2. Title: Insecticide Tolerance Level of Mosquitoes in Thailand.

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Objective: The development of resistance by mosquitoes to insecticides has posed a considerable threat to the success of public health efforts in combating mosquito-borne disease in many parts of the world. In Thailand, insecticides have been in use for several years against agricultural pests and more commonly in malaria eradication programs. The extent of resistance among mosquitoes in this country cannot be adequately assessed because relatively little information is available on the status of susceptibility of these species to insecticides. Therefore, this study is designed to determine the susceptibility of mosquitoes to insecticides with emphasis on vectors or potential vectors of human disease and to assess the possible consequences of resistance, whether physiological or behavioristic, on the disease incidence.

Progress: This report covers results obtained from (1) studies conducted by SMRL on the susceptibility of local mosquitoes to insecticides in current use and from (2) collaborative studies, with personnel of USDA and WRAIR, on field evaluation of newer insecticides and repellents.

I. SUSCEPTIBILITY OF MOSQUITOES TO INSECTICIDES.

The susceptibility levels of nine species of mosquitoes to DDT and/or dieldrin have been determined. These species included three culicines, Aedes aegypti, Culex gelidus and C. tritaeniorhynchus and six anophelines, Anopheles balabacensis, An. maculatus, An. minimus, An. splendidus, An. tessellatus and An. vagus. All of the species tested are known to feed freely on man. They have also been, except for An. splendidus, An. tessellatus and An. vagus, incriminated in the transmission of human disease in this country and elsewhere.

Material representative of all species tested was obtained from one or more of the following localities: the Experimental Farm of Kasetsart University in the Bang Khen District of Bangkok and from the Provinces of Saraburi, Cholburi, Nakornrajisima and Surathani. Dwellings in these Provinces have been treated for several years, under the malaria eradication program, with DDT residual sprays for one or two cycles per year. At the Kasetsart Experimental Farm, several insecticides ranging from parathion and chlorinated hydrocarbons to Paris green have been in continuous use. Specific locations for any single species tested will be mentioned under its appropriate section in the discussion. Anopheles balabacensis was the only species tested with material provided from a laboratory colony maintained by SMRL. The standard WHO

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adult and larval tests were used throughout, except when it was necessary to use the "time in concentration" technique to demonstrate low levels of resistance. Observed mortalities of mosquitoes obtained in the various tests were corrected for control mortality using Abbott's formula. Log–dose probit mortality regression lines were constructed and the method of Lichfield and Wilcoxon was followed for the calculation of the limits of confidences.

Culex gelidus.—Susceptibility tests on the adults showed that this species had developed an intermediate resistance to DDT and was completely resistant to dieldrin at Bang Khen District of Bangkok while it was still susceptible to DDT (the only insecticide tested) at Bang Phra, Cholburi Province. Resistant adults from Bang Khen showed a two-fold increase of the LC_{50} of DDT and from two to six-fold increase of LC_{90} value over the susceptible individuals from Bang Phra (Table I). Also the slope of the ld-p line as shown in Figure I, 6 was steeper for the susceptible adults than those constructed for the resistant ones of Bang Khen.

The appearance of resistance to DDT was first detected when 13% of the adults survived exposure to the highest concentration (4%) of the toxicant in the initial test (1). The percentage of survivors not only increased, in the follow–up test (no. 2) at the 4% DDT concentration but an increase in the LC_{50} and LC_{90} values was also recorded. This resulted in a shift in the position of the dosage–mortality line without change in slope indicating-in addition to increased resistance-an increase in vigour tolerance of the population tested (Fig. I, 1&2). Later tests conducted on populations of this species from Bang Khen continued to show the presence of resistant individuals though their percentages fluctuated from time to time (Table I, 3, 4&5).

Resistance of adults of C. gelidus from Bang Khen to dieldrin was discovered when this species was recently tested. Table 2 shows that approximately 68% of the adults survived one hour exposure to 4% dieldrin, and when adults were exposed to the same concentration for a two hour period, 47% of the total number tested survived through the 24 hours holding following exposure.

It is interesting to note that larvae tested from Bang Khen, where the resistant adults were found, continued to be susceptible to DDT. The LC_{50} value was 0.0085 ppm of DDT when tested in March 1967 (Fig. II, 2) compared with 0.0089 ppm obtained earlier in November, 1965 (Fig. II, 1). Larvae were also found susceptible to both dieldrin and BHC during 1965 with LC_{50} values of 0.010 and 0.24 ppm respectively. The dosage-mortality regression lines in Figure II show a slight increase in tolerance of the larvae to dieldrin and BHC than to DDT.

The absence of resistance among larvae and its appearance among adults from the same locality suggested the further testing of these larvae by the "time-in-concentration" technique. The reliability of the WHO test for resistance in larval populations, especially where resistance is not pronounced, has been open to criticism, and the "time-in-concentration" technique has been substituted in these studies for detection of resistance in larvae. Late fourth instar larvae were exposed to discriminating dosages of 8 ppm of dieldrin and 2.5 ppm of DDT. Mortality counts were made every 15 minutes after the larvae were placed in the test solutions. The percentage mortality versus time were plotted on log probit paper and the results are shown in Figure III.

In the dieldrin test the regression lines A and B reflected an almost identical genetic constitution of the population involved. The pronounced inflections of the regression lines clearly indicated the presence of two genotypes. Each regression line showed a platoau distinguishing the homozygous susceptible from the heterozygous resistant larvae. In this test approximately 72% of the larvae were susceptible and 28% were heterozygous resistant to dieldrin.

The regression line constructed for the DDT test also showed a pronounced inflection indicating the presence of two phenotypes. While 98% of the larvae appeared to be homozygous susceptible, the presence of heterozygous resistant larvae, though at a low level, was indicated.

Table 1. Results of DDT susceptibility tests on adult Culex gelidus from Bang Khen and Bang Phra (1966-1967)

Test No.	Date	Per cent mortality at each concentration 0.25%	0.5%	1%	2%	4%	Per cent control mortality	LC_{50}	95% confidence limits of LC_{50}	LC_{90}	Slope
				Bang Khen							
1	30Mar.-5 Apr. 66	15(104)	27(105)	29(105)	61(105)	87(105)	5(105)*	1.23%	1.03-1.46	6.75%	1.73
2	28Apr-4May66	7(70)	23(70)	42(70)	36(70)	84(70)	0(70)	1.59%	1.29-1.96	8.41%	1.77
3	10-12 May66	8(61)	17(60)	32(60)	50(59)	86(60)	2(60)	1.49%	1.19-1.87	6.61%	1.98
4	27May66	6(79)	21(77)	27(80)	57(80)	96(75)	1(79)	1.19%	1.003-1.41	3.81%	2.54
5	Mar.67	3(139)	6(140)	23(139)	69(136)	94(137)	4(138)	1.40%	1.22-1.60	3-81%	2.96
				Bang Phra							
6	Feb.67	7(80)	35(80)	79(80)	81(80)	100(80)	14(80)	0.66%	0.58-0.75	1.41%	3.89

* Figures shown in parenthesis represent the number of adults tested at each concentration.

Table 2.

Results of dieldrin susceptibility tests of adult C. gelidus from Bang Khen District, Bangkok, March 1967

Exposure time	Per cent mortality at each concentration							Per cent control mortality
	4	1.6	0.8	0.4	0.2	0.1	0.5	
1 hour	32(91)*	19(92)	17(92)	5(97)	0(85)	0(96)	0(98)	1(97)
2 hours	53(20)	10(21)	—	—	—	—	—	5(20)

* Figures shawn in parenthesis represent number of adults tested.

It may be concluded from those results that adults of C. gelidus from Bang Khen, showed intermediate resistance to DDT but were completely resistant to dieldrin. The appearance of this resistance was also confirmed by the "time-in-concentration" technique through which two genotypes were recognized. The development of this physiological resistance is probably the result of selection pressure of a high order imposed on the population through the extensive use of insecticides in the area.

Culex tritaeniorhynchus.—Only the adults of this species were tested for susceptibility to DDT. Adults were collected while biting cattle in the vicinity of houses near the Botanical Gardens at Saraburi. In addition, larval material was obtained for testing from Kasetsart's Experimental Farm at Bang Khen, Bangkok. These were reared through in the insectary and the sugar-fed females were tested when 4-5 days old. The LC_{50} for adults from Saraburi was 0.50% with 95% confidence limits of 0.4-0.6% and an LC_{90} of 1.8%. The LC_{50} for the Bang Khen adults was 0.57% with C.L. of 0.5-0.7% and an LC_{90} of 1.05%. Based on the LC_{50} values this species appeared to be equally susceptible to DDT at both locations. The high LC_{90} value determined for Saraburi adults resulted in a slightly flat dosage-mortality regression line (slope=2.05) indicating a heterogenous population (Fig IV). The steep line (slope=4.74) constructed for Bang Khen adults indicated a homogenous susceptible population. This reflected the uniformity in age and nutritional conditions of these adults in contrast with those wild collected females from Saraburi.

Aedes aegypti—Resistance of Aedes aegypti to DDT has been reported from differant areas of Thailand during the past few years. However, interest in the determination of its susceptibility status from Surathani Province arose following an epidemic of Thai Hemorrhagic Fever on the island of Koh Samui in that province. Eggs were collected from Ang Thong and Taling Ngam on the island of Koh Samui and the neighbouring islands of Koh Phaluai and Koh Phangan. Larvae were later tested in the laboratory against DDT. Resistance was detected in larvae representative of all locations considered. Larval mortality after 24 hours exposure to 2.50 ppm of DDT ranged from 63% at Ang Thong to a low level of 22% at Koh Phaluai (Table 3).

Larvae from three locations were also tested for resistance to dieldrin by the "time-in-concentration" technique. Larvae were exposed to an established discriminating dose of 5 ppm dieldrin and mortality was observed at hourly intervals over a 24 hours period. Results obtained by this method are expressed by the regression lines 1, 2 and 3 in figure V. These lines show the presence of three distinct genotypes in each of the samples tested. Each regression line shows a pronounced inflection and the presence of a plateau distinguishing the homozygous susceptibles from the heterozygous resistant larvae. The proportion of susceptible larvae was lowest (53%) at Koh Phangan and highest (80%) at Ang Thong. The survival of 3.8%, 17.8% and 6.3% of larvae from Ang Thong, Koh Phangan and Taling Ngam, respectively, to this discriminating

dose constituted the proportions of the homozygous resistant larvae present. The remaining proportions were the heterozygous resistant larvae. It was concluded that larvae of Aedes aegypti are resistant to both DDT and dieldrin at all locations tested from Surathani Province.

Table 3.

Results of DDT susceptibility tests on Aedes aegypti larvae from Surathani Province, 1967.

Concentration (ppm)	Percent larval mortality at each location			
	Koh Phaluai	Taling Ngam	Koh Phangan	Ang Thong
2.50	22 (60)*	32 (60)	30 (60)	63 (79)
0.5	2 (60)	2 (59)	0 (60)	8 (78)
0.1	0 (60)	0 (60)	0 (60)	0 (80)
0.02	0 (60)	0 (60)	0 (60)	0 (80)
0.004	0 (60)	0 (60)	0 (60)	0 (78)
Control	0 (80)	0 (80)	0 (80)	1 (79)

* Figures shown in paranthesis represent the number of larvae tested at each concentration.

Anopheles balabacensis—The susceptibility level of adults of this species to DDT was established previously with material collected at Kao Mai Kaeo, Cholburi Province. Since no information was available on its larval susceptibility, larvae from the SMRL laboratory colony were tested for this purpose. The LC_{50} was 0.015 ppm indicating the susceptibility of the larvae to this insecticide. It is interesting to note the small slope value (Table 4) of the mortality-regression line (Fig VI) obtained reflecting the heterogenity of the larvae of this colonized strain.

Anopheles maculatus.—Adults of this species were tested for their susceptibility of DDT and dieldrin. Blood-fed females were collected while biting man at Kho Mai Kaeo, Cholburi Province during February 1967. Some adults were held over for egg laying and as a result larval testing against DDT was possible. Results showed an LC_{50} of 0.056% of dieldrin with 95% confidence limits of 0.042-0.075%; an LC_{90} of 0.1% and a slope value of 4.32. These values indicated that adults of this species are highly susceptible to dieldrin.

In the DDT tests the LC_{50} values of 0.65% and 0.010 ppm obtained for the adults and larvae respectively pointed to the susceptibility of this species to this insecticide. The LC_{50} for the adult maculatus was two times greater than that (0.3%) determined for adult balabacensis tested from the same area by other workers. Whether this small amount of tolerance is interspecific or brought about by insecticide pressure is difficult to say. In the meantime, it was surprising to find that the LC_{50} of 0.010 ppm determined for maculatus larvae was the lowest recorded for any of the anopheline species tested (Table 4.)

Anopheles minimus—Both adults and larvae of this species were tested for their susceptibility to DDT. Blood-fed females were collected biting man in the vicinity of houses near the Botanical Gardens at Saraburi, Saraburi Province. Eggs were also recovered from some of the adults to support larval testing. The LC_{50} value of 0.31% is the lowest obtained for any of the adult anophelines tested, and falls in the range of normal

Table 4.

Results of DDT susceptibility tests on some Anopheles species from Thailand.

Species	Date tested	Locality	Stage tested	LC_{50}*	95% Confidence limits of LC_{50}	LC_{90}	Slope
Anopheles balabacensis	May, 66	Laboratory colony	L	0.015	0.013—0.018	0.052	2.42
An. maculatus	Feb.. 67	Kao Mai Kaea, Cholburi Province	A	0.65	0.51—0.84	1.35	3.86
		" "	L	0.010	0.005—0.021	0.023	3.63
An. minimus	Jan., 67	Botanical Gardens, Saraburi Province	A	0.31	0.25—0.37	0.49	6.41
		" "	L	0.016	0.0136—0.0198	0.036	3.82
An. splendidus	Feb., 67	" "	A	0.48	0.42—0.56	0.86	5.18
An. tessellatus	Dec., 66	" "	L	0.012	0.006—0.025	0.026	3.76
An. vagus	May, 67	I Bang Khen District, Bangkok	A	0.99	0.86—1.16	2.82	2.82
		II Mo Ban Takhop near Pak Thong Chai, Nakornrajisima Province	A	0.97	0.81—1.16	3.01	2.61

* LC values in ppm for larvae and in per cent for adults.

susceptible anopheles. The steep mortality regression line with a slope value of 6.41 also indicates that these adults are homogenous susceptible to DDT (Fig. VII) Although the LC_{50} of 0.016 ppm was the highest obtained among the anopheles larvae tested, the minimus larvae were still susceptible to DDT.

Anopheles splendidus.—Adults of this species were collected at the same location as those of minimus, and they were also tested for susceptibility to DDT. An LC_{50} of 0.48% indicated the susceptibility of this species to DDT. The confidence limits of the LC_{50}, LC_{90} and the slope values are given in Table 4.

Anopheles tessellatus: Only the larvae of this species were tested for their susceptibility to DDT. The few blood-fed females collected at Saraburi were held in the insectary to provide eggs for the eventual testing of the larvae. The LC_{50} of 0.012 ppm showed that this species is also susceptible to DDT in the larval stage.

Anopheles vagus.—Adults of this species obtained from two different sources were tested for their susceptibility to DDT. Females were collected while biting cattle at Kasetsart University Farm, in Bang Khen District of Bangkok and while biting man at Mo Ban Takop near Pak Thong Chai, Nakornrajisima Province. Almost identical LC_{50} values (0.97 and 0.99%) were obtained for this species at both locations. These values are greater than those obtained for the other susceptible anophelines tested (Table 4.). The slope of the regression line is flatter than those of the other species indicating considerable heterogeneity of vagus population (Fig. VII). Tests revealed the presence of 6% survivors to the highest DDT concentration (4%) in one of four tests made with adults from Mo Ban Thakop. Also in one of the replicates run with adults from Bang Khen, 10% of the mosquitoes survived exposure to the same high concentration. With this proportion of survivors, these tests indicate the presence of an intermediate level of resistance among population of this species, and suggest the possibility that we are dealing with a heterogenous population part of which is DDT resistant.

II COLLABORATIVE STUDIES ON INSECTICIDES AND REPELLENTS.

1. Laboratory larvicide tests:

Six insecticides, namely Abate, Dursban, fenthion, naled, malathion and baygon were tested against wild-collected larvae of Culex gelidus; malathion, baygon and naled were also tested against C. tritaeniorhynchus.

The calculated LC_{50} and LC_{90} values are given in table 5. These results showed that abate, dursban and fenthion were very effective against C. gelidus larvae. Based on the LC_{50}, Abate was 90 times, dursban was 24 times and fenthion was 12 times more toxic than the malathion standard. Naled was about as toxic as malathion, but Baygon was only one-fifth as toxic as this standard insecticide. Malathion was more toxic than Naled and Baygon against C. tritaeniorhynchus larvae.

Table 5.

Results on toxicity of insecticides to larvae of two Culicine species from Bang Khen District, Bangkok.

Insecticide	Culex gelidus		C. tritaeniorhynchus	
	LC_{50}	LC_{90}	LC_{50}	LC_{90}
Abate	.00012	.00023	—	—
Dursban	.00045	.00076	—	—
Fenthion	.00092	.0016	—	—
Malathion	.0108	.017	.0027	.012
Naled	.011	.022	.0038	.0073
Baygon	.058	.182	.054	.082

2. Fogging tests against a natural mosquito population:

Tests were conducted in the Klong Toey area of Bangkok to evaluate the effectiveness of fogs containing 1%, 2% or 4% malathion in diesel fuel in controlling mosquitoes, principally Culex quinquefasciatus. Results, presented in Table 6, showed that the mosquito population was reduced from 67% to 74% below the pretreatment counts in all the treatments. Of the mosquitoes collected the night of the treatment, 95% of these exposed to the 1% malathion died, and 99% of those exposed to the 2% and 4% malathion fogs were killed.

Table 6.

Results of malathion fogging tests in Bangkok against Culex quinquefasciatus.

Insecticide (%)	No. mosq/man/hr.		% Reduction	% mortality of captured mosquitoes after 24 hrs.
	Pretreatment	Posttreatment		
1	51	13	74	95
control	67	43	36	6
2	83	23	65	99
control	45	49	0	18
4	65	13	71	99
control	25	29	0	10

3. Toxicity of Abate and Malathion to fresh-water fish.

Tests were conducted in the laboratory to determine the toxicity of Abate and malathion to two species of edible fish, Cyprinus carpio and Tilapia mossambica, in rice fields and klongs. Both materials are of low mammalian toxicity and lend themselves to future mosquito control operations. Specimens of both

species were exposed for 24 hours to klong water containing concentrations of 10, 1. 0.1 and 0.01 ppm of the two chemicals, respectively. No kill was obtained except at the 10 ppm dosage of malathion against T. mossambica. In a second test, each species was tested in concentrations of Abate at 100 and 1,000 ppm. The higher concentration killed all the fish in 15 minutes and the lower killed them all in 2 hours.

4. Toxicity of Abate to fresh-water shrimp. A laboratory test was conducted to determine the toxicity of Abate to the edible shrimp (Caridina sp.) also commonly stocked in rice fields and klongs. Shrimps were tested in klong water containing Abate at concentrations of 0.01, 0.1, 1.0, and 10.0 ppm. Mortality counts taken after 24 hours gave the following results:

Dosage (ppm)	Per cent mortality
0.01	40
0.1	0
1.0	90
10.0	90
0 (control)	0

5. Toxicity of Abate Aedes aegypti larvae in concrete water jugs.

Abate was tested in different formulations and concentrations, against Adedes aegypti larvae in concrete water storage jugs. Each jug was filled with 45 gallons of water into which the insecticide was added to give the final concentration required. Table 7 gives a list of all the concentrations of the different formulations used. Fifty late third-or early fourth-instar larvae collected from the wild were introduced once a week into each jug and checked for mortality 3 days later. If larvae were present in any jug on two consecutive weeks, such a treatment was considered no longer effective. Two parrallel series were run simultaneously, one in which the water level was maintained constant ("Static") while in the other ("Fluctuating") 15 gallons of wates were dished out and replaced with fresh water every week to simulate normal usage. This test was run in triplicate with three jugs of fresh water as controls.

Table 7.

Toxicity of Abate to Aedes agypti larvae in concrete water jugs.

Formulation	Concentration (ppm)	Condition	Ave. weeks of effective residual toxicity
Emulsifiable concentrate	1.0	Static	> 34
	1.0	Fluctuating	> 34
	0.1	Static	19.0
	0.1	Fluctuating	16.3
	0.05	Static	20
	0.05	Fluctuating	13.7
	0.025	Static	15
	0.025	Fluctuating	11
Granules	1.0	Static	> 34
	1.0	Fluctuating	> 34
	0.1	Static	22.5
	0.1	Fluctuating	14.3
Impregnated concrete pellet	?	Static	11.7
	?	Fluctuating	8.3

Results obtained are expressed as the average numbers of weeks of effective residual toxicity for each treatment (Table 7). Abate when used as emulsifiable concentrate or as granules at a concentration of 1.0 ppm, gave an exceptionally long residual effect lasting over 34 weeks in both the static and fluctuating series. It is interesting to mention that the emulsifiable concentrate of Abate gave an effective control against aegypti for 11 weeks at the low concentration of 0.025 ppm in the fluctuating series. The least effective formulation was that of the impregnated pellets which gave an effective treatment lasting for only 11.7 weeks in the static series versus 8.3 weeks in the fluctuating ones.

6. Field tests with mosquito nets treated with repellents.

Tests were conducted on the evaluation of mosquito nets made of 1/4-inch mesh netting and treated with two repellents against Culex quinquefasciatus and Aedes aegypti in the Klong Toey area of Bangkok. Nets were treated with Deet and a mixture of M-1960 at the rate of 0.5 gram of repellent to 1 gram of netting. M-1960 consists of 30% benzyl benzoate, 30% N-butyl-acetanilide, 30% 2-butyl-2-ethyl-1, 3-propanediol, and 10% emulsifier. Nets were hung in selected houses and two subjects sat inside each of the treated and untreated nets for two hours. The mosquitoes that entered were collected and identified. One set of nets was tested between 1300 and 1500 hours, the time when Aedes aegypti was active, while the other set was checked between 1900 and 2100 hours when Culex quinquefasciatus was most prevalent. Results indicated that the nets treated with M-1960 gave complete protection from Aedes aegypti for 98 days versus 91 days by the Deet treated nets. M-1960 treated nets gave also complete protection from Culex quinquefasciatus for 126 days versus 113 days by the one treated with Deet. Another set of nets was also tested in forested areas south of Pak Thong Chai, Nakornrajisima Province. The anopheline population was low at the time, and only two Anopheles balabacensis entered the untreate net while none were collected in the treated ones.

7. Detection of Deet-treated subjects under jungle conditions.

Tests were conducted to determine whether Thai men and women could detect subjects treated with Deet when concealed in jungle bush. This test was run in a tropical evergreen forest south of Pak Thong Chai. Five Thai men and two Thai women served as detectors and nine Thai men and one American served as subjects. Each subject was treated with 3 ml. of deet, alcohol or water applied to their arms. Treated subjects were then stationed individually 5 or 10 feet from a trail, or in groups of five (all treated with the same material) 8 feet from the trail. Each detector walked down the trail and recorded the odor of deet at each numbered position at which it was detected. Replicate tests were made in the morning, afternoon, and night.

In the combined morning and night tests (when the humidity was high), with subjects located 10 feet from the trail, the male detectors made correct positive identification of deet 13% of the time (i.e., at 9 of 70 positions where the subjects were actually treated with deet) and false identification of deet 6% of the time (i.e., at 8 of 130 positions where the subjects were treated with alcohol or water). Results given in Table 8 showed that correct positives always exceeded false positives, except in the afternoon tests. However, when these detectors were allowed to smell treated arms the male detectors made 83% correct positive and 20% false positive determinations versus 54% correct positive and 8% false positive determinations by the female detectors.

Table 8.

Results of tests on the detection of deet under jungle conditions.

Test conditions	Male detectors		Female detectors	
	Correct positive%	False positive%	Correct positive%	False positive%
Morning & night tests				
Individual subjects at				
10 ft from trail	13	6	7	2
5 ft from trail	20	6	14	5
Groups of subjects at				
5 ft from trail	25	5	37	1
Afternoon tests				
Individual subjects at				
10 ft from trail	0	1	0	0
5 ft from trail	1	1	0	0
Groups of subjects at				
5 ft from trail	5	5	0	0

Summary: The susceptility status of nine species of mosquitoes from various localities in Thailand to DDT and/or dieldrin has been established. Adult Culex gelidus from Bang Khen, Bangkok showed intermediate resistance to DDT but were completely resistant to dieldrin. Aedes aegypti larvae from Suratani Province were also found resistant to both insecticides. The appearance of resistance among populations of these two species was confirmed by the "time in concentration" technique by which two and three genotypes were recognized for C. gelidus and A. aegypti respectively. The possibility that Anopheles vagus adults have developed resistance to DDT at two localities is suspected on basis of the appreciable increase observed in the LC-50 value and presence of survivors following exposures at the highiest concentration of 4% DDT. Adults and/or larvae of C. tritaeniorhynchus, Anopheles balabacensis, An. maculatus. An. minimus, An. splendidus, and An. tessellatus tested from different sources were found still susceptible to DDT.

Other studies on the evaluation of insecticides and repellents were conducted in collaboration with members of the USDA and WRAIR. These included testing of some promising insecticides for toxicity against larvae of three local mosquito species and to edible fresh water fish and shrimp. Fogs containing malathion were tested against natural mosquito populations and produced significant reduction in the populations. Special bed nets treated with repellents were effective against two species of mosquitoes for more than three months.

Fig. I Dosage-mortality regression lines of DDT against adult Culex gelidus from Bang Khen and Bang Phra.

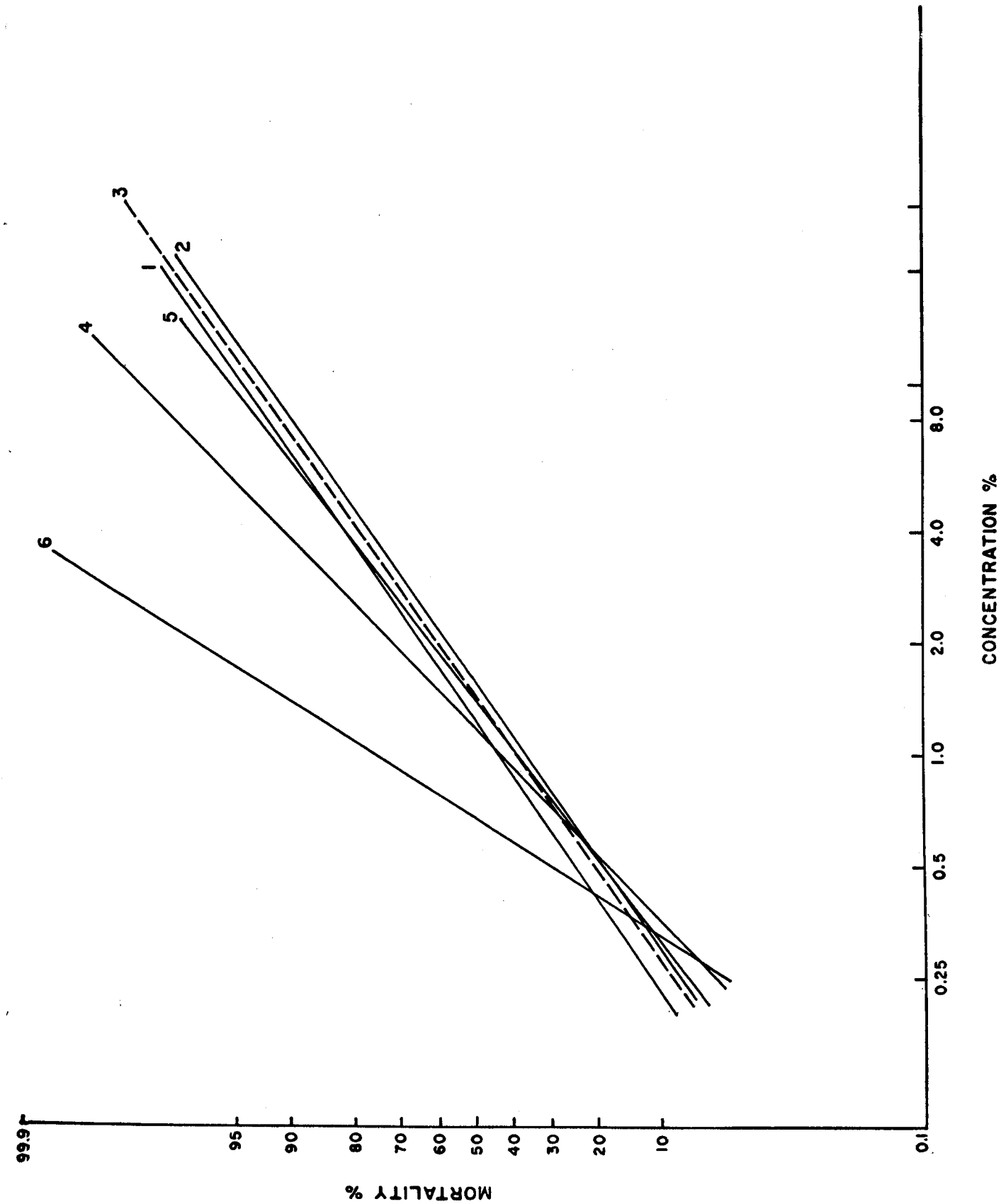


Fig. II Dosage-mortality regression lines of some chlorinated hydrocarbons against larvae of Culex gelidus from Bang Khen District, Bangkok.

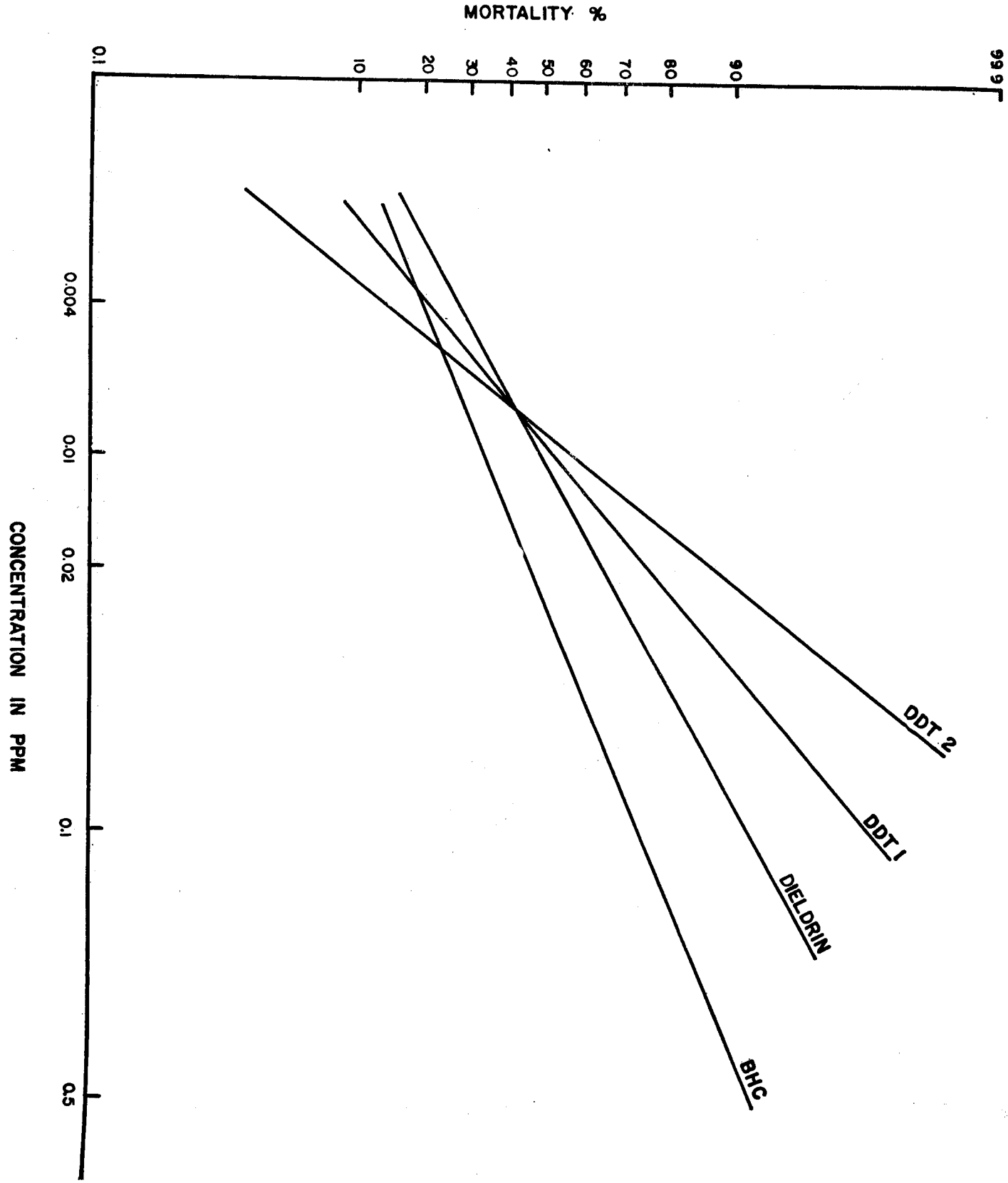


Fig. III Mortality regression lines of DDT and dieldrin when tested against Culex qelidus larvae by the "Time In Concentration" technique.

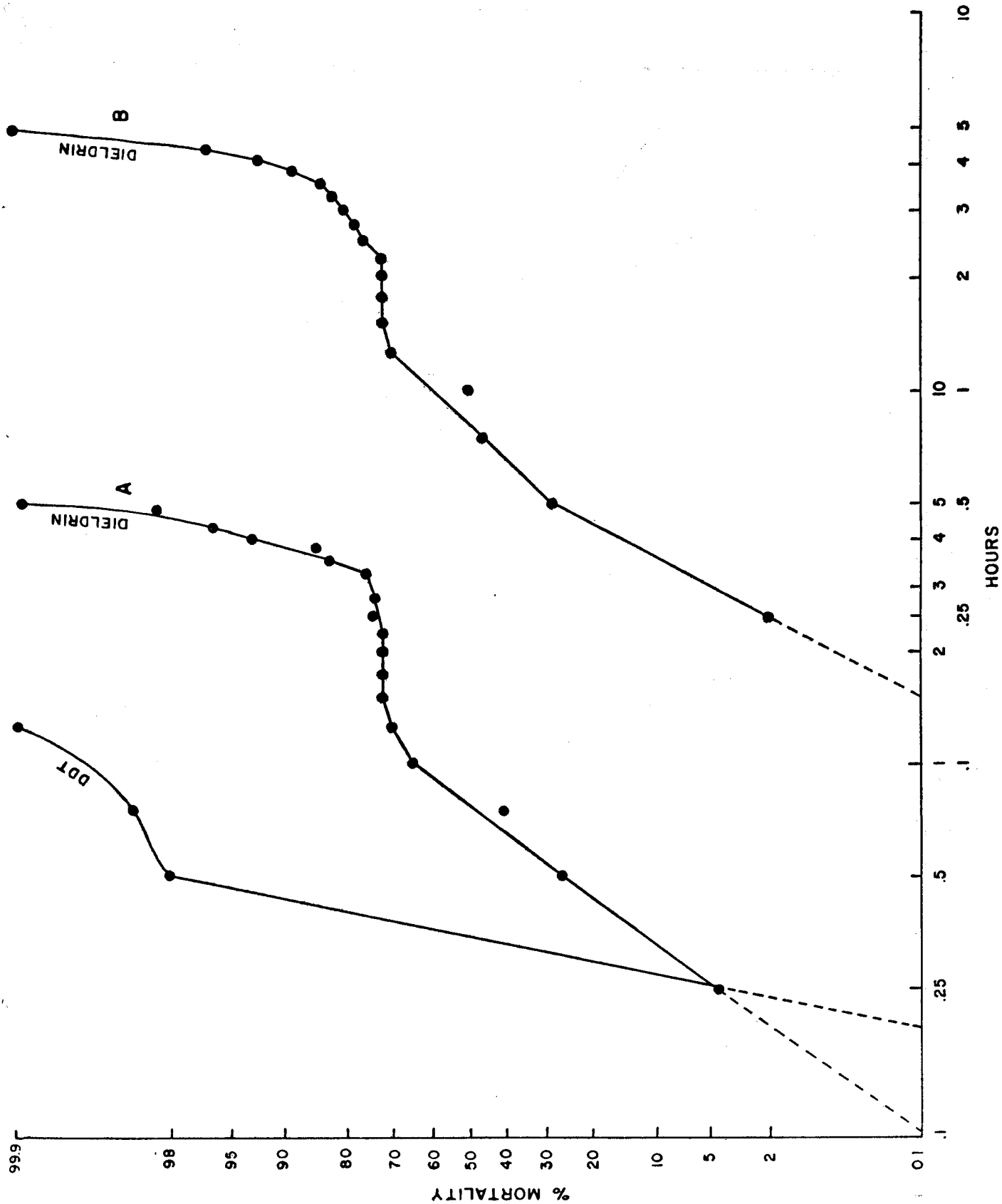


Fig. IV Dosage-mortality regression lines of DDT against adult Culex tritaeniorhynchus.

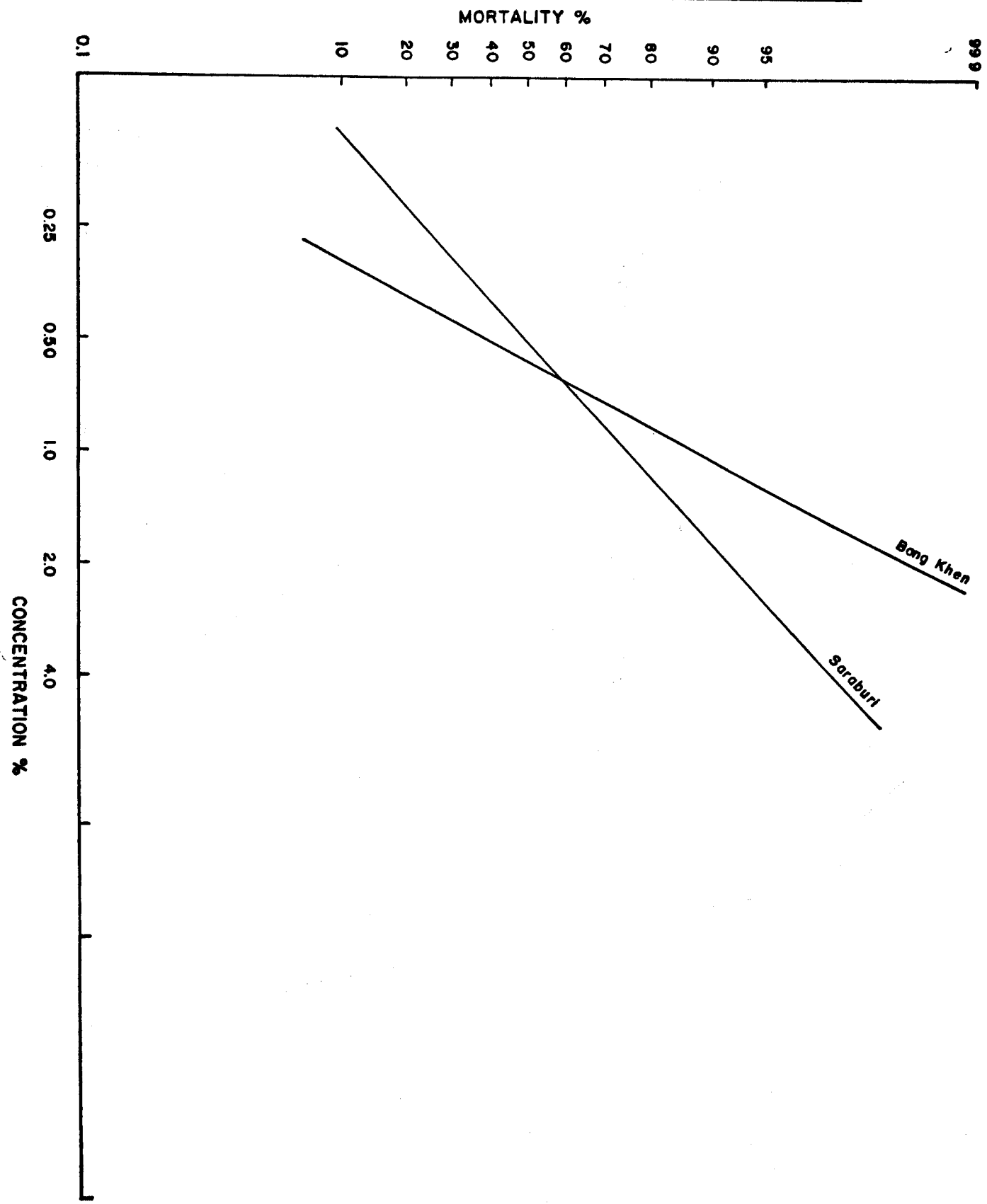


Fig. V. Mortality regression lines of dieldrin against Aedes aegypti larvae when tested by the "Time in Concentration" technique.

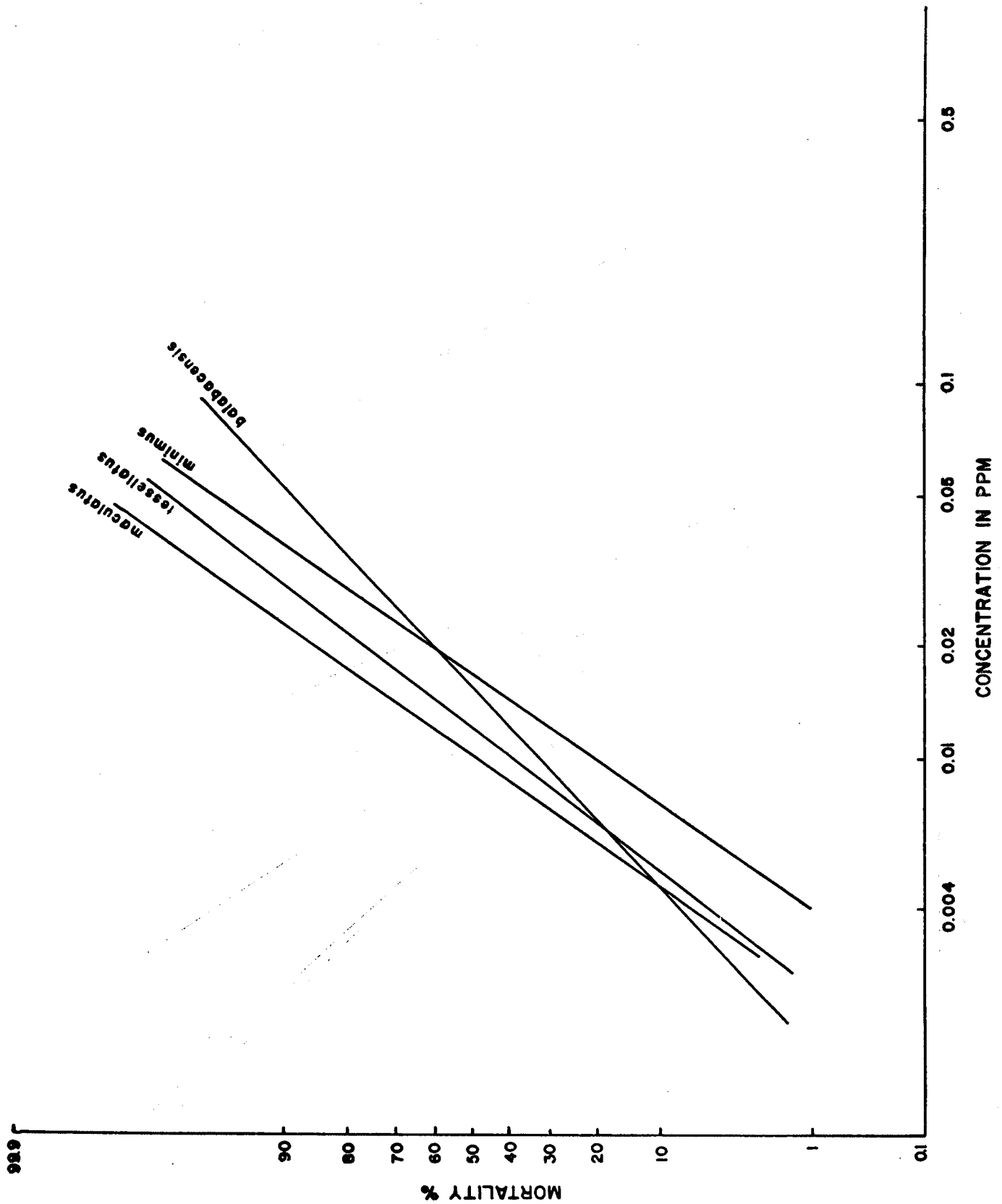
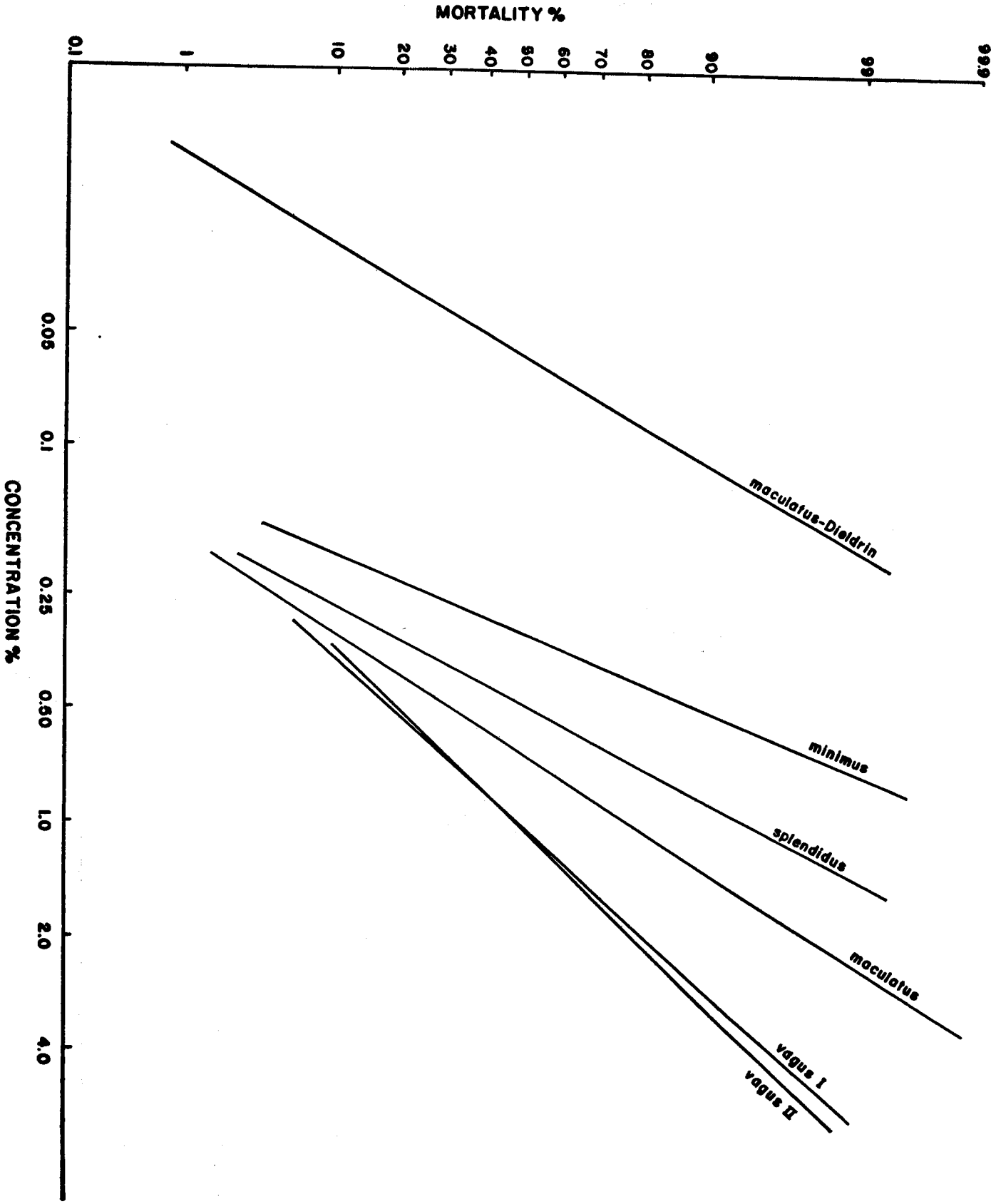


Fig. VI Dosage-mortality regression lines of DDT against larvae of some anopheles species.



Results obtained are expressed as the average numbers of weeks of effective residual toxicity for each treatment (Table 7). Abate when used as emulsifiable concentrate or as granules at a concentration of 1.0 ppm, gave an exceptionally long residual effect lasting over 34 weeks in both the static and fluctuating series. It is interesting to mention that the emulsifiable concentrate of Abate gave an effective control against aegypti for 11 weeks at the low concentration of 0.025 ppm in the fluctuating series. The least effective formulation was that of the impregnated pellets which gave an effective treatment lasting for only 11.7 weeks in the static series versus 8.3 weeks in the fluctuating ones.

6. Field tests with mosquito nets treated with repellents.

Tests were conducted on the evaluation of mosquito nets made of 1/4-inch mesh netting and treated with two repellents against Culex quinquefasciatus and Aedes aegypti in the Klong Toey area of Bangkok. Nets were treated with Deet and a mixture of M-1960 at the rate of 0.5 gram of repellent to 1 gram of netting. M-1960 consists of 30% benzyl benzoate, 30% N-butyl-acetanilide, 30% 2-butyl-2-ethyl-1, 3-propanediol, and 10% emulsifier. Nets were hung in selected houses and two subjects sat inside each of the treated and untreated nets for two hours. The mosquitoes that entered were collected and identified. One set of nets was tested between 1300 and 1500 hours, the time when Aedes aegypti was active, while the other set was checked between 1900 and 2100 hours when Culex quinquefasciatus was most prevalent. Results indicated that the nets treated with M-1960 gave complete protection from Aedes aegypti for 98 days versus 91 days by the Deet treated nets. M-1960 treated nets gave also complete protection from Culex quinquefasciatus for 126 days versus 113 days by the one treated with Deet. Another set of nets was also tested in forested areas south of Pak Thong Chai, Nakornrajisima Province. The anopheline population was low at the time, and only two Anopheles balabacensis entered the untreate net while none were collected in the treated ones.

7. Detection of Deet-treated subjects under jungle conditions.

Tests were conducted to determine whether Thai men and women could detect subjects treated with Deet when concealed in jungle bush. This test was run in a tropical evergreen forest south of Pak Thong Chai. Five Thai men and two Thai women served as detectors and nine Thai men and one American served as subjects. Each subject was treated with 3 ml. of deet, alcohol or water applied to their arms. Treated subjects were then stationed individually 5 or 10 feet from a trail, or in groups of five (all treated with the same material) 8 feet from the trail. Each detector walked down the trail and recorded the odor of deet at each numbered position at which it was detected. Replicate tests were made in the morning, afternoon, and night.

In the combined morning and night tests (when the humidity was high), with subjects located 10 feet from the trail, the male detectors made correct positive identification of deet 13% of the time (i.e., at 9 of 70 positions where the subjects were actually treated with deet) and false identification of deet 6% of the time (i.e., at 8 of 130 positions where the subjects were treated with alcohol or water). Results given in Table 8 showed that correct positives always exceeded false positives, except in the afternoon tests. However, when these detectors were allowed to smell treated arms the male detectors made 83% correct positive and 20% false positive determinations versus 54% correct positive and 8% false positive determinations by the female detectors.

Table 8.

Results of tests on the detection of deet under jungle conditions.

Test conditions	Male detectors		Female detectors	
	Correct positive%	False positive%	Correct positive%	False positive%
Morning & night tests				
Individual subjects at				
10 ft from trail	13	6	7	2
5 ft from trail	20	6	14	5
Groups of subjects at				
5 ft from trail	25	5	37	1
Afternoon tests				
Individual subjects at				
10 ft from trail	0	1	0	0
5 ft from trail	1	1	0	0
Groups of subjects at				
5 ft from trail	5	5	0	0

Summary: The susceptility status of nine species of mosquitoes from various localities in Thailand to DDT and/or dieldrin has been established. Adult Culex gelidus from Bang Khen, Bangkok showed intermediate resistance to DDT but were completely resistant to dieldrin. Aedes aegypti larvae from Suratani Province were also found resistant to both insecticides. The appearance of resistance among populations of these two species was confirmed by the "time in concentration" technique by which two and three genotypes were recognized for C. gelidus and A. aegypti respectively. The possibility that Anopheles vagus adults have developed resistance to DDT at two localities is suspected on basis of the appreciable increase observed in the LC-50 value and presence of survivors following exposures at the highiest concentration of 4% DDT. Adults and/or larvae of C. tritaeniorhynchus, Anopheles balabacensis, An. maculatus. An. minimus, An. splendidus, and An. tessellatus tested from different sources were found still susceptible to DDT.

Other studies on the evaluation of insecticides and repellents were conducted in collaboration with members of the USDA and WRAIR. These included testing of some promising insecticides for toxicity against larvae of three local mosquito species and to edible fresh water fish and shrimp. Fogs containing malathion were tested against natural mosquito populations and produced significant reduction in the populations. Special bed nets treated with repellents were effective against two species of mosquitoes for more than three months.

Fig. 1 Dosage-mortality regression lines of DDT against adult Culex gelidus from Bang Khen and Bang Phra.

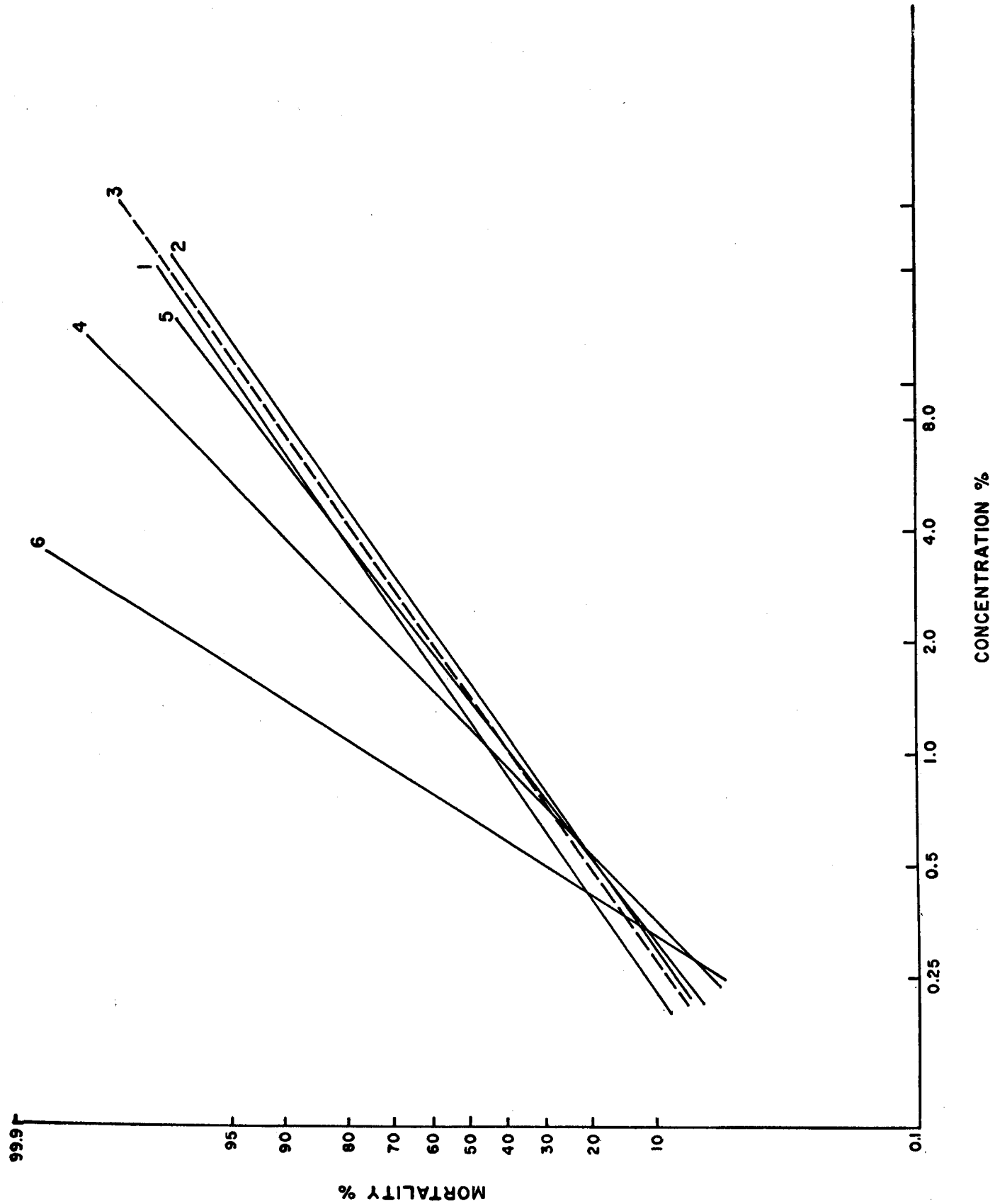


Fig. II Dosage-mortality regression lines of some chlorinated hydrocarbons against larvae of Culex gelidus from Bang Khen District, Bangkok.

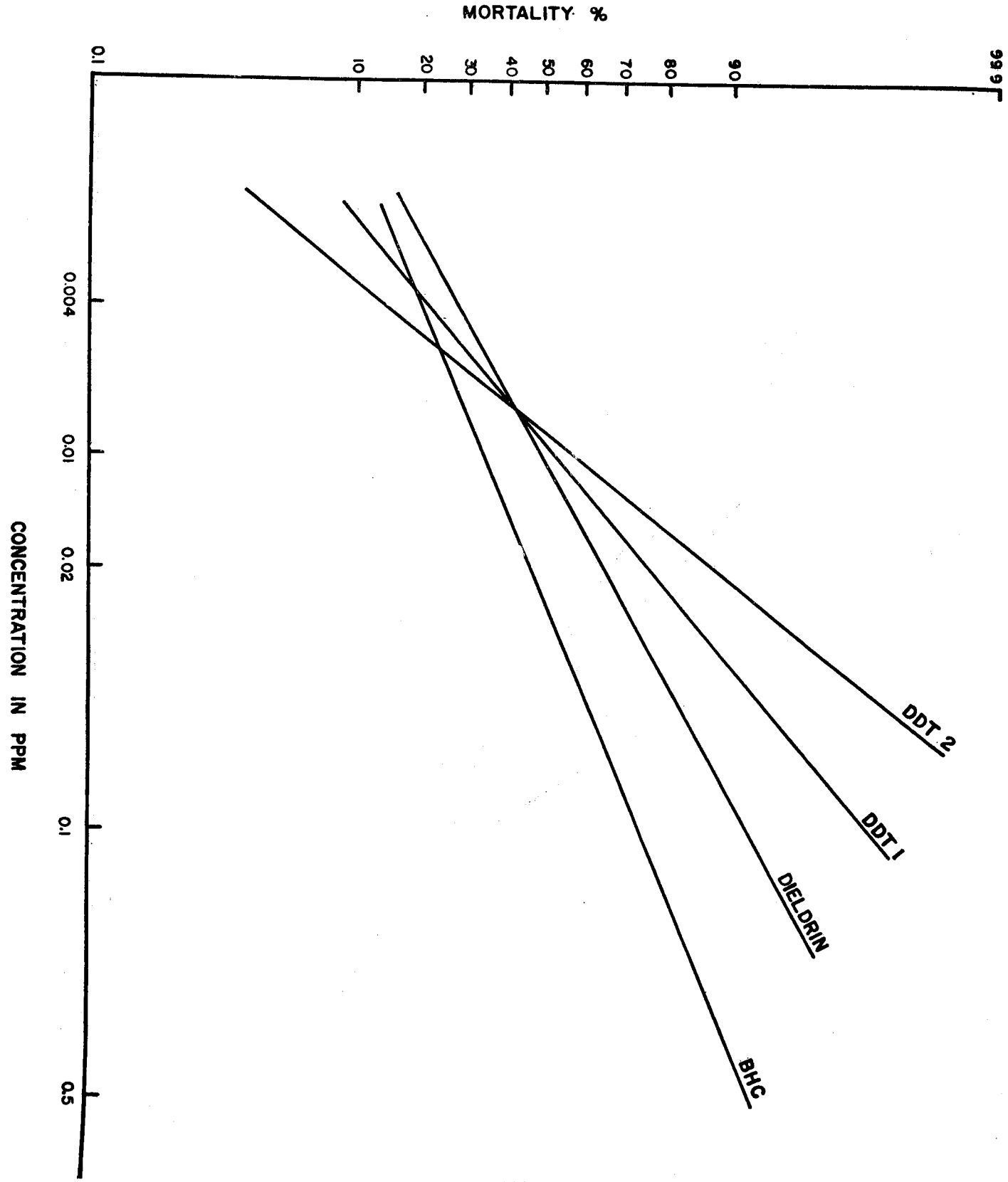


Fig. III Mortality regression lines of DDT and dieldrin when tested against Culex qelidus larvae by the "Time In Concentration" technique.

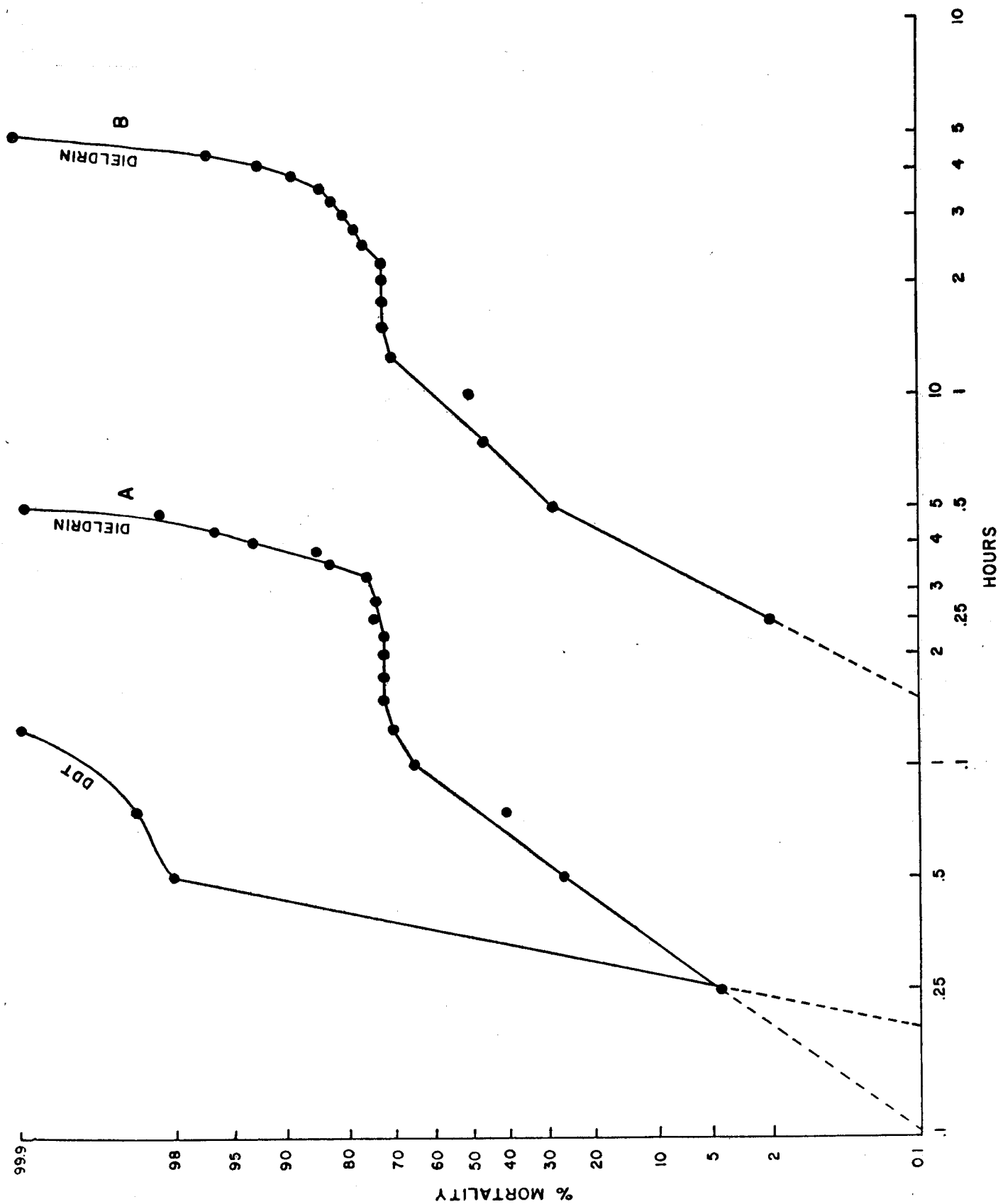


Fig. IV Dosage-mortality regression lines of DDT against adult Culex tritaeniorhynchus.

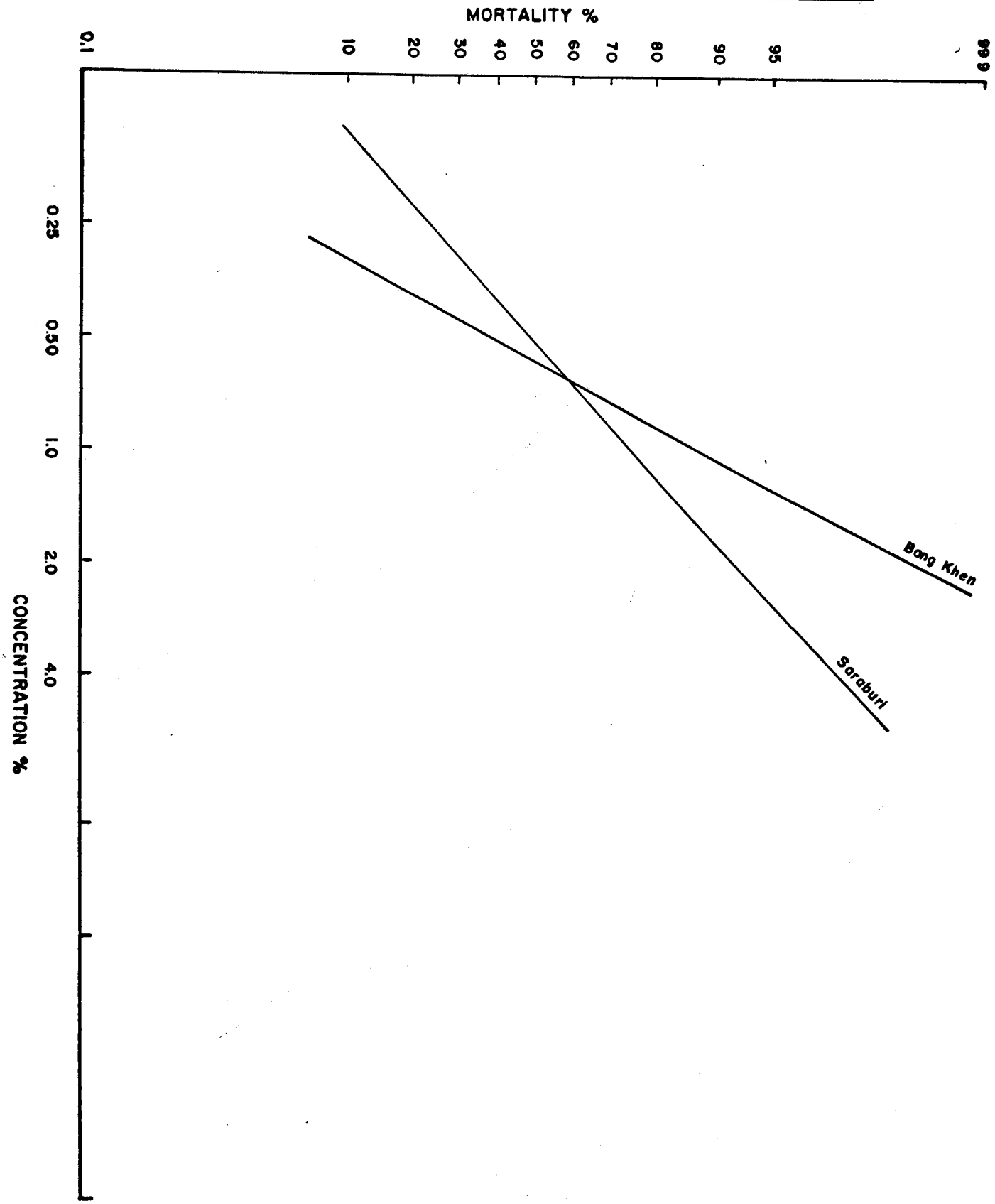


Fig. V. Mortality regression lines of dieldrin against Aedes aegypti larvae when tested by the "Time in Concentration" technique.

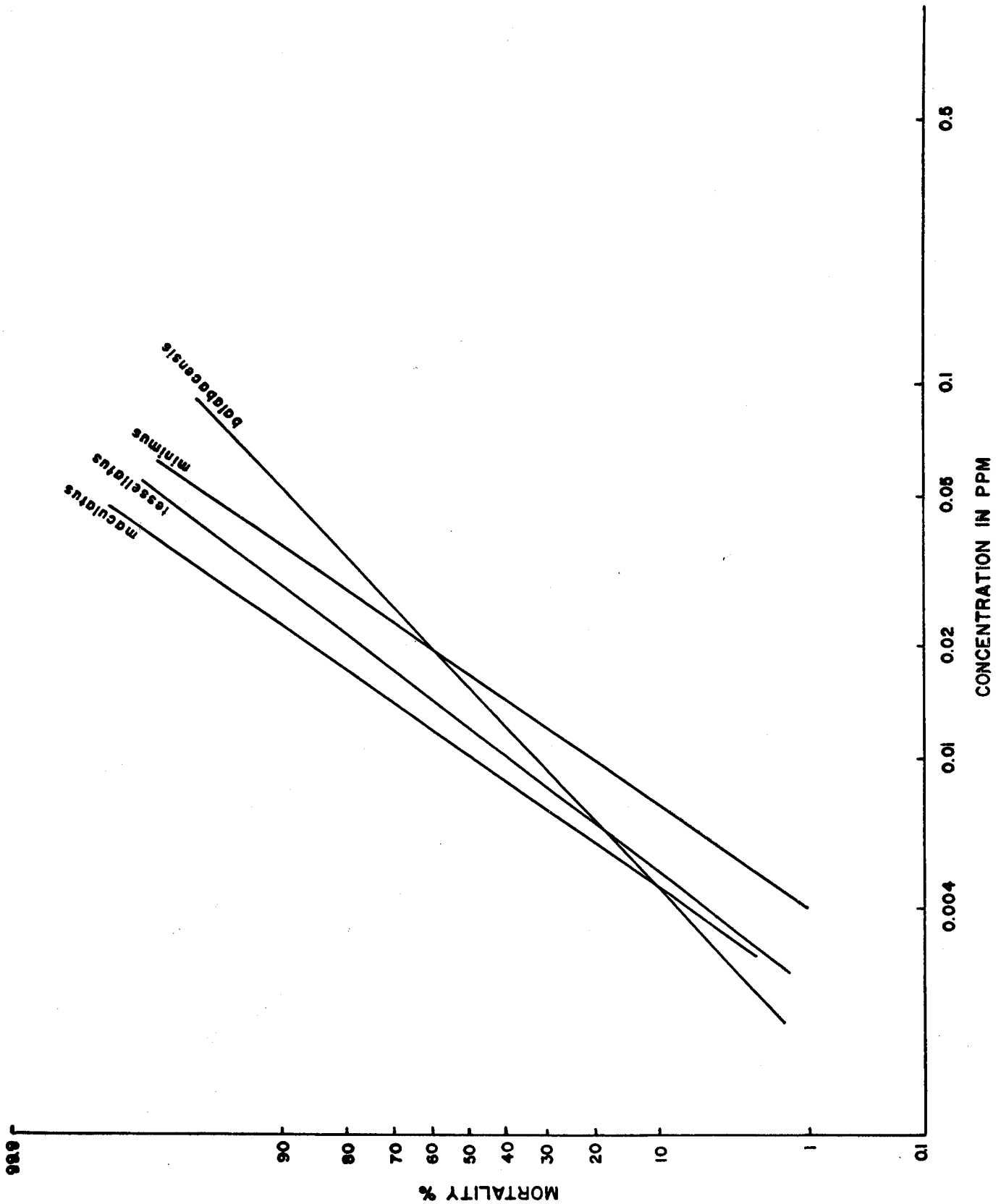


Fig. VI Dosage-mortality regression lines of DDT against larvae of some anopheles species.

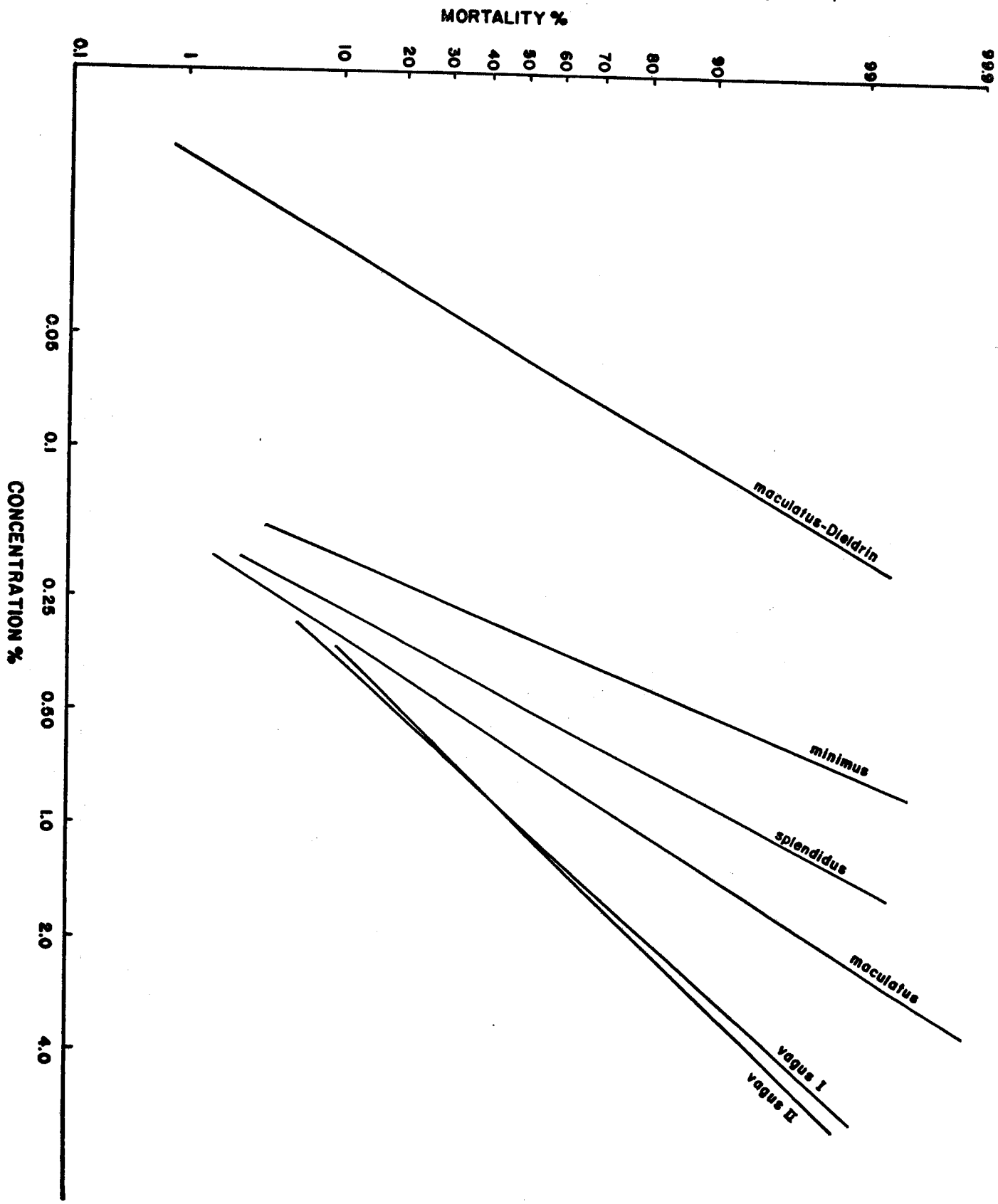


Fig. VII Dosage-mortality regression lines of DDT and dieldrin against adults of some anopheles species.