Effects of sublethal concentrations of dieldrin on *Culex pipiens fatigans*

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Introduction

IN ROUTINE CONTROL PROGRAMMES, it is possible that aquatic stages of mosquitoes in breeding grounds are exposed for prolonged periods of time to sublethal concentrations of various insecticides, including dieldrin. Information on the effects of such prolonged exposure of *Culex pipiens fatigans* larvae to sublethal concentrations of dieldrin, on their susceptibility or tolerance to dieldrin, on duration of their larval life and on reproductive potential of the adults which emerge from these larvae, is not available. This information is of great practical importance.

In comparison to the large proportion of research work on the development of resistance by insecticide selection, on the physiology of resistance and on the mode of inheritance of insecticide resistance, very little work has been done on the effects of sublethal concentrations of dieldrin on larvae of *C. p. fatigens*. The only work of this kind on *Culex pipiens* is that of Zaghloul and Brown (1968) on the effects of sublethal doses of DDT on the reproduction and susceptibility of this species. They have exposed one-day-old adults of three strains of *C. pipiens* to sublethal doses of DDT for one, two or 8 hours through 6 to 7 generations.

No such work has been done with any insecticide on the larvae or adults of *C.p. fatigans* in Malaysia although it is the most important vector of urban strain of *Wuchereria bancrofti* and the chief nuisance mosquito. This work, therefore, was undertaken to study the various effects of sublethal

Fig. 1

DOSAGE MORTALITY REGRESSION LINES FOR EARLY FOURTH-INSTAR LARVAE OF CULEX PIPIERS FATIGANS REARED IN SUBLETHAL CONCENTRATIONS OF DIELDRIN FOR 10 GENERATIONS TO DIELDRIN

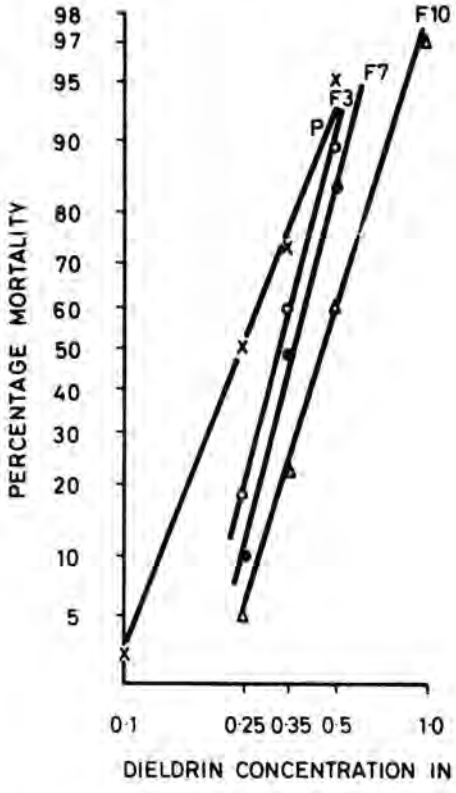
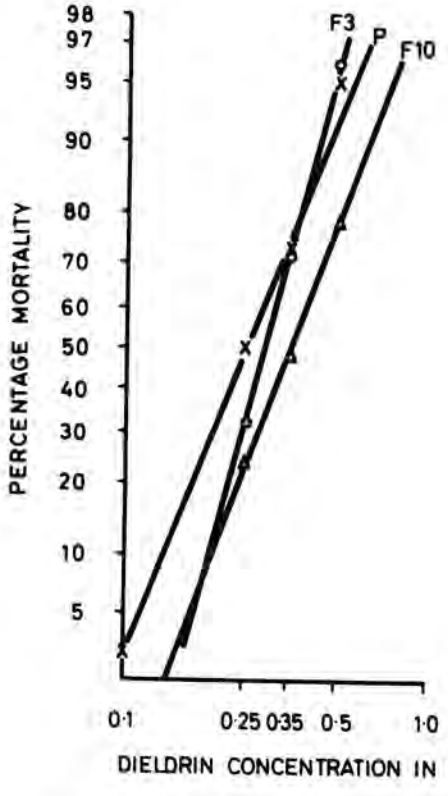


Fig. 2

DOSAGE MORTALITY REGRESSION LINES FOR EARLY FOURTH-INSTAR LARVAE OF CULEX PIPIERS FATIGANS FROM CONTROL SUBCOLONY TO DIELDRIN



doses of dieldrin on development of resistance, duration of larval life and fecundity of the adults when larvae were reared in truly sublethal concentrations of dieldrin.

Materials and Methods

Insecticide — Dieldrin

Dieldrin, which was used in these series, was obtained as standard solutions from the Vector Biology and Control Unit of the World Health Organisation, Geneva. Any intermediate strengths of insecticides needed were prepared from higher doses.

The term "sublethal" has been used to cover all concentrations of dieldrin which did not cause larval mortalities higher than those noticed among larvae in control cultures. As the effects of insecticides were thought to be proportional to the concentrations, it was necessary to keep the sublethal concentrations at the highest level. In order to do so, the concentrations used in older instars of larvae were progressively increased. The concentrations were also increased for some later generations. The concentrations used in these experiments varied from 0.004 ppm to 0.05 ppm dieldrin.

Mosquitoes

Larvae from a single colony of *Culex pipiens fatigans* were used for all experiments. Over 200 egg-rafts were collected from a natural breeding ground in Kuala Lumpur and these were put together for hatching. When the eggs hatched,

TABLE I
Effects of rearing larvae of Culex Pipiens Fatigans larvae in sublethal concentrations of Dieldrin

No. Pupated In		Percentage Pupation		Egg No/Raft For 20 Rafts					
				Control Culture			Sublethal Culture		
Culture Control	Sublethal Culture	Control Culture	Sublethal Culture	Minimum	Maximum	Mean	Minimum	Maximum	Mean
547	555	91·2	92·5	85	193	141·3	91	198	142·5
680	704	85·0	88·0	102	175	138·8	99	185	141·1
285	287	95	95·7	96	186	142·1	95	191	141·7
750	775	93·8	96·8	95	156	126·0	91	187	132·5
422	420	88·8	88·4	102	197	148·1	87	202	147·2
712	717	89	89·6	75	188	132·4	81	189	137·3
176	185	88	92·5	96	201	142·3	93	198	145·7
678	757	84·8	84·6	107	193	143·6	98	195	144·1
672	686	84	85·7	95	189	140·3	101	187	141·7
314	322	73·9	75·8	98	177	139·4	97	182	142·3
—	—	—	—	—	—	—	—	—	—

F5, F7 and F9 generations were 0.33 ppm, 0.36 ppm, 0.37 ppm and 0.43 ppm respectively compared to 0.29 ppm, 0.25 ppm, 0.34 ppm, and 0.34 ppm for control larvae in the same generations. In F10 the LC50 values for experimental larvae were 0.46 ppm as compared to that of 0.35 ppm for control larvae. This level was 1.8 times higher than that found for parents. Throughout this series of experiments, larvae from the sublethal cultures always showed a higher level of tolerance than those from the control cultures. In addition, there were some slight fluctuations in LC50 levels over 10 generations. A certain degree of fluctuations in LC50 levels to insecticides are common in *Culex pipiens fatigans*. Similar variations in LC50 levels among larvae of various generations of a laboratory colony of *C.p. fatigans* submitted to DDT-selection pressure has been observed (Thomas, 1966). These results show that rearing the larvae of *C.p. fatigans*, in sublethal concentrations of the insecticide, did not develop any significant degree of resistance. However, the larvae exhibited some increase in tolerance (1.8 times) levels. A lesser degree of increase in LC50 levels (1.4 times) was observed for larvae from control cultures also although these larvae have never been exposed to dieldrin. These slight variations and increase may then be a normal character and brought about by the optimum rearing conditions rather than by development of true resistance.

The results obtained in this series of experiments were very similar to those which have been noticed in a DDT-resistant and a DDT-tolerant strains of *Culex pipiens* which failed to show any material increase in resistance levels to this insecticide after sublethal exposure to DDT over 6 generations (Zaghloul and Brown, 1968). Similar results were obtained by Harrison (1952) who was unable to induce resistance in a housefly strain frequently exposed to very small doses of DDT. On the other hand, a susceptible strain of *C. pipiens* has developed a considerable degree of DDT-resistance when the adults were exposed to sublethal doses of DDT for 6-7 generations (Zaghloul and Brown, 1968). They concluded that the increase in resistance in this strain was due to hidden selection of eggs in ovary and of females which failed to oviposit. Lineva (1962) noticed that when houseflies were exposed to sublethal doses of DDT for successive generations, they became slightly more susceptible to DDT for the first four generations. In the subsequent 4 generations, the houseflies became steadily DDT-resistant. She also noticed disturbances in oogenesis in the first 4 generations which later disappeared.

Duration of larval life and larval mortality in sublethal cultures.

Subjection of larvae throughout their life to sublethal doses of dieldrin did not prolong the:

TABLE I
Effects of rearing larvae of Culex Pipiens Fatigans larvae in sublethal concentrations of Dieldrin

Generation	No. Of Larvae Reared In		Concentration Of Dieldrin (P.P.M.)		No. Larvae Used For LC 50 Tests From		No. Of Larvae Died In Cultures		Duration Of Larval Life In Days In	
	Control Culture	Sublethal Culture	Control Culture	Sublethal Culture	Control Culture	Sublethal Culture	Control Culture	Sublethal Culture	Control Culture	Sublethal Culture
F1	800	800	NONE	0·004 to 0.05 ppm	200	200	53	45	7-10	7-12
F2	800	800	"	"	—	—	120	96	6-11	6-11
F3	600	600	"	"	300	300	15	13	6-10	7-11
F4	800	800	"	"	—	—	50	25	6-10	6.10
F5	850	850	"	"	375	375	53	55·	6-12	7-12
F6	800	800	"	0·01 to 0.05 ppm	—	—	88	83	7-12	6-11
F7	400	400	"	"	200	200	24	+5	7-12	6-10
F8	800	800	"	"	—	—	123	43	6-14	6-13
F9	800	800	"	"	—	—	128	114	6-10	6-10
F10	800	800	"	"	375	375	111	103	7-11	—
F11	400	400	"	0·02 ppm	Heavy mortality in sublethal culture				—	—

larvae were allowed to remain together for a few hours for thorough mixing. Later, sufficient numbers of larvae were picked up and reared under normal laboratory conditions. The adults which emerged from these were kept as the stock colony (P). When females of this colony laid eggs (F1), the egg-rafts were all put together for hatching. A few hours after hatching, 2 sets of 800 larvae were picked up and were reared in two batches in measured quantities of water. One set of 800 larvae was reared normally without any insecticide (control subcolony) whereas the second batch of larvae was reared in weak solutions of dieldrin (sublethal subcolony).

In subsequent generations, newly-hatched out (3-5 hours old) larvae from experimental cultures were reared in sublethal concentrations of dieldrin. Larvae from the control subcolony were never exposed to dieldrin. The larvae from the sublethal subcolony were exposed throughout larval life to doses which were found to be sublethal to the entire population using modification of WHO method (1963). One cc of insecticide solution in absolute alcohol was added under water surface for every 250 cc of water. Twenty-five mosquito larvae were then reared in that amount of water. When larger numbers of larvae were reared, the same proportions (i.e. 10 cc water/larvae and 1 cc of dieldrin solution/250 cc of water) were used. The room temperature varied from 27°C to 29°C. The larvae in the sublethal cultures and those in the control cultures were fed daily on well-grounded farex, dried liver and yeast in a 3:1:1 ratio. The water in these cultures were changed twice during the larval life. When water in the sublethal cultures were changed, appropriate amount of dieldrin solution was added before the larvae were introduced. Mortality, if any, duration of larval life, number and percentage of pupation, etc., in both the sublethal and control cultures were recorded. LC50 levels of larvae were measured in various generations. Number of eggs per raft for 20 rafts collected in each generation were counted for both subcolonies.

Results and Discussion

Development of dieldrin tolerance in larvae

The number of first-instar larvae reared in sublethal and control cultures, the concentrations of dieldrin used, the larval mortalities in these cultures and the number and percentage of pupation are given in Table I. The LC50 levels of the larvae in alternate generations of control and sublethal subcolonies are given in Table II, and shown in Figures I and II.

The LC50 levels of the parent colony were 0.25 ppm (Table II, Figs. I & II). The LC50 values for larvae from the sublethal cultures were 0.35 ppm in F1 compared to that of 0.26 ppm for larvae from the control cultures. The LC50 levels for larvae from sublethal cultures in F3,

Table II:
Mortality Rates and LC 50 Values for Larvae of Culex Pipiens Fatigans from Control and Sublethal Subcolonies to Dieldrin.

Colony	Generation	Corrected percentage of larval mortality after 24 hrs. exposure to varying concentrations of dieldrin (p.p.m.)					
		0.1	0.25	0.35	0.5	1.00	LC50 (p.p.m.)
—	P	3	50	73	95	100	0.25
Control	F1	2	48	82	94	100	0.26
Sublethal		0	21	52	73	100	0.35
Control	F3	0	32	71	96	100	0.29
Sublethal		0	18	59	89	100	0.33
Control	F5	0	49	81	97	100	0.25
Sublethal		0	25	51	70	98	0.36
Control	F7	0	29	56	76	100	0.34
Sublethal		0	10	48	84	100	0.37
Control	F9	0	22	52	84	100	0.34
Sublethal		0	7	32	64	100	0.43
Control	F10	0	24	48	78	100	0.35
Sublethal		0	5	22	60	97	0.46

larval life (Table I). In most generations, larvae from both sublethal and control cultures which hatched out on the same day pupated on the same day. The larvae from control cultures pupated within 6 to 14 days and those from sublethal cultures pupated within 6 to 13 days (Table I). It was noticed, that in F10 generation, there were five or six larvae in experimental cultures, which remained as second stage larvae, while all other larvae pupated. Since this number was insignificant, these larvae were thrown off. These experiments clearly showed that true sublethal concentrations of dieldrin did not prolong the larval period of *Culex pipiens fatigens* or delayed the pupation. These concentrations did not have any apparent adverse effects on larvae or pupation.

The mortality rates in sublethal larval cultures were negligible in the first 10 generations (Table I). Number and percentage of pupation were very similar to those in control cultures. In F11 generation, the concentration of dieldrin used on first instar larvae was raised to 0.02 ppm from 0.01 ppm which was used on first instar larvae of F10 larvae and on larvae of a few other earlier generations (Table I). There was heavy mortality among first instar larvae of this generation. This was considered as selection rather than sublethal exposure and the experiments were discontinued.

These experiments showed that continuous exposure of all stages of larvae to truly sublethal concentrations of dieldrin for 10 generations did not increase the normal mortality rates of the larvae, pupae or adults which emerged from these larvae. Adults fed normally on pigeon and laid egg-rafts normally without any hesitation.

Effects of Sublethal Concentration of Dieldrin on Fecundity of Adults.

The mean numbers of eggs per raft for 20 rafts collected at random from adults which have emerged from sublethal and control cultures for 10 generations are given in Table I. In all generations except F3 and F5, the adults emerged from experimental cultures gave slightly larger rafts. In F1 generation, the mean number of eggs of control subcolony was 141.3 compared to that of 142.5 from experimental subcolony. In F3 and F5, the mean numbers of egg-rafts from adults of sublethal colony (141.7 and 147.2) were slightly fewer than those from adults of control colony (142.1 and 148.1) in corresponding generations. In all other generations, adults from experimental culture gave larger rafts. In F10 generation, the mean number of eggs from control females was 139.4 compared with the mean of 142.3 for adults from sublethal cultures. Thus the exposure of larvae of *Culex*

pipiens fatigans throughout their life to true sublethal concentrations of dieldrin did not reduce the fecundity of the adults. On the other hand, the adults laid on the average slightly larger eggs. The increase in the mean number of eggs varied from less than 1 per cent in F1 adults to about 5 per cent in F4 adults. It is doubtful whether this increase in the mean number showed any significant increase in the reproductive potential of the adults as the minimum-maximum range of egg numbers for 20 rafts has always been within the normal range of variations.

Increase in egg production has been reported in adult insects which as larvae have been subjected to sublethal doses of insecticides. Ouye and Knutson (1957) reported that adults from melathion-treated housefly larvae produced 12% more potential offspring, as a result of increased egg production and larval development rate. The adults of *Ae. aegypti* which emerged from larvae treated with sublethal concentrations of DDT developed 9 per cent more basal egg follicles (Sutherland, Bean and Gupta, 1967). Zaghloul and Brown (1968) noticed a 16-19 per cent increase in the basal follicles of adults which as larvae were reared for 24 hours in sublethal concentrations of DDT. When a normal housefly strain was exposed to DDT for successive generations, marked disturbances in oogenesis was induced for the first 4 generations (Lineva, 1962). Sublethal exposure of larvae of *Ae. aegypti* to DDT did not produce any significant overall effect upon the reproductive potential of this species (Havertz and Curtin 1967). All known effects of sublethal concentrations/doses of insecticides on larvae and adult mosquitoes, houseflies and other insects are reviewed by Brown and Pal (1971).

Summary

A colony of *Culex pipiens fatigans* was established in the laboratory from more than 200 egg-rafts collected from a natural breeding ground in Kuala Lumpur. The susceptibility level of the larvae (P) which hatched out from these egg-rafts to dieldrin was 0.25 ppm. In the F1 generation, the larvae were divided into control and sublethal subcultures. The larvae from control cultures were never exposed to dieldrin whereas those from sublethal cultures were exposed to true concentrations of dieldrin which varied from 0.004 to 0.05 ppm on various instars of larvae in different generations. The larvae from this subculture was exposed throughout their life for 10 generations. The LC50 levels of the larvae from these subcolonies were measured in different generations. There was a slight fluctuating increase in tolerance of about 1.8 times in the larvae of sublethal colony to dieldrin as compared to that of 1.4 times in the larvae from control culture during 10 generations.

Sublethal rearing of larvae did not cause any high mortality rates among larvae, pupae or adults. The duration of larval life has not increased or pupation delayed due to sublethal exposure throughout the life of larvae in 10 generations.

There was a slight increase in the mean fecundity rate of adults (except in F3 and F5) which emerged from larvae exposed to true sublethal concentrations of dieldrin. The rate of increase in mean number varied from less than 1 per cent in F1 adults to about 5 per cent in F4 adults, as compared to those in the control subcolony.

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