A COMPARATIVE STUDY OF THE TOXICITY OF ALDRIN, DIELDRIN AND DDT BY INJECTION INTO THE LARGE MILKWEED BUG, ONCOPELTUS FASCIATUS (DALL.)

by

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B.S., Trinity University, San Antonio, Texas, 1948

A THESIS

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

Department of Entomology

KANSAS STATE COLLEGE
OF AGRICULTURE AND APPLIED SCIENCE

1950

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INTRODUCTION

In the search for newer and better insecticides man has always asked the questions, "Would this compound be useful as an insecticide", and "How does this compound compare with other compounds now used as insect toxicants?" This problem deals with the second question, in that it attempts to compare the toxicity of three chemicals by their respective dosage-mortality curves.

Dr. Paul A. Dahm suggested in February, 1949, that the insecticides be injected into the coelomic cavity of the test insects. The writer acting upon that suggestion built a micro-injection apparatus, and the calibration and preliminary testing were started in March, 1949. One thousand insects were injected for the calibration of the injection apparatus, solvent toxicity studies, and preliminary dosage level mortality tests.

The three chemicals chosen for comparison were aldrin (recrystallized), dieldrin (recrystallized), and DDT. From the preliminary testing it was determined that six concentrations of each material would be enough to establish a dosage-mortality curve.

This study consisted of conducting three replications with each chemical. Each replication consisted of nineteen hundred insects. One hundred were injected with six concentrations of each of the three chemicals used, and one hundred insects were injected at the same time with the solvent only and were used as controls.

Literature Review

The injection procedure used in this problem was not a new one. Such techniques have been used by toxicologists and physiologists since the turn of the century. An excellent review of the literature covering the use of injection techniques is included in the paper by Heal and Menusan (1948). Other papers not included in the literature review by Heal and Menusan (1948) that are of particular interest in connection with this problem are: Beard (1949) who used the injection technique while studying the relation of species-specificity to toxicants and routes of administration, Beard (1949) who again used the injection technique in studies of time of evaluation and dosage-response curves, and Kearns et. al. (1949) who tested 10 species of insects with some of the new chlorinated organic insecticides using the injection technique in some cases.

Method of Application

The technique of injection has been used in some way in the work done by the authors listed in the literature review. The work of various authors indicates that generally the injection method administers the insecticides by the most effective route. Beard (1949, P. 298) states, "in 12 out of 20 possibilities, parenteral administration was more effective than other routes". Beard (1949, P. 297) also states that "the susceptibility of an insect to a compound when administered by the most effective route should serve as an index to the inherent toxicity of that compound". Assuming that variations in response are due not to differences in inherent toxicity, but to the relative ease with which the toxicants reaches the site of action.

Purpose

This investigation had as its purpose the accumulation of data that could be used in the development of dosage-mortality curves for aldrin, dieldrin and DDT.

Scope

The problem was limited by many factors; time, rearing facilities, and apparatus were the three main limiting factors. The time factor and the rearing facilities limited the number of replications. The injection apparatus presented factors that made it more desirable to use fewer insecticides with more individuals tested for each chemical than to use more chemicals with fewer individuals tested for each chemical.

The injection apparatus had mechanical limitations. The graduations of the machine were in 0.00008 ml, therefore no smaller volume than that could be delivered. Twenty-five graduations would dispense 0.002 ml, which was found to be the correct amount for injection into a one hundred milligram milkweed bug. Twenty-four graduations would dispense 0.00192 ml, the correct amount for a ninety-six milligram insect. Thus, the difference of one graduation on the injection apparatus delivered the calculated dosage for an insect that weighed four milligrams less than the one hundred milligram insect; therefore, the machine was capable of delivering dosages only to the nearest four milligrams of insect body weight. This fact was known at the outset of the investigation, and was recognized as a source of error. However, large numbers of insects tended to equalize this error.

The fluctuation in vigor of the test insect was a source of error that was reduced to a minimum by having the rearing procedure, environmental conditions and competition as uniform as possible for all groups of insects reared.

MATERIALS AND METHODS

Test Insect, The Large Milkweed Bug

The test insect used in this experiment was the large milkweed bug, Oncopeltus faciatus (Dall.). Only the female adults were used, the males were discarded after the insects were segregated according to sex (Ciocco, 1940). Insects used were 9 to 11 days old. The large milkweed bug was a desirable insect for this work because it was a convenient size, was easily reared, and it could be manipulated without anesthetization.

Chemicals and Solvent

The three chemicals used were aldrin (recrystallized), dieldrin (recrystallized), and DDT Lidov et al. (1950). "Aldrin (recrystallized), is a coined name for the insecticidal chemical containing not less than 99 per cent of the compound having the molecular formula $C_{12}H_8Cl_6$ and the structural formula corresponding to 1,2,3,4,10,10-hexachloro-1,4,4a,5,8,8a-hexahydro-1,4,5,8-dimethanonaphthalene. This material is a white crystalline solid having a melting point of 100-102°C.; it is very soluble in most organic solvents and is insoluble in water. It is stable in the presence of organic and inorganic alkalies and stable to the action of hydrated metallic chlorides" (Rohwer, 1949). "Dieldrin (recrystallized), is a coined name for the insecticidal chemical containing not less than 99 per cent of the compound having the molecular formula $C_{12}H_8Cl_6O$ and the structural formula corresponding to 1,2,3,4,10,10-hexachloro-6.7-epoxy-1,4,4a,5,6,7,8,8a-octahydro-1,4,5,8-dimethanonaphthalene. This material is a white crystalline solid having a melting point of 172-175° C.; is moderately soluble in the usual organic solvents except the aliphatic petroleum solvents and methanol in which it is sparingly soluble. It is insoluble in water. It is stable in the presence of organic and inorganic alkalies, and it reacts with strong mineral acids" (Rohwer, 1949a). The aldrin and dieldrin used in this study were supplied by Julius Hyman and Company, Denver, Colorado, and were labelled as compound 118, Sample #1337, and compound 497, Sample #1338, respectively.

The DDT used was the p,p' isomer of dichloro-diphenyl-trichloroethane, and it was supplied by the Geigy Company Inc., 89-91 Barclay Street, New York, New York.

All the chemicals used in this problem were assumed to be 100 per cent pure. The chemicals all dissolved readily in acetone; therefore acetone was the only solvent used and it constituted the liquid phase of the injection solution.

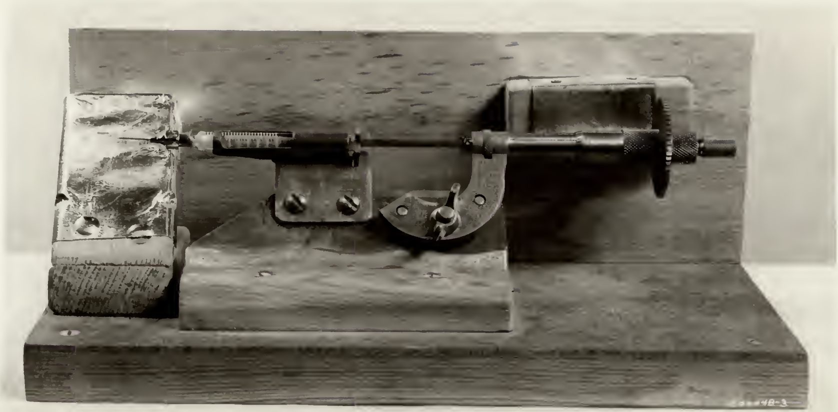
Apparatus

The apparatus used for the injecting was essentially a 0.25 cc tuberculin syringe, the plunger of which was activated by a micrometer screw. Plate I is a photograph of the injection apparatus. These component parts were supported by an iron frame mounted on a wooden base. The apparatus was fabricated by the writer. By removing the wing nut and the micrometer, access may be had to the syringe for removal, filling or cleaning. The

EXPLANATION OF PLATE I

The micro-injection apparatus used in this study.

PLATE I



syringe was held in place by a biramous iron key that fit in two rectangular slots in the frame, and was held rigid by a fixed iron pin that fit through a hole in the key.

The piece of metal behind the micrometer scale was a piece of thin sheet iron that engaged the rachet wheel and served as a pawl. The noise made by the sheet iron passing over the rachet wheel perimeter enabled the operator to count the number of clicks that represented certain dosages. Table 1 shows insect weights, number of clicks and dosage volumes dispensed. The graduations on the syringe and the micrometer scale were used only for calibrating the apparatus.

The other pieces of apparatus used were; laboratory glassware, balances, rearing and observation cages, and holding cages that permitted the operator to catch the insects with ease as the injection work was being done.

Rearing

The insect rearing room temperature was maintained between 74° and 84° F. with the relative humidity 50 per cent plus or minus five per cent. The insects were exposed to constant and equal amounts of incandescent light.

The insects were reared in crockery bowls 12 inches in diameter shown in Plate II. Four of the bowls were used for stock cultures, and from these bowls about 500 to 800 eggs were obtained every three days. The eggs were transferred from the stock culture bowls to bowls eight inches in diameter. Milkweed seeds and water absorbed in cellucotton provided food and moisture for the nymphs when they emerged. The nymphs, when 15 to 18 days

Table 1. Weight of the insect in milligrams, the dosage volume in milliliters, and the number of clicks on the apparatus to deliver the dosage desired.[1]

Weight of insect in milligrams	Volume of dosage in milliliters	Number of clicks on apparatus
35-38	0.00072	9
39-42	0.0008	10
43-46	0.00088	11
47-50	0.00096	12
51-54	0.00104	13
55-58	0.00112	14
59-62	0.0012	15
63-66	0.00128	16
67-70	0.00136	17
71-74	0.00144	18
75-78	0.00152	19
79-82	0.0016	20
83-86	0.00168	21
87-90	0.00176	22
91-94	0.00184	23
95-98	0.00192	24
99-102	0.002	25

1 One click on the apparatus delivers 0.00008 ml.

EXPLANATION OF PLATE II

Rearing cage showing screen wire cover, crockery bowl, with the watering device and some milkweed seeds shown on the right.

PLATE II

old, were transferred to 12 inch bowls. This transfer reduced the crowding and therefore reduced the competition for food and water. All the rearing cages were covered with screen wire covers to prevent the escape of the insects.

Insect Manipulation, Sexing and Counting

The sexing operation consisted of catching an adult milkweed bug and observing whether it was a male or female. The females were usually larger than the males, and the third abdominal sternite was pointed on the posterior margin. The female genitalia was enclosed within two smooth convex genital plates that met along the mid-line of the body. The caudal end of the female abdomen was shaped like the prow of a boat. The male had abdominal sternites with parallel edges, and the genital plates were cylindrical. The caudal end of the body was blunt. The females were placed in weighed flasks; 100 insects per flask. The counting was combined with the sexing to reduce the number of times the insects were handled.

Weighing

At the beginning of the problem some samples of insects were weighed in groups of 10, and the average weight determined. The insects of the sample were then weighed individually and the fluctuation of the individual weights from the average weight was found to be less than four milligrams. It was thought that the average weight would be accurate enough for the experiment.

The insects were weighed in tared 125 ml Erlenmeyer flasks with 100 insects per flask. After the weights were recorded, the groups were identified with numbers and placed in larger containers, each group being kept separate.

The top inside edges of all the containers used to contain insects had a thin film of mineral oil applied to them to prevent the escape of the insects. Although the adult milkweed bugs can fly, they only do so when agitated, but they will crawl away from the cage without provocation if given the opportunity.

Solution Preparation

The insecticide stock solutions were made up in acetone as five per cent by weight solutions. The dosage dilutions were made from these stock solutions. Acetone was used for the solvent for both the stock and dosage solutions, and pure acetone containing no toxicant was used as the check solution.

Dosage Volume Calculation

The dosage volume was based on the tolerances of the insect to certain volumes of the solvent. In the preliminary work it was found that the large milkweed bug would tolerate 0.002 ml of acetone per 0.1 gram of insect body weight. Heal (1948) found the American cockroach to tolerate from 0.002 ml to 0.004 ml per 0.1 gram of insect body weight, and these figures were used as a basis for the volumes tested for tolerance in the milkweed bug study.

Table 1 was prepared to give the dosage volume and the number of clicks on the rachet of the injection apparatus necessary to dispense the proper volume for any weight insect between 35 and 102 milligrams. Having these calculations already worked out it was only necessary to weigh the insects, calculate the average weight, then look up the dosage volume and the number of clicks on the table. The use of the table eliminated having to make calculations for each group of insects, and therefore reduced the amount of possible error that could have been made in these calculations, as well as saving time for the operator.

Injecting

The technique of injecting was not a difficult one; the insect was caught, placed on the inclined rest covered with aluminum foil, slid onto the needle, the dosage administered by turning the rachet wheel, the insect withdrawn from the needle and placed in an observation cage.

The insect rest could be adjusted so that the needle would be inserted in the insect body in approximately the same manner for each individual. It was found that the needle pierced the membrane between the third and fourth sternites easily and when inserted between these sternites, parallel to the longitudinal axis of the body, and a little to the left of the mid-line of the body the best results were obtained. Insects injected in such a manner tended to lose very little body fluid or insecticidal solution through the wound created by the needle insertion. During the injection process the insect was held between the thumb and forefinger of the left hand, with the ventral side of the insect up. The apparatus was operated with the right hand.

When the syringe was filled with the injection solution care was taken to have all the air that might be in the syringe expelled. The possibility of injecting air into an insect, and the inconsistency of dosage volume pressure due to the compression of the air were thus eliminated.

The needle was checked for obstructions during the injection procedure after about each five insects were injected. The needle was cleaned when necessary. The joint formed by the needle and the tip of the syringe that received the needle was watched carefully. The appearance of any of the injection solution at that joint indicated that the needle was partially or wholly clogged. The needles were cleaned with the fine wires that were supplied with them. The syringe and the needles were flushed with acetone before and after each use. The same syringe that was used for the calibration of the apparatus was also used for all the injecting done throughout the problem.

Observation

The insects were placed in wide-mouthed pint jars after injection. These jars were covered with gauze held in place by the metal screw band of the jar. Milkweed seeds, and water absorbed in cellucotton wrapped in aluminum foil were placed in each jar. See Plate III. Twenty insects were placed in each observation jar and held for a two day observation period. The observations were made at the end of 24 and 48 hours after injection, and the dead and moribund were counted and recorded. Since the insects were injected in groups of 100 each insect constituted one per cent, therefore the mortality count was numerically equal to the percentage mortality.

The gauze covering and all the contents of the observation jars were discarded after the 48 hour observation. The jars were washed in a synthetic detergent and water, rinsed in water and rinsed in acetone before reuse. These steps tended to eliminate possible contamination of insects later placed in the jars.

EXPLANATION OF PLATE III

Observation cage showing gauze cover. Watering device and milkweed seeds illustrated both inside and to the right of the jar.

PLATE III

RESULTS

The data obtained from the 5700 insects injected for the three replications were used to plot dosage-mortality curves for the three chemicals used. The amount of each chemical used and the percentage mortality for each dosage level for each replication may be found in Tables 2, 3 and 4. The mortality of the three replications was averaged and appears in Table 5.

The regression lines were derived from the average mortality of the three replications for each chemical. The regression equations, Table 6, were calculated, and the regression lines plotted by the Statistical Laboratory of the Kansas Agricultural Experiment Station, Manhattan. The regression lines for each chemical may be found in Figs. 1, 2 and 3. The regression lines for 24 hour mortality for each chemical are given in Fig. 4, and the regression lines for 48 hour mortality are given in Fig. 5.

Discussion of Results

The calculated LD_{50} and LD_{95} dosages for each chemical at 24 and 48 hours provided figures by which the toxic action of the three insecticides could be compared. These figures are given in Table 7. The limits based on the first deviation were determined graphically, and according to Dr. H. C. Fryer, experiment station statistician, the calculated LD_{50} and LD_{95} will fall within the limits two out of three times.

The comparison of the three insecticides was done in this manner. DDT was assigned a toxicity value of one, and the toxicities of the other two chemicals would be in the ratios given in Table 8. These comparisons were made using the calculated LD_{50} and LD_{95} values at 24 and 48 hours, therefore the comparisons were

Table 2. Dosage level, percentage mortality for each chemical and the check at 24 and 48 hours after injection for the first replication. One hundred insects were used for each mortality percentage-dosage level combination.

Micrograms of toxicant per gram of insect.	Percentage mortality							
	Aldrin		Dieldrin		DDT		Check	
	24 hr.	48 hr.	24 hr.	48 hr.	24 hr.	48 hr.	24 hr.	48 hr.
	per cent							
78					92	97		
62.4					68	79		
46.8	95	97	86	86	58	85		
31.2	90	91	98	100	38	56		
15.6	49	54	83	91	9	26		
11.7			68	70				
7.8	61	68	24	28				
1.56	6	12	4	8	10	14		
0.156	6	9						
0.00							3	5

Table 3. Dosage level, percentage mortality for each chemical and the check at 24 and 48 hours after injection for the second replication. One hundred insects were used for each mortality percentage-dosage level combination.

Micrograms of toxicant per gram of insect.	Percentage mortality							
	Aldrin		Dieldrin		DDT		Check	
	24 hr.	48 hr.	24 hr.	48 hr.	24 hr.	48 hr.	24 hr.	48 hr.
				per cent				
78					75	79		
62.4					28	64		
46.8	95	97	89	96	49	70		
31.2	79	88	94	99	37	68		
15.6	93	96	84	93	8	17		
11.7			80	91				
7.8	50	77	58	70				
1.56	28	40	13	37	11	29		
0.156	0	3						
0.00							3	26

Table 4. Dosage level, percentage mortality for each chemical and the check at 24 and 48 hours after injection for the third replication. One hundred insects were used for each mortality percentage-dosage level combination.

Micrograms of toxicant per gram of insect.	Percentage mortality							
	Aldrin		Dieldrin		DDT		Check	
	24 hr.	48 hr.	24 hr.	48 hr.	24 hr.	48 hr.	24 hr.	48 hr.
	per cent							
78					82	87		
62.4					68	77		
46.8	97	100	100	100	61	76		
31.2	90	97	66	85	74	87		
15.6	86	93	61	86	39	68		
11.7			70	84				
7.8	58	73	60	74				
1.56	12	36	21	35	14	30		
0.156	7	15						
							3	11

Table 5. Dosage level, average percentage mortality for each chemical and the check at 24 and 48 hours after injection for three replications.

Micrograms of toxicant per gram of insect.	Percentage mortality							
	Aldrin		Dieldrin		DDT		Check	
	24 hr.	48 hr.	24 hr.	48 hr.	24 hr.	48 hr.	24 hr.	48 hr.
	per cent							
78					83	87.6		
62.4					54.6	73.3		
46.8	95.6	98	91.6	94	56	77		
31.2	86.3	92	86	94.6	49.6	70.3		
15.6	76	81	76	90	18.6	37		
11.7			72.6	81.6				
7.8	56.3	72.6	47.3	57.3				
1.56	15.3	29.3	12.6	26.6	11.6	24.3		
0.156	4.3	9						
0.00							3	14

Table 6. Regression equations for aldrin, dieldrin, and DDT at 24 and 48 hours calculated from the average mortality values obtained when the chemicals were injected into the female large milkweed bug.

	Regression equations	
	24 hour	48 hour
Aldrin	Y= 5.48 + 1.37 X	Y= 5.81 + 1.32 X
Dieldrin	Y= 5.28 + 1.74 X	Y= 5.69 + 1.63 X
DDT	Y= 4.45 + 1.09 X	Y= 4.96 + 1.03 X

Table 7. Calculated LD_{50} and LD_{95} values for aldrin, dieldrin and DDT, at 24 and 48 hours, and the limits based on the first deviation, for the female large milkweed bug when injected with the chemicals. The values are in Micrograms per gram of insect.

Compound	LD_{50}, micrograms per gram of insect.			
	24 hours	Limits	48 hours	Limits
Aldrin	4.46	3.59 - 5.31	2.43	1.91 - 2.95
Dieldrin	6.90	6.31 - 7.6	3.77	3.02 - 4.39
DDT	31.3	22.4 - 48.4	10.9	7.24 - 15.1
	LD_{95}, micrograms per gram of insect.			
Aldrin	71.5	53.7 - 112	43.25	34.4 - 58.2
Dieldrin	61.3	55.0 - 72.5	38.8	30.2 - 46.8
DDT	1042.5	Not given	437.0	245.5 - 1780.0

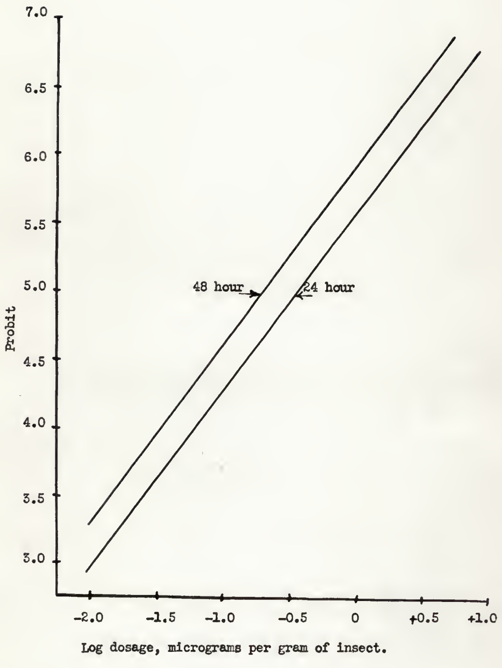


Fig. 1. Regression lines for the average 24 and 48 hour mortalities for the adult female large milkweed bug, Oncopeltus fasciatus (Dall.), injected with aldrin.

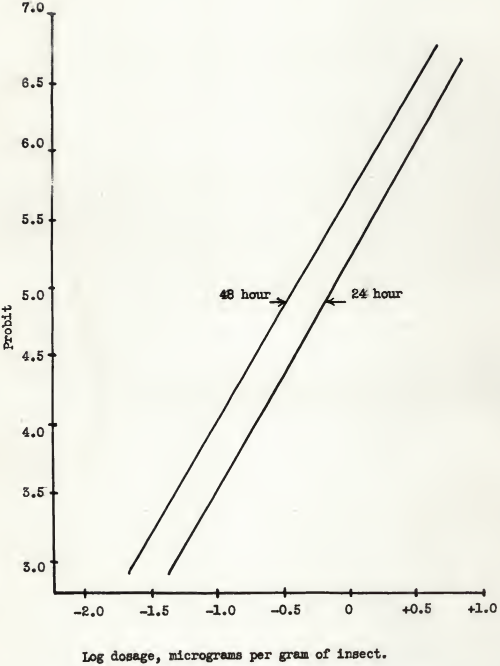


Fig. 2. Regression lines for the average 24 and 48 hour mortalities for the adult female large milkweed bug, <u>Oncopeltus fasciatus</u> (Dall.), injected with dieldrin.

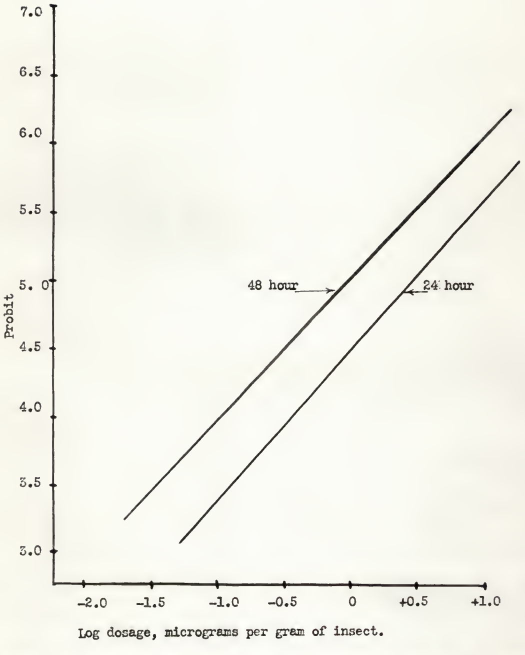


Fig. 3. Regression lines for the average 24 and 48 hour mortalities for the adult female large milkweed bug, *Oncopeltus fasciatus* (Dall.), injected with DDT.

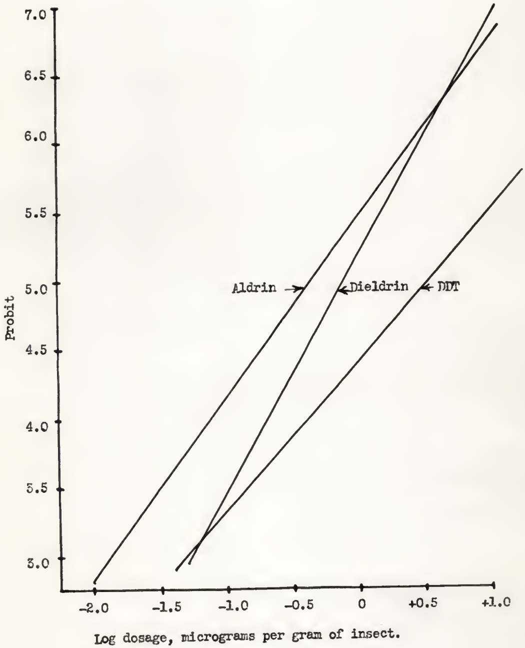


Fig. 4. Regression lines for the average 24 hour mortalities for the adult female large milkweed bug, Oncopeltus fasciatus (Dall.), injected with aldrin dieldrin and DDT.

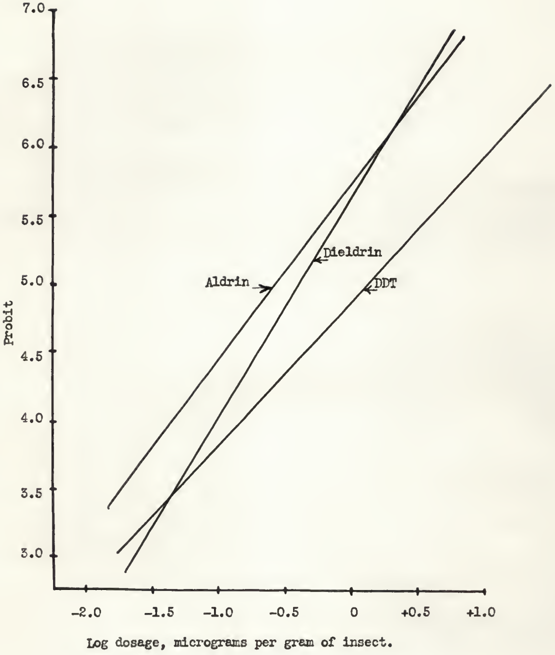


Fig. 5. Regression lines for the average 48 hour mortalities for the adult female large milkweed bug, Oncopeltus fasciatus (Dall.), injected with aldrin, dieldrin and DDT.

approximate since the positions of the calculated dosage values with relation to the extremes of the limits were not known.

The three chemicals in descending order of toxicity when compared by their LD_{50} values would be aldrin, dieldrin, and DDT. Tests by Kearns, Weinman and Decker (1949) on 10 species of insects show the overall toxicity order to be dieldrin, aldrin and DDT in descending order. The large milkweed bug was among the species tested, and the chemicals were administered to it by topical application.

The toxicity of the three chemicals when compared by their toxicity ratios computed from the calculated LD_{50} and LD_{95} values had these relationships. The descending order of toxicity for LD_{50} values at 24 hours was aldrin, 7.017; dieldrin, 4.536; and DDT, 1. The LD_{50} at 48 hours was aldrin, 4.485; dieldrin, 2,891; and DDT, 1. The descending order of toxicity for the LD_{95} values at 24 hours was dieldrin, 17.006; aldrin, 14.580; and DDT, 1. The LD_{95} at 48 hours was dieldrin, 11.262; aldrin, 10.104; and DDT, 1.

These figures indicated that in order to produce a 50 per cent mortality in 24 hours it would take approximately 7.0 times as much DDT as aldrin, and 4.5 times as much DDT as dieldrin. To produce a 50 per cent mortality in 48 hours it would take approximately 4.5 times as much DDT as aldrin, and 2.89 times as much DDT as dieldrin. To produce a 95 per cent mortality in 24 hours it would take approximately 14.58 times as much DDT as aldrin, and 17 times as much DDT as dieldrin. The amounts necessary to produce a 95 per cent mortality in 48 hours would be approximately 10.0 times

Table 8. Toxicity values and ratios for aldrin, dieldrin and DDT obtained by micro-injection into the adult female large milkweed bug (Oncopeltus fasciatus) (Dall.).

Compound	Toxicity values: Micrograms of chemical per gram of insect				Toxicity ratios			
	LD_{50}		LD_{95}		LD_{50}		LD_{95}	
	24 hrs	48 hrs	24 hrs	48 hrs	24 hrs	48 hrs	24 hrs	48 hrs
DDT	31.3	10.9	1042.5	437.0	1	1	1	1
Aldrin	4.46	2.43	71.5	43.25	7.017	4.485	14.580	10.104
Dieldrin	6.90	3.77	61.3	38.8	4.536	2.891	17.006	11.262

as much DDT as aldrin, and 11.0 times as much DDT as dieldrin.

Aldrin had an approximately normal picture when evaluated by the toxicity ratio values, since the LD_{50} and LD_{95} values at 24 hours were 7.0 and 14.58, respectively. The LD_{50} and LD_{95} values at 48 hours were 4.48 and 10.1 respectively. The dieldrin picture did not show a normal distribution since the LD_{50} and LD_{95} values at 24 hours were 4.5 and 17.0, respectively. The LD_{50} and LD_{95} values at 48 hours were 2.89 and 11.26, respectively.

The toxicity of aldrin and dieldrin when compared to each other by their toxicity ratios placed them in this order. At 24 hours aldrin appeared to be 1.54 times as toxic as dieldrin when compared at LD_{50} levels, while at LD_{95} dieldrin appeared to be 1.16 times as toxic as aldrin. At 48 hours aldrin appeared to be 1.58 times as toxic as dieldrin at the LD_{50} level, while at LD_{95} dieldrin appeared to be 1.114 times as toxic as aldrin.

Conclusions

These conclusions appear to be indicated by the results of these experiments.

Fifty per cent mortalities were produced at 24 hours when seven times as much DDT as aldrin or 4.5 times as much DDT as dieldrin was injected into the large milkweed bug. The fifty per cent mortalities were produced at 48 hours by injecting 4.5 times as much DDT as aldrin or 3 times as much DDT as dieldrin into the large milkweed bug.

Ninety-five per cent mortalities were produced at 24 hours when 14.5 times as much DDT as aldrin or 17 times as much DDT as dieldrin was injected into the large milkweed bug. The 95 per cent mortalities were produced at 48 hours by injecting 10 times as much DDT as aldrin, or 11times as much DDT as dieldrin into the large milkweed bug.

ACKNOWLEDGMENTS

The writer wishes to acknowledge the aid given by Dr. Paul A. Dahm, major instructor, in the planning of the problem, the techniques, and the apparatus used. Dr. H. C. Fryer advised the pattern and number of replications used, and the statistical analyses of the data were done under his direction. Mrs. Ted Stephens Brook aided materially in the manipulation of the insects, and in the preparation of the materials used during the preliminary investigations of the problem.

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