus que 66 % de mortalité. Le chlordane à 10 p.p.m. n'assurait que 14 % de mortalité. A la concentration de 2,5 p.p.m., le malathion, le Dipterex et le diazinon tuaient toutes les larves. Le Bayer 21/199 s'est montré le plus efficace (96% de mortalité à 0,5 p.p.m. et 100% de mortalité à 1 p.p.m.). La haute résistance de la souche de moustique au DDT et à la dieldrine a été confirmée sur des femelles adultes: mortalité de 11%-63% pour du DDT à 4%, et de 0%-22% pour la dieldrine à 1,6%.

De l'avis de l'auteur, il s'agit du premier exemple publié de résistance conjointe au DDT et à la dieldrine chez *A. aegypti*. On avait estimé jusqu'à maintenant que ce moustique pouvait être résistant à l'un ou à l'autre de ces insecticides, mais pas aux deux à la fois, et que ces deux types de résistance mettaient en jeu des facteurs biochimiques et génétiques différents.

Il est certain que, dans ces conditions, le traitement par le DDT à des concentrations supérieures, suffisantes pour dominer la résistance, est peu indiqué. Les essais avec le Bayer 21/199 doivent être poursuivis, avant que l'on puisse se prononcer sur sa valeur comme insecticide de remplacement.

L'auteur attire l'attention sur le fait qu'un type analogue de double résistance pourrait se manifester dans d'autres régions des Antilles, où se rencontrent des *A. aegypti* résistants au DDT.

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