THE INSECTICIDAL ACTIVITY OF THREE ANIMAL SYSTEMIC INSECTICIDES

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Bachelor of Science

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Stillwater, Oklahoma

1954

Submitted to the Faculty of the Graduate School of
the Oklahoma Agricultural and Mechanical College
in partial fulfillment of the requirements
for the degree of
MASTER OF SCIENCE
May, 1957

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PREFACE

Upon returning to Oklahoma Agricultural and Mechanical College from military service, the writer was interested in developing a research problem for the Master's Thesis; and after some consideration chose to work on the animal systemics, which Dr. Howell had suggested as a subject of study. At that time preliminary work had been done with three of Dow Chemical Company's insecticides to determine their approximate toxic effects on laboratory animals and cattle.

This situation left several avenues of approach open for evaluation of these materials. For the laboratory work, it was decided that to determine their insecticidal activity and duration of effectiveness in laboratory animals, bedbugs and mosquitoes would be used as indicators. The field work or evaluation with large animals was to be carried on to determine the activity of the three insecticides on the migrating cattle grubs, the effect of these materials on weight gains and toxic effects on the animals treated.

The success of chemotherapeutic agents such as these marks the beginning of positive control measures for the cattle grub.

The writer wishes to acknowledge the kind consideration and help of members of the staff of the Oklahoma Agricultural and Mechanical College and in particular Dr. D. E. Howell, the major professor, Dr. F. A. Fenton, Dr. D. A. Twohy, Prof. G. A. Bieberdorf, Prof. Quintin B. Graves, Mr. Orville Schomberg and Dr. C. E. Marshall of the statistical laboratory for assistance in preparing this manuscript; also the U. S. Dept. of Agri.,

Agricultural Research Service at Orlando, Florida and the U. S. Public Health Service, Communicable Disease Center at Savannah, Georgia for providing stock for the insect colonies used in the laboratory phase of this work.

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CHAPTER I

INTRODUCTION

One of the more important insect pests that the beef producer has to contend with is the cattle grub or heel fly. The activity of these insects results in damaged hides during the 6 to 8 month grub season, loss of meat in dressed carcasses amounting to as much as five dollars per head and loss of weight caused by the cattle running from flies during the fly season. These losses have been estimated by various authorities, (U. S. Dept. of Agr., Agricultural Research Service, 1954) at approximately 100 million dollars annually.

Throughout the United States and Canada several methods have been used or tried in fighting the grubs (Bishopp and Laake, 1949). Various repellents have been used against the heel flies. Hand extraction by pressing down with the thumb and fore finger of each hand or by use of forceps has been used. The treatment of the backs of the cattle with insecticides to kill the grubs has been used quite successfully in an eradication program where spray programs were properly enforced (Baker, Kingscote and Allan, 1955).

There are two distinct species of cattle grubs; the common cattle grub or southern heel fly Hypoderma lineatum (De Vill.) found throughout the United States and the northern cattle grub or northern heel fly Hypoderma bovis (Linn.) whose distribution is limited in the United States to the northern states.

Life History and Habits—The eggs of both species are laid on the hair of cattle; H. lineatum (De Vill.) attaches as many as a dozen eggs on one hair while H. bovis (Linn.) attaches only one egg per hair (Bishopp and Laake, 1949). As many as 800 eggs may be laid by a female of either species. The eggs are deposited on the legs from the hock to the knee of the standing animal, however in recumbent animals the eggs may be attached to hairs of other parts of the body close to the ground. Although no pain is inflicted at oviposition, the tickling and buzzing of the flies prompts the animals to shake or kick the flies away and to dash headlong for the protection of water or shade. This wild running away from the flies is considered to be one of the causes of loss of productivity attributable to these insects.

The eggs hatch in from 3 to 4 days and the larvae penetrate the skin causing considerable irritation to the cattle. During the following 7 or 8 months[1] they constantly burrow about the surface of the paunch, intestines, spleen and other organs. Grubs of the southern heel fly are especially numerous between the muscular and mucous layers of the esophagus or gullet; the grubs found in the gullet are slender and at this stage range from about 5 to 16 millimeters in length (Roth, 1957).

In the fall and winter the grubs migrate through the muscular tissue of the back and in a short time reach the skin where they cut a small hole through to the surface and lie with the two breathing pores, which are located at the posterior end, close to the surface. In this migration to the back, some of the grubs enter the spinal canal and burrow along the spinal cord.

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[1]Bishopp and Laake (1949) report 7 or 8 months as the average length of time, however it has been observed by several Oklahoma workers that apparently this length of time may be much less, possibly due to the eggs being laid over an extended period (Howell, 1957).

In 1 to 5 days after the grubs reach the back they molt a third time, after which the cuticles of the grubs are closely set with spines. The body of the host now begins to form cysts around the grubs to isolate them. From now on the grubs grow rapidly, with the fourth molt occurring about 25 days after the third. In this last larval stage, the grubs begin to darken and at the end of the development in the back, about 35 to 89 days, the grubs work their way out of the back and fall to the ground (Bishopp and Laake, 1949). The northern cattle grub develops in a similar way but requires from 50 to 100 days to complete development in the back. After dropping to the ground, the grubs transform into very hard blackish pupae; and, depending on the temperature, the southern heel fly will emerge in from 18 to 77 days and the northern heel fly from 15 to 25 days.

The adults do not feed, but seem to have adequate food stored from the grub stage to carry them through their very short adult life. The entire life cycle requires about a year.[2]

In Oklahoma the grubs of the southern heel fly are usually not found in the backs of cattle before August nor after the first part of April. To date, grubs of the northern heel fly have not been found in Oklahoma cattle.

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[2]This observation by Bishopp and Laake seems to be a general statement, subject to considerable variation.

CHAPTER II

REVIEW OF THE LITERATURE

Lindquist (1946) discovered that DDT fed to rabbits made the rabbit's blood toxic to Cimex lectularius Linn. which were fed on the treated rabbit. DeMeillon (1946) allowed the test insects, Cimex lectularius Linn., Aedes aegypti (Linn.) and Ornithodoros moubata (Murray) to engorge on a rabbit that had been given an oral dose of Gammexane[1] and concluded that "the possibility of interfering with the life processes of blood sucking arthropods by feeding insecticides to the host is an established fact."

Garnham (1947) reported that after an oral dose of 40 mg./kg. of Gammexane had been administered to a rabbit, the maximum period for complete kill of mosquitoes fed on the rabbit was four days; however when a lower oral dose was given to the rabbit, the effect lasted only two days.

In the beginning work on systemics for cattle grub control McGregor et al (1954) tested materials by removing first instar larvae from the gullets of cattle and adding to bovine serums, then treating the grubs with various materials. Guinea pigs infested with Callitroga hominivorax (Cqrl.) were also used to test various insecticides administered orally or sub-cutaneously. When an effective material was

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[1]The gamma insomer of BHC of not less than 99 per cent purity as named by British workers; Lindane, being the name of choice in America.

applied, screw-worms migrated from the pockets and stable flies Stomoxys calcitrans (Linn.) and other test insects which were put on the guinea pigs, were killed.

Later, it was found that lindane,[2] dieldrin[3] and aldrin[4] killed screw-worms, flies and late instar grubs but these insecticides were difficult to administer and created high residue problems (McGregor, 1955).

Phosphorus insecticides such as Bayer L 13/59[5] and diazinon[6] killed grubs in the back only; however, some of the Dow organic phosphates when administered to cattle showed some indications of controlling the migrating grubs. With the hope of finding a better material, with systemic activity, the Dow Chemical Company synthesized many phosphorus compounds. Among those compounds ET-57, ET-58 and ET-59, which are described on page 7, were selected as worthy of more extensive testing.

Preliminary work with these materials was conducted by the Dow laboratories to determine approximate LD_{50}'s and acute oral lethal doses of these materials by use on laboratory animals and a few cattle (Crenshaw, 1956).

Studies with P^{32}-labeled ET-57 were conducted by the U. S. Dept. of Agri., Entomology Research Branch laboratories at Kerrville, Texas and Corvallis, Oregon (Robbins, 1956). It was found that after an oral dose

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[2]Lindane--gamma isomer of BHC(1,2,3,4,5,6-hexachlorocyclohexane.

[3]Dieldrin-1,2,3,4,10,10-hexachloro-6-7-epoxy-1,4,4a,5,6,7,8,8a-octahydro-1,4-endo-exo-5,8-dimethanonaphtalene.

[4]Aldrin-1,2,3,4,10,10-hexachloro-1,4,4a,5,8,8a-hexahydro-1,4-endo-exo-5,8-dimethanonaphtalene.

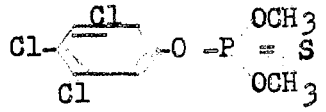
[5]Bayer L 13/59-0,0-dimethyl 2,2,2-trichloro-1-hydroxy-ethylphosphonate.

[6]Diazinon -,0-diethyl 0-(2-isopropyl-4-methyl-6-pyrimidinyl) thiophosphate.

of 100 mg./kg. of ET-57 to a guernsey bull calf, analysis of the blood, urine and feces showed that approximately 50 per cent of the total dose was accounted for in the urine at 30 hours and about 99 per cent in the urine and feces at the end of 10 days; unchanged Dow ET-57 was present in the extracts of blood and feces while only metabolic products were found in the urine.

CHAPTER III

MATERIALS AND METHODS

The three insecticides used in this study were as follows: 1. ET-57, O,O-dimethyl O-2,4,5-trichlorophenyl phosphorothioate has the following structural formula:  ET-57 is a white crystalline compound having a molecular weight of 321.56, a melting point of 41.0°C. and is soluble in acetone and chloroform. 2. ET-58, a coded compound,[1] is a tan crystalline form and may be dissolved in acetone. 3. ET-59, also a coded compound, is a white crystalline compound and may be dissolved in acetone.

Laboratory Phase

The laboratory evaluation of ET-57, ET-58 and ET-59 was done to determine the activity of the aforementioned materials when administered to laboratory animals.

Rearing of Aedes aegypti (Linn.)--This was accomplished with eggs obtained from the U. S. Public Health laboratories at Savannah, Georgia. The eggs, which were sent on paper toweling were submerged in tap water and hatched within 30 minutes. Approximately 250 newly hatched larvae were fed on Pablum,[2] finely ground with a mortar and pestle and distributed

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[1]The chemical names of ET-58 and ET-59 are not given in the interest of the Dow Chemical Company.

[2]Pablum is a pre-cooked mixed cereal made by Mead Johnson and Company.

with a salt shaker. Care was taken not to give excessive amounts of food, as a scum would result which would kill the larvae. Of course, too little food would prevent rapid development, therefore feeding had to be carefully controlled. As soon as pupae appeared in the culture pan, they were removed daily by means of an eye dropper and placed in a small, wide mouth, glass container. This container was dated and placed in an adult cage (figure 1). Adults were kept in this cage with an ample supply of fresh tap water and a 2 inch square, one-half inch thick piece of cotton soaked with 5 per cent sugar solution. After the third day the adults were offered a blood meal from a rabbit that had been shaved, placed in a restraining trough and inserted into the built in feeding chamber of the adult cage (figure 1). The rabbit was usually kept in the feeding chamber from one to three hours.

A petri dish containing a square of paper toweling and filled with water was placed in the cage to collect eggs. After a sufficient number of eggs were deposited, the toweling was taken out of the dish and allowed to dry. By this method the eggs could be preserved for as long as six months.

Rearing of Cimex lectularius Linn.--Stock was obtained from the U. S. Dept. of Agri. laboratory at Orlando, Florida. The bedbugs arrived sealed in small pill boxes and were transferred to Mason jars which were covered with nylon after the transfer. A few squares of paper toweling were placed in with the wool cloth squares sent with the colonies. The bedbugs were fed for 10 to 20 minute periods approximately every other day. A rabbit was held in the restraining trough previously mentioned and the jars containing the bedbug colonies were inverted on the rabbits shaved back so that they could feed through the nylon covering on the

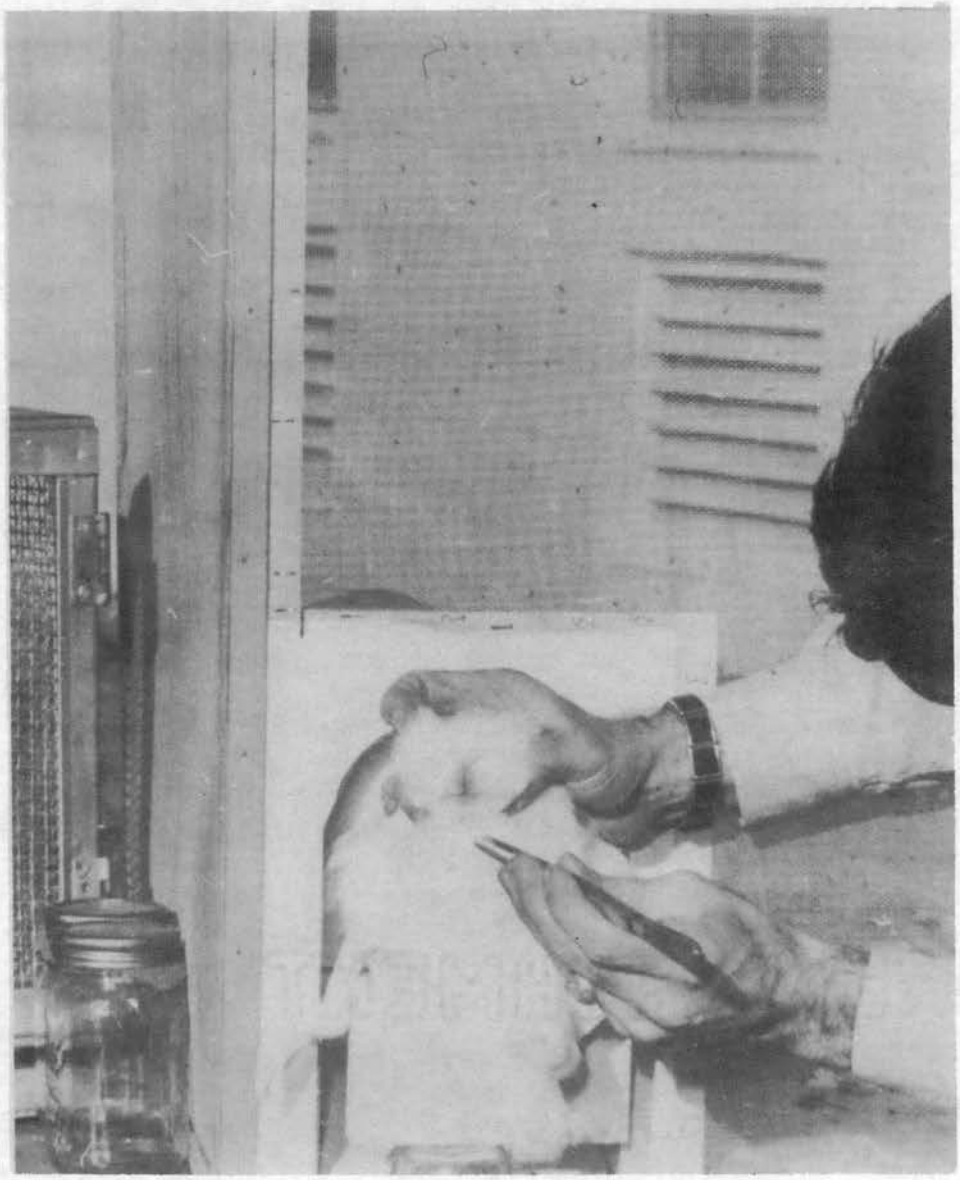


Figure 1. The Adult Cage for A. aegypti(Linn.) Showing a Rabbit in the Restraining Trough which had been inserted into the Feeding Chamber, also the Method of Capsule Administration.

top of the jar. The adults oviposited within the jars and the colonies continued to multiply to adequate numbers for the tests to be conducted.

Test #1--Before treating, four rabbits, which had not been fed for the previous eight hours, were put into restraining troughs and shaved. An ET-57 capsule of the pure material at the rate of 100 mg./kg. was given orally to the first rabbit. A second rabbit was treated with an ET-58 capsule at the rate of 40 mg./kg. A third rabbit was treated with an ET-59 capsule at the rate of 35 mg./kg. while a fourth rabbit remained untreated to serve as the control.

Bedbugs and mosquitoes were allowed to engorge on the rabbits 3,6,9,12,24 and 48 hours after treatment. The bedbugs were fed by inverting 5 dram vials containing 10 to 15 bedbugs on the shaved rabbit's backs. These vials were held in place during the 10 to 20 minute feeding period by a cardboard vial holder which had been strapped on the rabbit's backs. The vials were then set aside for 24 hour mortality counts. The rabbits were then placed one at a time into the feeding chamber of the adult mosquito cage and 5 to 10 mosquitoes were allowed to engorge on the rabbit, after which those engorged mosquitoes were removed from the cage and set aside for 24 hour mortality counts.

Test #2--This test was a duplicate of test #1 except that the materials, ET-57, ET-58 and ET-59 were dissolved in acetone and administered as a drench.

Test #3--In this test ET-57 was given at the rate of 150 mg./kg., ET-58 at the rate of 60 mg./kg. and ET-59 at the rate of 52.5 mg./kg. All three of the materials were dissolved in acetone and given as a drench. The methods of handling the rabbits and insects were as in tests #1 and #2.

Test #4--In this test ET-57 was given at the rate of 200 mg./kg., ET-58 at the rate of 80 mg./kg. and ET-59 at the rate of 70 mg./kg. The materials were dissolved in acetone and given as a drench; the rabbits and insects were handled as in the preceding tests.

Field Phase

After several workers had found ET-57, ET-58 and ET-59 to have systemic insecticidal activity when administered to laboratory animals, it was necessary to test the materials on an insect of real economic importance, namely the cattle grub.

In these field tests there were three methods used in oral administration of ET-57. The drench, made up of 25 per cent wettable powder, was given with a standard six ounce drench syringe (figure 2). The capsule, was made up of the technical material put into capsules of 5,10,20 and 30 gram sizes and administered with the standard equine balling gun (figure 3). The bolus was made up of the material compressed into pills ten grams in size and also administered with the standard balling gun.

All of the animals undergoing treatment with ET-57 in the form of drench, capsule and bolus, were given the material at the rate of 100 mg./kg. or as near thereto as possible.

In computing the amount of drench to be used, it was decided that the drench would be mixed so that one ounce of the material would treat 100 lbs. of animal, for example, to treat an animal that weighed 700 pounds, 7 ounces of the drench would be given. Doses were computed to the nearest fifty pounds.

All animals used in the field phase were treated either according to

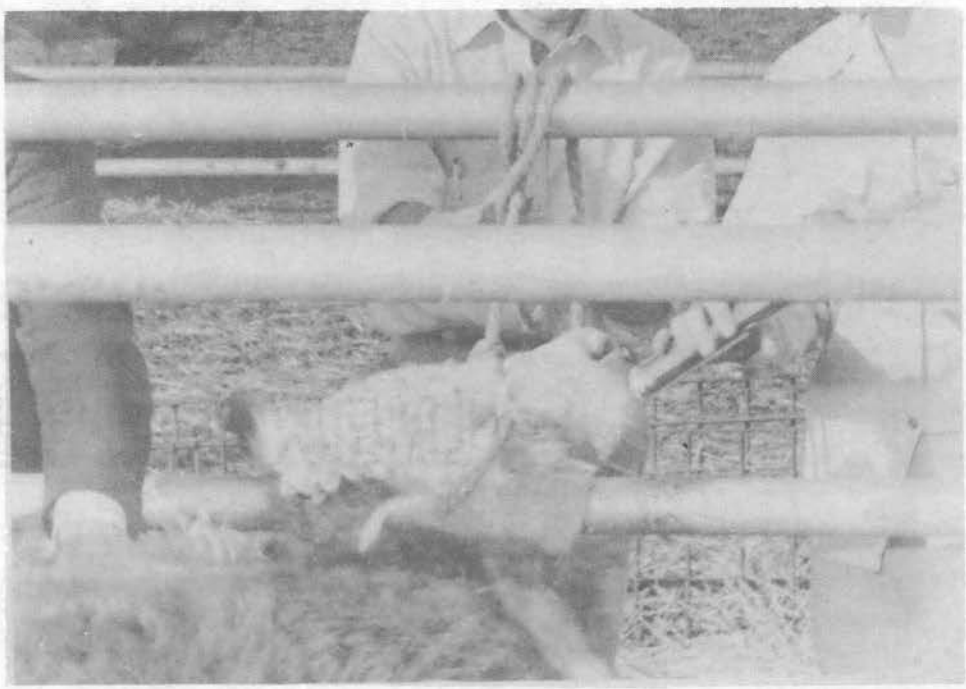


Figure 2. Dosing with the Standard 6 ounce Syringe; Also Showing the Method of Grub Counting.

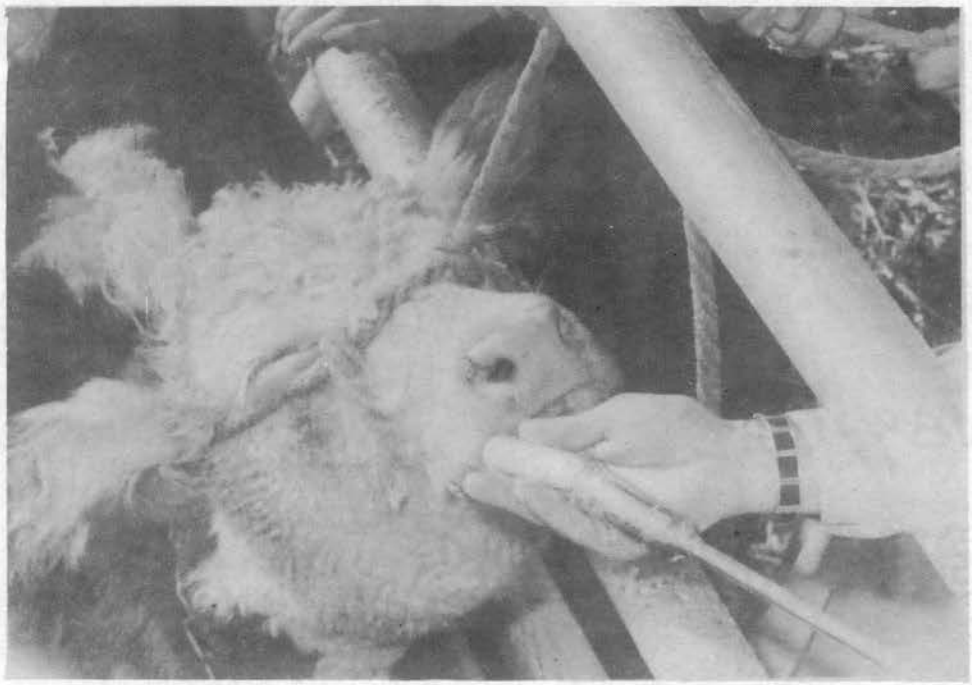


Figure 3. The Standard Equine Balling Gun and Its Use.

their weights on treatment day, or according to their last weight which was taken, in no case, more than two weeks prior to treatment.

With the hope of killing the highest numbers of migrating grubs with a single treatment, it was decided, for experimental purposes, that a treatment late in the year after the heel fly normally ceases its activity would be the time of choice.

Treatment at Stillwater, Oklahoma--This experiment was carried out at the Oklahoma Agricultural Experiment Station, experimental beef barn, which had been stocked with yearling steers obtained from Ft. Reno, Blackwell, and Wilburton, Oklahoma. The Animal Husbandry Department of the Oklahoma Agricultural and Mechanical College had obtained 80 steers for nutritional experiments and they agreed that the animals could be treated with these new Dow insecticides. It was decided that this test would be superimposed on nutritional studies by random selection. Lots 1 through 8 received feed consisting of silage, cotton seed hulls and other high protein supplements as well as stilbesterol, a thyroid depressant. Lots 9 and 10 received milo, alfalfa pellets, cotton seed hulls and cotton seed meal.

ET-57 was administered as a drench made from a 25 per cent wettable powder and in gelatin capsules at the rate of 100 mg./kg.; 20 animals receiving the drench and 20 animals receiving the capsules for a total of 40 animals.

ET-58 was administered as a drench made from a 25 per cent wettable powder at the rate of 40 mg./kg. and was mixed so that one ounce of the drench would treat 50 pounds of animal. By lowering the concentration of the material, it was expected that a more accurate dosage could be administered; thus giving a greater margin of safety. ET-58 was administered to 22 of the animals.

ET-59 was administered as a drench made from a 25 per cent wettable powder to 17 of the animals at the rate of 35 mg./kg. and was mixed so that one ounce of the drench would treat 50 pounds of animal as in the case of ET-58.

To study the toxic effect of high level dosages of ET-57, four steers of comparable weight and background to the preceding steers treated with ET-57, were chosen to fill that need. Before treatment, rumen pH's and blood samples were taken from each of the four steers; they then received an ET-57 drench at the rate of 150 mg./kg. Forty-eight hours later a second rumen sample was taken as well as a second blood sample. After that a blood sample was taken seven days after treatment and at weekly intervals for the next three weeks.

Treatment at Fort Supply, Oklahoma--These tests were carried out at the U. S. Southern Great Plains Field Station. There were 88 two year old cows, one-half of which were treated with ET-57 capsules at the rate of 100 mg./kg. while the remaining 44 cows were used as untreated controls. Also there were 84 yearling steers, one-half of which were treated with ET-57 capsules at the rate of 100 mg./kg. while the other half remained as untreated controls.

The cows were held in thirteen different pastures which had been under various treatments ranging from those lightly grazed to those heavily grazed. The yearling steers were held in 22 different pastures.

Treatment at Fort Reno, Oklahoma--These tests were carried out at the Oklahoma Experiment Station at Fort Reno. The cows, bulls and heifers used in this test were divided into groups according to age and sex and all received a feed of mixed supplement and native grass. Of the animals at Fort Reno, 185 were treated with a drench of ET-57 at the rate of 100 mg./kg. while 185 remained as untreated controls.

Treatment at Lake Carl Blackwell, Oklahoma--These tests were carried out at the Oklahoma Experiment Station at Lake Carl Blackwell, Oklahoma. Here, 50 three and one-half year old cows were treated with ET-57 at the rate of 100 mg./kg. while 50 remained untreated to serve as controls. The cows were on range pasture and in addition, received a mixed supplement.

In addition to the 100 three and one-half year old cows there were 79 five and one-half year old cows, of which 40 were treated with an ET-57 drench at the rate of 100 mg./kg. while 39 cows remained untreated to serve as controls.

Treatment at Coalgate, Oklahoma--The Coalgate test was carried out at the Oklahoma Agricultural Experiment Station there. This test was comprised of 79 weaning calves that received an ET-57 drench, a low level phenothiazine feeding of 2 grams per day per head or a phenothiazine drench of 4 ounces per head or a combination of one or more of these treatments. In addition to this, eight animals received no treatment of any kind. These calves were all on a similar diet of 2 pounds cotton seed cake per day plus 15 pounds mixed hay.

Treatment at Codding Ranch, Foraker and Perkins, Oklahoma--This test consisted of 526 animals which had been divided into five groups.

In the first group, which was located at Perkins, Oklahoma, 104 cows were treated with boluses of ET-57 at the rate of 100 mg./kg. and 23 cows were to serve as untreated controls.

Of the other groups, all located at Foraker, Oklahoma, the first group consisted of 43 cows which were treated with ET-57 capsules at the rate of 100 mg./kg. In the second group 39 cows were treated with ET-57 boluses and 81 cows were used as untreated controls. In the third group,

39 brood heifers were treated with an ET-57 drench at the rate of 100 mg./kg. and a fourth group consisted of 53 two year old heifers which had been treated with boluses at the rate of 100 mg./kg. and 104 two year old heifers that were left as untreated controls.

CHAPTER IV

RESULTS

Laboratory Phase

The laboratory phase of this research consisted of treating rabbits with ET-57, ET-58 and ET-59 and feeding test insects on those rabbits.

Test #1--In this test, rabbits received ET-57, ET-58 and ET-59 in a capsule which had been filled with the pure form of the compounds; the dosages were 100 mg./kg., 40 mg./kg. and 35 mg./kg. respectively.

No insects of either species, which had been fed on rabbits 3,6,9,12,24 and 48 hours after treatment, were killed as a result of that feeding.

Test #2--In this test which was identical to test #1 except that the materials had been dissolved in acetone, neither the insects which had been fed on rabbits receiving ET-58 or ET-59 nor the insects fed on the control rabbit were killed; however those mosquitoes and bedbugs which had been fed on the rabbit receiving ET-57 were killed in varying numbers (Table I).

Test #3--This test consisted of giving ET-57, ET-58 and ET-59, dissolved in acetone, at the rate of 150 mg./kg., 60 mg./kg. and 52.5 mg./kg. respectively.

In this test as in test #1, no insects of either species were killed as a result of feeding on rabbits treated with the test insecticides.

Test #4--This test consisted of giving ET-57, ET-58 and ET-59,

dissolved in acetone, at the rate of 200 mg./kg., 80 mg./kg. and 70 mg./kg. respectively.

In this test, no insects which had been fed on the control rabbit were killed. Those insects that had been fed on the rabbit receiving ET-57 were killed in varying numbers depending on the hour after treatment. A small number of the insects which were fed three hours after treatment on the rabbit that had received ET-58 were killed, while insects fed at the 6,9,12,24 and 48 hour feeding were not killed. The insects that had been fed on the rabbit receiving ET-59 reacted much the same as those that had been fed on the ET-58 treated rabbit (Table II).

Field Phase

Treatment at Stillwater, Oklahoma--On the day of treatment, an average of seven grubs per animal was found on the animals used in this test. Approximately 4 days after treatment, symptoms of severe toxicity developed in several of the steers that were treated with ET-59 and mild to no symptoms of toxicity appeared in the other animals. The symptoms were of the typical organic phosphate poisoning type consisting of lowering of tissue cholinesterase, weakness, stiffness, scouring and salivation. This toxicity seemed to appear in the first eight lots, only, which had been on a different diet than lots nine and ten. Those animals in lots nine and ten showed no symptoms of toxicity.

The animals that had been treated with the ET-57 drench were found to have an average of 7.5 grubs per animal on the day of treatment, an average of 1.0 grub per animal one month after treatment, an average of .80 grub per animal two months after treatment and an average of .15 grub

TABLE I

PERCENTAGE MORTALITY* OF A.AEGYPTI (LINN.) AND C.LECTULARIUS LINN. FED ON RABBITS TREATED WITH ET-57, ET-58 and ET-59 IN TEST #2

Test Material	Hours after treatment					
	3	6	9	12	24	48
ET-57	100	100	100	85	0	0
ET-58	0	0	0	0	0	0
ET-59	0	0	0	0	0	0
Control	0	0	0	0	0	0

*The percentages presented herein are based on the engorgement of 10 to 15 C. lectularius Linn. and 5 to 10 A. aegypti (Linn.) as a group since there was no difference in mortality between the two species.

TABLE II

PERCENTAGE MORTALITY* OF A. AEGYPTI (LINN.) AND C. LECTULARIUS LINN. FED ON RABBITS TREATED WITH ET-57, ET-58 and ET-59 IN TEST #4

Test Material	Hours after treatment					
	3	6	9	12	24	48
ET-57	70	100	100	100	85	0
ET-58	25	0	0	0	0	0
ET-59	20	0	0	0	0	0
Control	0	0	0	0	0	0

*The percentages presented herein are based on the engorgement of 10 to 15 C. lectularius Linn. and 5 to 10 A. aegypti (Linn.) as a group since there was no difference in mortality between the two species.

per animal three months after treatment (Table III). The weight gains in relation to other animals in this test appeared to be average (Table IV).

Animals drenched with ET-57 exhibited minor scouring and sluggishness.

Those animals that had been treated with the capsules were found to have an average of 6.6 grubs per animal on the day of treatment with a decrease to .65 grub per animal one month after treatment and at the counts made 2 and 3 months after treatment there were no grubs found on any of the animals (Table III). The weight gains of these animals appeared to be average, in relation to other animals in this test (Table IV).

There were no symptoms of toxicity noted in any of the animals treated with ET-57 capsules.

The animals treated with ET-58 showed an average of 5.2 grubs per animal on treatment day and a decrease of grubs per animal one month after treatment to 2.77. Two months after treatment 1.3 grubs per animal were found and three months after treatment .22 grub per animal was found (Table III). Weight gains seemed to be average in comparison to the rest of the animals under test (Table IV).

Signs of moderate toxicity were noted in some of the animals treated with ET-58.

Treatment with ET-59--An average of 6.3 grubs per animal was found on treatment day. At the count made one month after treatment there was .36 grub per animal and at the counts made 2 and 3 months after treatment no grubs were found on any of the animals (Table III).

Two of the animals treated with ET-59 died showing severe symptoms of phosphate poisoning while fifteen other steers treated with ET-59 showed mild to severe symptoms. The seven animals showing the most

TABLE III

AVERAGE NUMBER OF GRUBS PER ANIMAL FOUND ON ANIMALS TREATED AT STILLWATER, OKLAHOMA, ON 21 NOV 56

Treatment	Average no. grubs per animal			
	21 Nov 56	21 Dec 56	21 Jan 57	21 Feb 57
ET-57 Drench	7.5	1.00	0.00	0.15
ET-57 Capsule	6.6	0.65	0.00	0.00
ET-58 Drench	5.2	2.77	1.30	0.22
ET-59 Drench	6.3	0.36	0.00	0.00

TABLE IV

AVERAGE WEIGHTS OF ANIMALS IN LOTS ONE THROUGH EIGHT AT STILLWATER, OKLA.

Treatment	Average weights			
	21 Nov 56	21 Dec 56	21 Jan 57	21 Feb 57
ET-57 Drench	560	613	685	759
ET-57 Capsule	539	600	671	759
ET-58 Drench	552	613	685	766
ET-59 Drench	529	534	633	709

severe symptoms were treated with atropine and 5 per cent glucose and electrolytes within 5 days after treatment with ET-59. Approximately 8 days after treatment with ET-59 those animals were treated with Pyridine Aldoxine Methiodide, an antidote for nerve gas and phosphate poisoning. However, two of the animals died while the other five recovered along with the rest of the animals under test. The five animals showing the most severe symptoms were found to have a definite decrease in weight as well as 100 per cent reduction of grubs (Table VI).

In the toxicity study of ET-57 given at the rate of 150 mg./kg. to four steers, rumen pH's taken before and after treatment were not changed and the choline esterase level was little affected by this treatment. No symptoms of toxicity appeared in any of the animals treated.

Treatment at Fort Supply, Oklahoma--On the day of treatment, a rough survey indicated that grubs had not yet begun to come into the backs of any of the animals in this test. There were 44 cows and 42 steers treated with ET-57 capsules at the rate of 100 mg./kg. and a like number of cows and steers remained untreated to serve as controls.

The cows treated in this test showed 92 per cent control one month after treatment, 92 per cent control two months after treatment and 94 per cent control three months after treatment (Table VII).

Weight gains in the treated group were not significantly greater.

In the steers in this test, 85 per cent control was found one month after treatment, 94 per cent control two months after treatment and 100 per cent control three months after treatment (Table VIII).

Weight gains in the treated group, here again, were not significantly greater.

Treatment at Fort Reno, Oklahoma--In this test 185 animals which had

TABLE V

AVERAGE WEIGHTS OF ANIMALS IN LOTS NINE AND TEN
AT STILLWATER, OKLAHOMA

Treatment	Average weights			
	21 Nov 56	21 Dec 56	21 Jan 57	21 Feb 57
ET-57 Drench	467	490	550	568
ET-57 Capsule	450	447	543	560
ET-58 Drench	465	475	551	579
ET-59 Drench	482	415	565	595

TABLE VI

AVERAGE WEIGHTS AND GRUBS PER ANIMAL ON ANIMALS SHOWING
TOXICITY SYMPTOMS AFTER TREATMENT WITH ET-59
AT STILLWATER, OKLAHOMA

	21 Nov 56	21 Dec 56	21 Jan 57	21 Feb 57
Average weights	512	505	579	643
Grubs per animal	6.5	0.0	0.0	0.0

TABLE VII

COWS AT THE U.S. SOUTHERN GREAT PLAINS FIELD STATION,
FORT SUPPLY, OKLA., TREATED 31 OCT 56
WITH ET-57 CAPSULES

Lot number		Total number of grubs per lot			
		31 Oct 56	30 Nov 56	30 Dec 56	31 Jan 57
17 & 19	Treated (8)	0	2	12	3
	Control (8)	0	12	45	29
18 & 20	Treated (8)	0	3	5	1
	Control (8)	0	30	46	17
21 & 24	Treated (8)	0	1	5	1
	Control (8)	0	37	41	13
37 a&c	Treated (4)	0	3	6	2
	Control (4)	0	9	30	20
37 b&d	Treated (4)	0	1	1	1
	Control (4)	0	15	24	16
38s	Treated (4)	0	1	1	9
	Control (4)	0	16	46	38
Mod.Res.	Treated (5)	0	1	1	0
	Control (5)	0	24	91	67
Hvy.Res.	Treated (3)	0	5	0	0
	Control (3)	0	9	31	17
Average grubs per animal					
	Treated (44)	0.0	0.3	0.7	0.3
	Control (44)	0.0	3.4	8.0	4.9

TABLE VIII

STEERS AT THE U.S. SOUTHERN GREAT PLAINS FIELD STATION, FORT SUPPLY, OKLA., TREATED 31 OCT 56 WITH ET-57 CAPSULES

Lot number		Total number of grubs per lot			
		31 Oct 56	30 Nov 56	30 Dec 56	31 Jan 57
51	Treated (2)	0	3	0	0
	Control (2)	0	2	8	0
52	Treated (3)	0	0	0	0
	Control (3)	0	0	10	0
54	Treated (3)	0	7	4	0
	Control (3)	0	2	25	0
55	Treated (3)	0	0	0	0
	Control (3)	0	9	55	0
56	Treated (3)	0	0	0	0
	Control (3)	0	14	30	0
57	Treated (3)	0	3	0	0
	Control (3)	0	1	0	0
58	Treated (3)	0	7	0	0
	Control (3)	0	0	0	0
59	Treated (3)	0	1	1	0
	Control (3)	0	61	38	0
60	Treated (3)	0	2	3	0
	Control (3)	0	18	19	0
4e	Treated (2)	0	0	0	0
	Control (2)	0	17	30	0
1w	Treated (1)	0	0	0	0
	Control (1)	0	0	0	0
1e	Treated (1)	0	0	0	0
	Control (1)	0	0	0	0
2	Treated (1)	0	0	0	0
	Control (1)	0	2	12	0
3w	Treated (1)	0	0	0	0
	Control (1)	0	8	25	0

TABLE VIII (Continued)

Lot number		Total number of grubs per lot			
		31 Oct 56	30 Nov 56	30 Dec 56	31 Jan 57
4w	Treated (1)	0	0	0	0
	Control (1)	0	0	0	0
6	Treated (1)	0	0	2	0
	Control (1)	0	0	0	0
7n	Treated (1)	0	0	0	0
	Control (1)	0	0	2	0
9w	Treated (1)	0	0	0	0
	Control (1)	0	3	6	0
14	Treated (1)	0	0	2	0
	Control (1)	0	17	29	2
26	Treated (1)	0	4	8	0
	Control (1)	0	13	24	0
32	Treated (1)	0	0	0	0
	Control (1)	0	2	9	0
Average total grubs					
	Treated (42)	0.0	0.64	0.47	0.0
	Control (42)	0.0	4.02	7.66	0.04

been divided into five different groups were treated with 100 mg./kg. of ET-57 drench while 185 animals remained untreated to serve as controls.

In the first group which consisted of mature cows, there was 71 per cent control two months after treatment and 59 per cent control three months after treatment (Table IX).

In the second group which consisted of mature cows (4-9 year old), 49 cows were treated with an ET-57 drench and 47 cows remained untreated to serve as controls. There was 87 per cent control at a count made one month after treatment and 79 per cent control two months after treatment (Table X).

In the third group, 16 two-year old heifers were treated with an ET-57 drench and 17 heifers remained untreated. One month after treatment there was 86 per cent control and 72 per cent control was found two months after treatment (Table XI).

The fourth group consisted of 19 yearling heifers which had been treated with a drench of ET-57 and 18 yearling heifers which remained untreated to serve as controls. At a grub count made one month after treatment there was 92 per cent control with a drop to 68 per cent control approximately two months after treatment (Table XIII).

The fifth group was made up of bull calves of which 24 were treated and 25 untreated. At a grub count made one month after treatment there was 96 per cent control and two months after treatment there was also 96 per cent control. Four months after treatment there was 56 per cent control (Table XIII).

In the fifth group of animals there was no evidence of additional weight gains in the treated group (Table XIV).

TABLE IX

MATURE COWS AT FORT RENO, OKLAHOMA, TREATED 11 OCT 56 WITH ET-57 AS A DRENCH

Lot number		Total number of grubs per lot		
		4 Dec 56	9 Jan 57	4 Feb 57
1.	Treated (20)	32	20	16
	Control (20)	82	75	20
2.	Treated (20)	22	40	10
	Control (21)	40	53	13
3.	Treated (16)	11	18	9
	Control (15)	37	52	26
4.	Treated (20)	33	31	17
	Control (19)	171	81	16
Average total grubs				
	Treated (76)	1.28	1.43	.68
	Control (75)	4.40	3.48	1.00

TABLE X

MATURE COWS (4-9 YEARS OLD) AT FORT RENO, OKLAHOMA, TREATED 18 OCT 56 WITH ET-57 AS A DRENCH

Lot number		Total number of grubs per lot		
		20 Nov 56	18 Jan 57	14 Feb 57
1.	Treated (16)	1	10	5
	Control (16)	50	42	5
2.	Treated (10)	6	11	1
	Control (10)	22	35	4
3.	Treated (10)	5	4	3
	Control (9)	10	18	10
4.	Treated (13)	5	11	2
	Control (11)	31	54	7
Average total grubs				
	Treated (49)	0.34	0.75	0.26
	Control (47)	2.45	3.46	0.59

TABLE XI

TWO YEAR OLD HEIFERS AT FORT RENO, OKLAHOMA, TREATED 18 OCT 56 WITH ET-57 AS A DRENCH

Lot number		Total number of grubs per lot		
		20 Nov 56	18 Jan 57	14 Feb 57
1.	Treated (4)	5	9	4
	Control (4)	38	29	2
2.	Treated (2)	2	6	1
	Control (3)	13	21	3
3.	Treated (6)	6	20	4
	Control (6)	39	71	4
4.	Treated (4)	1	4	0
	Control (4)	14	23	1
Average total grubs				
	Treated (16)	0.87	2.43	0.56
	Control (17)	6.11	8.47	0.58

TABLE XII

YEARLING HEIFERS AT FORT RENO, OKLAHOMA, TREATED 18 OCT 56 WITH ET-57 AS A DRENCH

Lot number		Total number of grubs per lot			
		29 Nov 56	10 Jan 57	7 Feb 57	7 Mar 57
1.	Treated (7)	0	6	2	1
	Control (7)	31	17	5	0
2.	Treated (5)	1	8	3	0
	Control (5)	15	33	20	0
3.	Treated (4)	0	1	8	3
	Control (4)	10	23	1	1
4.	Treated (3)	5	14	7	1
	Control (2)	8	10	2	0
Average total grubs					
	Treated (19)	0.31	1.52	1.05	0.26
	Control (18)	3.55	4.61	2.59	0.05

TABLE XIII

BULL CALVES AT FORT RENO, OKLAHOMA, TREATED 18 OCT 56
WITH ET-57 AS A DRENCH

Lot number		Total number of grubs per lot				
		29 Nov 56	13 Dec 56	7 Feb 57	21 Feb 57	7 Mar 57
1.	Treated (7)	1	0	4	2	0
	Control (7)	33	69	16	4	1
2.	Treated (5)	2	9	13	7	3
	Control (6)	69	89	16	11	0
3.	Treated (8)	1	1	1	1	1
	Control (8)	53	83	6	6	1
4.	Treated (4)	3	0	5	6	1
	Control (4)	11	14	11	3	0
Average total grubs						
	Treated (24)	0.29	0.41	0.96	0.67	0.21
	Control (25)	6.6	9.04	1.96	1.00	0.91

TABLE XIV

BULL CALVES AT FORT RENO, OKLAHOMA, TREATED 18 OCT 56
WITH ET-57 AS A DRENCH

Lot number		Total number of grubs per lot					
		2 Oct 56	29 Nov 56	13 Dec 56	7 Feb 57	21 Feb 57	7 Mar 57
1.	Treated (7)	481	635	713	811	852	879
	Control (7)	470	617	667	763	820	889
2.	Treated (5)	434	574	624	731	770	805
	Control (6)	418	559	602	701	766	761
3.	Treated (8)	565	721	766	891	928	948
	Control (8)	516	675	715	846	878	903
4.	Treated (4)	475	623	667	790	828	852
	Control (4)	653	928	696	767	792	876
Average total weights							
	Treated (24)	538	638	692	806	844	871
	Control (25)	514	695	670	769	814	857

Treatment at Lake Carl Blackwell, Oklahoma--This test consisted of two groups of cows, those three and one-half years old and those five and one-half years old. Both of these groups had been treated with an ET-57 drench at the rate of 100 mg./kg.

In the 50 three and one-half year old cows that had been treated there was 67 per cent control one month after treatment and 59 per cent control two months after treatment (Table XV).

The five and one-half year old cows consisted of 40 treated animals and 38 control animals. One month after treatment there was 79 per cent control in the treated cows with a rise to 84 per cent control three months after treatment (Table XVI).

Treatment at Coalgate, Oklahoma--In this test which was done on 78 weaning calves that received an ET-57 drench, a low level phenothiazine feeding of 2 grams per day per head or a phenothiazine drench of 4 ounces per head or a combination of one or more of these treatments, eight groups were necessary to obtain the desired results. Each group was made up of ten animals and grub counts were made over a five month period (Table XVII).

In group #1, which had received a combination of all three treatments, 78 per cent control was found.

In group #2, 35 per cent control was found; group no. 2, had received the phenothiazine drench and low level phenothiazine feeding of 2 grams per day per head but received no ET-57.

Group #3 showed 70 per cent control; this group received the ET-57 drench and the low level feeding of phenothiazine.

Group #4 showed 28 per cent control. Group no. 4 received the low level phenothiazine feeding only.

TABLE XV

THREE AND ONE-HALF YEAR OLD COWS AT LAKE CARL BLACKWELL, OKLAHOMA, TREATED 9 OCT 56 WITH ET-57 AS A DRENCH

Lot number		Total number of grubs per lot				
		3 Nov 56	1 Dec 56	1 Jan 57	1 Feb 57	1 Mar 57
1.	Treated (10)	8	5	28	33	3
	Control (10)	4	19	47	37	2
2.	Treated (10)	3	2	9	11	0
	Control (10)	2	27	63	26	0
3.	Treated (10)	0	7	16	9	2
	Control (10)	0	15	39	40	0
4.	Treated (10)	0	6	20	3	0
	Control (10)	1	35	48	10	0
5.	Treated (10)	6	24	31	16	0
	Control (10)	0	25	34	24	0
Average total grubs						
	Treated (50)	0.34	0.88	2.12	1.46	0.10
	Control (50)	0.15	2.63	5.05	2.97	0.04

TABLE XVI

FIVE AND ONE-HALF YEAR OLD COWS AT LAKE CARL BLACKWELL, OKLAHOMA, TREATED 10 OCT 56 WITH ET-57 AS A DRENCH

Lot number		Total number of grubs per lot			
		24 Nov 56	29 Dec 56	1 Feb 57	1 Mar 57
1.	Treated (10)	5	5	7	0
	Control (10)	17	22	26	1
2.	Treated (10)	2	2	4	0
	Control (10)	3	8	7	0
3.	Treated (10)	1	5	2	0
	Control (10)	15	14	18	2
4.	Treated (10)	3	4	1	0
	Control (9)	13	22	22	2
Average total grubs					
	Treated (40)	0.27	0.42	0.35	0.00
	Control (39)	1.26	1.94	2.08	0.14

TABLE XVII

WEANING CALVES AT COALGATE, OKLAHOMA

Group number	Total number of grubs per lot					
	2 Nov 56	30 Nov 56	30 Dec 56	1 Feb 57	1 Mar 57	Total
1. (10) 1,2,3*	9	44	32	16	4	105
2. (10) 2,3	29	140	115	21	5	310
3. (10) 1,3	13	35	64	22	10	144
4. (10) 3	43	132	114	45	9	343
5. (10) 1,2,3	7	15	38	12	5	77
6. (10) 2,3	50	134	124	21	10	339
7. (10) 1	0	44	42	15	3	104
8. (10)	43	228	164	26	12	473

*1-ET-57 Drench at the rate of 100 mg./kg. on 13 Oct 56.
2-Phenothiazine drench of 4 oz per head on 13 Oct 56.
3-Low level Phenothiazine feeding of 2 grams per day per head.

TABLE XVIII

WEANING CALVES AT COALGATE, OKLAHOMA

Group number	Average weights				
	2 Nov 56	30 Nov 56	30 Dec 56	1 Feb 57	1 Mar 57
1. (10)	468	504	547	564	581
2. (10)	546	561	618	632	642
3. (10)	513	542	601	609	625
4. (10)	502	524	572	587	608
5. (10)	485	518	564	578	603
6. (10)	515	535	591	620	640
7. (10)	480	504	555	581	598
8. (10)	521	539	590	609	629

Group #5 showed 84 per cent control; this group received the combination of all three treatments as did group no. 1.

In group #6 29 per cent control was found; this group received the phenothiazine drench and low level phenothiazine feeding of 2 grams per day per head but no ET-57 as in the case of group no. 2.

Group #7 showed 79 per cent control; this group received the ET-57 treatment only.

Group #8, also made up of 10 animals which had received no treatment of any kind, was found to have a total of 473 grubs over the five month grub counting period.

There appeared to be no significance in the weight gains of the preceding groups (Table XVIII).

Treatment at Codding Ranch, Foraker and Perkins, Oklahoma--This test consisted of 526 animals which had been divided into five different groups.

In the first group, located at Perkins, Oklahoma, 104 mature cows were treated with boluses of ET-57 at the rate of 100 mg./kg. and 23 cows remained untreated to serve as controls. At a grub count made on January 5, 1957, 74 per cent control was found. These cows had been treated on October 10, 1956 (Table XIX).

Of the other groups, all located at Foraker, Oklahoma, the first group consisting of 43 cows which were treated with ET-57 capsules at the rate of 100 mg./kg. showed 82 per cent control. In the second group consisting of 39 bolus treated cows 67 per cent control was found. The second group had been treated on October 10, 1956 and the count made on January 5, 1957. The third group which also received treatment and grub counts at the same time the other groups had, was treated with an ET-57

TABLE XIX

COWS AT THE CODDING RANCH, PERKINS, OKLAHOMA, TREATED 13 OCT 56 WITH ET-57 BOLUSES

	Average grubs per animal
	5 Jan 57
Bolus treated cows (104)	1.83
Untreated controls (23)	6.80

TABLE XX

COWS AND HEIFERS AT THE CODDING RANCH, FORAKER, OKLAHOMA, TREATED 13 OCT 56

		Average grubs per animal
		5 Jan 57
Capsule treated cows	(43)	2.5
Controls	(81)	13.5
Bolus treated cows	(39)	4.5
Controls	(81)	13.5
Drench treated Heifers	(39)	1.5
Controls	(39)	12.8
Bolus treated Heifers	(53)	2.0
Controls	(104)	18.3

Drench. The third group was made up of 39 treated brood heifers and 39 untreated brood heifers and showed 89 per cent control. The fourth group was made up of 53 two year old heifers which had been treated with boluses at the rate of 100 mg./kg. and 104 two year old heifers which were untreated controls. There was 90 per cent control in the fourth group (Table XX).

CHAPTER V

DISCUSSION

Laboratory Phase

The laboratory work was comprised of four different tests, each of which consisted of giving the Dow materials ET-57, ET-58 and ET-59 in capsule and drench form at various concentrations.

Test #1 consisted of administering ET-57 at 100 mg./kg., ET-58 at 40 mg./kg. and ET-59 at 35 mg./kg., all in a capsular form. None of the C. lectularius, Linn., or A. aegypti, (Linn), were killed when fed on the rabbits 3,6,9,12,24 and 48 hours after the rabbits had been treated; therefore, it appeared that the materials did not go into the blood of the rabbits or, if there, they were not at a sufficiently high level to cause mortality in the test insects.

Consequently it was decided that the materials would best be absorbed by the rabbits if they were first dissolved then given as a drench, as was done in test #2. Test #2 resulted in 100 per cent mortality of the test insects fed 3,6,& 9 hours after treatment of the host rabbit with ET-57. There was 85 per cent mortality 12 hours after treatment and no mortality 24 and 48 hours after treatment. No insects fed on rabbits receiving ET-58 and ET-59 and of course the control rabbit were killed.

This indicated that ET-57 became systemic in the rabbit when administered in a dissolved form at the rate of 100 mg./kg.; at least to the extent that C. lectularius, Linn. and A. aegypti, (Linn) were killed

3,6,9, and 12 hours after the treatment. However, the mortality dropped to 85 per cent at the 12th hour indicating that the material was losing its activity. At the 24 and 48 hour feedings no mortality occurred, indicating a complete loss of insecticidal activity.

In test #3, the dosages of ET-57, ET-58 and ET-59 were increased to 150 mg./kg., 60 mg./kg., and 52.5 mg./kg. respectively. In this experiment no mortality occurred in any of the test insects.

ET-57 was not active at 150 mg./kg. when it was active at 100 mg./kg. possibly because of individual differences between rabbits or experimental error.

In test #4 the original dosages of ET-57, ET-58 and ET-59 were doubled to 200 mg./kg., 80 mg./kg. and 70 mg./kg. respectively.

The test insects fed on the ET-57 treated rabbit began to die in the amount of 70 per cent at the 3 hour feeding and 100 per cent mortality occurred at the 6,9 and 12 hour feeding. At the 24 hour feeding 85 per cent of the insects were killed and none were killed at the 48 hour feeding. In the case of ET-57 this higher dosage became insecticidally active more slowly than the lower dosage but stayed active a few hours longer. ET-58 and ET-59 showed slight insecticidal activity at this higher dosage, but only at the 3 hour feeding. It would seem that a far larger dosage of these materials could be given, possibly close to the 200 mg./kg. level, with results closely resembling ET-57's insecticidal activity in rabbits.

Field Phase

At Stillwater, Oklahoma, ET-57 was given as a drench and in capsular form and ET-58 and ET-59 were given as a drench. In addition to this,

ET-57 was given at a high level to induce symptoms of poisoning in four steers.

It was noted that the ET-57 drench at 100 mg./kg., ET-58 at 40 mg./kg. and ET-59 at 35 mg./kg. caused poisoning symptoms in the steers in this test. However, ET-59 appeared to be the most toxic of the compounds to steers with much less severe symptoms being produced in the animals treated with ET-57 and ET-58. As the poisoning symptoms came on four days after treatment, which is a longer time than in the case of other organic phosphate poisonings, it might be postulated that the toxicity was not due entirely to the chemicals but due, in part, to the type of feed that the steers were on. The silage which had made up a large part of the ration had been found to have a high content of cyanide tied up in it.

The results with ET-57, ET-58 and ET-59 were quite positive, in that all three of the materials reduced the grub population. A statistical analysis illustrated with the multiple range test showed ET-57 drench, ET-57 capsules and ET-59 drench to be significantly different in their ability to control grubs as compared to the ET-58 drench (Figure 4).

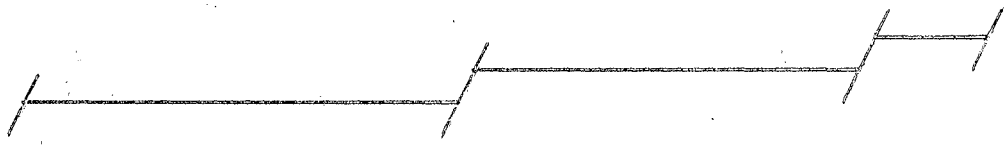
ET-59 gave the most dramatic results with close to 100 per cent grub control; however, there was a marked decrease in weight of the animals treated with ET-59 when weighed one month after treatment.

At Fort Supply, Oklahoma, 44 cows and 42 steers were treated with ET-57 capsules at the rate of 100 mg./kg. and a like number were untreated controls. As is shown in Tables VII and VIII, it might be said almost conclusively, that the reason for fewer grubs in the treated animals was that they had received treatment with ET-57. A statistical analysis of variance which recognized each treatment in each lot as a replicate of

	ET-59 Drench	ET-57 Capsules	ET-57 Drench	ET-58 Drench
Mean	.77	1.22	1.24	2.07

Figure 4. The Multiple Range Test which uses the Mean in Grub Population to Illustrate Significant Differences Between Groups, Here Indicates that ET-58 Controlled Fewer Grubs than the Other Three Materials. At Stillwater, Oklahoma

Group No.	5	7	1	3	6	4	2	8
Treatment*	1,2	1	1,2,3	1,3	2	3	2,3	0
Mean	8.67	10.50	10.50	16.00	33.90	34.30	34.44	55.25



*1-ET-57 Drench; 2-Phenothiazine Drench; 3-Low Level Phenothiazine Feeding

Figure 5. The Multiple Range Test here Illustrates the Significant Differences Between the ET-57 Treated Animals, the Phenothiazine Treated Animals and the Untreated Animals. At Coalgate, Oklahoma.

Group No.	5	7	1	3	6	4	2	8
Treatment*	1,2	1	1,2,3,	1,3	2	3	2,3	0
Mean	95.11	105.70	111.38	111.78	112.80	117.44	118.40	124.50

*1-ET-57 Drench; 2-Phenothiazine Drench; 3-Low Level Phenothiazine Feeding

Figure 6. The Multiple Range Test here Illustrates the fact that there was no Significant Difference in Weight Gains Between the Different Groups of Animals at Coalgate, Oklahoma

the experiment, further substantiates this. In both the cows and steers in this test, control of grubs was well over 80 per cent.

At Fort Reno, Oklahoma, 185 animals were treated with an ET-57 drench and 185 animals remained untreated to serve as controls. These animals were divided into five different groups, each of which was divided into several lots or replicates as is illustrated in Tables IX through XIII. In each case the analysis of variance pointed to the fact that ET-57 was the reason for the grub control which ranged from 75 per cent in the mature cows to well over 80 per cent in the bull calves. Table XIV, which gives the weights of the bull calves, shows no significant difference in their weights.

At Lake Carl Blackwell, Oklahoma, 50, 3 1/2 year old cows and 40, 5 1/2 year old cows were treated with ET-57 as a drench while 50, 3 1/2 year old and 38, 5 1/2 year old cows were untreated controls. It is shown in Tables XV and XVI that each lot appears as a replicate of 10 animals each. In this test it is clear that grubs were controlled by ET-57, however the percentage control was lower in these older animals.

At Coalgate, Oklahoma, the experiment was divided into eight groups, each of which consisted of 10 animals. Table XVII illustrated the various treatments and the gross grub counts which indicates that the animals which had received ET-57 had fewer grubs than those that had not. In statistical analysis, illustrated with the multiple range test (Figure 5), significantly better control of grubs in the odd numbered group of animals which received only ET-57 or it in combination with a phenothiazine drench or the low level feeding of phenothiazine as compared to even numbered groups of animals which received a phenothiazine drench or low level feeding of phenothiazine, a combination of the two or no

treatment of any kind. Again, there was a significant difference in grub population between the three groups receiving a phenothiazine drench, low level feeding or both in combination as compared to the group receiving no treatment.

There was no significant difference in weight gains in all groups as illustrated in the multiple range test (Figure 6).

The treatment at the Codding Ranch, Foraker and Perkins, Oklahoma, consisting of bolus, capsule and drench administration of ET-57, gave very good control of grubs as is shown in Table XIX and XX. This experiment, which was conducted as part of a private enterprise, seems to point out the fact that ET-57 appears to be an effective tool in grub control when in the hands of the cattle producer.

Throughout these tests a marked difference in control of grubs was found when ET-57 was given to animals under two years old as compared to animals over 2 years old. In the animals under two years old there was an average of 82 per cent control while in the animals over two years old there was an average of 75 per cent control.

There was also, in most cases, a high percentage control one month after treatment with a relative drop in control in the following months. This indicated that some of the grubs were not killed when the materials were given. It is likely that the very young grubs escaped poisoning because of their small fat content as compared to the grubs which are migrating to the back and are storing great amounts of fat for the coming pupal and adult stages.

CHAPTER VI

SUMMARY AND CONCLUSIONS

Three compounds designated as ET-57, ET-58 and ET-59 were administered orally in capsule form to rabbits at 100, 40 and 35 mg./kg. respectively. Bioassay tests in which the bedbugs and yellow fever mosquitoes were allowed to feed on the rabbits up to 48 hours after treatment indicated either that insufficient amounts were present in the blood stream to kill the test insects or that the compounds had not entered the blood stream. On the other hand when the ET-57 was dissolved in acetone and administered orally to rabbits all of the test insects were killed as a result of feeding 3, 6 and 9 hours after treatment. The mortality dropped to 85 per cent control 24 and 48 hours after treatment. ET-58 and ET-59 proved to be non toxic to the test insects at this dosage.

When ET-57, ET-58 and ET-59, which had been dissolved in acetone, were administered to rabbits at 200, 80 and 70 mg./kg. respectively the mortality was the same as in the previous test but in the case of the ET-57 treatment came on more slowly. ET-58 and ET-59 caused only slight mortality in the test insects.

When ET-57 was administered to cattle at the rate of 100 mg./kg. in several different forms, 82 per cent control was the result in animals under two years old. In older animals there was an average of 75 per cent control.

ET-58 administered at the rate of 40 mg./kg. gave approximately 70 per cent control which is well below the control in ET-57 treated animals. There were also symptoms of mild toxicity appearing in ET-58 treated animals.

There was well over 90 per cent control of grubs in animals receiving ET-59 at the rate of 35 mg./kg. but symptoms of severe toxicity appeared in these animals. Two steers died as a result of that treatment.

Although, much is not known about the mode of action of animals systemic insecticides, these experiments point out that ET-57 is a material capable of controlling the cattle grub while being relatively non-toxic to animals. ET-58 seemed to be less capable of controlling the cattle grubs; however, a higher dosage of the material in a larger group of animals may point to it as being an even more effective material than ET-57. ET-59 gave dramatic results in cattle grub control but was dangerously toxic in this test.

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