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DIELDRIN RESISTANCE IN *CULEX PIPIENS FATIGANS* IN MALAYA

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SYNOPSIS

Resistance to insecticides in *Culex pipiens fatigans* has already been reported from two areas in Malaya. In Penang two years' use of BHC as a larvicide resulted in the development of a strain which was found to have acquired a tenfold resistance to BHC, and also to dieldrin to which it had not been exposed. In Singapore, when larval control became unsatisfactory after 6 months' use of a dieldrin emulsion, laboratory experiments confirmed that active resistance to dieldrin had developed. The present observations report the finding of two further dieldrin-BHC resistant strains of *C. p. fatigans* in Malaya, but differ from the previous reports in that resistance, in one strain at least, was developed as a result of house-spraying with dieldrin against adult mosquitos. In this strain resistance to dieldrin was about 100 times in both adults and larvae, resistance to gamma-BHC in larvae was about 20 times, while resistance to DDT was slight.

Residual house-spraying with dieldrin at 100 mg per ft^2 (1.1 g per m^2) of a small isolated Malay settlement in East Pahang, Malaya, was started in November 1954, as part of an experiment aimed at the control of filariasis caused by *Wuchereria malayi*. The vectors of filariasis in the area are *Mansonia (Mansonioides)* mosquitos. To check the effect of dieldrin on the mosquito population in houses, pyrethrum knock-down sprays were made before and after spraying with dieldrin. In pre-spray catches fairly large numbers of *Culex pipiens fatigans* were noted, but in the six months following dieldrin spraying very few were found. No further knock-down sprays were carried out until March 1957, when large numbers of *C. p. fatigans* were recovered from houses which had been sprayed five times with dieldrin, the age of the most recent deposit being four months. A repeat check in June 1957, following the sixth dieldrin spray in May, again showed the presence of large numbers of *C. p. fatigans*. Susceptibility tests were therefore made to see whether resistance had developed.

Materials and Methods

The Ubai settlement, where dieldrin is being sprayed, consists of some 300 people living in 60 houses, rather scattered, but separated from any other settlement by about two miles (3 km) of swamp forest. Blood-fed

C. p. fatigans were collected from the walls of sprayed houses and allowed to lay eggs in the laboratory. At the same time mosquitos were collected from the walls of houses in Lamir, the nearest settlement to Ubai, where residual insecticides had never been used. Laboratory colonies of each strain, Ubai and Lamir, were established, but all insecticide tests were carried out with first-generation larvae or adults bred from eggs laid by wild-caught females. The larvae were raised in the manner described by Reid [9] and tested when 3 days old as early Stage IV larvae. Adults were allowed to emerge in one foot-cube (30.5 × 30.5 × 30.5 cm) cages; they were provided with raisins, and the females were tested when 2-3 days old.

During the experiments the supply of adults from the unsprayed area was difficult to maintain, so mosquitos were caught from unsprayed houses in Kuantan town. It was hoped that they would prove to be susceptible but in fact they provided a second strain of mosquito resistant to dieldrin.

Tests to determine the susceptibility of adults and larvae were made according to the recommendations of the World Health Organization, using their materials and kits. The method for testing adult susceptibility is based on that of Busvine & Nash,[3] simplified and standardized by the issue of papers already impregnated with known concentrations of DDT or dieldrin in a non-toxic mineral oil. Adults were exposed at 26°C approximately and the holding-tubes were maintained at 24°-29°C and 75%-90% humidity. The method for testing larval susceptibility followed the recommendations of Brown,[1] using 1000 ml of rainwater in glass jars with a surface area of 300 cm^2. Larval tests were run at room temperature, the water varying from 24°-28°C over the 24-hour period of exposure. The results were analysed by the probit method and the 50% mortality level (LC_{50}) was estimated visually from graphs.

Results

Tests against adults

The results are summarized in Table I. It will be seen that the Ubai strain was found to be highly resistant to dieldrin. All adults survived exposure for 1 hour to a concentration of 4% whereas the LC_{50} for the Lamir strain was 0.49%. In an attempt to measure the degree of resistance in the Ubai strain the exposure period was increased. After 16 hours' continuous exposure to 4% dieldrin the mortality was 27%. Exposure of the susceptible strain to 0.1% dieldrin for this period killed 87%. These data were graphed and a regression line was drawn to make the LC_{90}/LC_{50} ratio equal to 2.0, thus allowing a very rough estimate of the LC_{50} to be calculated. For the Ubai strain the LC_{50} was 5.6%, which, compared with 0.06% for the Lamir strain, indicates that this strain was about 100 times as resistant to dieldrin as the Lamir strain.

TABLE I. PERCENTAGE MORTALITY AMONG SUSCEPTIBLE AND RESISTANT STRAINS OF UNFED ADULT FEMALES OF *CULEX PIPIENS FATIGANS* AGED 2-3 DAYS EXPOSED FOR ONE HOUR AND 16 HOURS TO VARIOUS CONCENTRATIONS OF DIELDRIN AND DDT

Insecticide	Mosquito strain	Number of replicates (25 adults)	Concentration of insecticides (%)									LC_{50}
			nil	0.1	0.2	0.4	0.5	0.8	1.0	2.0	4.0	
1-hour exposures												
Dieldrin	Ubai	4	0	—	—	—	—	0	—	—	0	—
	Lamir	5	0	1	4	38	—	78	—	—	—	0.49
16-hour exposures												
Dieldrin	Ubai	5	1	—	—	—	—	—	—	—	27	5.6*
	Lamir	3	0	87	—	—	—	—	—	—	—	0.06*
DDT	Ubai	4	0	—	—	—	3	—	11	57	81	1.95
	Lamir	3	0	—	—	—	22	—	54	80	91	0.98

* Estimation from a regression line drawn so as to make LC_{90}/LC_{50} ratio equal to 2.0

Previous tests by Wharton [10] showed that *C. p. fatigans* has a natural insusceptibility to DDT; concentrations up to 5% killed very few exposed for 1 hour. In the present experiments exposure for 16 hours allowed an LC_{50} for the Ubai and Lamir strains to be determined: 1.95% for Ubai and 0.98% for Lamir, indicating that the dieldrin-resistant strain was slightly resistant to DDT also.

A few tests only were made with adults of the Kuantan strain, and these indicated that this strain also was resistant to dieldrin; one out of 47 was killed after exposure for 16 hours to 0.1% dieldrin.

Tests against larvae

The results are summarized in Table II. Comparing first the Ubai (dieldrin-resistant) and Lamir (susceptible) strains, it will be seen that larvae of the Ubai strain were about 100 times as resistant to dieldrin as were Lamir larvae—LC_{50} 0.62% as compared to 0.006%. Similarly Ubai larvae were also highly resistant to gamma-BHC—LC_{50} 0.87% as compared to 0.043%, a resistance of about 20 times the normal. The LC_{50} for Ubai larvae exposed to DDT was 0.18%, about three times the LC_{50} for Lamir larvae.

The Kuantan *C. p. fatigans* larvae were also found to be resistant to dieldrin and gamma-BHC. Compared with the Lamir strain, the degree of resistance was about 60 times for dieldrin and 7 times for gamma-BHC; resistance to DDT was again slight.

TABLE II. PERCENTAGE MORTALITY OF SUSCEPTIBLE AND RESISTANT STRAINS OF EARLY STAGE-IV LARVAE OF *CULEX PIPIENS FATIGANS* AFTER EXPOSURE FOR 24 HOURS TO VARIOUS CONCENTRATIONS OF DIELDRIN, GAMMA-BHC AND DDT

Insecticide	Mosquito strain	Number of replicates (30 larvae)	Concentration of insecticide (p.p.m.)												LC_{50}
			nil	0.002	0.004	0.01	0.02	0.05	0.10	0.25	0.50	0.625	0.75	1.0	1.25	
Dieldrin	Ubai	10-12	0	—	—	—	—	—	—	0	24	—	76	96	—	0.62
	Kuantan	4	0	—	—	—	2	—	13	18	69	—	—	—	—	0.37
	Lamir	6	3	14	60	70	79	—	—	—	—	—	—	—	—	0.006
Gamma-BHC	Ubai	6	0	—	—	—	—	—	—	—	2	4	—	—	96	0.87
	Kuantan	4	0	—	—	—	—	—	5	—	77	—	—	—	96	0.31
	Lamir	4	0	—	3	—	34	—	66	—	96	—	—	—	—	0.043
DDT	Ubai	8	0	—	—	—	0	3	18	73	—	—	—	—	—	0.18
	Kuantan	4	0	—	—	—	2	16	32	71	—	—	—	—	—	0.15
	Lamir	8	2	—	—	—	7	52	77	91	—	—	—	—	—	0.065

Discussion

The general background is of some importance to the discussion of these dieldrin- and gamma-BHC-resistant strains of *C. p. fatigans*. The area where the investigations took place is on the east coast of Malaya, some 150 miles (240 km) from Kuala Lumpur, and where in recent years a programme of filariasis research has been in progress. Endemic filariasis is caused by *W. malayi*, and *C. p. fatigans* is of no epidemiological significance in the area; the spraying of Ubai with dieldrin was directed at *Mansonia (Mansonioides)* mosquitos which rest in houses by day only to a small extent. Pyrethrum knock-down sprays were made in sprayed houses after the initial spray, mainly to confirm the residual effect of the dose applied, and the finding that a single application of 100 mg dieldrin per ft^2 (1.1 g per m^2) practically eliminated the house-resting population of *C. p. fatigans* was incidental to the filariasis control programme. A chance inquiry, about two and a half years after the initial spray, showed that *C. p. fatigans* was again present in sprayed houses, and led to the present investigations. The resistant strain is extremely localized since mosquitos collected from the nearest settlement, Lamir, provided the susceptible strain. This localization of the resistant strain may seem strange in view of the fact that a second dieldrin- and gamma-BHC-resistant strain was found in Kuantan. Ubai and Lamir, however, are Malay settlements (kampongs) situated in swamp-forest areas and with comparatively little contact with modern life, whereas Kuantan is a rapidly-growing town of some 30 000 inhabitants, 20 miles (about 30 km) from the two Malay settlements. The history of this Kuantan strain is somewhat obscure. The mosquitos were collected from houses two to three years old in a residential area where there had been no organized house-spraying, though a few houses have been sprayed in recent months with gamma-BHC. The vegetation close to the houses had been fogged once with dieldrin about three months previously, and commercial Flit-sprays containing DDT, dieldrin or gamma-BHC are used by the residents. DDT, dieldrin and gamma-BHC have not been used in routine larvicidal operations in the Kuantan area.

The main features of the results are:

(1) The degree of resistance exhibited by *C. p. fatigans*, roughly 100 times and 60 times for the Ubai and Kuantan strains respectively with dieldrin, and 20 and 7 times for the same strains with gamma-BHC.

(2) Although larvae were not apparently exposed to the insecticide in the field, resistance in the larvae was of the same order as in the adults and the degree of resistance developed was in fact more accurately and easily measured by tests against larvae than against the adults. Comparatively little attention appears to have been paid to this aspect of resis-

tance, and workers in the field who suspect resistance, but are unable to obtain satisfactory numbers of adults, or test-papers with a high enough concentration of the insecticide, might well be able to use larvae instead.

(3) Although cross-resistance between dieldrin and gamma-BHC was clearly shown for both the Ubai and Kuantan strains, cross-resistance to DDT was very slight. Adults from Ubai appeared to have a resistance of twice that of the susceptible strain, while larvae from Ubai and Kuantan had LC_{50}'s of twice to three times that of the susceptible strain. The results, therefore, are consistent with the belief that development of a marked resistance to dieldrin and gamma-BHC conferred a slight degree of resistance to DDT. Some doubt must remain, however, since in earlier larval tests on susceptible strains of *C. p. fatigans* in Malaya, LC_{50}'s of 0.224 parts per million (p.p.m.) for a Kuala Lumpur strain [8] and 0.145 p.p.m. for a Tampin strain [10] were recorded; these are considerably higher than the LC_{50} of 0.065 p.p.m. recorded for the susceptible strain in the present experiments, and cover a wider range than the LC_{50}'s of 0.18 (Ubai) and 0.15 (Kuantan) for the dieldrin-resistant strains. Though the earlier results were obtained with a slightly different technique from that used in the present experiments[a] the probable explanation lies in local strain differences. It is possible also that *C. p. fatigans* from Kuala Lumpur at least could have had some contact with DDT in the field before it was tested.

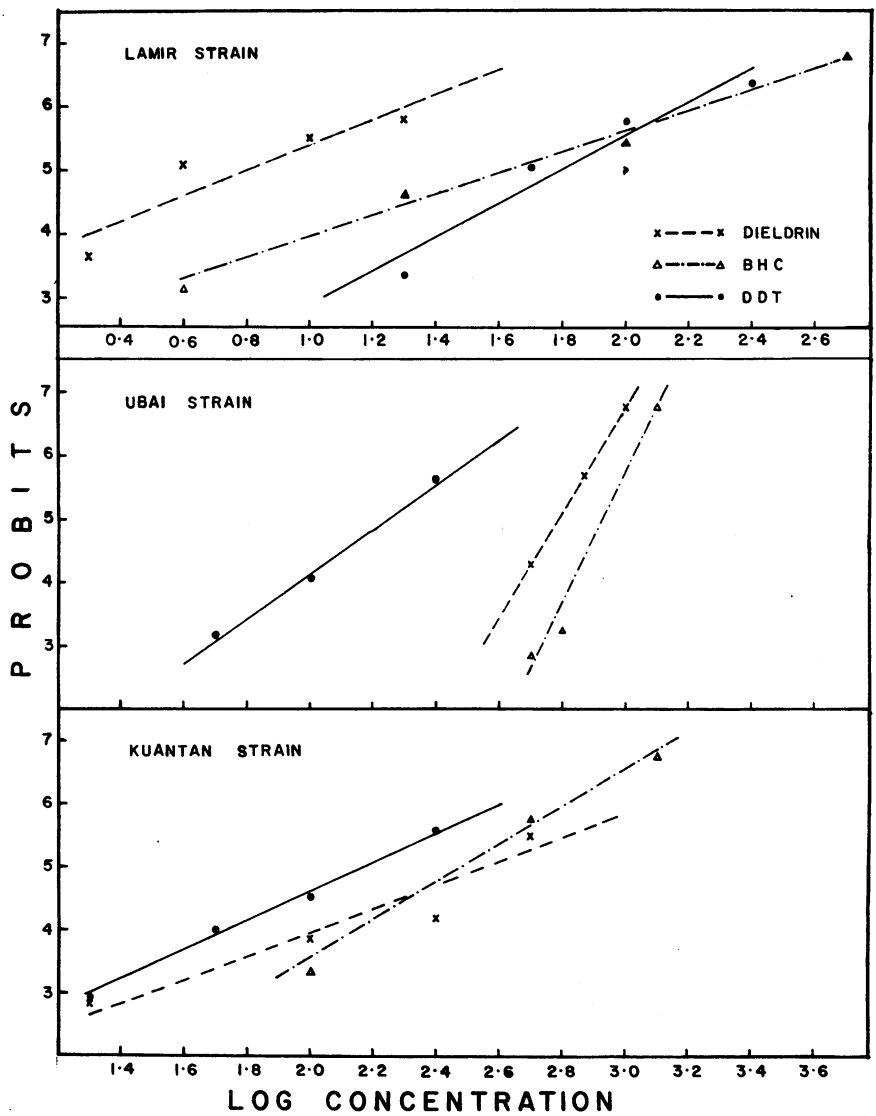
A feature of the dose-mortality regression lines was the steep slope of those drawn for dieldrin and gamma-BHC against the Ubai strain. This is illustrated in Fig. 1 which compares the regression lines of dieldrin, gamma-BHC and DDT for the Lamir (susceptible), Ubai (resistant) and Kuantan (resistant) strains. The regression lines show that the susceptible strain has a much wider range of susceptibility to dieldrin and gamma-BHC than has the Ubai strain. This is at first thought contrary to expectations, in view of the belief that resistant strains produced in the field are generally heterogeneous, and should therefore be expected to show a wider range of susceptibility than normal strains. In Ubai, however, a high degree of selection must have taken place. Dieldrin had been sprayed in houses at the high dose of 100 mg per ft^2 (1.1 g per m^2) at six-monthly intervals for more than two years before resistance was noticed (and it seems likely that resistance had been present for some time beforehand). The effect of the first spray had been to reduce the house-resting *C. p. fatigans* population to a very low level, and it is known that the vast majority rest in houses by day. Pre-

[a] A few tests were run to compare the LC_{50} of Lamir *fatigans* when using enamel pans containing 500 ml of rainwater (surface area 225 cm^2) and half the quantity of insecticide solution but the same numbers of larvae, with the LC_{50} when using glass jars with 1000 ml of water. The results were as follows:

	DDT	*BHC*	*Dieldrin*
1000 ml in glass jar	0.056	0.048	0.0060
500 ml in enamel pan	0.068	0.041	0.0065

Clearly, the type of container and the quantity of water used made no significant difference to the results.

FIG. 1. THE LOG-CONCENTRATION/PROBIT REGRESSION LINES OF LARVAL TESTS WITH DIELDRIN, BHC AND DDT AGAINST THREE STRAINS OF *CULEX PIPIENS FATIGANS*



sumably, therefore, in this small area there has been continued selection at a high level for over two years resulting in the production of an extremely homogeneous resistant strain (it should be remembered that the tests were made on the progeny of numerous wild-caught adults, not from a colony established from a few females). The regression lines for DDT are much the same for all three strains. Those for dieldrin and gamma-BHC with the Kuantan strain do not show the same steepness as do those of the Ubai strain, but at the same time they do not indicate that the population was any more heterogeneous than the more susceptible Lamir strain. Crossings between the various strains would be of obvious interest; other commitments make it impossible for these to be carried out in Malaya at present but material would be gladly provided for any interested worker.

This high degree of dieldrin resistance developed by *C. p. fatigans* under field conditions over a period of two and a half years illustrates again how little value residual insecticides have in the control of this mosquito. This was the conclusion of Reid [9] in Malaya and of Chow & Thevasagayam [4] in Ceylon. House-spraying with DDT has proved ineffective in many areas and since the review by Busvine [2] on resistance in this species further reports have come from Malaya,[5] Taiwan,[7] and Okinawa.[6]

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RÉSUMÉ

Une résistance aux insecticides chez *Culex pipiens fatigans* a déjà été signalée dans deux régions de Malaisie. Au Penang, après avoir employé pendant deux ans de l'HCH comme larvicide, on a constaté l'existence d'une souche qui avait acquis une résistance dix fois plus forte à l'HCH, et même à la dieldrine, sans pourtant avoir été exposée à cette dernière. A Singapour, la lutte larvicide à l'aide d'émulsion de dieldrine est devenue inefficace au bout de six moix; les expériences en laboratoire ont alors confirmé le fait qu'une résistance manifeste à la dieldrine s'était développée. D'après les observations rapportées ici, deux autres souches de *C. p. fatigans* résistantes à la dieldrine et à l'HCH ont été trouvées en Malaisie. Mais, dans ce cas, chez l'une tout au moins, la résistance s'est développée à la suite de pulvérisations de dieldrine contre les moustiques adultes dans les maisons. Dans cette même souche, la résistance à la dieldrine a centuplé aussi bien chez les adultes que chez les larves, la résistance au gamma-HCH est devenue vingt fois plus forte chez les larves, tandis que la résistance au DDT n'était que faible.

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