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The comparative toxicity of developmental inhibitors and organophosphates on mosquitoes

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THE COMPARATIVE TOXICITY OF DEVELOPMENTAL INHIBITORS AND ORGANOPHOSPHATES ON MOSQUITOES

A Thesis

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by

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INTRODUCTION

In the majority of mosquito abatement programs conventional insecticides are universally used. However, chemical insecticides are not totally acceptable because of extensive ecological damage, magnification within food chains and insecticide resistance (Hoope, Isler and Vogel 1974).

For several years holometabolous insects have been controlled with juvenile hormones (JH) which disrupt normal metamorphosis. Larvae constantly require the presence of specific amounts of JH throughout their development. The corpus allatum suppresses the production of JH before mature larvae can metamorphose into adults (Williams 1967). Several investigators (Spielman and Williams 1966, Jakob and Schoof 1972) reported that in both laboratory and field trials JH and its analogues were effective in killing mosquitoes in the pupal stage. The hazards imposed by conventional insecticides may be avoided by the use of JH analogues in mosquito abatement programs.

Field testing of a JH analogue is necessary prior to large field applications of the compound. The efficiency of growth regulators varies according to the environment, resistance to insecticides and species of mosquito tested (Hsieh and Steelman 1974, Kadri 1975).

In Utah Valley the synthetic insecticides used are Parathion and Abate. Parathion is applied in the majority of the problem areas

except where damage might result from its high toxicity to warm-blooded animals. In that case the more expensive Abate is used. If JH analogues are to compete successfully with existing insecticides, they must exhibit high pest mortality, a reduction of mortality on non-target organisms, and compete economically with conventional insecticides.

The objectives of this study were to (1) determine the lethal concentration for 50 percent (LC_{50}) and 95 percent (LC_{95}) kill for four growth regulators and two organophosphates when applied to Aedes aegypti, (2) determine the effectiveness of Altosid SR-10 against natural populations of Culiseta inornata in Utah Valley, and (3) provide base-line information for future bioassay experiments by comparing the performance of JH analogues with the efficiency of the standard organophosphorous compounds.

MATERIALS AND METHODS

Field Experiments

Growth Regulator. Altosid SR-10 was obtained from Zoecon Corporation, 975 California Avenue, Palo Alto, California. The ingredients are:

Active ingredient: Methoprene (Isopropyl (E,E)-11-methoxy-3,7,11-trimethyl 2,4-dodecadianoate)	10%
Inert ingredients	90%

General Description of Study Area. Both study plots and their controls were located near the south shore of Mud Lake, a subdivision of Utah Lake in Utah County, Utah (Fig. 1).

The water source was a well located within plot 2. Plot 1 was downstream about .8 km (along the water course) from plot 2 (Fig. 2). Controls were located between plot 1 and plot 2. Both controls were environmentally similar to their respective plots.

The surrounding area served as a grazing pasture for cattle. Large numbers of water and marsh birds were seen in the second plot. The substrate of both areas consisted of a fine black silt that, when disturbed, remained suspended within the water for a considerable length of time. Emergent vegetation and filamentous green algae occurred near the margins of plot 2 and were continuous throughout plot 1. Plot 1 was partly protected from sun, but plot 2

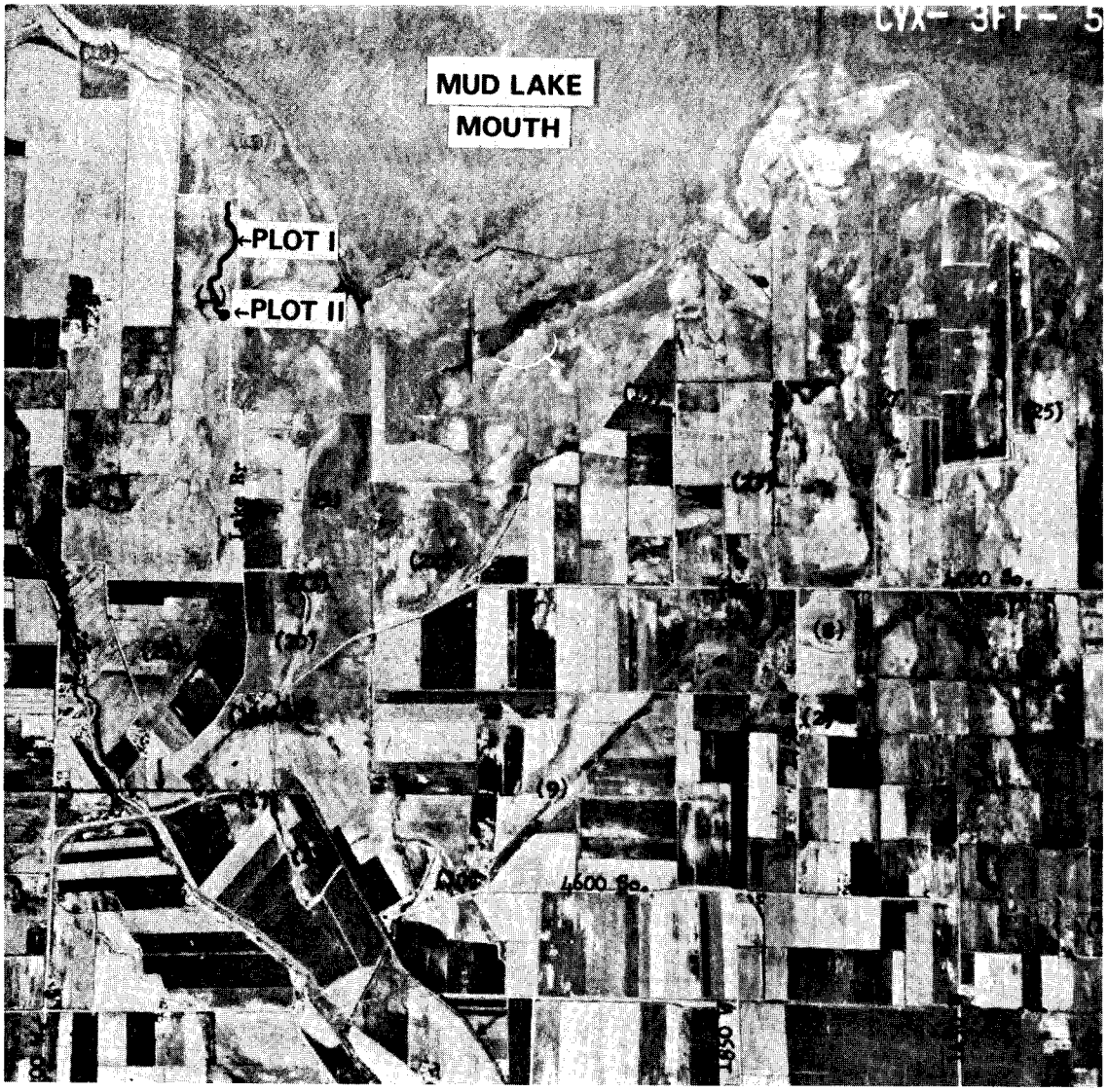
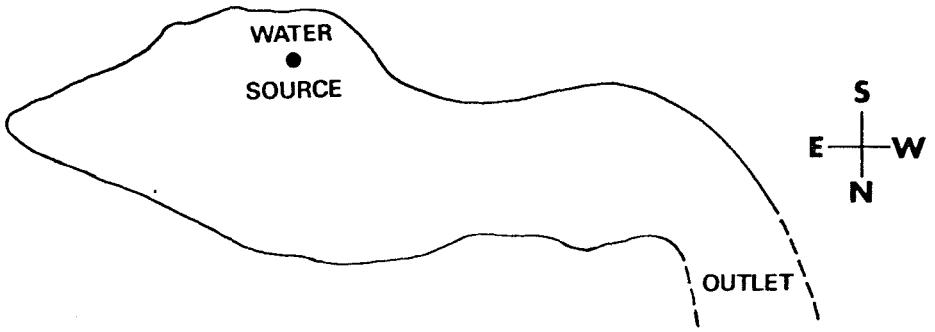


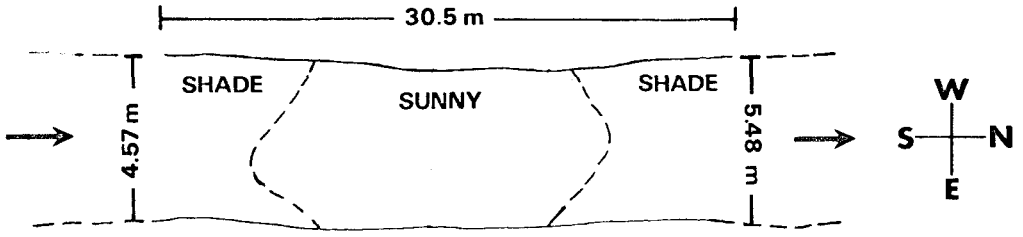
Figure 1. Aerial Photograph Showing Location of Plot 1 and Plot 2

PLOT II



AVERAGE DEPTH..... 30.5-35.5 CENTIMETERS
AREA..... 486.86 SQUARE METERS
TOTAL VOLUME..... 146.58 CUBIC METERS
BOTTOM TYPE ..... SILT (MUD)
VEGETATION..... ..... PERIPHERAL ONLY
CURRENT..... SMALL MOVEMENT NORTH OUTLET
(NOT MEASURABLE)

PLOT I



AVERAGE DEPTH..... 12.7 CM
AREA..... 153.45 SQUARE METERS
TOTAL VOLUME..... 19.25 CUBIC METERS
BOTTOM TYPE ..... SILT (MUD)
VEGETATION ..... THICKLY EMERGENT THROUGHOUT
CURRENT..... MOVEMENT NORTH
(NOT MEASURABLE)

Figure 2. Physical Information and Map of Plot 1 and Plot 2

was fully exposed. Identification of the vascular plants and aquatic insects is shown in the appendix.

The most numerous animals within the plots were cladocerans and copepods. Snails of the genus Physa and a few amphipods were also present. Fish and amphibians were not seen in the plots.

Three species of mosquitoes were found within the study area—Anopheles freeborni, Culex tarsalis and Culiseta inornata. The distribution of these larvae is shown in Figure 3.

Neither plot received any previous insecticidal treatment for the control of mosquitoes. The collecting and testing was done from August 16 to October 10, 1974.

Plot One. Forty-five ml of Altosid SR-10 was mixed with 3.8 liters and applied on August 24, 1974.

Ten traps made from rectangular sheets of screen, 30.7 cm by 92.1 cm, were stapled into the form of cylinders. Mosquito netting was placed over the screen, with one circular opening in the bottom of each trap.

Five traps were placed in the sprayed plot and five in the control. The control plot was located upstream from the sprayed area. A distance of 9.2 m separated the closest traps between the plot and its control (Fig. 4).

Culiseta inornata was chosen for the field experiments for two reasons: (1) high concentrations of Culiseta were easily available in cow hoofprints, and (2) data had been reported on its resistance to Altosid. Hsieh and Steelman (1974) claimed that the LC_{90} of 3rd instar Culiseta inornata was 1.6357 ppm, the highest

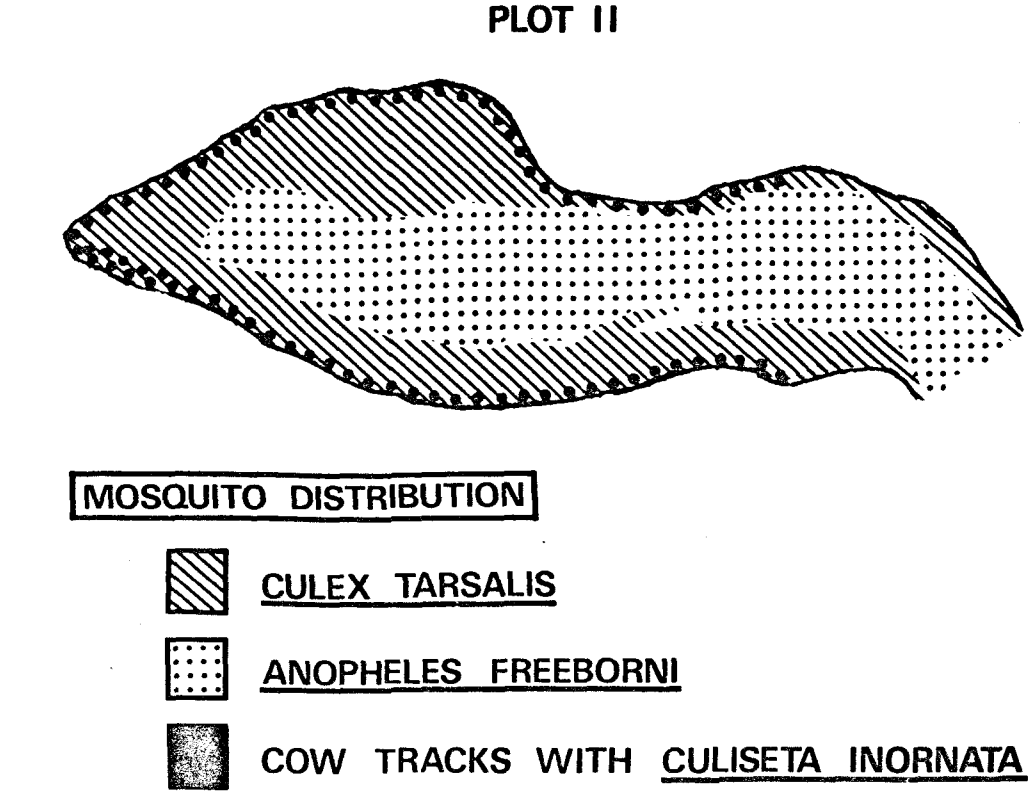


Figure 3. Mosquito Distribution in Plot 1 and Plot 2

KEY:

TREATED TRAPS: (1) (2) (3) (4) (5)

CONTROL TRAPS: (6) (7) (8) (9) (10)

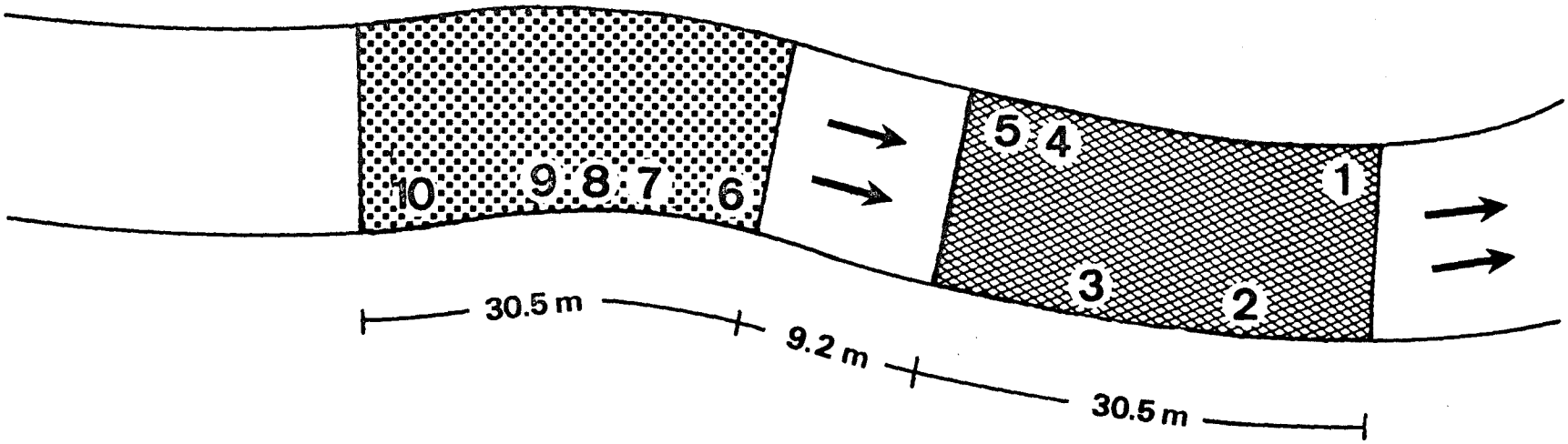


Figure 4. Location of Emergence Traps in Plot 1 and its Control

LC_{90} of the twelve different species of mosquitoes tested with Altosid.

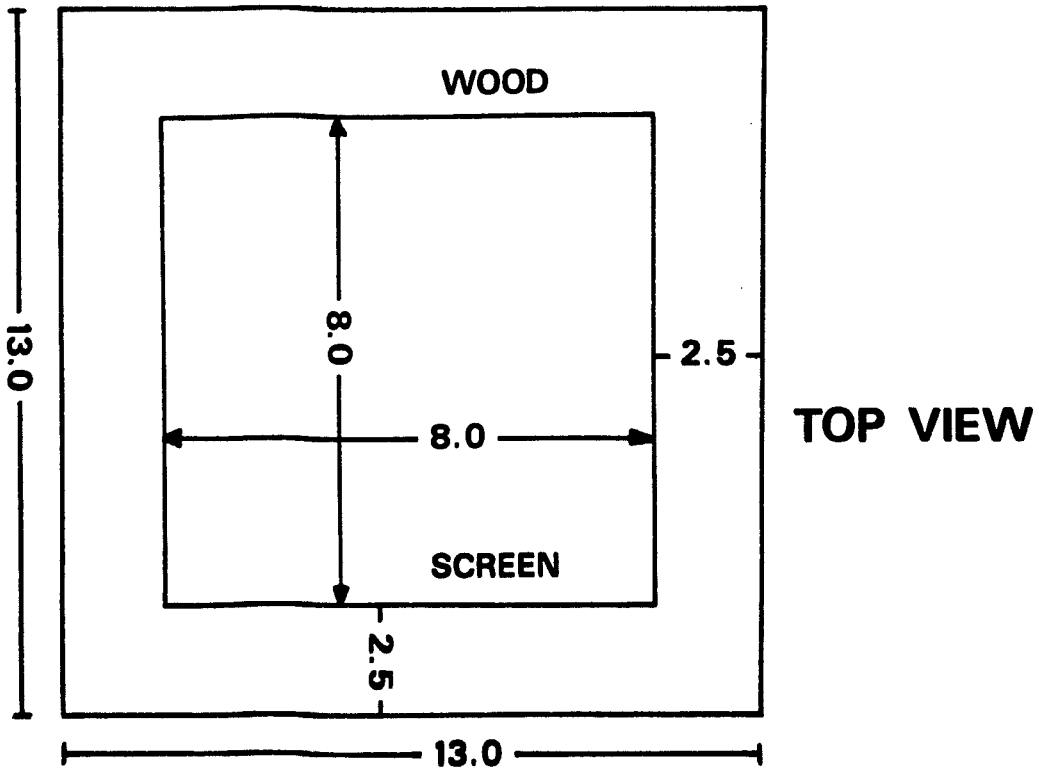
The traps were placed over cow hoofprints. All of the hoofprints were isolated from the main source of water flow. The prints were selected after the area was sprayed. All of the hoofprints used in both the control and sprayed plots contained 8 to 12 fourth-instar larvae per dip (200 ml) and greater numbers of 3rd, 2nd and 1st instar larvae plus eggs. Each print was approximately 20 cm in diameter and partially exposed to the sun.

To test the effects of Altosid on non-target organisms, 10 dips of 200 ml each were taken from both the control and the treated plot. Sampling was conducted six days before, two hours after and four days after spraying. The number and species of organisms found in each dip were recorded. This served to detect reductions of non-target organisms within plot 1.

All emergences within the traps were recorded from August 25 to September 8, 1974.

Plot Two. The plot was sprayed September 15, 1974 with 142 ml of Altosid SR-10. A maximum dosage of 118.3 ml per acre was used, mixed with 3.8 liters of water obtained from the plot. Three traps, each with 20 Culiseta inornata, were placed in the plot. The traps (Fig. 5) were modeled after those of Mulla (1973) and Hoppe, Isler and Vogel (1974). Locations of the traps are shown in Figure 6.

The control plot, started at the same time, was located just south of plot 1. Exposed areas of the control plot were picked to



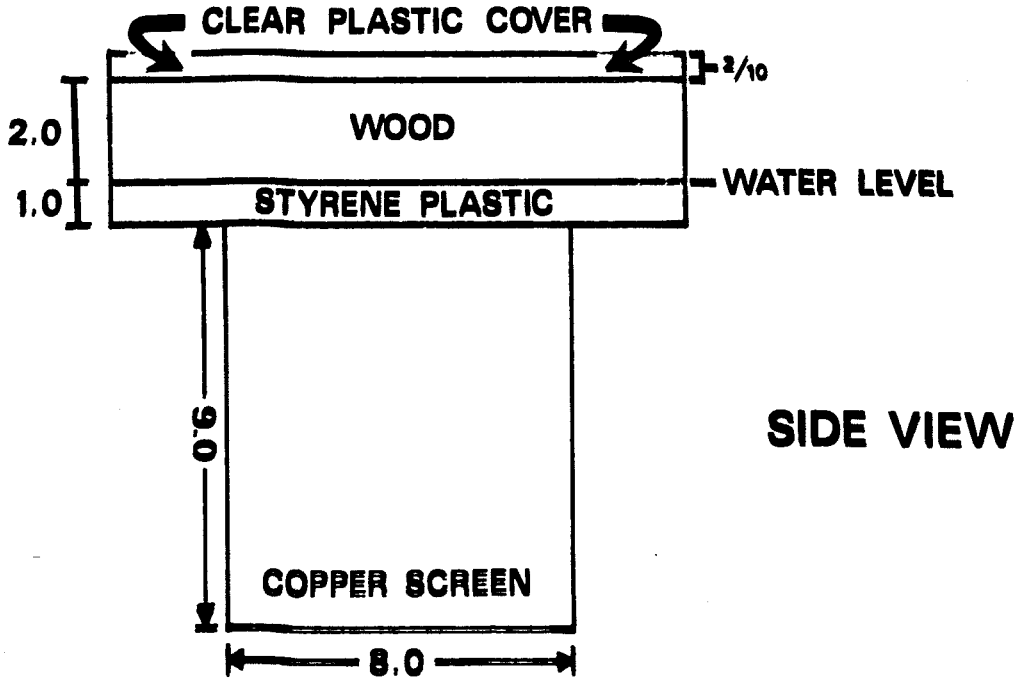


Figure 5. Dimensions in cm of a Plot 2 Trap

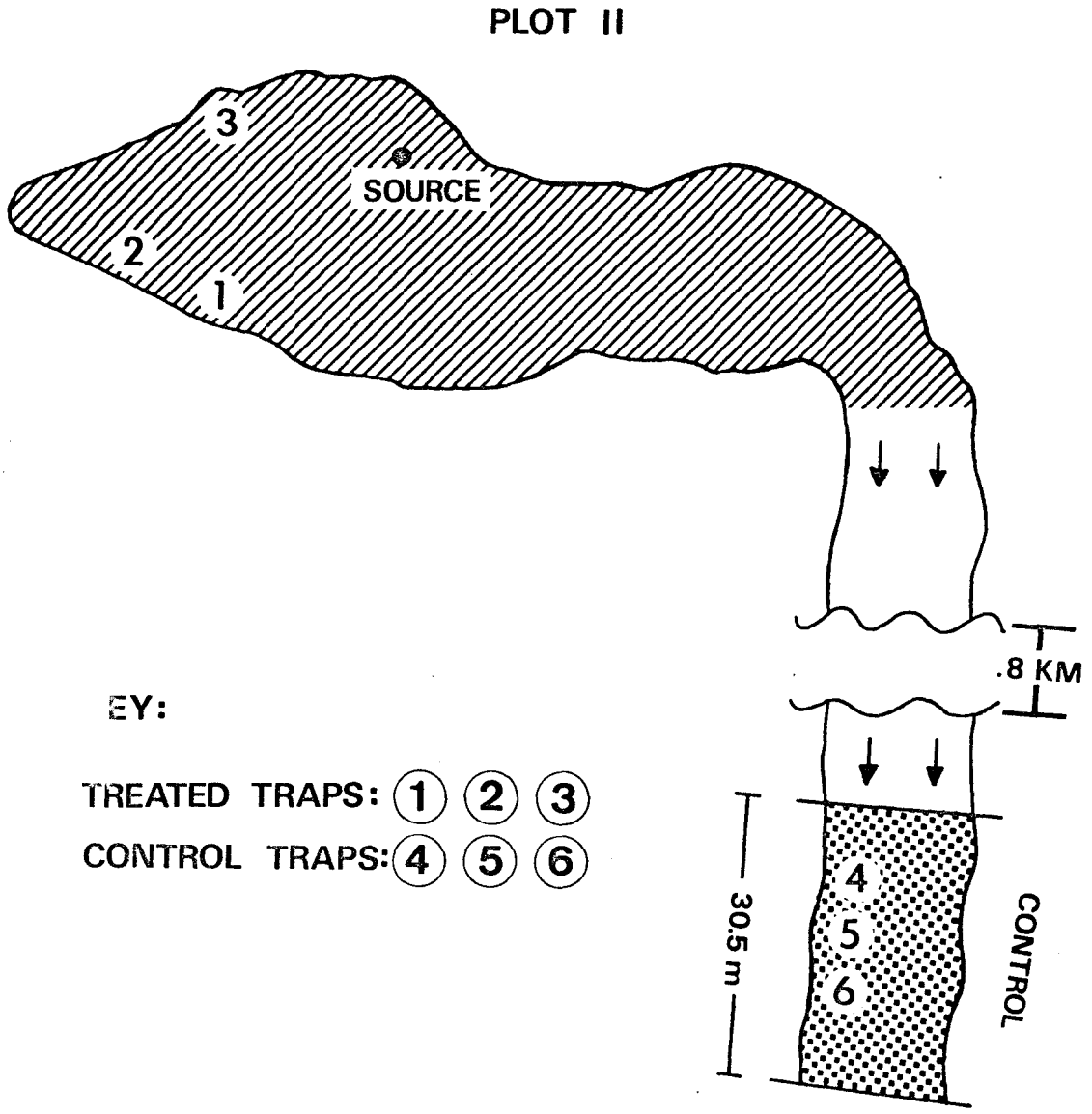


Figure 6. Location of Traps in Plot 2 and its Control

conform with the plot 2 environment. Three identical traps were placed in the control area.

Fourth-instar Culiseta inornata were used in the field tests. All of the mosquito larvae were obtained from the control plot. On September 1, 1974 one trap containing 20 fourth-instar C. inornata collected in the control area was placed in plot 2 before it was treated. This experiment tested the effects of the environment of plot 2 on mosquitoes collected in the control area.

The field experiment ended when all of the mosquitoes in the traps were dead or had emerged.

Laboratory Experiments

Growth Regulators and Organophosphates. The growth regulators and organophosphates used are listed below, and the trade name for each is shown on the right.

Isopropyl (E,E)-11-methoxy-3,7,11-trimethyl-2,4-dodecadienoate	Altosid
Ethyl (2E,4E) 3,7,11-trimethyl-2,4-dodecadienoate	Altozar
Prop-2-ynyl 3,7,11-trimethyl-(2E,4E)-dodecadienethiolate	ZR-777
Ethyl n-methoxy-3,7,11-trimethyl-(2E,4E)-dodecadienethiolate	AR-619
0,0-diethyl 0-p-nitrophenyl phosphorothiolate	Parathion
0,0-dimethyl phosphorothiolate 0,0 diester with 4,4' thiodiphenol	Abate

Operation of Bioassay. Stock solutions of 1% compound and 99% reagent acetone in concentrations of 10,000 ppm were made for the bioassays.

Serial dilutions with aged tap water were made for each of the compounds tested. The bioassays were conducted in plastic-coated paper cups which were 5 cm high, 8 cm in diameter at the bottom, and 9.5 cm in diameter at the top. A total of 100 ml of the tested concentration was placed in each cup. If the experiment lasted over three days the lost volume was replaced with distilled water every three days.

Tests were run on fourth-instar larvae and pupae with no distinction of sex. When enough larvae or pupae were present, two cups were assayed for each concentration tested. Each cup contained 20 or 25 mosquitoes. Details of each bioassay are located in the appendix. Two bioassays were conducted for each of the JH analogues. The first served to determine the approximate range of the lethal concentrations. The second bioassay's concentrations were within the lethal range determined from the first bioassay. This provided enough points to plot a log-dosage probit mortality curve. The bioassays were conducted under the same temperature and photoperiod as that of the larval incubator. On alternate days small amounts of yeast were added to the cups containing larvae used for the JH bioassays. All pupal bioassays and 48-hour Parathion and Abate bioassays were free of yeast.

Controls were conducted to determine if reagent acetone or the coating on the paper cups interfered with the mortality readings.

To test the organophosphates, 24 and 48-hour mortalities were recorded. Because the growth regulator analogues that were used kill mosquitoes only at the pupal or emerging adult stage (Jakob 1972, Schaefer and Wilder 1972, Mulla, Darwazeh and Norland 1974), growth regulator bioassays were terminated only when the cup was empty of living mosquitoes. Percentage mortality was recorded when the experiment was terminated for the analogues. All control mortalities were corrected with the following formula (March and Metcalf 1949):

$$\text{Corrected Mortality} = \frac{\text{observed \% mortality - check \% mortality}}{\text{100 - check \% mortality}}$$

Log-dosage probit mortality curves for the bioassays were plotted for each chemical to determine LC_{50} and LC_{95} values.

Rearing Technique for Aedes aegypti

Eggs. Egg deposition occurred in a glass bowl 10 cm in diameter and 6 cm high. The bowl was filled with distilled water and placed in the cage containing adults. The water level was not allowed to drop one-fourth or more below the rim of the bowl so that the adults were able to escape from the dish. The eggs were removed by carefully pouring the contents of the bowl onto a filter paper 20.4 cm in diameter. Distilled water was then used to wash the remaining eggs from the bowl and concentrate the eggs in the center of the paper. The filter paper was then folded flat and stapled shut. The eggs were dried for three hours in the larvae incubator, and then stored in a tightly sealed gallon jar (Gerberg 1970). The jar contained a supply of water in an open container which kept the humidity at approximately 80%. The eggs were stored at room temperature (about 30°C) for as long as 12 months.

Larvae. The eggs were brushed from the filter paper into photographic trays (45 cm x 25 cm x 7.5 cm) filled to the depth of 5 cm with tap water. Hot tap water was cooled to room temperature prior to the addition of the eggs. The food--1 g of whole wheat bread, .5 g of yeast and 1 g tetramin-- was added at the time of incubation. Additional yeast was added every three days as needed. The trays were placed in an incubator regulated for 32°C and 80% humidity. A photoperiod of 15 daylight hours was maintained and a fan used to circulate the air within the incubator. Eggs hatched within three hours to three days after placement in the incubator.

Pupae. Pupae appeared within four to five days after hatching. The males pupated first. The pupae were removed daily from the tray to prevent emergence of adults in the incubator, and transferred by means of a pipette into a 250 ml petri dish filled with distilled water. The petri dish, with a bubble cover, was placed into the cage containing adults.

Adults. The cage containing the adult mosquitoes was regulated at approximately 30°C with a photoperiod identical to that of the incubator. Adults began emerging within two days; the males emerged first. Adults were removed daily from the bubble cover and freed within the cage. Cotton balls soaked in a solution of one part sugar to three parts distilled water were placed on plastic dishes to serve as food for the emerged adults. The cotton balls were moistened daily with distilled water and changed every three days. Females accepted a blood meal three to four days after they

emerged. They were allowed to feed for five minutes on alternate days on a human arm, and within four days they began to lay eggs.

RESULTS

Field Experiments

Plot One. Emergence data are recorded in Table 1. Most of the insects captured in the traps belonged to the families Culicidae, Dolichopodidae and Chironomidae. Total emergence of these families in the control and sprayed plot is shown below.

	Treated areas	Control areas
Culicidae	2	26
Dolichopodidae	1	11
Chironomidae	11	9

Figures 7, 8 and 9 compare the emergence time for the same three families. Results of the non-target organism samples are shown in Tables 2, 3 and 4.

Plot Two. The experiment, conducted from September 1 to 15, tested the effects of the environment of plot 2 on the mosquitoes taken from the control. Fourteen adults emerged (70% emergence) and three pupae developed. However, the pupae were long over-due for emergence and would not have survived to the adult stage.

A comparison between Culiseta emergence in plot 2 and its control is shown in Table 5 and Figure 10. The control showed 66.5% emergence compared to 0% emergence in the treated plot.

Table 1

Plot 1 Data on Total Insect Emergence per Trap[1]

Trap number	M C D O	M C D O	M C D O	M C D O	M C D O
Date	8/25	8/27	8/28	8/29	8/30
Plot 1					
1	0 0 0 0	0 0 0 0	0 0 0 0	0 0 0 0	0 0 0 0
2	1 0 0 0	0 0 0 0	0 1 0 0	0 0 0 0	0 1 0 0
3	0 0 0 0	0 0 0 0	0 0 0 0	0 0 0 0	0 0 0 0
4	0 0 0 0	0 0 0 0	0 0 0 0	0 1 0 0	0 0 0 0
5	0 0 0 0	X X X X	0 0 0 0	0 0 0 1‡	0 0 1 0
Control					
6	0 1 0 0	1 0 2 0	3 0 0 0	0 0 0 0	0 1 0 0
7	0 0 1 0	0 0 0 0	0 0 0 0	0 0 0 0	1 0 1 0
8	0 0 0 0	3 0 0 0	1 0 0 0	2 0 0 0	0 1 0 0
9	0 0 2 0	2 0 1 0	1 0 1 0	1 0 0 0	1 1 0 0
10	0 0 0 0	0 0 0 0	0 0 0 0	0 0 0 0	0 0 2 0

Date	9/1	9/3	9/5	9/8	Total
Plot 1					
1	0 0 0 0	X X X X	0 0 0 0	0 0 0 0	0 0 0 0
2	0 0 0 0	0 1 0 0	1 1 0 0	0 2 0 0	2 6 0 0
3	0 0 0 2*	0 0 0 0	0 0 0 0	X X X X	0 0 0 2
4	0 0 0 1*	0 1 0 0	0 2 0 0	0 1 0 0	0 5 0 1
5	0 0 0 1+	0 0 0 0	X X X X	0 0 0 0	0 0 1 2
Control					
6	8 0 0 0	6 0 0 0	8 0 0 0	1 0 0 0	27 2 2 0
7	0 1 0 0	7 0 0 0	3 0 0 0	1 0 0 0	12 1 2 0
8	9 1 0 0	5 0 0 0	8 0 0 0	11 0 0 0	39 2 0 0
9	1 1 0 0	0 0 0 0	1 0 0 0	1 0 0 0	8 2 4 0
10	0 1 0 1*	0 0 1 0	0 1 0 0	X X X X	0 2 3 1

[1]Key to symbols:

M = Culicidae (Mosquitoes)
C = Chironomidae (Midges)
D = Dolichopodidae (Long-Legged Flies)
O = Other Families of Insects
X = Trap Knocked Over by Cow (prints not damaged)
* = Tipulidae (Crane Flies
+ = Baetidae (Mayflies)
‡ = Coenagrionidae (Damselflies)

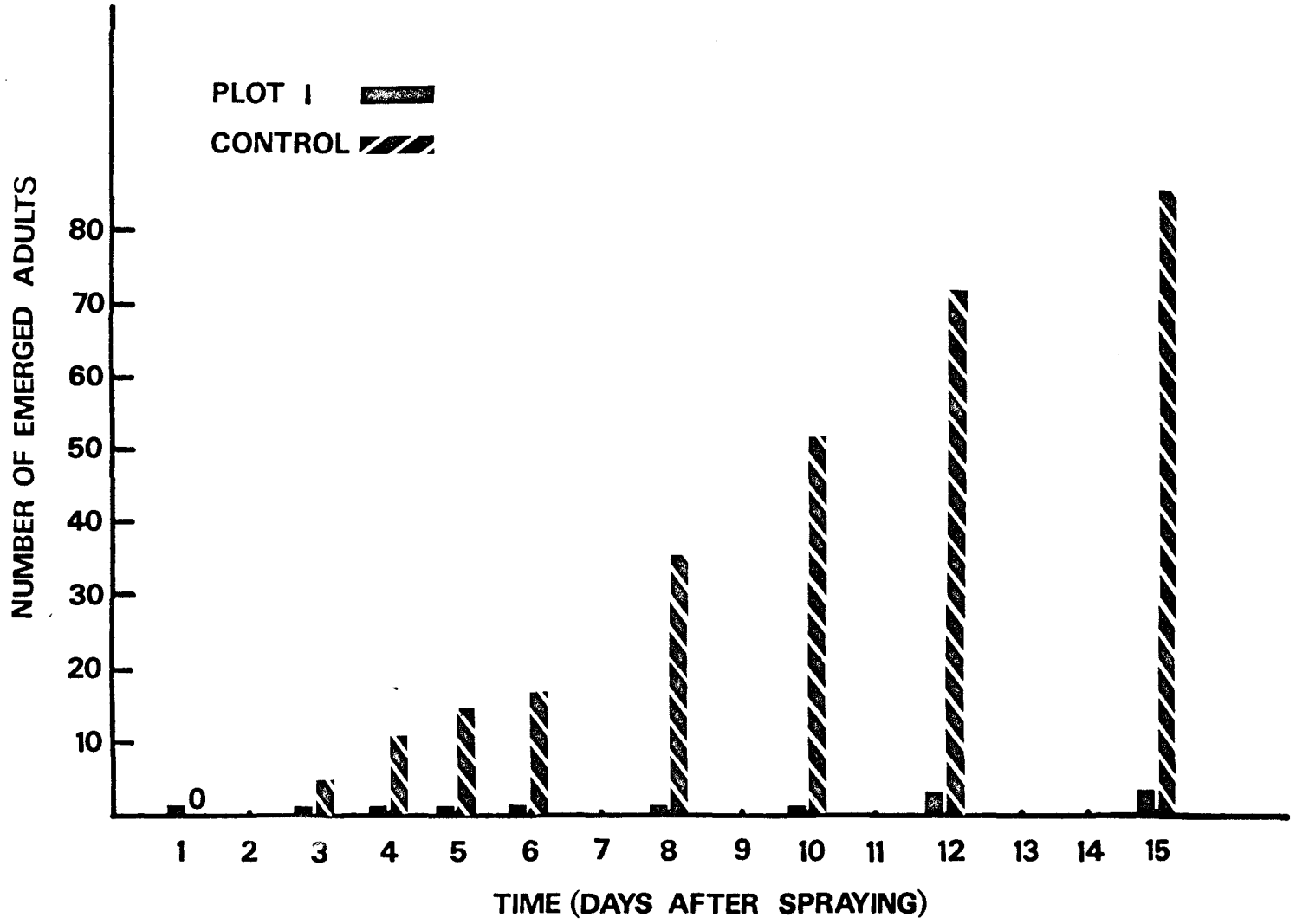


Figure 7. Mosquito Emergence from Plot 1 and its Control

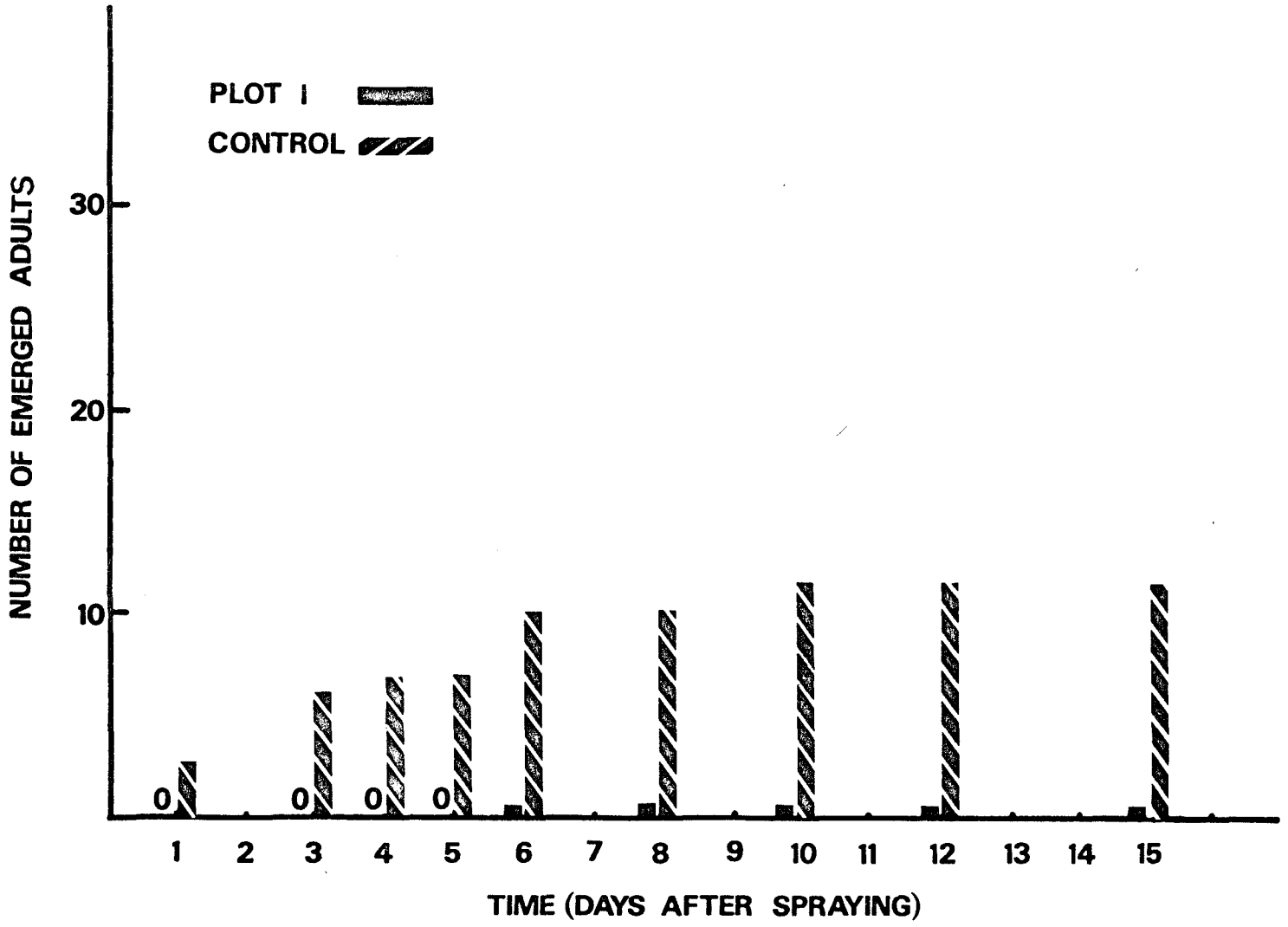


Figure 8. Dolichopodidae Emergence from Plot 1 and its Control

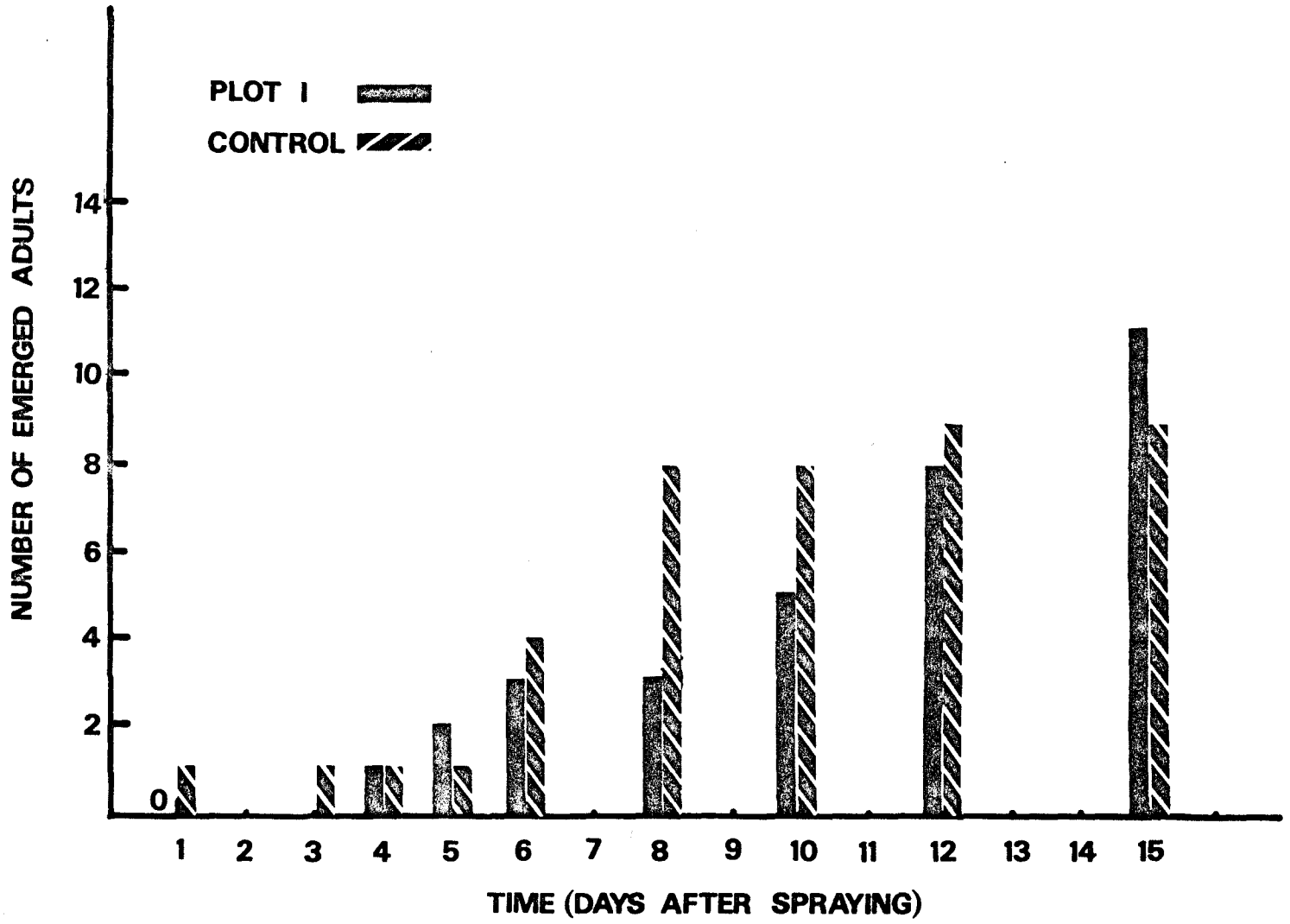


Figure 9. Chironomidae Emergence from Plot 1 and its Control

Table 2

Non-Target Organisms Sampled August 18, 1974, Six Days Before Spraying

Dip 1		Dip 2		Dip 3		Dip 4		Dip 5	
Organism	Number	Organism	Number	Organism	Number	Organism	Number	Organism	Number
				Plot 1					
Anopheles	1	Anopheles	1	Anopheles	3	Anopheles	3	Coleoptera	3
Callibaetis	4	Callibaetis	1	Callibaetis	1	Callibaetis	2	Callibaetis	2
Cladocera	many	Cladocera	many	Cladocera	many	Cladocera	many	Cladocera	many
Copepods	many	Copepods	many	Copepods	many	Copepods	many	Copepods	many
Culex	1	Corixidae	1	Ischura	1	Culex	2	Culex	4
Gerris	1	Microvelia	2			Gerris	1	Ischura	3
				Control					
Anopheles	3	Anopheles	1	Coleoptera	1	Callibaetis	6	Anopheles	1
Cladocera	many	Coleoptera	3	Callibaetis	2	Cladocera	many	Cladocera	many
Copepods	many	Callibaetis	3	Cladocera	many	Copepods	many	Copepods	many
Sciomyzidae	1	Cladocera	many	Copepods	many	Culex	3		
		Copepods	many	Physa	1				
		Corixidae	2						

Table 3

Non-Target Organisms Sampled August 24, 1974, Two Hours After Spraying

Dip 1		Dip 2		Dip 3		Dip 4		Dip 5	
Organism	Number	Organism	Number	Organism	Number	Organism	Number	Organism	Number
				Plot 1					
Copepods	10	Copepods	13	Callibaetis	1	Anopheles	1	Anopheles	4
Culex	2	Corixidae	1	Cladocera	many	Cladocera	15	Cladocera	many
Ischura	3	Culex	4	Copepods	17	Copepods	10	Copepods	many
		Cytiscidae	1	Culiseta	1	Gerris	1	Dytiscidae	1
				Ischura	2	Microvelia	25	Microvelia	5
				Microvelia	3				
				Control					
Anopheles	2	Cladocera	18	Anopheles	2	Cladocera	many	Cladocera	12
Cladocera	16	Copepods	11	Cladocera	many	Copepods	many	Copepods	14
Copepods	12	Ischura	1	Copepods	many	Microvelia	6	Physa	1
Microvelia	1	Microvelia	4	Culiseta	5	Physa	3		
		Physa	2						

Table 4

Non-Target Organisms Sampled August 28, 1974, Four Days After Spraying

Dip 1		Dip 2		Dip 3		Dip 4		Dip 5	
Organism	Number	Organism	Number	Organism	Number	Organism	Number	Organism	Number
				Plot 1					
Callibaetis	1	Callibaetis	1	Cladocera	11	Anopheles	4	Callibaetis	2
Cladocera	19	Cladocera	many	Corixidae	8	Cladocera	15	Cladocera	18
Corixidae	2	Copepods	18	Culex	2	Copepods	12	Copepods	16
Culex	2	Culex	2			Corixidae	1	Physa	1
Culiseta	1	Microvelia	1			Culex	1		
Mosquito pupae	1					Ischura	1		
				Control					
Callibaetis	2	Callibaetis	1	Callibaetis	2	Cladocera	16	Cladocera	many
Cladocera	19	Cladocera	19	Physa	2	Copepods	11	Collembola	1
Copepods	12	Copepods	17	Sciomyzidae	1	Culex	2	Copepods	many
Microvelia	1	Culex	2			Spider	1		
Physa	1								

Table 5

Emergences of Culiseta inornata
Within Traps of Plot 2 and the
Control

	Number of emergences up to date indicated[1]										
Date	9/20	9/23	9/25	9/27	9/29	10/1	10/3	10/6	10/10	10/13	10/15
Control	3	11	15	27	30	32	37	41	41	41	41
Plot 2	0	0	0	0	0	0	0	0	0	0	0

[1]Percentage emergence: Control = 66.5%, Plot 2 = 0%.

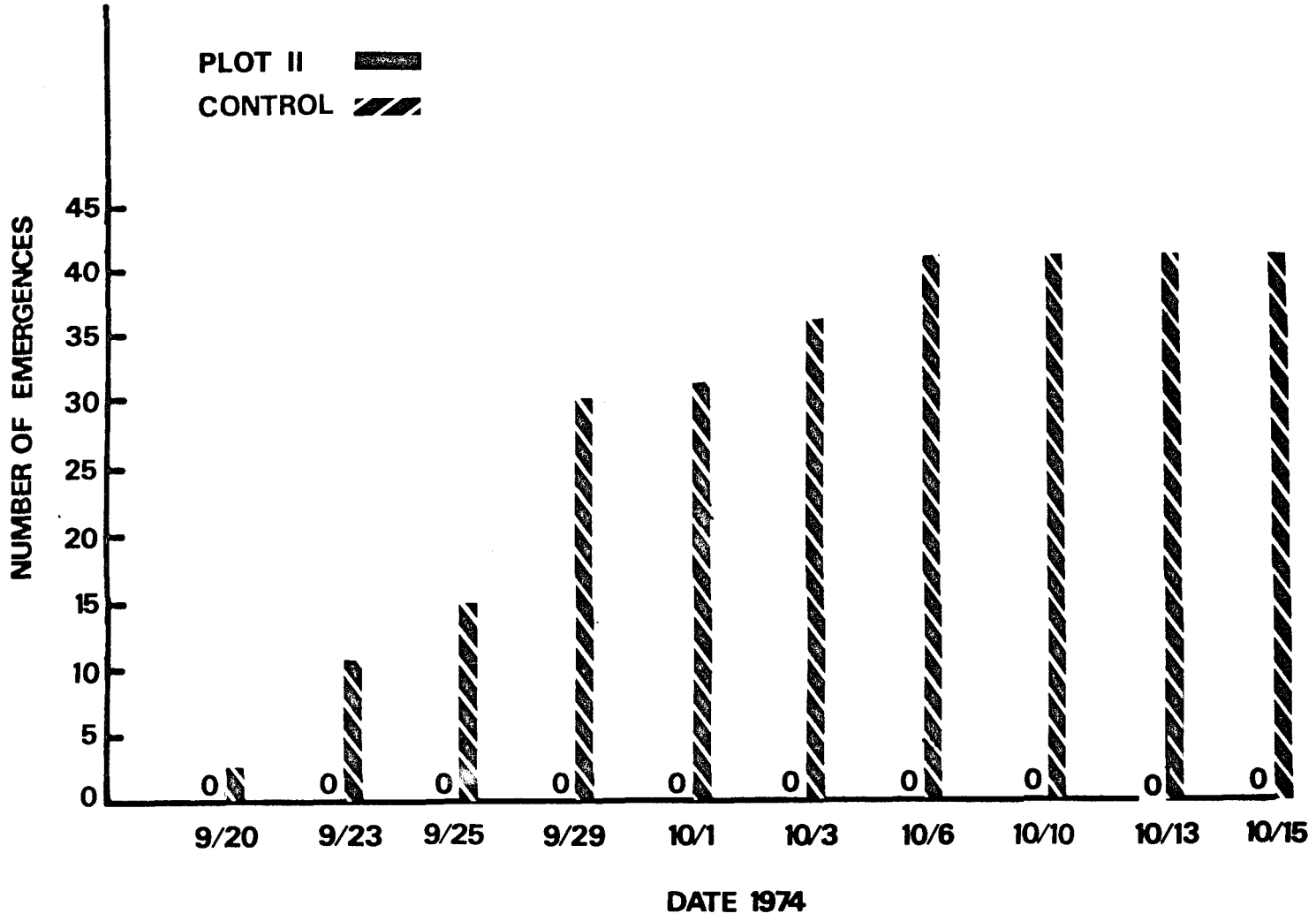


Figure 10. Mosquito Emergence from Plot 2 and its Control

Laboratory Experiments

The results of the experiments testing the intrinsic toxicity of the cups and acetone showed insignificant mortality (Table 22, Appendix C).

Details of the results of the bioassays are shown in Appendix C. Totals and corrected mortalities from the bioassays are presented in Tables 7 through 18. Figures 11 through 22 show the log-probit mortality curves derived from the second bioassay of the JH analogues and the 48-hour corrected mortalities from the organophosphates. A comparison of the log-probit mortality curves is illustrated in Figures 23 (larvae) and 24 (pupae). The LC_{50} and LC_{95} determined from the log-probit mortality curves are summarized below.

Table 6

LC_{50} and LC_{95} of Compounds Tested

Compound	Development stage	PPM LC_{50}	PPM LC_{95}
Altosid	larva	0.027	0.34
Altosid	pupa	9.2	38.0
ZR-619	larva	0.033	0.28
ZR-619	pupa	16.0	55.0
Altozar	larva	0.09	1.15
Altozar	pupa	10.3	18.5
ZR-777	larva	0.17	5.1
ZR-777*	pupa	11.1	38.0
Parathion	larva	0.0034	0.0205
Parathion	pupa	0.056	0.225
Abate	larva	0.011	0.077
Abate	pupa	6.0	270.0

*Three bioassays were used in the log-probit mortality curve for determining LC_{50} and LC_{95} (Table 14).

Table 7

Corrected Mortalities and Totals
of Aedes aegypti Larvae
With Altosid

PPM	Alive	Dead	Percentage mortality	Corrected percentage mortality
		First bioassay		
10.0	0	40	100.0	100.00
1.0	2	38	95.0	94.29
0.1	10	30	75.0	71.43
0.01	28	12	30.0	20.00
0.001	17	3	15.0	2.86
Control	35	5	12.5	-----
		Second bioassay		
1.0	2	18	90.0	89.47
0.5	2	18	90.0	89.47
0.1	5	35	87.5	86.84
0.05	11	29	72.5	71.05
0.01	12	8	40.0	36.84
Control	19	1	5.0	-----

Table 8

Corrected Mortalities and Totals
of Aedes aegypti Pupae
With Altosid

PPM	Alive	Dead	Percentage mortality	Corrected percentage mortality
		First bioassay		
100	0	50	100.0	100.00
10	19	31	62.0	60.42
1	26	24	48.0	45.83
Control	24	1	4.0	-----
		Second bioassay		
40	2	38	95.0	94.87
20	5	35	87.5	87.18
10	22	18	45.0	43.59
8	24	16	40.0	38.46
6	24	16	40.0	38.46
Control	39	1	2.5	-----

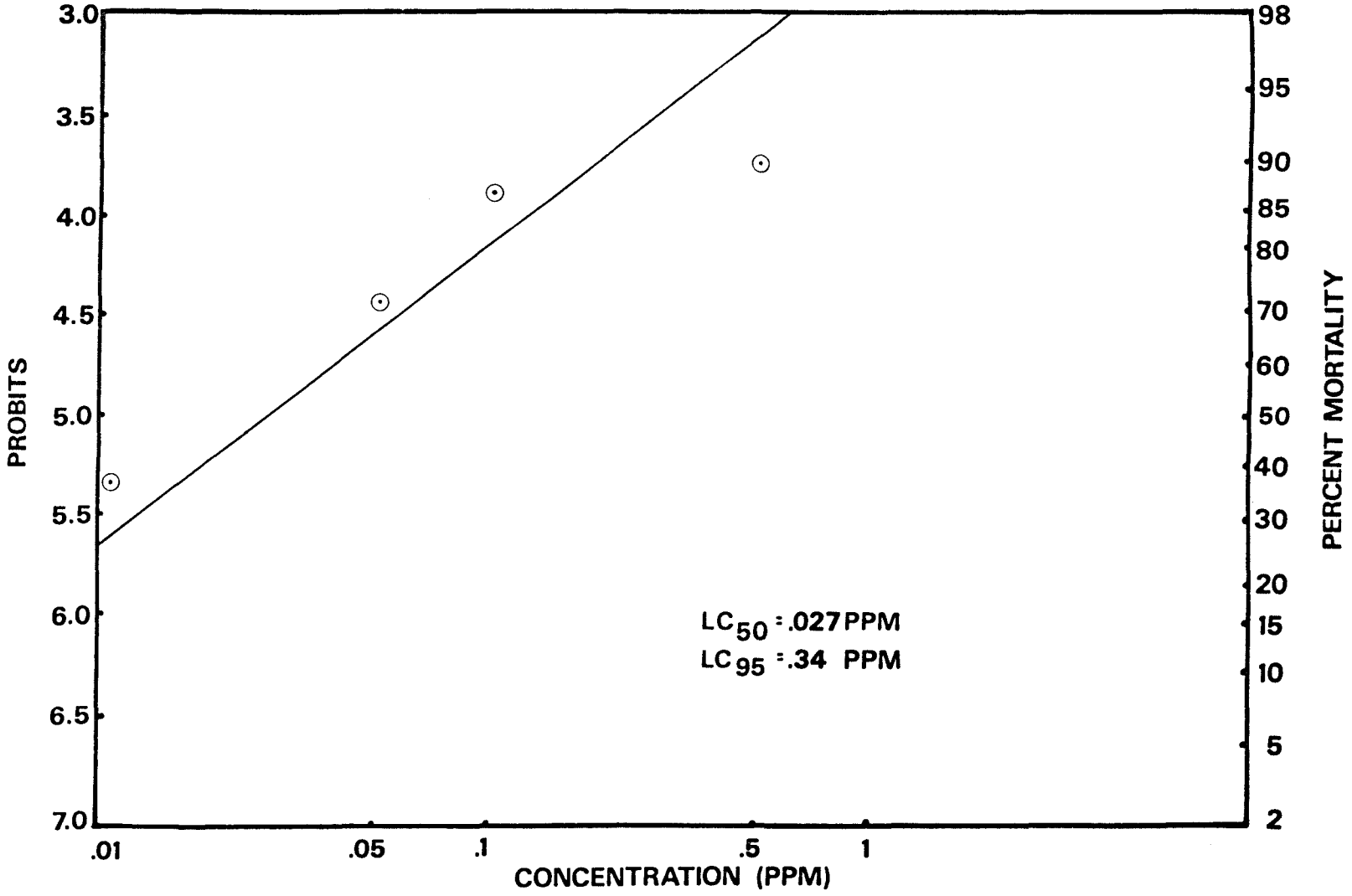


Figure 11. Log-Probit Mortality Curve Showing Effects of Altosid on Fourth-Instar Larvae

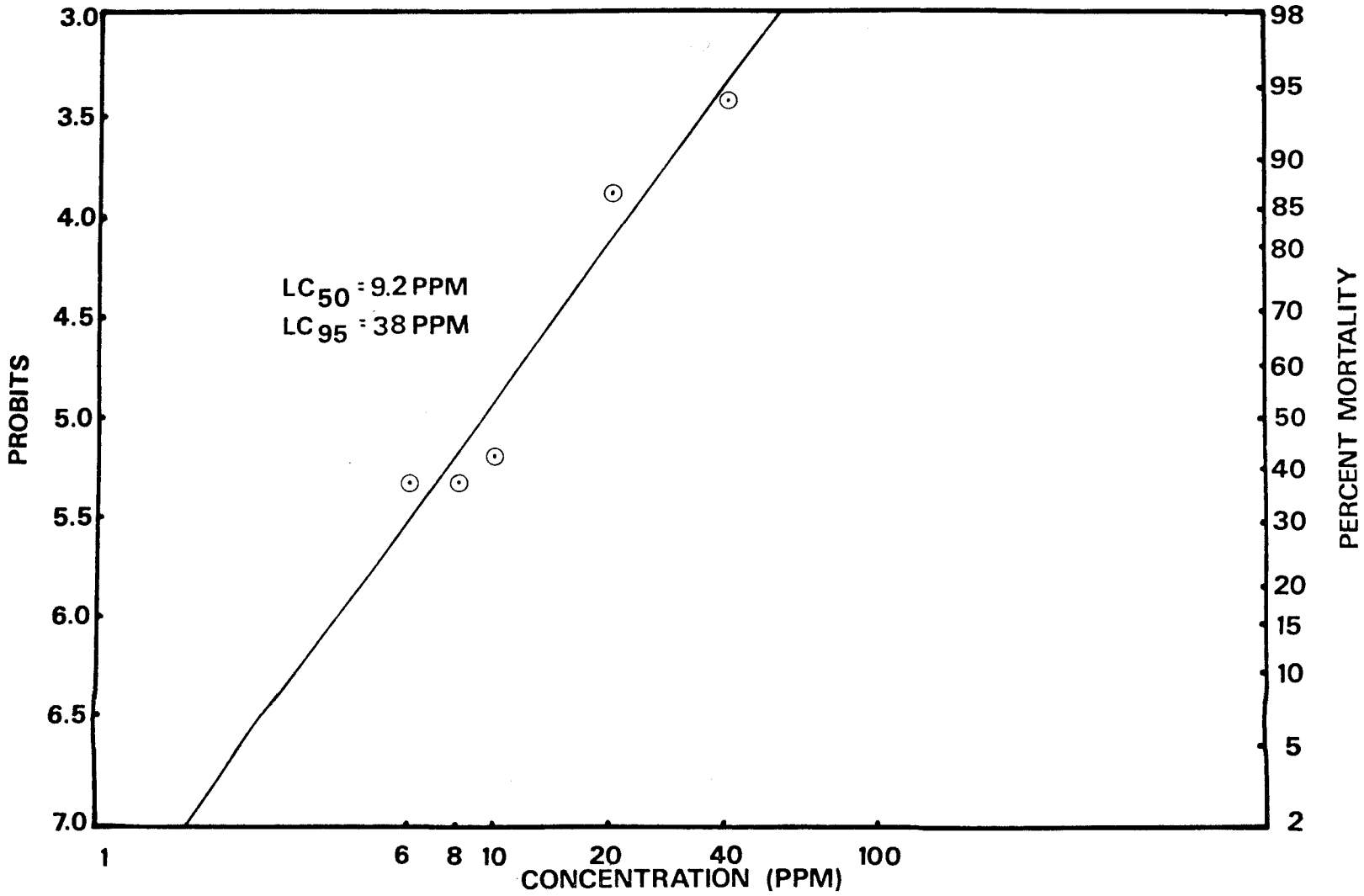


Figure 12. Log-Probit Mortality Curve Showing Effects of Altosid on Pupae

Table 9

Corrected Mortalities and Totals
of Aedes aegypti Larvae
With ZR-619

PPM	Alive	Dead	Percentage mortality	Corrected percentage mortality
		First bioassay		
10.0	1	39	97.5	97.06
1.0	3	37	92.5	91.18
0.1	8	32	80.0	76.47
0.01	31	9	22.5	8.82
0.001	36	4	10.0	-----
Control	34	6	15.0	-----
		Second bioassay		
1.0	0	40	100.0	100.00
0.5	0	40	100.0	100.00
0.1	5	35	87.5	83.33
0.05	14	26	65.0	53.33
0.01	24	16	40.0	20.00
Control	30	10	25.0	-----

Table 10

Corrected Mortalities and Totals
of *Aedes aegypti* Pupae
With ZR-619

PPM	Alive	Dead	Percentage mortality	Corrected percentage mortality
		First bioassay		
100.0	0	50	100.0	100.00
10.0	37	13	74.0	74.00
1.0	49	1	2.0	2.00
0.1	49	1	2.0	2.00
Control	75	0	0.0	-----
		Second bioassay		
100.0	0	40	100.0	100.00
80.0	0	40	100.0	100.00
60.0	2	38	95.0	94.29
40.0	3	37	92.5	91.43
20.0	7	33	82.5	80.00
10.0	28	12	30.0	20.00
Control	35	5	12.5	-----

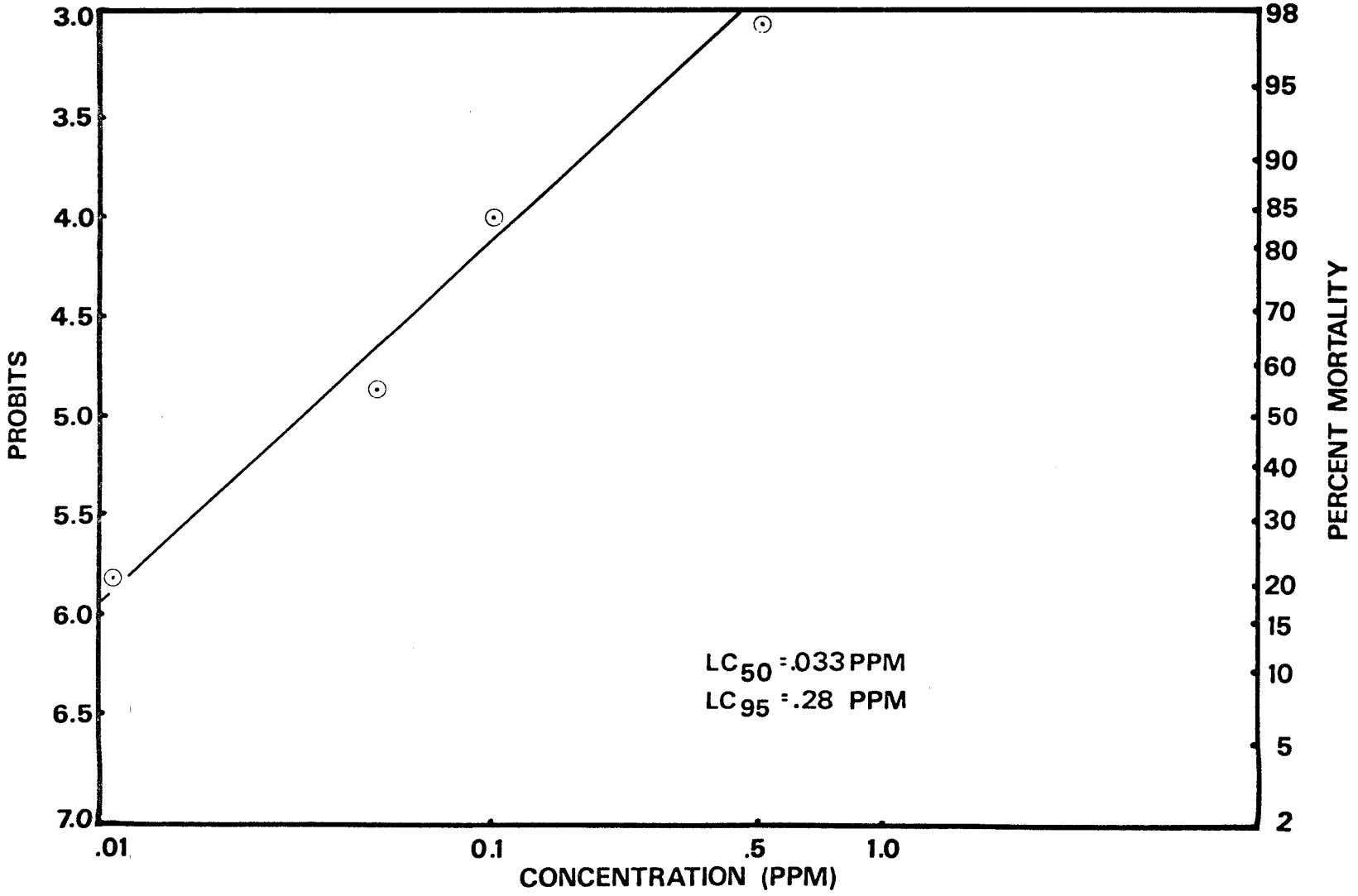


Figure 13. Log-Probit Mortality Curve Showing Effects of ZR-619 on Fourth-Instar Larvae

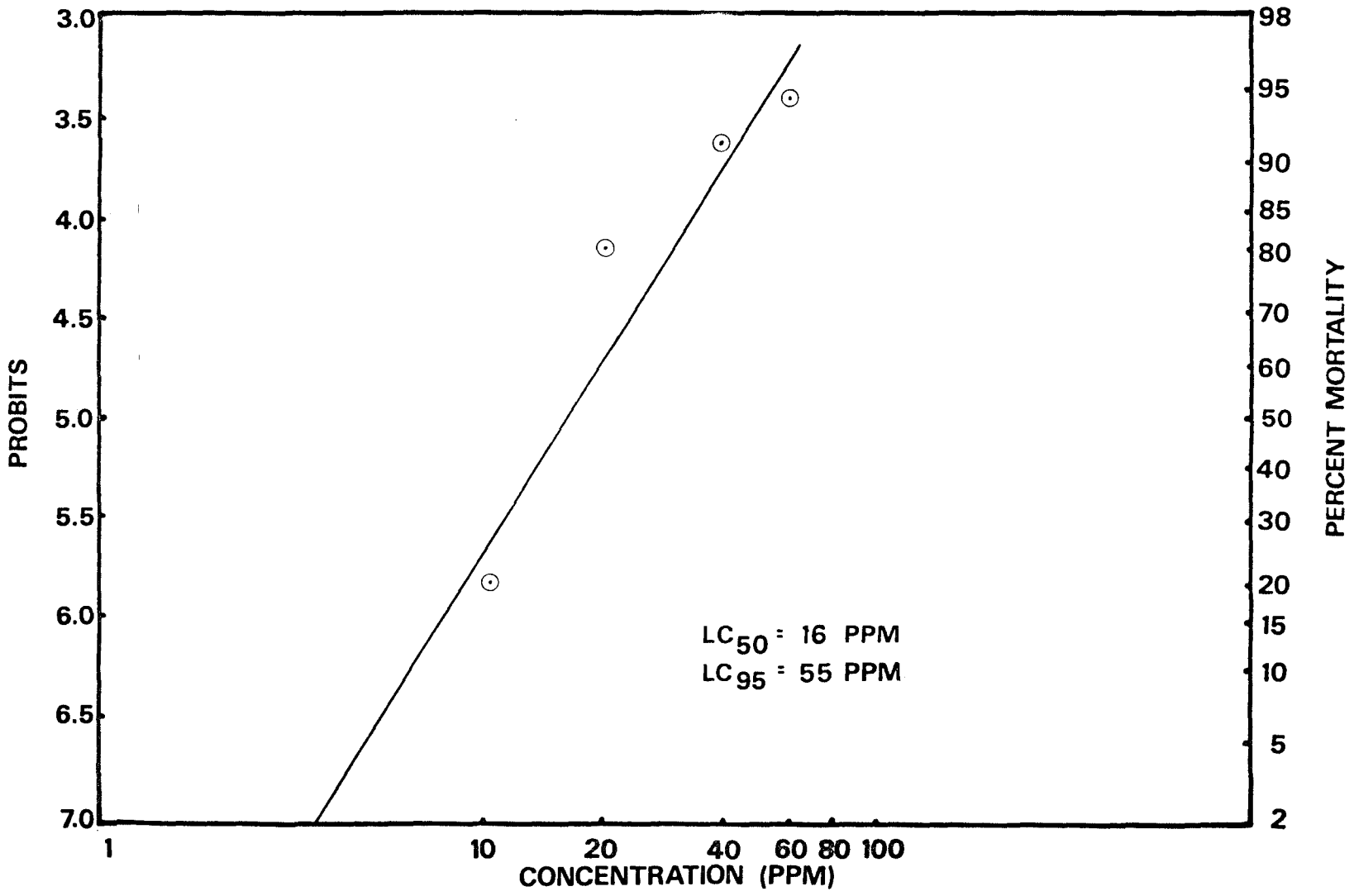


Figure 14. Log-Probit Mortality Curve Showing Effects of ZR-619 on Pupae

Table 11

Corrected Mortalities and Totals
of Aedes aegypti Larvae
With Altozar

PPM	Alive	Dead	Percentage mortality	Corrected percentage mortality
		First bioassay		
10.0	1	39	97.5	96.88
1.0	10	30	75.0	68.75
0.1	15	25	62.5	53.13
0.01	29	11	27.5	9.38
0.001	33	7	17.5	-----
Control	32	8	20.0	-----
		Second bioassay		
1.0	2	38	95.0	94.29
0.5	6	34	85.0	82.86
0.1	8	32	80.0	77.14
0.05	28	12	30.0	20.00
0.01	31	9	22.5	11.43
Control	35	5	12.5	-----

Table 12

Corrected Mortalities and Totals
of Aedes aegypti Pupae
With Altozar

PPM	Alive	Dead	Percentage mortality	Corrected percentage mortality
		First bioassay		
100	0	50	100.0	100.00
10	15	10	40.0	40.00
Control	25	0	0.0	-----
		Second bioassay		
40	0	40	100.0	100.00
20	1	39	97.5	97.37
10	21	19	47.5	44.74
8	28	12	30.0	26.32
6	36	4	10.0	5.26
Control	38	2	5.0	-----

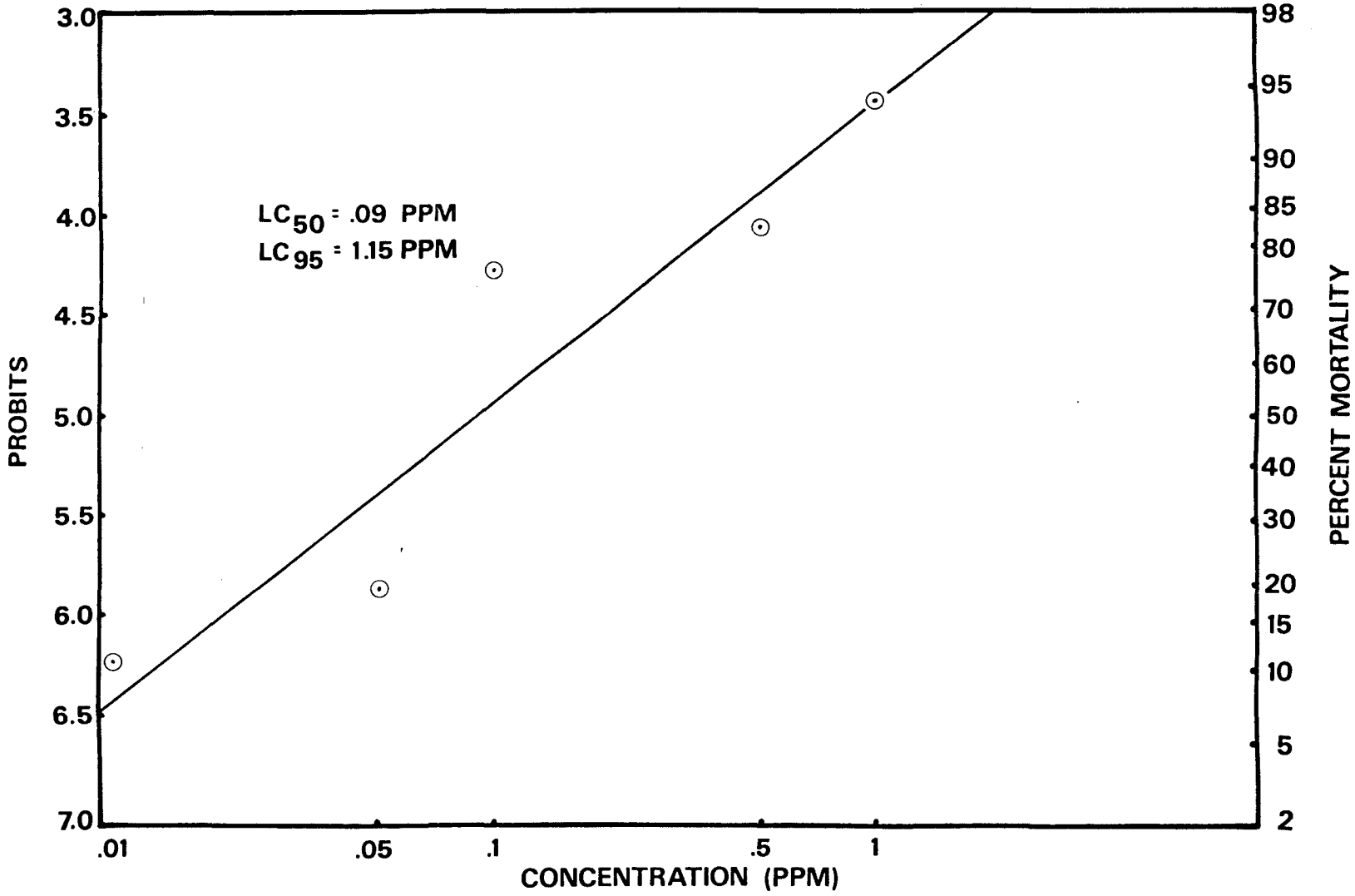


Figure 15. Log-Probit Mortality Curve Showing Effects of Altozar on Fourth-Instar Larvae

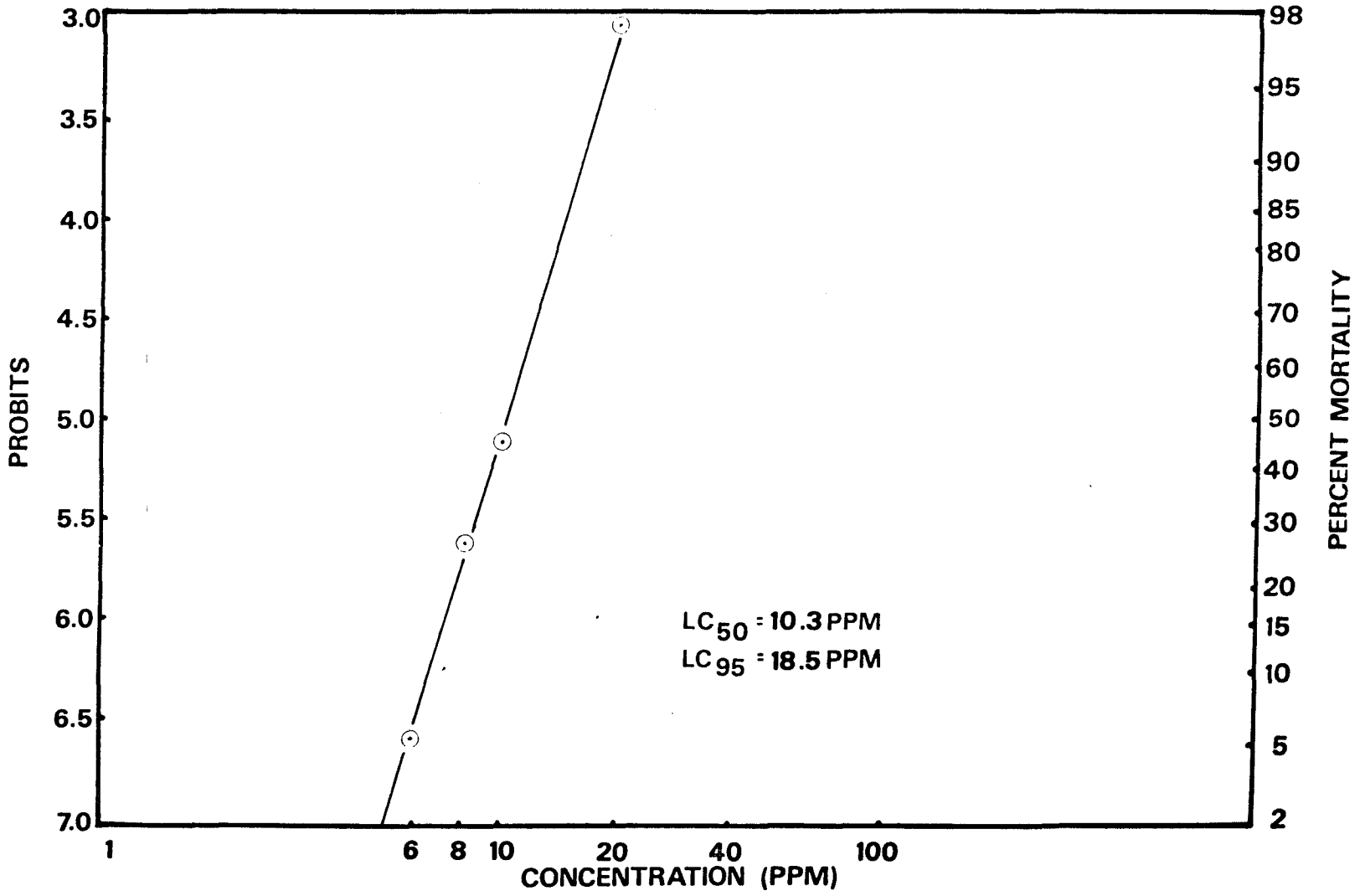


Figure 16. Log-Probit Mortality Curve Showing Effects of Altozar on Pupae

Table 13

Corrected Mortalities and Totals of *Aedes aegypti* Larvae With ZR-777

PPM	Alive	Dead	Percentage mortality	Corrected percentage mortality
		First bioassay		
10.0	0	40	100.0	100.00
1.0	3	37	92.5	91.18
0.1	16	24	60.0	52.94
0.01	33	7	17.5	2.94
0.001	31	9	22.5	8.82
Control	34	6	15.0	———
		Second bioassay		
1.0	8	32	80.0	79.50
0.5	6	31	77.5	76.92
0.1	12	8	40.0	38.46
0.05	33	7	17.5	15.38
0.01	35	5	12.5	10.26
Control	39	1	2.5	———

Table 14

Corrected Mortalities and Totals
of Aedes aegypti Pupae
With ZR-777

PPM	Alive	Dead	Percentage mortality	Corrected percentage mortality
		First bioassay		
100	0	40	100.0	100.00
80	0	40	100.0	100.00
60	1	39	97.5	97.44
40	1	39	97.5	97.44
20	5	35	87.5	87.18
10	23	17	42.5	41.03
Control	39	1	2.5	———
		Second bioassay		
8	29	11	27.5	22.16
6	32	8	20.0	13.51
4	34	6	15.0	8.11
2	32	8	20.0	13.51
Control	37	3	7.5	———
		Third bioassay		
10	22	18	45.0	40.54*

*Control of second bioassay used to determine the corrected percentage mortality of the third bioassay.

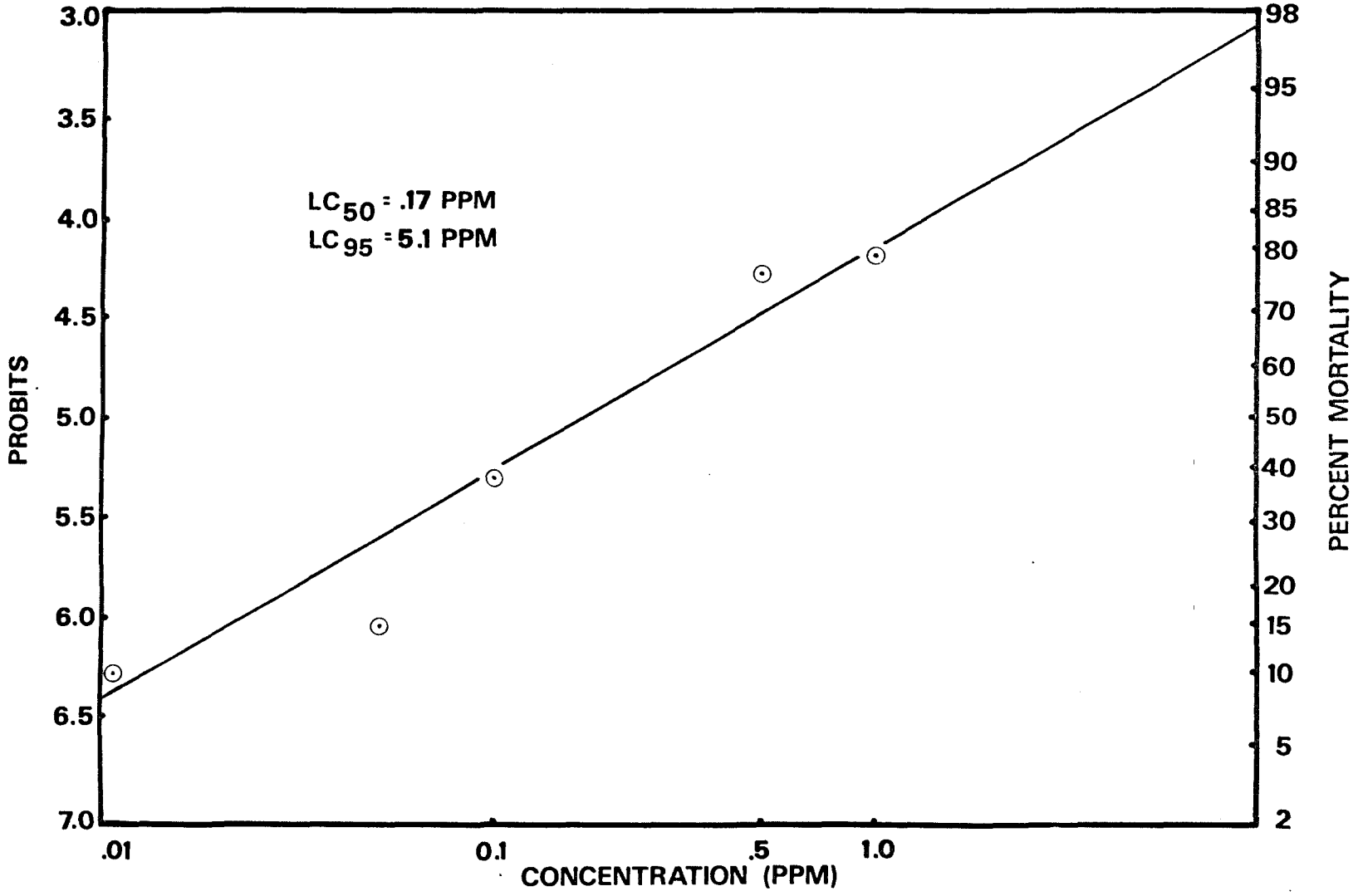


Figure 17. Log-Probit Mortality Curve Showing Effects of ZR-777 on Fourth-Instar Larvae

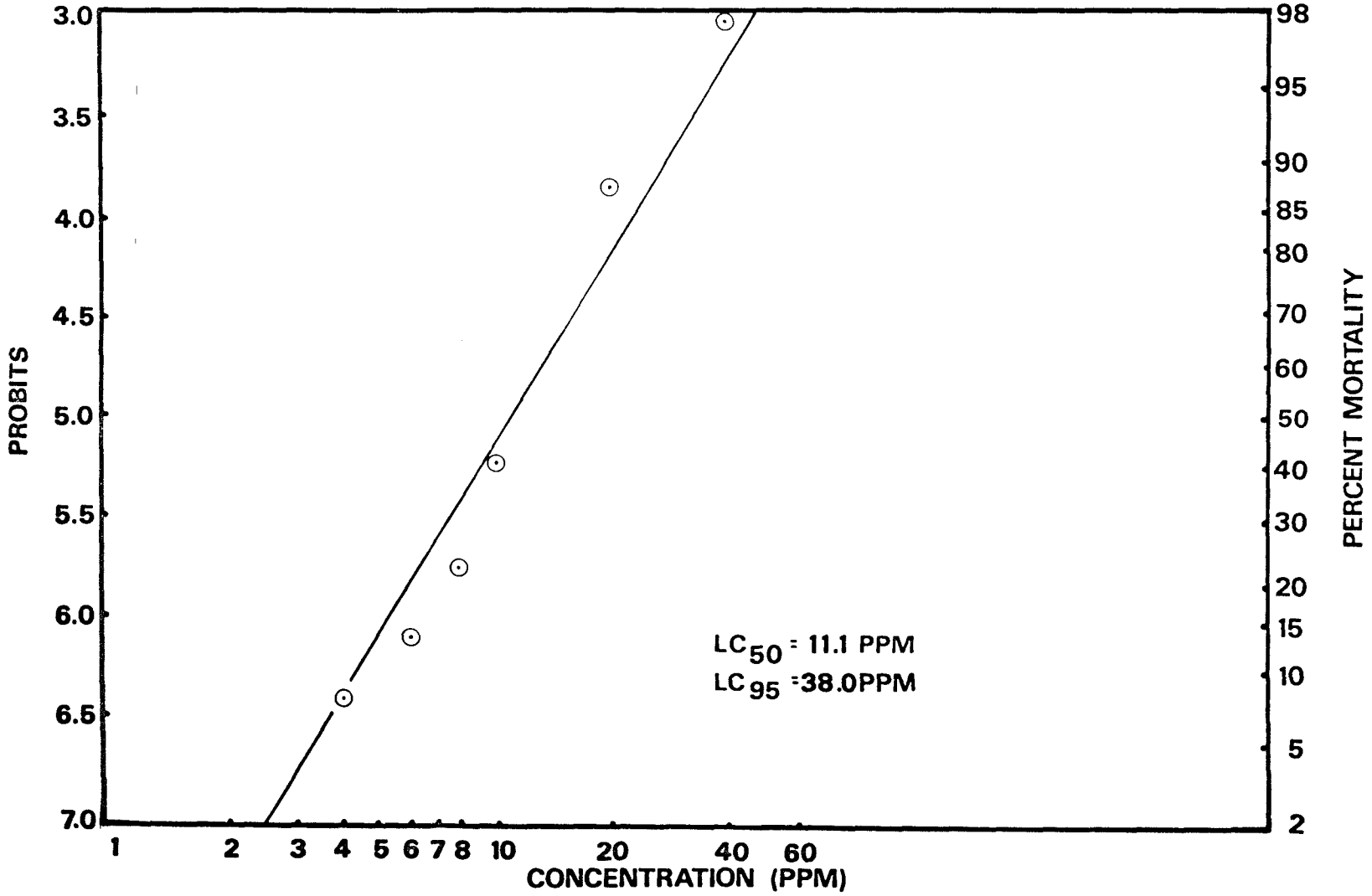


Figure 18. Log-Probit Mortality Curve Showing Effects of ZR-777 on Pupae

Table 15

Corrected Mortalities and Totals
of Aedes aegypti Larvae
With Parathion

PPM	Alive	Dead	Percentage mortality	Corrected percentage mortality
		24 hours		
0.01	33	7	17.5	15.38
0.005	40	0	0.0	-----
0.001	39	1	2.5	0.00
0.0005	39	1	2.5	0.00
0.0001	40	0	0.0	-----
Control	39	1	2.5	-----
		48 hours		
0.01	4	36	90.0	89.74
0.005	23	17	42.5	41.03
0.001	32	8	20.0	17.95
0.0005	37	3	7.5	5.13
0.0001	40	0	0.0	-----
Control	39	1	2.5	-----

Table 16

Corrected Mortalities and Totals
of Aedes aegypti Pupae
With Parathion

PPM	Alive	Dead	Percentage mortality	Corrected percentage mortality
		24 hours		
100.0	0	50	100.0	100.00
10.0	0	50	100.0	100.00
1.0	3	47	94.0	94.00
0.1	46	4	8.0	8.00
0.01	50	0	0.0	0.00
Control	50	0	0.0	-----
		48 hours		
100.0	0	50	100.0	100.00
10.0	0	50	100.0	100.00
1.0	0	50	100.0	100.00
0.1	12	38	76.0	76.00
0.01	49	1	2.0	2.00
Control	50	0	0.0	-----

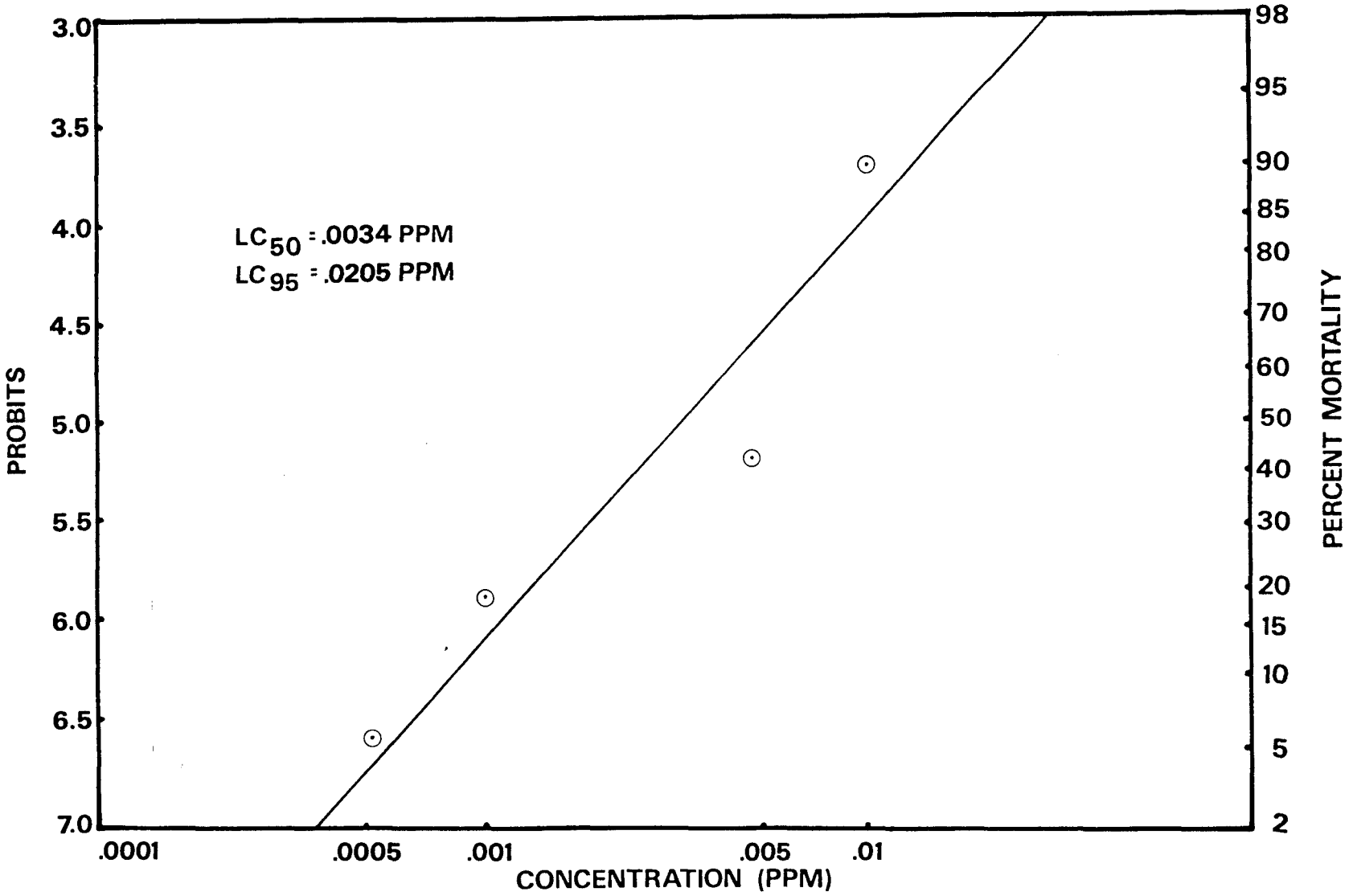


Figure 19. Log-Probit Mortality Curve Showing Effects of Parathion on Fourth-Instar Larvae

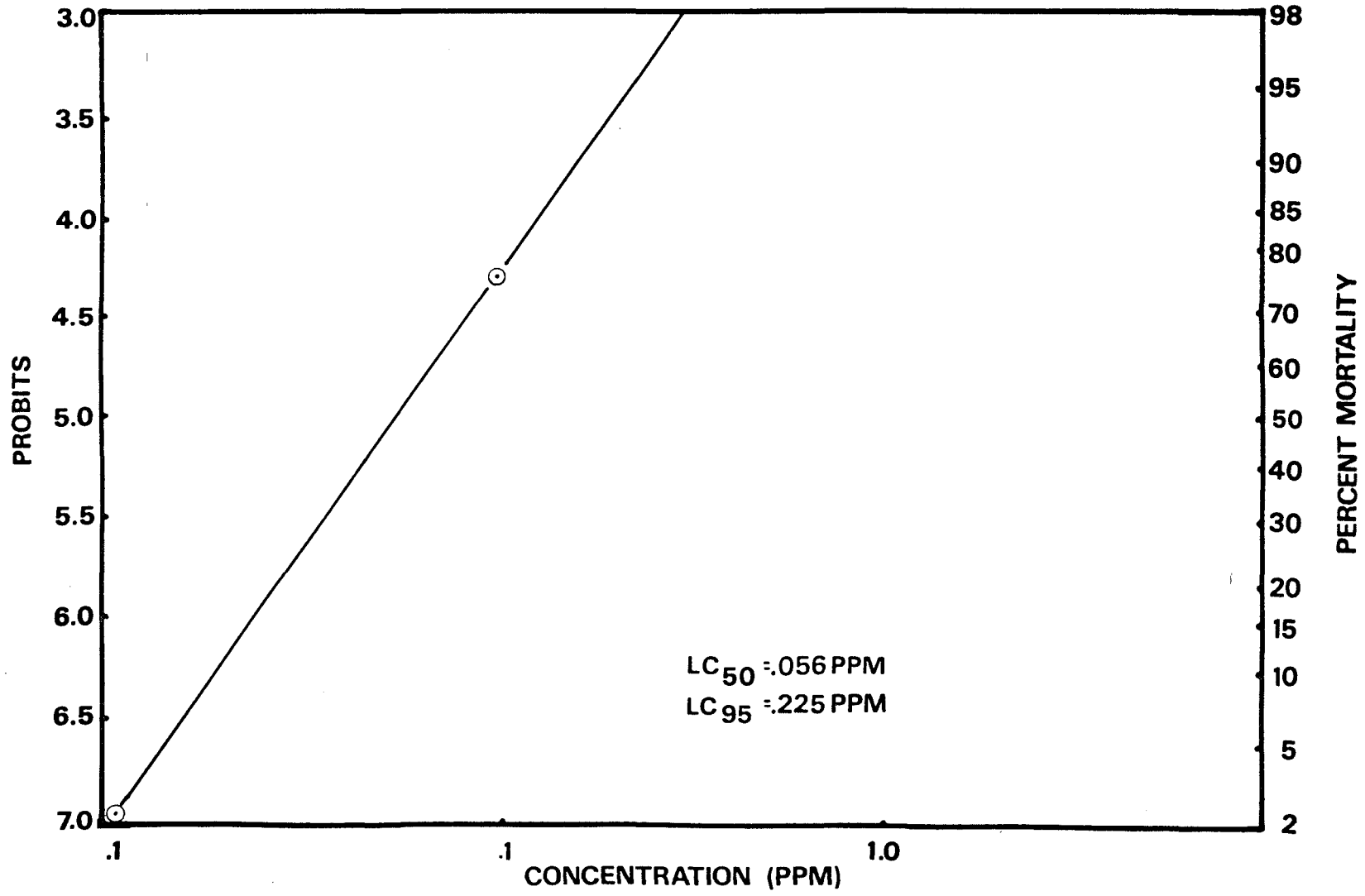


Figure 20. Log-Probit Mortality Curve Showing Effects of Parathion on Pupae

Table 17

Corrected Mortalities and Totals
of Aedes aegypti Larvae
With Abate

PPM	Alive	Dead	Percentage mortality	Corrected percentage mortality
		24 hours		
0.1	3	37	92.5	92.31
0.08	5	35	87.5	87.18
0.05	6	34	85.0	84.62
0.02	11	29	72.5	71.79
0.01	26	14	35.0	33.33
Control	39	1	2.5	-----
		48 hours		
0.1	1	39	97.5	97.37
0.08	3	37	92.5	92.11
0.05	4	36	90.0	89.47
0.02	10	30	75.0	73.68
0.01	17	23	57.5	55.26
Control	38	2	5.0	-----

Table 18

Corrected Mortalities and Totals
of Aedes aegypti Pupae
With Abate

PPM	Alive	Dead	Percentage mortality	Corrected percentage mortality
		24 hours		
100	13	37	74.0	74.00
10	45	5	10.0	10.00
1	46	4	8.0	8.00
Control	25	0	0.0	-----
		48 hours		
100	4	46	92.0	91.67
10	28	22	44.0	41.67
1	36	14	28.0	25.00
Control	24	1	4.0	-----

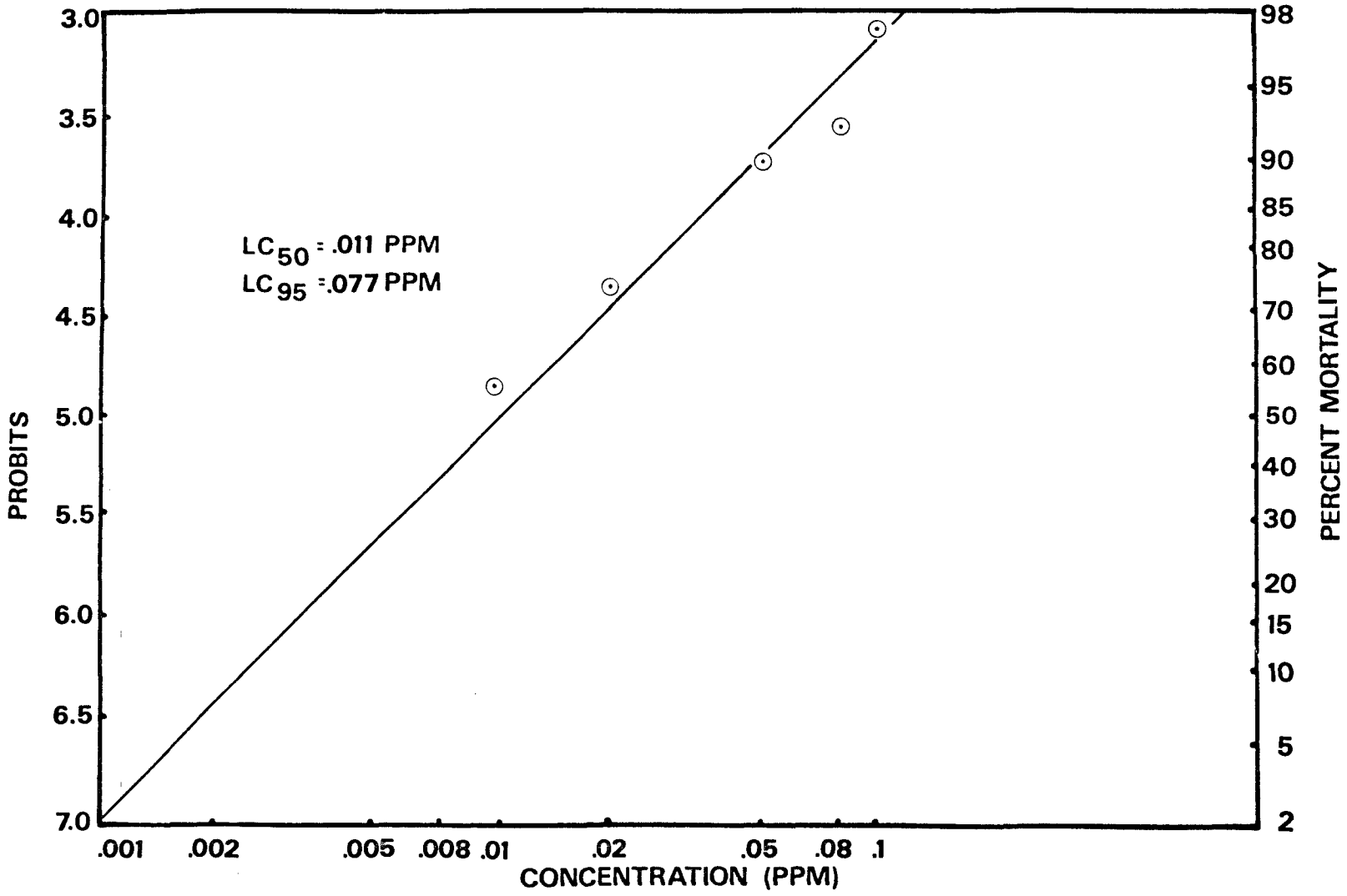


Figure 21. Log-Probit Mortality Curve Showing Effects of Abate on Fourth-Instar Larvae

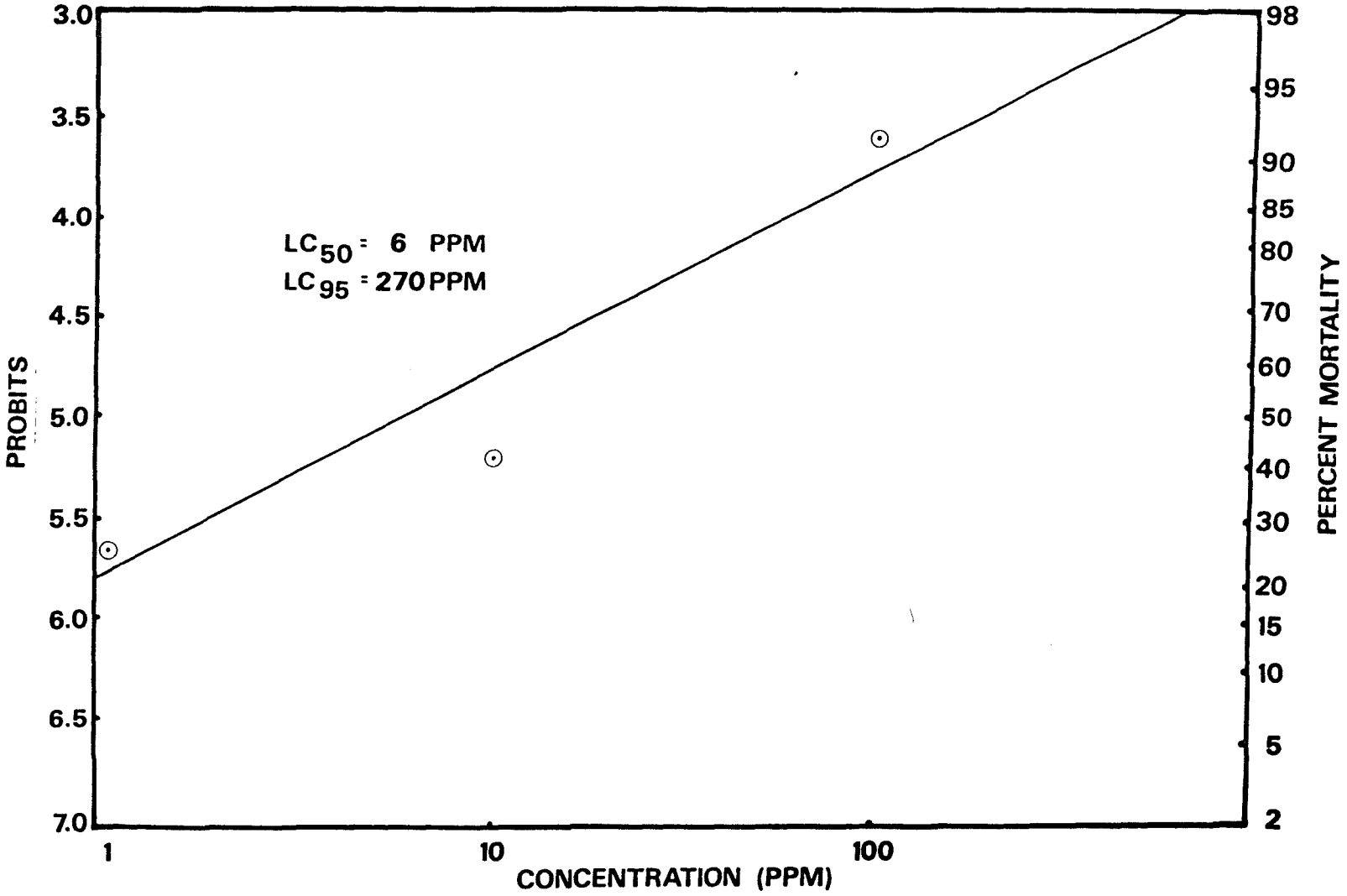


Figure 22. Log-Probit Mortality Curve Showing Effects of Abate on Pupae

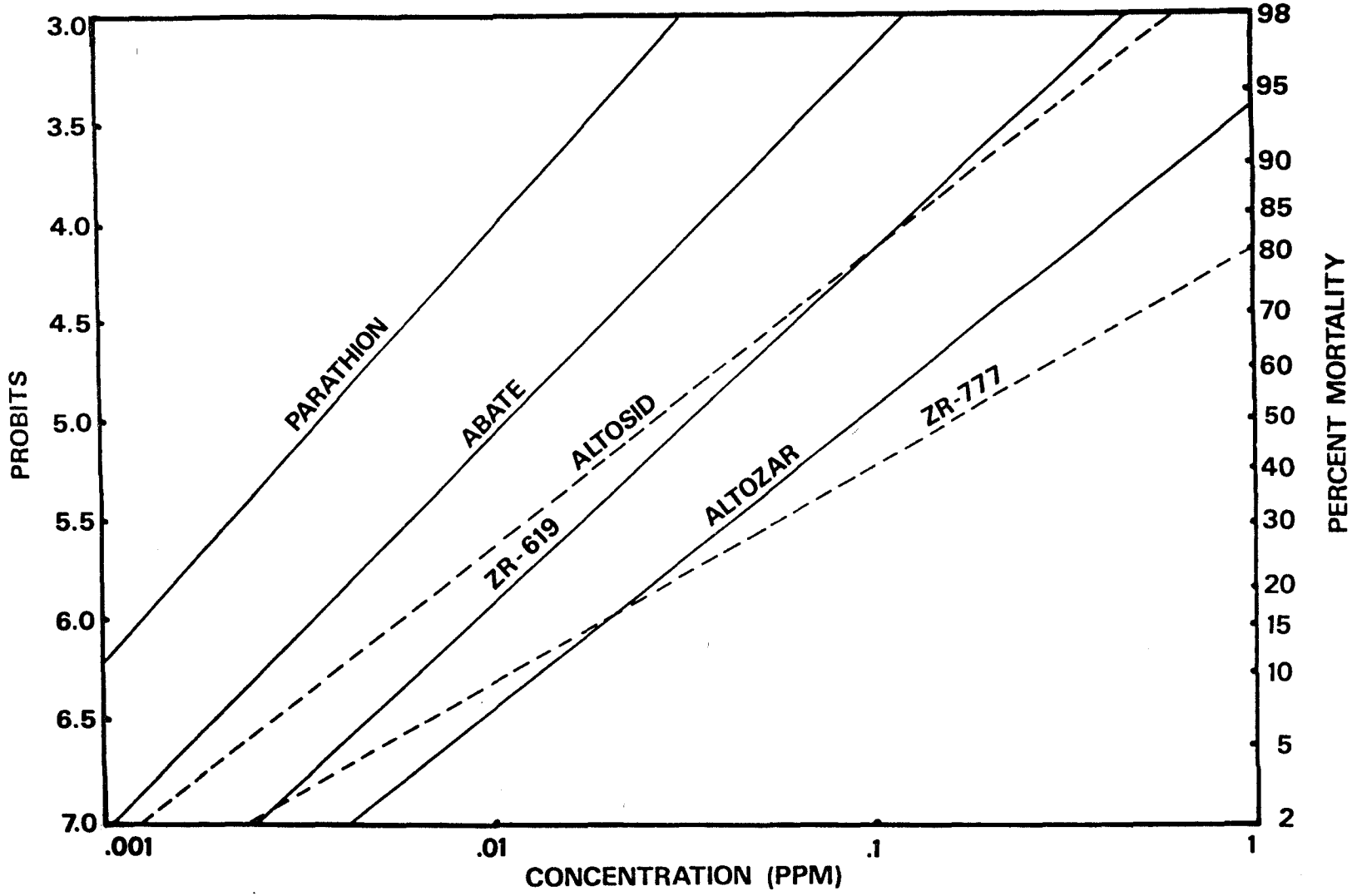


Figure 23. Log-Probit Mortality Curves Showing Comparison of Effects of Five Chemicals on Fourth-Instar Larvae

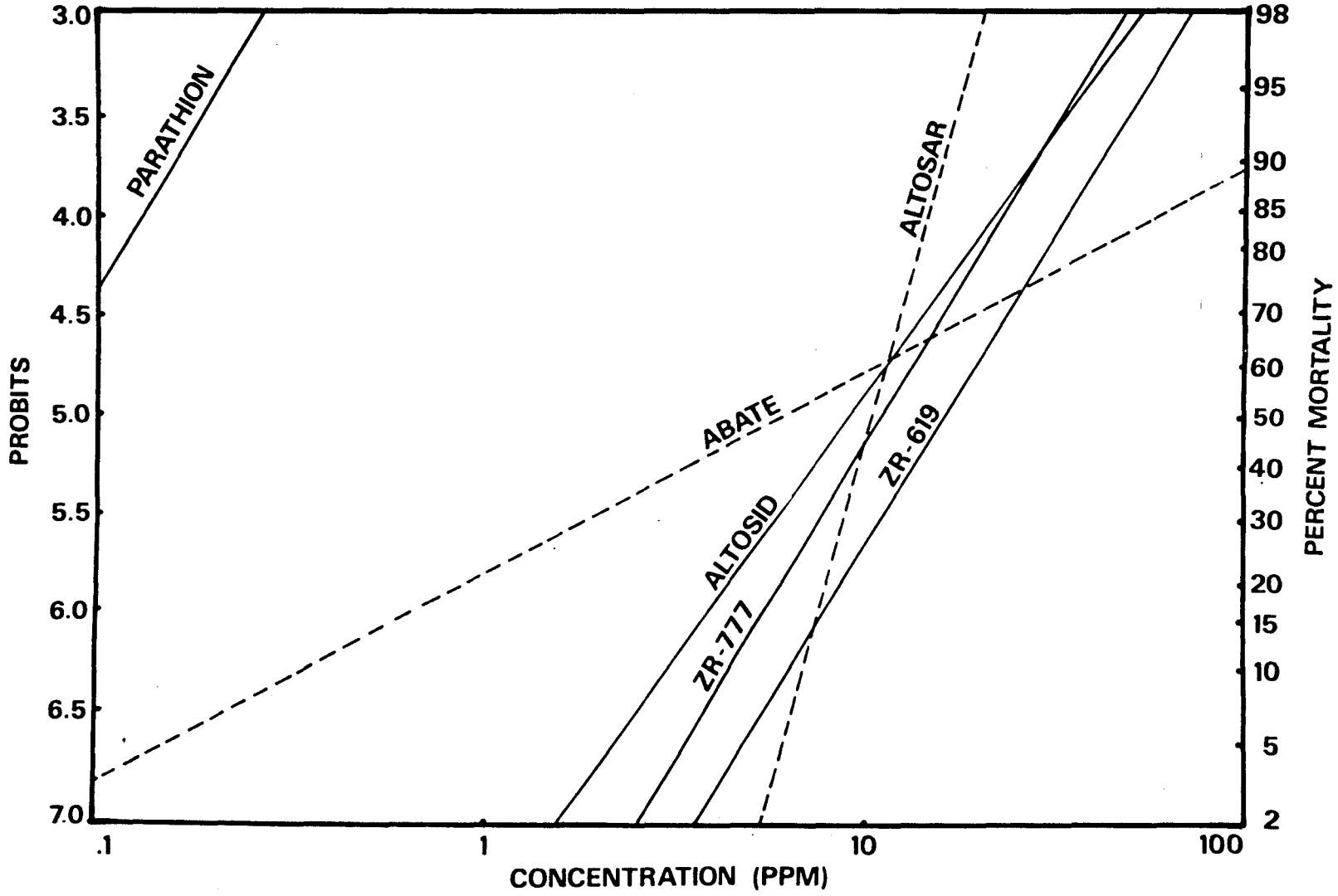


Figure 24. Log-Probit Mortality Curves Showing Comparison of Effects of Five Chemicals on Pupae

DISCUSSION AND CONCLUSION

The acetone and the coating on the containers were insignificant causes of mortality. The deaths in the bioassays resulted from the compounds tested, except for those which occurred in the controls of that particular bioassay. The compounds tested were ineffective against pupae of Aedes aegypti under normal-control spray concentrations. For this reason the discussion centers on larval mortalities.

Parathion (LC_{95} = 0.0205 ppm) and Abate (LC_{95} = 0.077 ppm), the chemicals used for mosquito control in Utah Valley, were more effective against Aedes aegypti under laboratory conditions than the four growth regulators tested. Altosid (LC_{95} = 0.34 ppm) and ZR-619 (LC_{95} = 0.28 ppm) caused a higher mortality than ZR-777 (LC_{95} = 5.1 ppm) and Altozar (LC_{95} = 1.15 ppm) against Aedes aegypti. Previous experiments with JH analogues have shown that Aedes aegypti is more resistant than other mosquito species to Altosid (Hsieh and Steelman 1974).

A number of analogues were previously tested on mosquito larvae, but except for Altosid, the analogues tested in this study have not been reported in the literature. Altosid has been tested against Aedes aegypti by Jakob and Schoof (1971; LC_{95} = 0.5 ppm) and Hsieh and Steelman (1974; LC_{50} = 0.1532 ppm, LC_{90} = 0.7799). In my experiments a lower concentration of Altosid was required. The reason for the higher concentration reported in the literature was possibly due to their use of third-instar larvae instead of fourth-instar

larvae. Juvenile hormone analogues, unlike conventional insecticides, are more effective against larvae of the fourth-instar stage just prior to pupation (Spielman and Williams 1966, Sacher 1971). Jakob (1972) reported that fourth-instar larval Aedes aegypti was 100 times more susceptible to Altosid than the third-instar.

The mosquitoes tested in this study showed resistance to Parathion (LC_{95} = 0.205 ppm, LC_{50} = 0.0034 ppm) and Abate (LC_{95} = 0.079 ppm, LC_{50} = 0.011 ppm). Aedes aegypti mortality values for Parathion and Abate are well established. Pass and Knapp (1966) rated Parathion mortality in 48 hours as LC_{50} = 0.0043 and LC_{90} = 0.0145 ppm, and Lofgren, Scanlon and Israngura (1967) gave Abate mortality in 48 hours as LC_{50} = 0.0026 ppm and LC_{90} = 0.0040 ppm. This resistance could effect the mortality data of the JH analogues. Cross-resistance for organophosphates and JH compounds have not been reported, but cross-resistance has been demonstrated between the analogues and chlorinated hydrocarbon insecticides. Scafer and Wilder (1972) showed that S-strains of Aedes nigromaculis are more susceptible to ZR-515, a juvenile hormone analogue, than are resistant strains. Anopheles gambiae demonstrates cross-resistance between DDT/dieldrin and ZR-515 (Kadri 1975).

Altosid SR-10, when applied at the maximum recommended concentration of 118.3 ml per acre (approximately .5 ppm), controlled Culiseta inornata for two weeks in the field. Hsieh and Steelman (1974) reported that 3rd-instar Culiseta inornata had the highest LC_{90} of the twelve species of mosquitoes tested in their laboratory. Consequently, under field conditions, Altosid SR-10 can control any species of mosquito so far tested in laboratory studies.

Although Parathion and Abate are as effective as Altosid against Culiseta inornata at lower concentrations and subsequently less cost (Mulla 1963, Dixon and Brust 1971), the advantage of growth regulators over organophosphates is that JH analogues are not poisons in the generic sense. Williams (1967) suggested that JH analogues cause mortality by upsetting the normal mechanism of insect metamorphosis.

Altosid, the first JH analogue to be tested by the Environmental Protection Agency, is safe to warm-blooded animals (Dickman 1973). Tests with white rats showed that the lethal dose required for 50% kill was greater than 34,600 ppm on a short-term basis. Dogs receiving 5,000 ppm in food in long-term tests of 90 days showed a slight enlargement of the liver. Pregnant mammals were fed Altosid without noticeable effects on their progeny. Birds fed Altosid exhibited no signs of eggshell thinning or behavioral change. Altosid passed through the gut of a cow without leaving residues in the milk (Dickman 1973).

Neither Parathion nor Abate is as selective as Altosid, but under correct field application neither of these organophosphate compounds build up concentrations great enough to poison warm-blooded animals. However, to measure the effects of a compound on vertebrates is not enough. Invertebrates are the major non-target organisms that come in contact with insecticides. Invertebrates occupy important niches in an ecosystem, and their elimination may cause undesirable environmental effects.

The sampling of aquatic invertebrates taken from plot 1 was not designed as a statistical experiment but as an observation to

note any obvious reduction in the non-target invertebrate population due to Altosid SR-10. No reduction was noted. Emergence from the cow hoofprints (plot 1) showed little effect on non-target organisms except Dolichopodidae (long-legged flies), a family in the Order Diptera. Chironomidae (midges, Diptera) showed little mortality in the test plots. Chironomidae have been controlled by this compound, but only when it has been applied by the 10% slow-release formulation (Mulla, Norland, Ikeshoji and Kramer 1974). The families Chironomidae, Ephydridae and Psycodidae in the Order Diptera have demonstrated sensitivity to JH compounds (Miura and Takahashi 1973). The selectivity of JH compounds is further reported by Steelman, Farlow, Breaud and Schilling (1975).

Most conventional organophosphates, including Parathion, show little selectivity when applied to invertebrates, but Abate is unique among organophosphates because it shows some selectivity. Copepods, ostracods, Hydrophilidae and Physa are not effected by Abate at 1.13 kg/0.405 hectare (2.5 lbs/acre), but cladocerans and the dipterans were eradicated (Didia, LaSalle and Liem 1975). Wingeguth and Patterson (1966) reported that under field conditions, application of 0.113 kg of Abate per 0.405 hectare (0.10 lb/acre) caused no mortality to Odonata, copepods, ostracods, Chaoborus and shrimp. However, Porter and Gajmirae (1967) claimed that at 0.0136 kg of Abate per 0.405 hectare (0.03 lb/acre), cladocera were eradicated along with a species of Trichoptera (Limnephilus indivisus). Toxic effects were also shown in the same paper for libellulid naiads.

Although the reports for Abate are somewhat contradictory, the compound does cause mortality to organisms other than dipterans. Juvenile hormone compounds show more selectivity than the presently used organophosphates. The JH analogues may prove to be more advantageous than organophosphates in the areas where significant environmental damage overshadows economic considerations.

SUMMARY

Parathion (LC_{95} = 0.0205 ppm) and Abate (LC_{95} = 0.077 ppm) showed greater control of Aedes aegypti under laboratory conditions than did the JH analogues tested. Altosid (LC_{95} = 0.34 ppm) and ZR-619 (LC_{95} = 0.28 ppm) had higher percentage kills than ZR-777 (LC_{95} = 5.1 ppm) and Altozar (LC_{95} = 1.15 ppm). The field studies indicate that Culiseta inornata in Utah Valley can be controlled by Altosid SR-10 with no visible effects on the non-target organisms, with the exception of certain dipterans. Members of the non-target family Dolichopodidae were virtually irradicated with field application of Altosid SR-10. Although Altosid requires a higher concentration and greater expense for control, it has the advantage of inflicting a minimum amount of damage to aquatic ecosystems.

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LITERATURE CITED

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APPENDIXES

APPENDIX A
PLANT IDENTIFICATION

Table 19

Plant Identification and Abundance
Within the Study Area

Genus and species	Common name	Area[1]	Abundance[2]
	Plot 1		
Lemna minor	Lesser Duckweed	S	1
Sagittaria cuneata	Arrowhead	E	2
Many species	Grass	E,A	1
Carex sp.	Sedge	E	4
Populus angustifolia	Narrowleaf Cottonwood	A,E	3
Rumex sp.	Dock	A,E	4
Polygonum amphibium	Knotweed	E	2
Chenopodium sp.	Goosefoot	A	1
Rorippa sp.	Water Cress	A	1
Solanum dulcamara	Nightshade	A	3
Xanthium strumarium	Cocklebur	A,E	4
Grindelia squarrosa	Gumplant	A	1
	Plot 2		
Lemna minor	Lesser Duckweed	S	1
Lemna trisulca	Greater Duckweed	S	3
Sagittaria cuneata	Arrowhead	E	4
Many species	Grass	E,A	1
Carex sp.	Sedge	E	4
Chenopodium sp.	Goosefoot	A	1
Xanthium strumarium	Cocklebur	A	3
Grindelia squarrosa	Gumplant	A	2
Helianthus anuus	Sunflower	A	3

[1]Area: A = edge of land near the water, E = emergent vegetation, S = surface of the water, U = under the water.

[2]Abundance: 1 = very common, 2 = common, 3 = few, 4 = rare.

APPENDIX B
INSECT IDENTIFICATION

Table 20

Aquatic Insect Adults and Insect Adults Emerging in the Study Area

Genus and species	Family	Order	Abundance
	Plot 1		
Ishnura sp.	Coenagrionidae	Odonata	Common
Libellula sp.	Libellulidae	Odonata	Common
Gerris sp.	Gerridae	Hemiptera	Common
Unknown	Corixidae	Hemiptera	Common
Microvelia sp.	Veliidae	Hemiptera	Very Common
Tropisternum sp.	Hydrophilidae	Coleoptera	Rare
Unknown	Dytiscidae	Coleoptera	Rare
Peltodytes callosus	Haliplidae	Coleoptera	Rare
Two species	Tipulidae	Diptera	Rare
Tetanocerinae (subfamily)	Sciomyzidae	Diptera	Rare
Dolichopus sp.	Dolichopodidae	Diptera	Common
Unknown	Chironomidae	Diptera	Very Common
Chrysops sp.	Tabanidae	Diptera	Few
Hybomitra sp.	Tabanidae	Diptera	Rare
Unknown	Ephydridae	Diptera	Common
Culex tarsalis	Culicidae	Diptera	Common
Anopheles freeborni	Culicidae	Diptera	Common
Culiseta inornata	Culicidae	Diptera	Common
	Plot 2		
Ishnura sp.	Coenagrionidae	Odonata	Common
Libellula sp.	Libellulidae	Odonata	Common
Gerris sp.	Gerridae	Hemiptera	Common
Unknown	Corixidae	Hemiptera	Common
Microvelia sp.	Veliidae	Hemiptera	Common
Lethocerus americanus	Belostomatidae	Hemiptera	Rare
Tropisternus sp.	Hydrophilidae	Coleoptera	Rare
Unknown	Dytiscidae	Coleoptera	Rare
Two species	Tipulidae	Diptera	Rare
Tetanocerinae (subfamily)	Sciomyzidae	Diptera	Rare
Dolichopus sp.	Dolichopodidae	Diptera	Common
Unknown	Chironomidae	Diptera	Common
Chrysops sp.	Tabanidae	Diptera	Common
Culex tarsalis	Culicidae	Diptera	Common
Anopheles freeborni	Culicidae	Diptera	Common
Culiseta inornata	Culicidae	Diptera	Common

Table 21

Aquatic Insect Immatures
in the Study Area

Genus and species	Family	Order	Abundance
	Plot 1		
Callibaetis sp.	Baetidae	Ephemeroptera	Common
Ishnura sp.	Coenagrionidae	Odonata	Common
Libellula sp.	Libellulidae	Odonata	Few
Gerris sp.	Gerridae	Hemiptera	Common
Unknown	Corixidae	Hemiptera	Very Common
Microvelia sp.	Veliidae	Hemiptera	Very Common
Tropisternus sp.	Hydrophilidae	Coleoptera	Few
Rhantus sp.	Dytiscidae	Coleoptera	Few
Unknown	Tipulidae	Diptera	Rare
Unknown	Sciomyzidae	Diptera	Rare
Unknown	Dolichopodidae	Diptera	Common
Unknown	Chironomidae	Diptera	Common
Culex tarsalis	Culicidae	Diptera	Common
Anopheles freeborni	Culicidae	Diptera	Common
Culiseta inornata	Culicidae	Diptera	Common
	Plot 2		
Callibaetis sp.	Baetidae	Ephemeroptera	Common
Ishnura sp.	Coenagrionidae	Odonata	Common
Libellula sp.	Libellulidae	Odonata	Few
Gerris sp.	Gerridae	Hemiptera	Common
Lethocerus americanus	Belostomatidae	Hemiptera	Rare
Microvelia sp.	Veliidae	Hemiptera	Common
Unknown	Corixidae	Hemiptera	Common
Tropisternus sp.	Hydrophilidae	Coleoptera	Few
Rhantus sp.	Dytiscidae	Coleoptera	Few
Eretes stricticus	Dytiscidae	Coleoptera	Rare
Unknown	Tipulidae	Diptera	Rare
Unknown	Sciomyzidae	Diptera	Rare
Unknown	Dolichopodidae	Diptera	Few
Unknown	Chironomidae	Diptera	Common
Culex tarsalis	Culicidae	Diptera	Common
Anopheles freeborni	Culicidae	Diptera	Few
Culiseta inornata	Culicidae	Diptera	Few

APPENDIX C
INDIVIDUAL BIOASSAYS

Table 22

Major Controls Testing the Effect of Cups and Acetone on *Aedes aegypti* Larval Mortality (Started May 2, 1975)

Cup number	Contents of cups	Number of larvae per cup	Number dead in 48 hours
1	Water	25	1
2	Water	25	0
3	Water	25	0
4	Water	25	2
5	Water	25	0
6	Water	25	0
7	Acetone 100 ppm	25	1
8	Acetone 100 ppm	25	1
9	Acetone 10 ppm	25	0
10	Acetone 10 ppm	25	0
11	Acetone 1 ppm	25	1
12	Acetone 1 ppm	25	0

Table 23

First Bioassay Using Altosid on Fourth-Instar Larvae (Started March 1, 1975)

Cup number	Number of larvae per cup	PPM	Date 3/7 AE[1]	3/7 DE[1]	3/10 AE	3/10 DE	3/12 AE	3/12 DE	3/14 AE	3/14 DE	Total AE	Total DE	D[2]
1	20	10.0	0	0	0	0	0	0	0	0	0	0	20
2	20	10.0	0	0	0	0	0	0	0	0	0	0	20
3	20	1.0	0	0	0	0	0	0	0	0	0	0	20
4	20	1.0	0	0	0	0	0	1	2	0	2	1	17
5	20	0.1	0	0	0	1	0	0	0	0	0	1	19
6	20	0.1	1	0	5	0	3	0	1	0	10	0	10
7	20	0.01	0	0	7	0	5	0	4	0	16	0	4
8	20	0.01	0	0	9	0	3	0	0	0	12	0	8
9	20	0.001	0	0	7	0	10	0	0	0	17	0	3
10	20	Control	0	0	5	0	9	0	3	0	17	0	3
11	20	Control	0	0	15	0	3	0	0	0	18	0	2

[1]AE = alive emergence--able to free itself from the pupal case; DE = died during emergence--unable to free itself from the pupal case.

[2]D = dead pupae and larvae--over 90% consisted of dead pupae.

Table 24

Second Bioassay Using Altosid on Fourth-Instar Larvae (Started April 10, 1975)

Cup number	Number of larvae per cup	PPM	Date 4/17 AE[1]	4/17 DE[1]	4/20 AE	4/20 DE	4/22 AE	4/22 DE	Total AE	Total DE	D[2]
1	20	1.0	0	0	0	0	2	0	2	0	18
2	20	0.5	0	0	0	0	2	0	2	0	18
3	20	0.1	0	0	3	0	0	0	3	0	17
4	20	0.1	0	0	0	0	2	0	2	0	18
5	20	0.05	0	0	3	0	2	0	5	0	15
6	20	0.05	0	0	0	0	6	0	6	0	14
7	20	0.01	3	0	3	0	6	0	12	0	8
8	20	Control	1	0	15	0	3	0	19	0	1

[1]AE = alive emergence--able to free itself from the pupal case; DE = died during emergence--unable to free itself from the pupal case.

[2]D = dead pupae and larvae--over 90% consisted of dead pupae.

Table 25

First Bioassay Using Altosid on Pupae
(Started July 12, 1974)

Cup number	Number of pupae per cup	PPM	Date 7/14 AE[1]	7/14 DE[1]	7/15 AE	7/15 DE	Total AE	Total DE	D[2]
1	25	100	0	21	0	1	0	22	3
2	25	100	0	13	0	3	0	16	9
3	25	10	9	7	1	8	10	15	0
4	25	10	9	7	0	8	9	15	1
5	25	1	11	1	3	9	14	10	1
6	25	1	9	0	3	13	12	13	0
7	25	Control	14	0	9	1	24	1	0

[1]AE = alive emergence--able to free itself from the pupal case; DE = died during emergence--unable to free itself from the pupal case.

[2]D = dead pupae.

Table 26

Second Bioassay Using Altosid on Pupae
(Started July 30, 1974)

Cup number	Number of pupae per cup	PPM	Date 7/31 AE[1]	7/31 DE[1]	8/1 AE	8/1 DE	8/2 AE	8/2 DE	Total AE	Total DE	D[2]
1	20	40	0	0	0	11	0	7	0	18	2
2	20	40	2	1	0	11	0	3	2	15	0
3	20	20	1	1	0	16	0	2	1	19	0
4	20	20	1	0	3	14	0	1	4	15	1
5	20	10	3	0	5	5	3	3	11	6	3
6	20	10	2	0	8	4	1	2	11	6	3
7	20	8	1	0	10	1	4	2	15	3	2
8	20	8	2	0	3	5	4	4	9	9	2
9	20	6	1	0	7	5	3	3	11	8	1
10	20	6	1	0	9	1	3	3	13	4	3
11	20	Control	0	0	19	1	0	0	19	1	0
12	20	Control	1	0	19	0	0	0	20	0	0

[1]AE = alive emergence--able to free itself from the pupal case; DE = died during emergence--unable to free itself from the pupal case.

[2]D = dead pupae.

Table 27

First Bioassay Using ZR-619 on Fourth-Instar Larvae (Started January 27, 1975)

Cup number	Number of larvae per cup	PPM	Date 2/3 AE[1]	2/3 DE[1]	2/6 AE	2/6 DE	2/10 AE	2/10 DE	2/12 AE	2/12 DE	2/14 AE	2/14 DE	Total AE	Total DE	D[2]
1	20	10.0	0	0	0	0	0	0	0	0	0	0	0	0	20
2	20	10.0	0	0	0	0	0	0	0	0	1	0	1	0	19
3	20	1.0	0	0	0	0	0	0	0	0	0	0	0	0	20
4	20	1.0	0	0	0	0	0	0	1	0	2	0	3	0	17
5	20	0.1	0	0	0	0	0	0	0	0	0	0	0	0	20
6	20	0.1	0	0	3	0	4	1	1	0	0	0	8	1	11
7	20	0.01	4	0	3	0	7	0	0	0	0	0	14	0	6
8	20	0.01	5	0	7	0	3	1	2	0	0	0	17	1	2
9	20	0.001	9	0	0	0	3	0	0	0	4	0	16	0	4
10	20	0.001	7	0	6	0	1	0	1	0	0	0	20	0	0
11	20	Control	5	0	4	2	2	0	5	0	2	0	18	2	0
12	20	Control	4	0	6	0	2	0	1	0	3	1	16	1	3

[1]AE = alive emergence--able to free itself from the pupal case; DE = died during emergence--unable to free itself from the pupal case.

[2]D = dead pupae and larvae--over 90% consisted of dead pupae.

Table 28

Second Bioassay Using ZR-619 on Fourth-Instar Larvae (Started March 1, 1975)

Cup number	Number of larvae per cup	PPM	Date 3/7 AE[1]	3/7 DE[1]	3/10 AE	3/10 DE	3/12 AE	3/12 DE	3/14 AE	3/14 DE	3/17 AE	3/17 DE	Total AE	Total DE	Total D[2]
1	20	1.0	0	0	0	0	0	0	0	0	0	0	0	0	20
2	20	1.0	0	0	0	0	0	0	0	0	0	0	0	0	20
3	20	0.5	0	0	0	0	0	0	0	0	0	0	0	0	20
4	20	0.5	0	0	0	0	0	0	0	0	0	0	0	0	20
5	20	0.1	0	0	0	0	3	0	0	0	0	0	3	0	17
6	20	0.1	0	0	0	0	2	0	0	0	0	0	2	0	18
7	20	0.05	0	0	3	0	1	0	1	0	0	0	5	0	15
8	20	0.05	0	0	1	0	3	0	4	0	1	0	9	0	10
9	20	0.01	0	0	7	0	1	0	1	0	1	0	10	0	10
10	20	0.01	0	0	8	3	1	0	5	0	0	0	14	3	3
11	20	Control	1	0	7	0	2	0	2	0	1	0	13	0	7
12	20	Control	1	0	10	0	1	0	5	0	0	0	17	0	3

[1]AE = alive emergence--able to free itself from the pupal case; DE = died during emergence--unable to free itself from the pupal case.

[2]D = dead pupae and larvae--over 90% consisted of dead pupae.

Table 29

First Bioassay Using ZR-619 on Pupae
(Started July 19, 1974)

Cup number	Number of Pupae per cup	PPM	Date 7/20 AE[1]	7/20 DE[1]	7/21 AE	7/21 DE	Total AE	Total DE	D[2]
1	25	100.0	0	4	0	21	0	25	0
2	25	100.0	0	6	0	15	0	21	4
3	25	10.0	18	3	3	0	21	3	1
4	25	10.0	13	3	3	6	16	9	0
5	25	1.0	24	1	0	0	24	1	0
6	25	1.0	25	0	0	0	25	0	0
7	25	0.1	25	0	0	0	25	0	0
8	25	0.1	24	1	0	0	24	1	0
9	25	Control	25	0	0	0	25	0	0
10	25	Control	25	0	0	0	25	0	0
11	25	Control	25	0	0	0	25	0	0

[1]AE = alive emergence--able to free itself from the pupal case; DE = died during emergence--unable to free itself from the pupal case.

[2]D = dead pupae.

Table 30

Second Bioassay Using ZR-619 on Pupae
(Started July 29, 1974)

Cup number	Number of pupae per cup	PPM	Date 7/30 AE[1]	7/30 DE[1]	7/31 AE	7/31 DE	8/1 AE	8/1 DE	Total AE	Total DE	D[2]
1	20	100	0	1	0	10	0	7	0	18	2
2	20	100	0	6	0	10	0	3	0	19	1
3	20	80	0	5	0	7	0	6	0	18	2
4	20	80	0	0	0	13	0	7	0	20	0
5	20	60	0	2	1	2	1	14	1	18	1
6	20	60	1	2	0	10	0	7	1	19	0
7	20	40	0	1	0	13	1	5	1	19	0
8	20	40	0	3	2	9	0	6	2	18	0
9	20	20	1	4	2	5	0	7	3	16	1
10	20	20	0	4	4	5	0	7	4	16	0
11	20	10	3	0	9	4	1	3	13	7	0
12	20	10	4	0	10	0	1	5	15	5	0
13	20	Control	4	0	6	3	7	0	17	3	0
14	20	Control	4	0	11	0	3	1	18	1	1

[1]AE = alive emergence--able to free itself from the pupal case; DE = died during emergence--unable to free itself from the pupal case.

[2]D = dead pupae.

Table 31

First Bioassay Using Altozar on Fourth-Instar Larvae (Started January 27, 1975)

Cup number	Number of larvae per cup	PPM	Date 2/3 AE[1]	2/3 DE[1]	2/6 AE	2/6 DE	2/10 AE	2/10 DE	2/12 AE	2/12 DE	2/14 AE	2/14 DE	Total AE	Total DE	Total D[2]
1	20	10.0	0	0	0	0	0	0	0	0	0	0	0	0	20
2	20	10.0	0	0	0	0	0	0	0	0	1	0	1	0	19
3	20	1.0	1	0	0	2	4	3	0	0	0	0	5	5	10
4	20	1.0	1	0	2	0	0	0	0	0	2	0	5	0	15
5	20	0.1	0	0	0	0	7	3	1	0	3	0	11	3	6
6	20	0.1	0	0	0	0	0	0	1	0	3	0	4	0	16
7	20	0.01	5	0	3	2	3	0	3	0	0	0	14	2	4
8	20	0.01	5	0	6	0	3	0	1	0	0	0	15	0	5
9	20	0.001	3	0	7	1	5	0	0	0	3	0	18	0	2
10	20	0.001	5	0	6	2	1	0	3	0	0	0	15	2	3
11	20	Control	2	0	3	0	5	1	1	0	3	0	14	1	5
12	20	Control	4	0	4	0	4	0	3	0	3	0	18	0	2

[1]AE = alive emergence--able to free itself from the pupal case; DE = died during emergence--unable to free itself from the pupal case.

[2]D = dead pupae and larvae--over 90% consisted of dead pupae.

Table 32

Second Bioassay Using Altozar on Fourth-Instar Larvae (Started March 1, 1975)

Cup number	Number of larvae per cup	PPM	Date 3/7 AE[1]	3/7 DE[1]	3/10 AE	3/10 DE	3/12 AE	3/12 DE	3/14 AE	3/14 DE	Total AE	Total DE	D[2]
1	20	1.0	0	0	0	0	1	0	1	0	2	0	18
2	20	1.0	0	0	0	0	0	1	0	0	0	1	19
3	20	0.5	0	0	1	1	0	0	2	0	3	1	16
4	20	0.5	0	0	2	0	0	0	1	0	3	0	17
5	20	0.1	0	0	1	0	1	0	3	0	5	0	15
6	20	0.1	0	0	3	1	0	0	0	0	3	1	16
7	20	0.05	3	0	4	0	3	0	3	0	12	0	8
8	20	0.05	1	1	9	0	3	0	3	0	16	1	3
9	20	0.01	0	0	10	0	6	0	0	0	16	0	4
10	20	0.01	0	0	9	0	6	0	0	0	15	0	5
11	20	Control	1	0	10	0	3	0	5	0	19	0	1
12	20	Control	2	0	8	0	3	0	3	0	16	0	4

[1]AE = alive emergence--able to free itself from the pupal case; DE = died during emergence--unable to free itself from the pupal case.

[2]D = dead pupae and larvae--over 90% consisted of dead pupae.

Table 33

First Bioassay Using Altozar on Pupae
(Started January 26, 1974)

Cup number	Number of pupae per cup	PPM	Date 1/28 AE[1]	1/28 DE[1]	1/29 AE	1/29 DE	Total AE	Total DE	D[2]
1	25	100	0	9	0	1	0	10	15
2	25	100	0	9	0	1	0	10	15
3	25	10	12	2	3	5	15	7	3
4	25	Control	12	0	13	0	25	0	0

[1]AE = alive emergence--able to free itself from the pupal case; DE = died during emergence--unable to free itself from the pupal case.

[2]D = dead pupae.

Table 34

Second Bioassay Using Altozar on Pupae
(Started July 31, 1974)

Cup number	Number of pupae per cup	PPM	Date 8/1 AE[1]	8/1 DE[1]	8/2 AE	8/2 DE	8/3 AE	8/3 DE	Total AE	Total DE	D[2]
1	20	40	0	0	0	3	0	0	0	3	17
2	20	40	0	0	0	9	0	0	0	9	11
3	20	20	0	2	0	10	0	2	0	14	6
4	20	20	0	1	1	11	0	1	1	13	6
5	20	10	2	0	7	0	0	4	9	4	7
6	20	10	2	0	8	4	2	0	12	4	4
7	20	8	16	3	0	1	0	0	16	4	0
8	20	8	0	0	11	5	1	2	12	7	1
9	20	6	14	3	3	0	0	0	17	3	0
10	20	6	19	1	0	0	0	0	19	1	0
11	20	Control	13	0	5	1	0	0	18	1	1
12	20	Control	16	0	4	0	0	0	20	0	0

[1]AE = alive emergence--able to free itself from the pupal case; DE = died during emergence--unable to free itself from the pupal case.

[2]D = dead pupae.

Table 35

First Bioassay Using ZR-777 on Fourth-Instar Larvae (Started January 27, 1975)

Cup number	Number of larvae per cup	PPM	Date 2/3 AE[1]	2/3 DE[1]	2/6 AE	2/6 DE	2/10 AE	2/10 DE	2/12 AE	2/12 DE	2/14 AE	2/14 DE	Total AE	Total DE	Total D[2]
1	20	10.0	0	0	0	0	0	0	0	0	0	0	0	0	20
2	20	10.0	0	0	0	0	0	0	0	0	0	0	0	0	20
3	20	1.0	0	1	0	0	0	0	0	0	0	0	0	1	19
4	20	1.0	0	0	1	0	2	4	0	0	0	0	3	4	13
5	20	0.1	0	0	0	0	0	0	3	2	3	2	6	4	10
6	20	0.1	0	0	3	0	3	0	2	0	1	0	10	0	10
7	20	0.01	2	0	5	2	6	0	1	0	2	0	16	2	2
8	20	0.01	7	0	5	0	5	0	0	0	1	0	16	0	4
9	20	0.001	3	1	5	0	5	0	1	0	4	0	18	1	1
10	20	0.001	3	0	6	1	1	0	3	0	0	0	13	1	6
11	20	Control	5	0	6	0	2	0	2	0	1	0	16	0	4
12	20	Control	2	0	4	0	8	1	0	1	4	0	18	2	0

[1]AE = alive emergence--able to free itself from the pupal case; DE = died during emergence--unable to free itself from the pupal case.

[2]D = dead pupae and larvae--over 90% consisted of dead pupae.

Table 36

Second Bioassay Using ZR-777 on Fourth-Instar Larvae (Started February 28, 1975)

Cup number	Number of larvae per cup	PPM	Date 3/5 AE[1]	3/5 DE[1]	3/7 AE	3/7 DE	3/10 AE	3/10 DE	3/12 AE	3/12 DE	3/14 AE	3/14 DE	Total AE	Total DE	Total D[2]
1	20	1.0	0	0	4	0	0	0	0	0	0	0	4	0	16
2	20	1.0	1	0	3	0	0	0	0	0	0	0	4	0	16
3	20	0.5	1	1	1	2	0	0	1	0	0	0	3	3	14
4	20	0.5	0	0	3	0	0	0	0	0	0	0	3	0	17
5	20	0.1	1	0	–	–	–	–	–	–	–	–	(cup spilled)		
6	20	0.1	2	0	3	3	4	0	1	0	2	0	12	3	5
7	20	0.05	0	0	0	0	13	0	0	0	1	0	14	0	6
8	20	0.05	0	0	3	0	11	0	3	0	2	0	19	0	1
9	20	0.01	0	0	3	0	10	0	0	0	2	0	15	0	5
10	20	0.01	1	0	8	0	7	0	4	0	0	0	20	0	0
11	20	Control	6	0	3	0	10	0	0	0	0	0	19	0	1
12	20	Control	1	0	1	0	18	0	0	0	0	0	20	0	0

[1]AE = alive emergence—able to free itself from the pupal case; DE = died during emergence—unable to free itself from the pupal case.

[2]D = dead pupae and larvae--over 90% consisted of dead pupae.

Table 37

First Bioassay Using ZR-777 on Pupae
(Started July 20, 1974)

Cup number	Number of pupae per cup	PPM	Date 7/21 AE[1]	7/21 DE[1]	7/22 AE	7/22 DE	Total AE	Total DE	D[2]
1	20	100	0	2	0	15	0	17	3
2	20	80	0	1	0	19	0	20	0
3	20	60	0	2	0	18	0	20	0
4	20	40	0	3	1	15	1	18	1
5	20	20	2	5	1	11	3	16	1
6	20	10	3	0	4	12	7	12	1
7	20	100	0	3	0	17	0	20	0
8	20	80	0	5	0	15	0	20	0
9	20	60	1	19	0	0	1	19	0
10	20	40	0	7	0	13	0	20	0
11	20	20	2	15	0	3	2	18	0
12	20	10	16	4	0	0	16	4	0
13	20	Control	20	0	0	0	20	0	0
14	20	Control	19	1	0	0	19	1	0

[1]AE = alive emergence--able to free itself from the pupal case; DE = died during emergence--unable to free itself from the pupal case.

[2]D = dead pupae.

Table 38

Second Bioassay Using ZR-777 on Pupae
(Started November 15, 1974)

Cup number	Number of pupae per cup	PPM	Date 11/16 AE[1]	11/16 DE[1]	11/18 AE	11/18 DE	11/19 AE	11/19 DE	Total AE	Total DE	D[2]
1	20	8	2	1	9	0	6	1	17	2	1
2	20	8	0	0	5	2	7	6	12	8	0
3	20	6	0	0	4	1	14	1	18	2	0
4	20	6	1	0	1	2	12	4	14	6	0
5	20	4	0	1	12	1	3	3	15	5	0
6	20	4	0	0	9	0	10	0	19	0	1
7	20	2	3	0	5	4	6	2	14	6	0
8	20	2	0	0	5	0	13	2	18	2	0
9	20	Control	0	0	6	0	13	1	19	1	0
10	20	Control	1	0	5	0	12	2	18	2	0

[1]AE = alive emergence--able to free itself from the pupal case; DE = died during emergence--unable to free itself from the pupal case.

[2]D = dead pupae.

Table 39

Third Bioassay Using ZR-777 on Pupae
(Started November 19, 1974)

Cup number	Number of pupae per cup	PPM	Date 11/20 AE[1]	11/20 DE[1]	11/21 AE	11/21 DE	Total AE	Total DE	D[2]
1	20	10	11	8	0	0	11	8	1
2	20	10	10	9	1	0	11	9	0

[1]AE = alive emergence--able to free itself from the pupal case; DE = died during emergence--unable to free itself from the pupal case.

[2]D = dead pupae.

Table 40

Bioassay Using Parathion on Fourth-Instar Larvae (Started January 20, 1975)

Cup number	Number of larvae per cup	PPM	Number dead after 24 hours	Number dead after 48 hours
1	20	0.01	5	20
2	20	0.01	2	16
3	20	0.005	0	7
4	20	0.005	0	10
5	20	0.001	0	4
6	20	0.001	1	4
7	20	0.0005	0	0
8	20	0.0005	1	3
9	20	0.0001	0	0
10	20	0.0001	0	0
11	20	Control	0	0
12	20	Control	1	1

Table 41

Bioassay Using Parathion on Pupae
(Started May 20, 1974)

Cup number	Number of pupae per cup	PPM	Number dead after 24 hours	Number dead after 48 hours
1	25	100.0	25	25
2	25	100.0	25	25
3	25	10.0	25	25
4	25	10.0	25	25
5	25	1.0	23	25
6	25	1.0	24	25
7	25	0.1	3	21
8	25	0.1	1	17
9	25	0.01	0	1
10	25	0.01	0	0
11	25	Control	0	0
12	25	Control	0	0

Table 42

Bioassay Using Abate on Fourth-Instar Larvae (Started January 20, 1975)

Cup number	Number of larvae per cup	PPM	Number dead after 24 hours	Number dead after 48 hours
1	20	0.1	18	20
2	20	0.1	19	19
3	20	0.08	18	18
4	20	0.08	17	17
5	20	0.05	17	19
6	20	0.05	17	17
7	20	0.02	17	17
8	20	0.02	12	13
9	20	0.01	9	12
10	20	0.01	5	11
11	20	Control	0	1
12	20	Control	1	1

Table 43

Bioassay Using Abate on Pupae
(Started May 7, 1974)

Cup number	Number of pupae per cup	PPM	Number dead after 24 hours	Number dead after 48 hours
1	25	100	21	21
2	25	100	16	25
3	25	10	1	11
4	25	10	4	11
5	25	1	1	7
6	25	1	3	7
7	25	Control	0	1

VITA

Richard L. Orr

THE COMPARATIVE TOXICITY OF DEVELOPMENTAL INHIBITORS AND ORGANOPHOSPHATES ON MOSQUITOES

Richard L. Orr

Department of Zoology

M.S. Degree, April 1976

ABSTRACT

Four developmental inhibitors (Altosid, Altozar, ZR-619 and ZR-777) and two organophosphates (Parathion and Abate) were tested on Aedes aegypti under laboratory conditions. When applied to fourth-instar larvae, the organophosphates were more toxic than the developmental inhibitors. Concentrations required for standard kills (LC_{50} and LC_{95}) were lower for Altosid and ZR-619 than for Altozar and ZR-777.

Field applications of Altosid SR-10 controlled Culiseta inornata for two weeks at 4 ounces per acre. With the exception of Dolichopodidae, most non-target aquatic organisms showed little response to Altosid SR-10.

Although the developmental inhibitors Altosid and ZR-619 require higher concentrations for mosquito control, they inflict a minimum amount of damage to non-target aquatic organisms.

COMMITTEE APPROVAL: