THE EFFECT OF ALFALFA DEHYDRATION UPON INSECTICIDE RESIDUES OF ALDRIN, CHLORDANE, PARATHION, AND TOXAPHENE

by

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INTRODUCTION

Alfalfa is grown in the United States on approximately 15 million acres of land and produces over 100 million tons of hay per year. Alfalfa is rated as the most important forage crop grown in the United States.

The year 1949 found farmers using, in an ever increasing amount, a wide variety of new synthetic organic insecticides for the control of insects attacking their alfalfa. With the use of these new insecticides came the problem of insecticide residues. These residues are known to remain on the foliage because of their persistent toxicity to insects. The problem of how long these residues last and what finally becomes of them has been studied by a number of workers. This problem is important to the United States public, for alfalfa is fed to livestock whose flesh and milk, in the case of dairy cows, are consumed ultimately by man.

The problem of what happens to insecticide residues on alfalfa when it undergoes dehydration was suggested to the writer by Dr. Paul A. Dahm, Assistant Professor of Entomology, Kansas State College. Dehydrated alfalfa is used solely by the mixed-feeds industry for the production of high protein, high vitamin A feeds. These feeds are used to put a finish on livestock before they are sent to market, therefore, it is highly possible that any insecticide residue present in the alfalfa meal may find its way to market in the animal tissues.

It is the purpose of this study to observe the effect of dehydration of alfalfa on the residues of aldrin, chlordane, parathion and toxaphene.

REVIEW OF LITERATURE

Aldrin (Lidov et al. 1950) is one of the latest of the new insecticides to be placed on the market by Julius Hyman and Company, Denver, Colorado.[1] The compound is of such recent origin that very little information concerning it exists in the literature. Danish and Lidov (1950) have developed a colorimetric method for estimating small amounts of aldrin.

Chlordane, which has been produced commercially for several years, has been used to control several insects attacking alfalfa.[2] Brett and Rhoades (1948) used a five percent chlordane dust to control grasshoppers (Melanoplus mexicanus and M. differentialis) in alfalfa. Since then chlordane has become one of the insecticides recommended by the U.S.D.A., Bureau of Entomology and Plant Quarantine (1948) for the control of grasshoppers. Studies of chlordane toxicity to white rats were made by Ingle (1947). He found that weight for weight chlordane was of the same order of toxicity to white rats as DDT. DDT has an LD_{50} of 225-250 mg per kg to white rats. Ingle states that the lapse of time between the acute lethal dose and death was longer for chlordane. The exact reason for

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[1] Aldrin is the name for an insecticidal product having not less than 95 percent of its principal constituent, the chemical 1, 2, 3, 4, 10, 10-hexachloro -1, 4, 4a, 5, 8, 8a- hexahydro -1, 4, 5, 8- dimethanonaphthalene.

[2] Chlordane is the common name for a chlorinated hydrocarbon having the chemical name 1, 2, 4, 5, 6, 7, 8, 8- octachloro -4, 7- methano -3a, 4, 7, 7a- tetahydroindans.

the toxicity of the polychloro hydrocarbons to insects is unknown. Martin and Wain (1944) advanced the hypothesis that the insecticidal activity of a polychloro hydrocarbon compound is due to the ability of the compound to liberate HCl at the site of action. A study was made by Cristol (1950) of the insecticidal activity and the rate of dehydroclorination of some polychloro insecticides. Among the compounds he studied were chlordane and aldrin. Cristol could find no relationship between the rate of dehydrochlorination of these two compounds and their insecticidal activity. Stohlman and Smith (1950) injected white rats and rabbits intravenously with chlordane and checked the urine for an increase in the chlorine content. They established that an unidentified chlorine compound was excreted but this degradation product was not identified.

Ginsburg et al. (1949) experimented with parathion residues on alfalfa.[3] They applied a five percent parathion dust to alfalfa and analyzed for the residue at various intervals after application. They used the Averell and Norris (1948) method in their analysis of parathion residues. Only 0.53 ppm (parts per million) of parathion was found when they cut, dried for three or four days, and then analyzed the sample. In addition, they found 30.4 ppm of parathion on alfalfa which had not been subjected to air drying before analysis and 7.72 ppm on alfalfa which was air dried. Thus a considerable amount of the parathion

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[3]Parathion is the common name for O, O- diethyl O, p-nitrophenyl thiophosphate.

residue appears to be lost in the drying process. Ginsburg et al. (1950) continued their studies in 1949 and found a residue of 47.2 ppm of parathion on alfalfa which had been dusted with a one percent parathion dust, sampled and analyzed on the same day. Samples from the same plot analyzed 6 days later showed only 0.32 ppm of parathion.

Hoskins (1949) analyzed alfalfa for parathion which had been applied by an airplane. A one percent parathion dust was applied to the alfalfa at the rate of 0.25 lb of actual parathion per acre; the alfalfa was analyzed five days later and showed the presence of 0.70 ppm of parathion. A two percent parathion dust was applied at the rate of 0.4 lb of actual parathion per acre; the alfalfa was analyzed five days later and showed the presence of 1.8 ppm of parathion. These data indicate that residues of parathion applied at normal rates for the control of insects after a period of 10 to 16 days are almost nil.

Dahm et al. (1950) fed parathion in capsules to dairy cows at the rates of one and five ppm of parathion, based upon the roughage dry matter intake of the cows, and checked to see if parathion was excreted in the milk. Parathion was not found in the milk at either of these feeding levels or when the rate of parathion was increased for a short period to 40 ppm.

Laakso and Johnson (1949) studied the residues of toxaphene on alfalfa.[4]

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[4]Toxaphene is a chlorinated camphene having an average empirical formula of $C_{10}H_{10}Cl_8$. It contains from 67 to 69 percent chlorine and melts in the range of 70° to 95° C.

Two experimental plots at Bozeman, Montana, sprayed with two pounds of actual toxaphene per acre (emulsifiable concentrate) and sampled within 24 hours showed residues of 0.50 lb and 0.42 lb per acre. Samples taken 31 days later from one of these plots showed a residue of 0.18 of a pound of toxaphene per acre. This indicates that residues of toxaphene on alfalfa persist for long periods of time. Diephius and Dunn (1949) found that when steers and sheep were fed alfalfa hay, sprayed with toxaphene at various rates, the toxaphene accumulated in the muscles and fatty tissues of their bodies. When the same steers and sheep were no longer fed toxaphene sprayed hay they gradually eliminated the accumulated toxaphene from their body tissues. This is an important fact for it shows that, if the intake of toxaphene is stopped, the animals can eliminate the residues from their bodies; thus the build up of toxaphene residues in the body tissues for infinite periods of time is averted.

MATERIALS AND METHODS

The procedures used for applying and analyzing the residues are essentially the same for the four insecticides, aldrin, chlordane, parathion and toxaphene. The general procedure is described and any deviations from that procedure are mentioned under the corresponding insecticide.

Application of the Insecticides

In order to study the effect of alfalfa dehydration on insecticide residues, the insecticides were applied to the alfalfa immediately ahead of the cutter. By applying the insecticide in this manner, the maximum amount of the insecticide would be present on the foliage at the time of dehydration. Each of the insecticides was applied at the recommended rate for the control of alfalfa insects and these application rates were as follows: aldrin, 0.5 lb per acre (25% emulsifiable concentrate from Julius Hyman & Co., Denver, Colorado); chlordane, 1.5 lb per acre (74% emulsifiable concentrate from Julius Hyman & Co., Denver, Colorado); parathion, 0.5 lb per acre (15% wettable powder formulated by Thompson-Hayward Chemical Company, Kansas City, Missouri); and toxaphene, 2.25 lb per acre (65% emulsifiable concentrate from William Cooper & Nephews, Inc., Chicago, Illinois). Application was made on August 24, 1949, with a John Bean hydraulic sprayer (Farm Protector model 4-E) equipped with a five foot boom and operated at a pressure of approximately 50 pounds per square inch. The insecticides were applied in the following order: chlordane, toxaphene, aldrin, and parathion. To avoid contamination of one insecticide with the other insecticides they were applied to the alfalfa field in the following manner. The cutter started at the edge of the field and cut counter-clockwise around the periphery of the field, gradually working its way to the center of the nearly rectangular field. One load was cut and taken to the dehydrator

before the first insecticide was applied. The first insecticide was applied and while this load of sprayed alfalfa was being taken to the dehydrator a load of unsprayed alfalfa was cut. This placed a load of untreated alfalfa between each load of treated alfalfa. With a five foot boom a distance of 2.4 miles had to be driven in order to cover an area of one and one half acres. Speedometer readings taken before and after each application showed that all applications were made within 0.1 of a mile of that distance. This provided enough alfalfa for sampling before and after dehydration.

The Alfalfa Dehydrating Process

The standard procedure in dehydrating alfalfa (Griffiths, 1949) is to cut and chop the alfalfa in the field. The chopped hay is then blown into a truck and hauled to the dehydrator where it is dumped into an automatic feeder. All operations are automatic after it reaches the dehydrator. The alfalfa is fed into a rotating drum into which is drawn a blast of hot air. The alfalfa passes the length of the drum three times by a moving air stream before being drawn out the opposite end. The temperature of the feeder end of the drum ranges from 900° F. to 1100° F., depending upon the moisture content of the alfalfa. The temperature at the outlet end of the drum ranges from 250° F. to 270° F. The temperatures at the feeder end of the drum, when the sprayed alfalfa was dehydrated, are as follows: for

chlordane, 925° F.; for aldrin, parathion and toxaphene, 900° F. The dried alfalfa passes through a series of pipes and collectors which cool the alfalfa before it is pulverized by a hammermill and sacked.

Sampling Methods

The process of chopping and blowing the alfalfa into the truck thoroughly mixed the sprayed alfalfa. The alfalfa was hauled to the dehydrator and dumped into the automatic feeder. Here, by the use of a pitch fork, the load was divided into four parts and large samples of approximately 800 grams were taken from each part. These samples were labeled "Field Samples 1, 2, 3, and 4." The time required for alfalfa fed into the rotating drum to reach the hammermill was approximately three minutes. An interval of five minutes was allowed from the time sprayed alfalfa started into the drum until samples were taken. The dehydrated samples were taken from the delivery spout just before the dried alfalfa entered the hammermill. As near as possible a sample was taken from each of the four parts of the load. These were labeled "Dehydrated Samples 1, 2, 3, and 4."

Two untreated alfalfa samples were taken and two untreated dehydrated samples were taken. These samples were from the same field of alfalfa and were treated in the same manner as the sprayed samples.

Extraction of the Insecticide Residues from the Alfalfa

All samples were extracted the same day they were sprayed. The longest period of time from cutting the alfalfa in the field until the time of extraction was six hours. Each sample was treated in the following manner. The approximately 800 gram sample of alfalfa was spread on a table, thoroughly mixed, quartered and a weighed 200 gram portion was placed in a five quart glass jar. To the jar were added 800 grams of benzene, the jar was sealed, and fastened on the extracting machine, (Plate I). The lids of the jars had replaceable aluminum foil discs which were replaced each time the lids were used so one insecticide would not contaminate another. The extracting machine had a capacity of eight jars and rotated at the rate of approximately 50 revolutions per minute. The jars were rotated for one hour; the jars were removed and the benzene extract filtered into a one quart fruit jar. The weight of the filtered extract was recorded, then the extraction jar and the filter were rinsed with 100 grams of benzene. The fruit jar containing extract and the rinsings was then labeled, sealed with a lid provided with aluminum foil and stored in the absence of light until biological assays could be made.

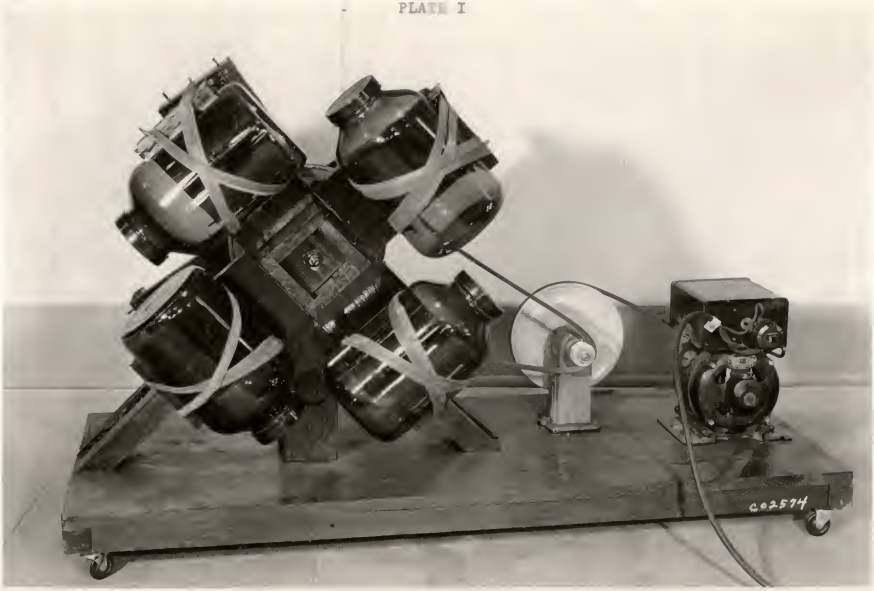
Determination of the Moisture Content of the Samples

The moisture content of the samples was determined as follows. The portions of each set (a set was considered to

EXPLANATION OF PLATE I

The Extracting machine used to wash the residues from the foliage of the alfalfa.

PLATE I



be the four samples of any insecticide) of field samples not used for extraction with benzene were mixed and a 200 gram sample was weighed and placed in an electric oven set at 105° C for 36 hours. It was determined that this period of time and this temperature were sufficient to dry the sample to a constant weight. After 36 hours the moisture content was calculated and recorded. Three field samples of alfalfa were dried. The moisture contents for these samples were 71.0, 69.8 and 69.3 percent; the average moisture content was 70.1 percent. Therefore, a value of 30 percent dry weight was used for all calculations involving field samples. The dehydrating process dried the samples to such a low moisture content that further drying was not possible. Therefore, the actual sample weights of the dehydrated samples were used as dry weight in all calculations involving dehydrated samples.

Preparation of Sample for Biological Assays

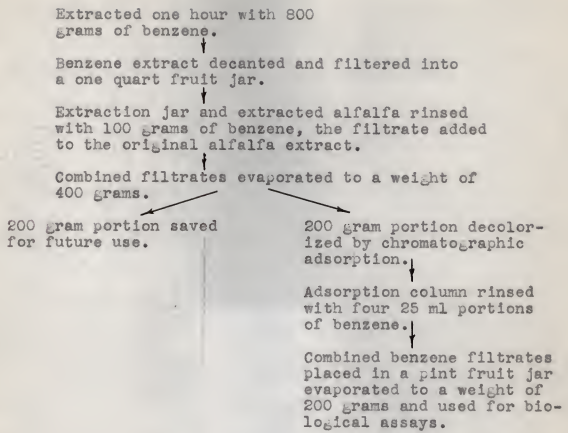
The fruit jars containing the alfalfa extract and the rinsings were prepared for biological assay in the following manner. The initial benzene extract and the rinsings were evaporated to 400 grams by the use of a gentle jet of air. This concentrated extract was then divided into two 200 gram portions, one portion was saved for future use and the other portion was decolorized. It was necessary to decolorize the extracts to remove the green and yellow-green material which sometimes interferes with the

insecticide residues. The extract was decolorized by filtering (Averell and Norris, 1948) through 25 grams of an absorbent mixture consisting of two parts of Attapulgus clay and one part of Hyflo-Super-Cel which had been well mixed.[5] The adsorption column was prepared by placing a small cotton plug in the bottom of a 500 ml seperatory funnel, and 25 grams of the Attapulgus clay mixture were added, then 80 ml of benzene were added and the mixture stirred until the clay was completely wetted and free of air bubbles. The benzene was drawn through by suction almost to the surface of the adsorbent. The 200 gram portion of the alfalfa extract was then poured into the funnel and drawn through the adsorbent. The adsorbent was then rinsed with four 25 ml portions of benzene. The decolorized extract was poured into a pint fruit jar, labeled and then evaporated to the original 200 grams. The jar was sealed with a lid which had an aluminum foil disc to prevent contamination. The alfalfa extracts were then ready to be used for biological assays. The following diagram summarizes the extraction and preparation procedures used for the field samples, the dehydrated samples and the untreated check samples:

200 gram sample of alfalfa ↓ (sprayed field alfalfa (dehydrated alfalfa (untreated field alfalfa

(continued on next page)

[5] Hyflo-Super-Cel. A product of Johns Manville Company. Attapulgus clay (200/up mesh) manufactured by the Attapulgus Clay Company, Philadelphia, Pennsylvania.



Biological Assay Method

The biological assay of an insecticide consists essentially of a comparison of the percentage kill produced on the test insects by the toxicants of unknown concentration with the percentage kill produced by known concentrations of the same toxicants. The standard solutions (known concentrations) used in these biological assays consisted of a solution of each insecticide in benzene. The solvent used to make the standard solutions should be the same as the solvent used to extract the unknown solutions (Gnadinger, 1945). Several different dosages

of the standard solution were run with each set of assays. The regression of the percent mortality on dosage of the standard solution was calculated by the method of least squares and plotted on graph paper (Snedecor, 1946). It was found that the regression of mortality-probits on dosage of the standard solution gave a better regression line than did the regression of percentage mortality on dosage of the standard solution, therefore, in all subsequent tests the percent mortality was converted to probits, then the regression line was calculated by the method of least squares. The mortality probits produced by benzene solutions of the alfalfa extracts were then compared with this standard regression line to obtain an estimation of the strength of the insecticide present in the benzene extracts of alfalfa. The insecticides used to make up the standard solutions were: aldrin, technical grade (95 percent aldrin) sample #4156, furnished by Julius Hyman & Company, Denver, Colorado; chlordane, technical grade (refined) no sample number, furnished by Julius Hyman & Company, Denver, Colorado; parathion, technical grade (98.2 percent parathion) batch #66, furnished by the American Cyanimid Company, New York, New York; and toxaphene, technical grade (67-69 percent Cl) #X6508-47, furnished by the Hercules Powder Company, Wilmington, Delaware. The temperature of the benzene was kept at 25° C. while the standard solutions were made.

The test insect used in these biological assays was the common housefly (Musca domestica Linn). The flies were reared

according to the official method of the National Association of Insecticide and Disinfectant Manufacturers, Inc. (Soap Blue Book, 1948). Three to four day old adult flies were used in all the tests.

Preliminary tests, using aldrin as the insecticide, were run to establish a uniform method for biological assay of the alfalfa extracts. The biological assay method outlined by Dahm and Pankaskie (1949) was tried. Excessive kills were obtained in the jars containing 3 ml to 10 ml of decolorized benzene extracts of untreated alfalfa; however, no mortalities occurred in jars containing 5 ml to 15 ml of a benzene-corn oil solution (20 mg corn oil/ml benzene). This led the writer to believe that either some compound was present in the decolorized alfalfa extract which was toxic to the flies or that small amounts of waxes and oils in the alfalfa extract prevented full evaporation of the benzene which in turn was lethal to the flies. Small amounts of alfalfa extract which were not decolorized were not only lethal to flies, but also gave erratic results if known amounts of aldrin were added.

The writer found that by using the bottoms of 100 mm petri dishes the alfalfa extract was much more easily evaporated and that the petri dish provided a more even surface over which the residue could be spread. The petri dishes were fitted with a wire cage (A and B in Plate II). This wire cage was three and three-eighths inches in diameter and eight inches high. There was no special reason for the selection of this size cage,

EXPLANATION OF PLATE II

Fig. A. Position of the cage while the flies were confined near the surface of the residue.

Fig. B. Position of cage during the holding period of 48 hours.

Fig. C. A one-pint Sealrite carton with a screen lid.

Fig. D. The can used for anesthetization of the flies in the one-pint containers.

Fig. E. The container used for collecting the flies to be used for the entire number of tests.

Fig. F. The by-pass in the vacuum line to control the amount of vacuum.

Fig. G. The aspirator used to pick up the flies.

Fig. H. The perforated plastic disc on which the anesthetized flies were spread and counted. The flow of CO_2 can be controlled by the hose clamps to the right.

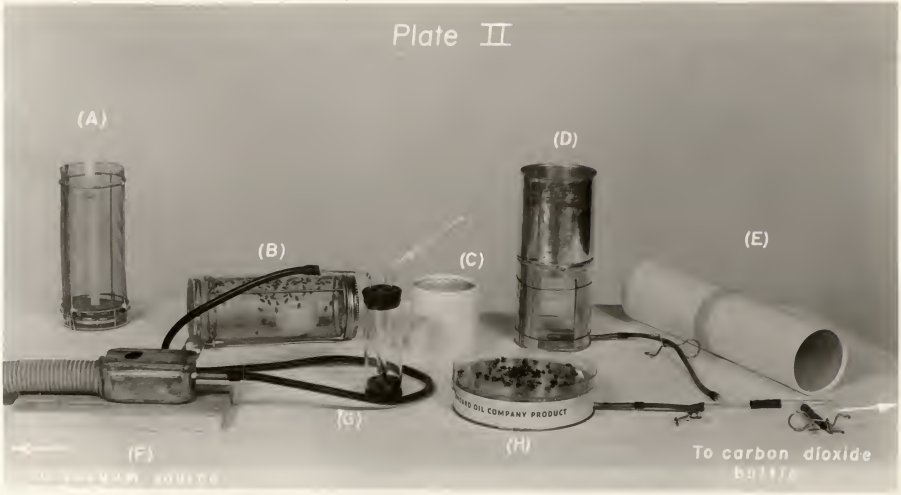


Plate II
(A)
(B)
(C)
(D)
(E)
(F)
(G)
(H)
To carbon dioxide

however, it did supply enough area so that food could be supplied to the flies in souffle cups yet left enough room so the dead flies could be counted easily.

Further investigations were made as to the effect of confining the flies close to the residue in the petri dish for a period of 30 minutes, 1 hour and 2 hours. These tests indicated that very little additional kill was produced by confining the flies close to the residue for more than one hour; therefore, the one hour exposure period was used in all subsequent tests. Preliminary tests were run also to determine the optimum number of flies to use. It was found that when 125 to 160 flies were used per test practically no kill was obtained with residue deposits ranging from 10 to 18 micrograms of aldrin; when 75 to 100 flies were used, kills were obtained but the results at the lower dosages were erratic; when from 40 to 60 flies were used per test the deviations from the calculated regression line were least. Fifty flies per test were used, therefore, in all the biological assays of the insecticide residues.

Tests were run, using aldrin, chlordane, parathion and toxaphene as the insecticides, to determine the value of adding 1 ml of corn oil solution (20 mg/ml corn oil solution) to the decolorized alfalfa extract residues as a sticker. The results showed that with residues of high dosages of the insecticides no significant difference occurred; however, in the medium and lower dosages the addition of corn oil definitely increased the mortality. Therefore, in all subsequent assays, 1 ml of corn

oil solution was added to each test residue.

The normal dosage mortality curve is sigmoidal. This S-shaped curve makes the extreme high and low values, for both dosage and mortality, difficult to use. It was the aim to use as much of each decolorized alfalfa extract to produce a kill between 20 percent and 80 percent; this range would avoid those extreme values. For best results the high and the low dosages of the standard solution should equal, or slightly exceed, the range of percentage kills obtained by the various dosages of the decolorized extracts. To find the approximate dosage, in the case of the standard solutions, and amounts, in the case of the decolorized field and dehydrated samples, trial assays were always run. When these dosages and amounts were determined, the actual assays for the residues were run.

The decolorized field extracts and the decolorized dehydrated extracts for one of the insecticides were assayed on the same day. This made it possible to compare them to the same regression line. Usually 15 to 16 tests were run on the same day. This day's "set" of tests comprised four decolorized field samples, four decolorized dehydrated samples, a decolorized untreated field sample, a decolorized untreated dehydrated sample, and five or six dosages of the standard solution. The general procedure was to number the clean petri dishes (washed with benzene, Dreft, then rinsed with acetone) with a waxed pencil; these numbers were recorded. One ml of corn oil solution was added to each petri dish. Predetermined amounts of the

decolorized field and dehydrated samples were added to each petri dish. The amount used and the sample name and number were recorded along with the corresponding number of the petri dish. Predetermined dosages of the standard solution were added to the petri dishes and the dosage recorded. At this point, in the handling of the extracts, volumetric measure replaced gravimetric measure. Because of this change, the temperature of the extracts and the standard solution were always brought to 25° C., then the solutions were measured with a pipette and transferred to the petri dishes. The extracts and the standard solutions were evaporated by a gentle stream of air. The extracts were let stand for one hour even though it took only from 10 to 20 minutes for them to evaporate. From time to time, the unevaporated portion of the benzene solutions were shaken to distribute the residue evenly over the bottom of the petri dish. After one hour the petri dishes were each fitted to a cage (a rubber band held the petri dish on the cage) and the cages were then ready to receive the flies.

Three to four day old adult flies designated for tests were collected in a container (E in Plate II). The container used was made by removing the bottoms from two one-quart Sealrite containers then taping them together. Each end was provided with a screen lid. The flies were anesthetized with carbon dioxide (Williams, 1946). The anesthetized flies were then distributed among 16 one-pint Sealrite containers, each of which had been provided with a screen lid (C in Plate II).

The flies revived quickly and were checked to make sure none had been injured during the transfer. When flies were needed, a pint container containing approximately 60 flies was placed in a can (D in Plate II) and the carbon dioxide valve to the can opened. The CO_2 would flow into the can and within a few seconds the flies were again anesthetized. The container was removed and the flies were poured on a perforated plastic disc (H in Plate II). By regulating the flow of CO_2 the flies remained quiet so they could be counted. Fifty flies were counted as they were picked up by an aspirator (G in Plate II). The vacuum for the aspirator was furnished by a vacuum cleaner. The amount of vacuum could be regulated with a by-pass (F in Plate II). The flies were then emptied from the aspirator into the test cage. The cage was set on end and a plunger confined the flies within one inch of the residue on the petri dish (A in Plate II). This procedure was repeated until all of the tests for that day had flies on the residues. After one hour the flies were again anesthetized, the plunger removed, the cage laid on its side and a souffle cup (Lily-Tulip Souffle Cup No. 50PB) containing sugar water absorbed in Cellu-cotton to prevent drownings was placed in the cage. The cage was covered by a wire lid. The cages were then placed in a cabinet. This cabinet maintained a temperature of 80 degrees Fahrenheit ($\pm 2^{\circ}$ F.) and a relative humidity of 50 percent ($\pm 5\%$).

Twenty-four and fourty-eight hour mortality counts were made and recorded.

Estimation of the Amount of Insecticide per Gram Dry Weight of Alfalfa

The percent mortality produced by a known volume of decolorized extract of either the field or dehydrated alfalfa was always converted to probits. This probit value could be substituted in the regression equation or read from the regression line; either method gave the estimated amount of insecticide present in the known volume of the decolorized extract. Varying amounts of the decolorized extracts were used; therefore, in order to compare assays to each other, a common unit was needed. The best method was to express each test in terms of the amount of insecticide present per gram dry weight of the alfalfa. The following formula was used.

$$\frac{A \times 400}{B \times 0.87} = \text{the estimated amount of insecticide present in 400 grams of alfalfa benzene extract.}$$

Where:

A = micrograms of insecticide in B as determined by referring the resulting mortality in probits to the calculated standard regression line.
B = the number of ml of extract used in the test.
400 = the number of grams of alfalfa extract.
0.87 = the specific gravity of benzene at 25° C.

This gave the amount of insecticide present in 400 grams of alfalfa benzene extract. The 400 grams represents 200 grams of dry alfalfa in the case of dehydrated alfalfa samples. Thus, the amount of insecticide estimated to be present in the 400 grams of the alfalfa benzene extract divided by 200 gives the estimated amount of insecticide present per gram dry weight of dehydrated alfalfa. In the case of field alfalfa samples, the

amount of insecticide present in 400 grams of alfalfa extract represents the amount of insecticide present on 60 grams of dry alfalfa (i.e. 30% of 200 grams field alfalfa). So this figure is divided by 60 grams to obtain the estimated amount of insecticide present per gram dry weight of field alfalfa.

RESULTS

Biological assays of the aldrin samples were made on February 21, 22, and 27, 1950 and March 4, 1950. The results of individual assays are shown in Tables 1 to 4. The regression lines for the aldrin standard are shown in Fig. 1. A summary of the parts per million of aldrin present in each sample is shown in Table 5. These data show that alfalfa dehydration decreased the aldrin content of the alfalfa from 45 ppm to 14 ppm.

Biological assays of the chlordane samples were made on March 7, 13, 19, 22, 25, 1950, and April 8, 16, and 17, 1950. Eight biological assays were made on the chlordane extracts for several reasons. The field sample assays on March 7th and March 13th were not valid because the failure of the writer to evaporate the decolorized field extract plus the 100 ml of benzene rinsings to a weight of 200 grams. The error was noticed, the samples were evaporated to the correct weight, and biological assaying was resumed on March 19, 1950. This summary showed a large decrease in the chlordane content of the alfalfa. To be absolutely sure the decrease was due to the samples and

Table 1. Data for aldrin residue tests on Feb. 22, 1950

A. Determination of the regression curve from dosage-mortality data obtained by using various dosages of the standard benzene solution.

Dosage of standard solution :	Mortality		:Calculated regression : curve values
(X) micrograms	%	(Y) Probits	$\hat{Y}$
3	12	3.83	3.84
5	16	4.01	4.32
7	58	5.20	4.79
9	66	5.41	5.27
11	70	5.52	5.75

B. Regression equation:[1] $\hat{Y} = 0.239X + 3.121$

C. Data.

	% Mortality	Probits $\hat{Y}$	Micrograms of insecticide as read from the standard curve X
1.0 ml Field sample #1	38	4.69	6.6
1.5 ml Field sample #2	52	5.02	8.0
1.0 ml Field sample #3	20	4.16	4.4
1.5 ml Field sample #4	22	4.23	4.7
1.0 ml Dehydrated sample #1	38	4.69	6.6
1.5 ml Dehydrated sample #2	36	4.64	6.4
1.0 ml Dehydrated sample #3	40 -	4.75	6.8
1.0 ml Dehydrated sample #4	30	4.48	5.7

Micrograms per gram dry weight of alfalfa

Field sample #1	50.6	Dehydrated sample #1	15.2
Field sample #2	40.9	Dehydrated sample #2	9.8
Field sample #3	33.7	Dehydrated sample #3	15.6
Field sample #4	24.0	Dehydrated sample #4	13.1
Total	148.2	Total	53.7
Average	37.0	Average	13.4

[1] Calculated by method of least squares.

Table 2. Data for aldrin residue tests on February 24, 1950

A. Determination of the regression curve from dosage-mortality data obtained by using various dosages of the standard benzene solution.

Dosage of standard solution	Mortality		Calculated regression curve values
(X) micrograms	%	(Y) Probits	$\hat{Y}$
3	14	3.92	4.00
5	28	4.42	4.39
7	48	4.95	4.78
9	52	5.05	5.17

B. Regression equation:[1] $\hat{Y} = 0.196X + 3.409$

C. Data.

	% Mortality	Probits	Micrograms of insecticide as read from the standard curve
0.5 ml Field sample #1	16	4.00	3.8
0.5 ml Field sample #2	6	3.45	0.3
1.0 ml Field sample #3	20	4.16	4.3
0.5 ml Field sample #4	10	3.72	1.7
0.5 ml Dehydrated sample #1	24	4.29	4.9
0.5 ml Dehydrated sample #2	10	3.72	1.7
0.5 ml Dehydrated sample #3	30	4.76	7.0
0.5 ml Dehydrated sample #4	20	4.16	3.9

Micrograms per gram dry weight of alfalfa

Field sample #1	58.2	Dehydrated sample #1	22.5
Field sample #2	4.6	Dehydrated sample #2	0.8
Field sample #3	32.9	Dehydrated sample #3	32.2
Field sample #4	26.0	Dehydrated sample #4	17.9
Total	121.7	Total	73.4
Average	30.4	Average	18.3

[1] Calculated by method of least squares.

Table 3. Data for aldrin residue tests on February 27, 1950

A. Determination of the regression curve from dosage-mortality data obtained by using various dosages of the standard benzene solution.

Dosage of standard solution :	Mortality		:Calculated regression : curve values
(X) micrograms	%	(Y) Probits	$\hat{Y}$
3	30	4.48	4.53
5	50	5.00	4.81
7	52	5.05	5.10
9	54	5.10	5.38
11	80	5.84	5.66

B. Regression equation:[1] $\hat{Y} = 0.141X + 4.108$

C. Data

	% Mortality	Probits	Micrograms of insecticide as read from the standard curve
1.0 ml Field sample #1	70	5.52	10.4
1.5 ml Field sample #2	66	5.41	9.2
1.0 ml Field sample #3	50	5.00	6.3
1.5 ml Field sample #4	48	4.95	6.0
1.0 ml Dehydrated sample #1	38	4.69	4.1
1.5 ml Dehydrated sample #2	70	5.52	10.4
1.5 ml Dehydrated sample #3	68	5.47	9.7
1.0 ml Dehydrated sample #4	52	5.05	6.7

Micrograms per gram dry weight of alfalfa

Field sample #1	79.7	Dehydrated sample #1	9.4
Field sample #2	47.0	Dehydrated sample #2	15.9
Field sample #3	48.3	Dehydrated sample #3	14.9
Field sample #4	30.6	Dehydrated sample #4	15.4
Total	205.6	Total	55.6
Average	51.4	Average	13.9

[1] Calculated by method of least squares.

Table 4. Data for aldrin residue tests on March 4, 1950

A. Determination of the regression curve from dosage-mortality data obtained by using various dosages of the standard benzene solution.

Dosage of standard solution :	Mortality :		Calculated regression curve values
(X) micrograms	%	(Y) Probits	$\hat{Y}$
3.0	36	4.64	4.93
5.5	76	5.71	5.39
8.0	80	5.84	5.86
10.5	94	6.55	6.32
13.0	94	6.55	6.79

B. Regression equation:[1] $\hat{Y} = 0.186X + 4.368$

C. Data

	% Mortality	Probits	Micrograms of insecticide as read from the standard curve
1.0 ml Field sample #1	84	5.99	8.8
1.5 ml Field sample #2	94	6.55	11.8
1.0 ml Field sample #3	84	5.99	8.8
1.5 ml Field sample #4	90	6.28	10.4
1.0 ml Dehydrated sample #1	64	5.36	5.3
1.5 ml Dehydrated sample #2	72	5.58	6.6
1.5 ml Dehydrated sample #3	84	5.99	8.8
1.0 ml Dehydrated sample #4	58	5.20	4.5

Micrograms per gram dry weight of alfalfa

Field sample #1	67.4	Dehydrated sample #1	12.2
Field sample #2	60.3	Dehydrated sample #2	10.0
Field sample #3	61.3	Dehydrated sample #3	13.5
Field sample #4	53.1	Dehydrated sample #4	10.3
Total	242.1	Total	46.0
Average	60.5	Average	14.0

[1] Calculated by method of least squares.

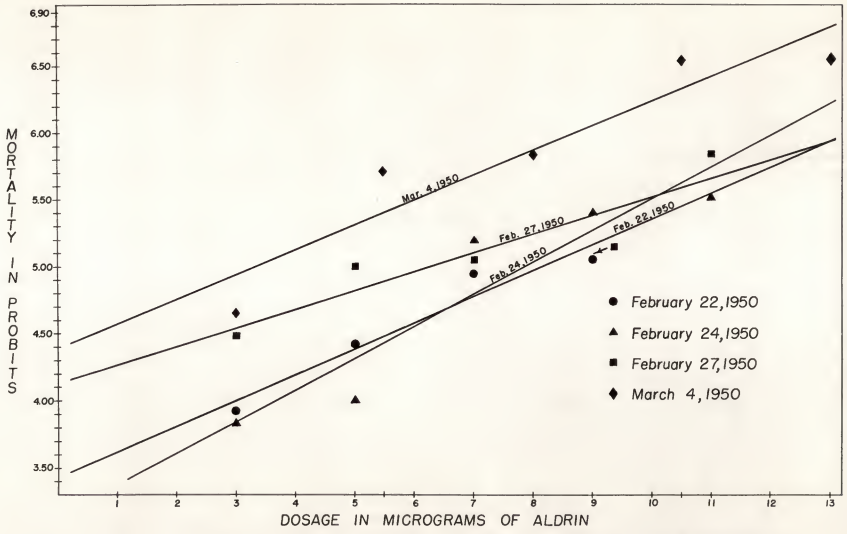


Fig. 1. Regression of mortality-probits on dosage of aldrin - used as standards.

Table 5. A summary of the average PPM of aldrin present in each sample (dry weight of alfalfa).

Sample No.	22 Feb. 50	24 Feb. 50	27 Feb. 50	4 Mar 50	Average
Field sample #1	50.6	58.2	79.9	67.4	64
Field sample #2	40.9	4.6	47.0	60.3	38
Field sample #3	33.7	32.9	48.3	61.3	44
Field sample #4	24.0	26.0	30.6	53.1	33
Average					45
Dehydrated sample #1	15.2	22.5	9.4	12.2	15
Dehydrated sample #2	9.8	0.8	15.9	10.0	9
Dehydrated sample #3	15.6	32.2	14.9	13.5	19
Dehydrated sample #4	13.1	17.9	15.4	10.3	14
Average					14

not to an error in technique, the second 200 gram portion of the alfalfa extract was decolorized and evaporated. Biological assays of these extracts on April 16 and 17 correlated with the other assays made previously. The results of each day's tests are shown in Tables 6 to 13. Figure 2 shows the regression lines for the chlordane standard. Table 14 gives a summary of all the chlordane tests. These data show that the alfalfa dehydrating process reduced the chlordane content from 233 ppm before dehydration to 45 ppm after dehydration.

The application of parathion was poor for the 15 percent wettable powder clogged the spray nozzles and frequent stops to clean out the nozzles were necessary. During the extraction process, field sample #4 came off the extracting machine and the glass jar was broken. The sample could not be repeated for the remaining field sample #4 had been mixed with other alfalfa. Therefore, only three field samples of parathion were used. Biological assays of parathion samples were made on the following dates: March 29 and 31, 1950, and April 3 and 5, 1950. The results of individual assays are shown in Tables 15 to 18. The regression lines for the parathion standard are shown in Fig. 3. A summary of the parts per million present in each sample is shown in Table 19. These data show that the alfalfa dehydration process decreased the parathion content from 43 ppm before dehydration to 7 ppm after dehydration.

Biological assays of toxaphene samples were made on April 10, 11, 12 and 13, 1950. The results of individual assays are

Table 6. Data for chlordane residue tests on Mar. 7, 1950.

A. Determination of the regression curve from dosage-mortality data obtained by using various dosages of the standard benzene solution.

Dosage of standard solution :	Mortality		:Calculated regression curve values
(X) micrograms	%	(Y) Probits	$\hat{Y}$
14	14	3.92	3.81
20	16	4.00	4.16
26	32	4.53	4.51
32	46	4.90	4.86

B. Regression equation:[1] $\hat{Y} = 0.058X + 3.003$

C. Data

	% Mortality	Probits Y	Micrograms of insecticide as read from the standard curve X
1.0 ml Dehydrated sample #1	22	4.23	21
1.0 ml Dehydrated sample #2	28	4.42	24
1.0 ml Dehydrated sample #3	12	3.82	14
1.0 ml Dehydrated sample #4	22	4.23	21

Micrograms per gram dry weight of alfalfa

Dehydrated sample #1	48.3
Dehydrated sample #2	55.2
Dehydrated sample #3	32.2
Dehydrated sample #4	48.3
Total	184.0
Average	46.0

[1] Calculated by method of least squares.

Table 7. Data for chlordane residue tests on March 13, 1950.

A. Determination of the regression curve from dosage-mortality data obtained by using various dosages of the standard benzene solution.

Dosage of standard solution	Mortality		Calculated regression curve values
(X) micrograms	%	(Y) Probits	$\hat{Y}$
20	44	4.85	4.75
26	48	4.95	4.98
32	54	5.10	5.21
38	62	5.30	5.44
42	78	5.77	5.59

B. Regression equation:[1] $\hat{Y} = 0.038X + 3.993$

C. Data

	% Mortality	Probits	Micrograms of insecticide as read from the standard curve
1.5 ml Dehydrated sample #1	56	5.15	30
1.5 ml Dehydrated sample #2	46	4.90	24
2.0 ml Dehydrated sample #3	50	5.00	27
1.5 ml Dehydrated sample #4	54	5.10	28

Micrograms per gram dry weight of alfalfa

Dehydrated sample #1	46.0
Dehydrated sample #2	36.8
Dehydrated sample #3	31.0
Dehydrated sample #4	42.9
Total	156.7
Average	39.2

[1] Calculated by method of least squares.

Table 8. Data for chlordane residue tests on March 19, 1950.

A. Determination of the regression curve from dosage-mortality data obtained by using various dosages of the standard benzene solution.

Dosage of standard solution	Mortality		Calculated regression curve values
(X) micrograms	%	(Y) Probits	$\hat{Y}$
20	28	4.42	4.50
26	48	4.95	4.84
32	60	5.25	5.19
38	64	5.36	5.53
42	80	5.84	5.76

B. Regression equation:[1] $\hat{Y} = 0.058X + 4.344$

C. Data

	% Mortality	Probits	Micrograms of insecticide as read from the standard curve
1.5 ml Field sample #1	80	5.84	43
1.5 ml Field sample #2	74	5.64	40
1.5 ml Field sample #3	76	5.71	41
1.5 ml Field sample #4	86	6.08	48
1.5 ml Dehydrated sample #1	62	5.30	34
1.5 ml Dehydrated sample #2	50	5.00	29
2.0 ml Dehydrated sample #3	72	5.58	39
1.5 ml Dehydrated sample #4	66	5.41	36

Micrograms per gram dry weight of alfalfa

Field sample #1	219.7	Dehydrated sample #1	52.1
Field sample #2	204.3	Dehydrated sample #2	44.4
Field sample #3	209.4	Dehydrated sample #3	44.8
Field sample #4	245.2	Dehydrated sample #4	55.2
Total	878.6	Total	196.5
Average	219.6	Average	49.1

[1] Calculated by method of least squares.

Table 9. Data for chlordane residue tests on March 22, 1950.

A. Determination of the regression curve from dosage-mortality data obtained by using various dosages of the standard benzene solution.

Dosage of standard solution :	Mortality		:Calculated regression curve values
(X) micrograms	%	(Y) Probits	$\hat{Y}$
20	30	4.48	4.35
26	38	4.69	4.75
32	50	5.00	5.15
38	68	5.47	5.55
42	84	5.99	5.82

B. Regression equation:[1] $\hat{Y} = 0.067X + 3.015$

C. Data

	% Mortality	Probits	Micrograms of insecticide as read from the standard curve
1.0 ml Field sample #1	38	4.69	25
1.0 ml Field sample #2	64	5.36	35
1.0 ml Field sample #3	32	4.53	23
1.0 ml Field sample #4	24	4.29	19
1.5 ml Dehydrated sample #1	22	4.23	18
1.5 ml Dehydrated sample #2	40	4.75	26
1.5 ml Dehydrated sample #3	42	4.80	27
1.5 ml Dehydrated sample #4	42	4.80	27

Micrograms per gram dry weight of alfalfa

Field sample #1	191.6	Dehydrated sample #1	27.6
Field sample #2	268.2	Dehydrated sample #2	39.8
Field sample #3	176.2	Dehydrated sample #3	41.4
Field sample #4	145.6	Dehydrated sample #4	41.4
Total	781.6	Total	150.2
Average	195.4	Average	37.5

[1] Calculated by method of least squares.

Table 10. Data for chlordane residue tests on March 25, 1950.

A. Determination of the regression curve from dosage-mortality data obtained by using various dosages of the standard benzene solution.

Dosage of standard solution : micrograms (X)	Mortality : %	Mortality : Probits (Y)	Calculated regression curve values $\hat{Y}$
20	32	4.53	4.51
26	46	4.90	4.80
32	50	5.00	5.09
38	60	5.25	5.38
42	76	5.71	5.57

B. Regression equation:[1] $\hat{Y} = 0.048X + 3.551$

C. Data

	% Mortality	Probits	Micrograms of insecticide as read from the standard curve
1.0 ml Field sample #1	44	4.85	27
1.0 ml Field sample #2	46	4.90	28
1.0 ml Field sample #3	48	4.95	29
1.0 ml Field sample #4	68	5.47	42
1.5 ml Dehydrated sample #1	40	4.75	25
1.5 ml Dehydrated sample #2	38	4.69	24
1.5 ml Dehydrated sample #3	20	4.16	13
1.5 ml Dehydrated sample #4	22	4.23	14

Micrograms per gram dry weight of alfalfa

Field sample #1	206.9	Dehydrated sample #1	38.3
Field sample #2	214.6	Dehydrated sample #2	36.8
Field sample #3	222.2	Dehydrated sample #3	19.9
Field sample #4	321.8	Dehydrated sample #4	21.5
Total	965.5	Total	116.5
Average	241.4	Average	29.1

[1] Calculated by method of least squares.

Table 11. Data for chlordane residue tests on April 8, 1950.

A. Determination of the regression curve from dosage-mortality data obtained by using various dosages of the standard benzene solution

Dosage of standard solution	Mortality		Calculated regression curve values
(X) micrograms	%	(Y) Probits	$\hat{Y}$
16	16	4.00	3.00
24	20	4.16	4.01
32	52	5.05	5.02
40	80	5.84	6.02
48	96	6.75	7.03

B. Regression equation:[1] $\hat{Y} = 0.126X + 0.994$

C. Data

	% Mortality	Probits	Micrograms of insecticides as read from the standard curve
1.0 ml Field sample #1	76	5.71	38
1.0 ml Field sample #2	82	5.91	39
1.0 ml Field sample #3	46	4.90	31
1.0 ml Field sample #4	50	5.00	32
1.5 ml Dehydrated sample #1	78	5.77	38
1.5 ml Dehydrated sample #2	70	5.52	36
1.5 ml Dehydrated sample #3	64	5.36	35
1.5 ml Dehydrated sample #4	58	5.20	33

Micrograms per gram dry weight of alfalfa

Field sample #1	291.2	Dehydrated sample #1	58.2
Field sample #2	298.8	Dehydrated sample #2	55.2
Field sample #3	237.5	Dehydrated sample #3	53.6
Field sample #4	245.2	Dehydrated sample #4	50.6
Total	1072.7	Total	217.6
Average	268.2	Average	54.4

[1]Calculated by method of least squares.

Table 12. Data for chlordane residue tests on April 16, 1950

A. Determination of the regression curve from dosage-mortality data obtained by using various dosages of the standard benzene solution

Dosage of standard solution	Mortality		Calculated regression curve values
(X) micrograms	%	(Y) Probits	$\hat{Y}$
16	18	4.08	4.22
24	36	4.64	4.74
32	68	5.47	5.27
40	74	5.64	5.79
48	92	6.40	6.31

B. Regression equation:[1] $\hat{Y} = 0.065X + 3.170$

C. Data

	% Mortality	Probits	Micrograms of insecticides as read from the standard curve
1.0 ml Field sample #1	70	5.52	35
1.0 ml Field sample #2	50	5.00	27
1.0 ml Field sample #3	46	4.90	26
1.0 ml Field sample #4	58	5.20	31
1.5 ml Dehydrated sample #1	52	5.05	28
1.5 ml Dehydrated sample #2	66	5.41	34
1.5 ml Dehydrated sample #3	48	4.95	27
1.5 ml Dehydrated sample #4	38	4.69	23

Micrograms per gram dry weight of alfalfa

Field sample #1	268.2	Dehydrated sample #1	42.9
Field sample #2	206.9	Dehydrated sample #2	52.1
Field sample #3	199.2	Dehydrated sample #3	41.3
Field sample #4	237.5	Dehydrated sample #4	35.2
Total	911.8	Total	171.5
Average	227.9	Average	42.9

[1] Calculated by method of least squares.

Table 13. Data for chlordane residue tests on April 17, 1950.

A. Determination of the regression curve from dosage-mortality data obtained by using various dosages of the standard benzene solution.

Dosage of standard solution : micrograms (X)	Mortality : %	Probits (Y)	Calculated regression curve values : $\hat{Y}$
16	36	4.64	4.51
24	38	4.69	4.95
32	70	5.52	5.39
40	80	5.84	5.84
48	90	6.28	6.28

B. Regression equation:[1] $\hat{Y} = 0.055X + 3.621$

C. Data

	% Mortality	Probits	Micrograms of insecticides as read from the standard curve
1.0 ml Field sample #1	42	4.80	20
1.0 ml Field sample #2	82	5.91	41
1.0 ml Field sample #3	50	5.00	25
1.0 ml Field sample #4	82	5.91	41
1.5 ml Dehydrated sample #1	78	5.77	39
1.5 ml Dehydrated sample #2	88	6.17	45
1.5 ml Dehydrated sample #3	80	5.84	40
1.5 ml Dehydrated sample #4	60	5.25	28

Micrograms per grams dry weight of alfalfa

Field sample #1	153.3	Dehydrated sample #1	59.8
Field sample #2	314.2	Dehydrated sample #2	69.0
Field sample #3	191.6	Dehydrated sample #3	61.3
Field sample #4	314.2	Dehydrated sample #4	42.9
Total	973.3	Total	233.0
Average	243.3	Average	58.2

[1] Calculated by method of least squares.

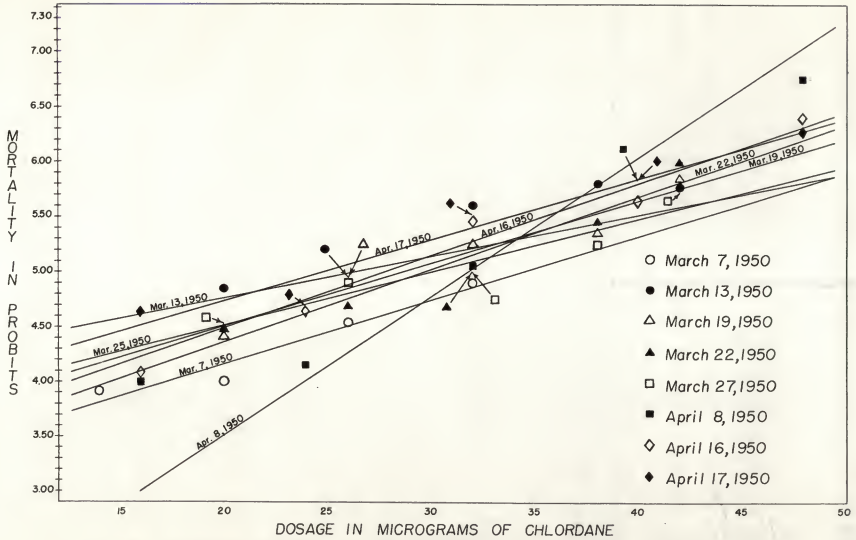


Fig. 2. Regression of mortality-probits on dosage of chlordane - used as standards.

Table 14. A summary of the ppm of chlordane present in each sample (dry weight of alfalfa).

Sample No.	March 1950					April 1950			Average
	7	13	19	22	25	8	16	17	
Field sample #1			219.7	191.6	206.9	291.2	268.2	153.3	222
Field sample #2			204.3	268.2	214.6	298.8	206.9	314.2	251
Field sample #3			209.4	176.2	222.2	231.5	199.2	191.6	206
Field sample #4			245.2	145.6	321.8	245.2	237.5	314.2	252
Average									233
Dehydrated sample #1	48.3	46.0	52.1	27.6	38.3	58.2	42.9	59.8	47
sample #2	55.2	36.8	44.4	39.8	36.8	55.2	52.1	69.0	49
sample #3	32.2	31.0	44.8	41.4	19.9	53.6	41.9	61.3	41
sample #4	48.3	42.9	55.2	41.4	21.5	50.6	35.2	42.9	42
Average									45

Table 15. Data for parathion residue tests on March 29, 1950.

A. Determination of the regression curve from dosage-mortality data obtained by using various dosages of the standard benzene solution.

Dosage of standard solution :	Mortality		:Calculated regression : curve values
(X) micrograms	%	(Y) Probits	$\hat{Y}$
1	4	3.25	3.24
3	6	3.44	3.93
5	56	5.15	4.63
7	72	5.58	5.33
10	86	6.08	6.37

B. Regression equation:[1] $\hat{Y} = 0.348X + 2.891$

C. Data

	% Mortality	Probits $\hat{Y}$	Micrograms of insecticide as read from the standard curve X
1.5 ml Field sample #1	48	4.95	5.9
1.5 ml Field sample #2	98	7.05	11.9
1.5 ml Field sample #3	96	6.75	11.1
2.0 ml Dehydrated sample #1	32	4.53	4.7
2.0 ml Dehydrated sample #2	44	4.85	5.6
2.0 ml Dehydrated sample #3	72	5.58	7.7
2.0 ml Dehydrated sample #4	58	5.20	6.6

Micrograms per gram dry weight of alfalfa

Field sample #1	30.1	Dehydrated sample #1	5.4
Field sample #2	60.8	Dehydrated sample #2	6.4
Field sample #3	56.7	Dehydrated sample #3	8.8
		Dehydrated sample #4	7.6
Total	147.6	Total	28.2
Average	49.2	Average	6.5

[1] Calculated by method of least squares.

Table 16. Data for parathion residue tests on March 31, 1950.

A. Determination of the regression curve from dosage-mortality data obtained by using various dosages of the standard benzene solution.

Dosage of standard solution :	Mortality		:Calculated regression curve values
(X) micrograms	%	(Y) Probits	$\hat{Y}$
2	6	3.44	3.60
4	28	4.42	4.51
6	82	5.91	5.42
8	90	6.28	6.33
10	98	7.05	7.24

B. Regression equation:[1] $\hat{Y} = 0.454X + 2.696$

C. Data

	% Mortality	Probits	Micrograms of insecticide as read from the standard curve
		$\hat{Y}$	X
1.5 ml Field sample #1	68	5.47	6.1
1.5 ml Field sample #2	90	6.28	7.9
1.5 ml Field sample #3	67	5.44	6.0
2.0 ml Dehydrated sample #1	26	4.36	3.7
2.0 ml Dehydrated sample #2	52	5.05	5.2
1.5 ml Dehydrated sample #3	26	4.36	3.7
2.0 ml Dehydrated sample #4	36	4.64	4.3

Micrograms per gram dry weight of alfalfa

Field sample #1	31.2	Dehydrated sample #1	4.2
Field sample #2	40.4	Dehydrated sample #2	6.0
Field sample #3	30.7	Dehydrated sample #3	5.7
		Dehydrated sample #4	4.9
Total	102.3	Total	20.8
Average	34.1	Average	5.2

[1] Calculated by method of least squares.

Table 17. Data for parathion residue tests on April 3, 1950.

A. Determination of the regression curve from dosage-mortality data obtained by using various dosages of the standard benzene solution.

Dosage of standard solution	Mortality		Calculated regression curve values
(X) micrograms	%	(Y) Probits	$\hat{Y}$
2	4	3.25	3.66
4	52	5.05	4.59
6	70	5.52	5.52
8	96	6.75	6.45
10	98	7.05	7.38

B. Regression equation:[1] $\hat{Y} = 0.465X + 2.734$

C. Data

	% Mortality	Probits	Micrograms of insecticide as read from the standard curve
		$\hat{Y}$	X
1.5 ml Field sample #1	26	4.36	3.5
1.0 ml Field sample #2	66	5.41	5.8
1.0 ml Field sample #3	76	5.71	6.4
2.0 ml Dehydrated sample #1	28	4.42	3.6
2.0 ml Dehydrated sample #2	84	5.99	6.2
1.5 ml Dehydrated sample #3	30	4.48	3.8
2.0 ml Dehydrated sample #4	44	4.85	4.6

Micrograms per gram dry weight of alfalfa

Field sample #1	17.9	Dehydrated sample #1	4.1
Field sample #2	44.4	Dehydrated sample #2	7.1
Field sample #3	49.0	Dehydrated sample #3	5.8
		Dehydrated sample #4	5.3
Total	111.3	Total	22.3
Average	37.1	Average	5.1

[1] Calculated by method of least squares.

Table 18. Data for parathion residue tests on April 5, 1950.

A. Determination of the regression curve from dosage-mortality data obtained by using various dosages of the standard benzene solution.

Dosage of standard solution :	Mortality		:Calculated regression curve values
(X) micrograms	%	(Y) Probits	$\hat{Y}$
2	20	4.16	4.06
4	60	5.25	4.82
6	64	5.36	5.58
8	86	6.08	6.34
10	98	7.05	7.10

B. Regression equation:[1] $\hat{Y} = 0.380X + 3.297$

C. Data

	% Mortality	Probits	Micrograms of insecticide as read from the standard curve
		$\hat{Y}$	X
1.5 ml Field sample #1	82	5.91	6.9
1.0 ml Field sample #2	88	6.17	7.6
1.0 ml Field sample #3	86	6.08	7.3
2.0 ml Dehydrated sample #1	64	5.36	5.4
2.0 ml Dehydrated sample #2	90	6.28	7.9
1.5 ml Dehydrated sample #3	48	4.95	4.4
2.0 ml Dehydrated sample #4	90	6.28	7.9

Micrograms per gram dry weight of alfalfa

Field sample #1	35.2	Dehydrated sample #1	6.2
Field sample #2	58.2	Dehydrated sample #2	9.1
Field sample #3	55.9	Dehydrated sample #3	6.7
		Dehydrated sample #4	9.1
Total	149.3	Total	31.1
Average	49.8	Average	7.8

[1] Calculated by method of least squares.

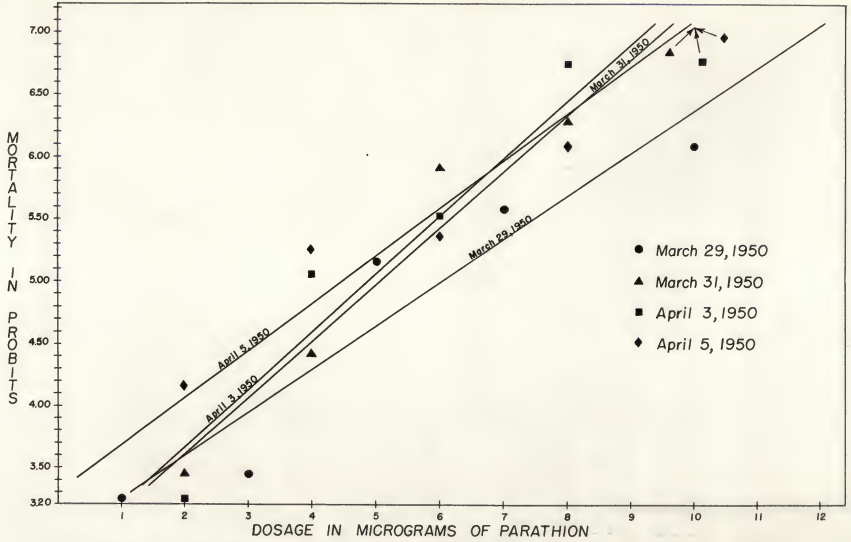


Fig. 3. Regression of mortality-probits on dosage of parathion - used as standards.

Table 19. A summary of the average ppm of parathion present in each sample (dry weight of alfalfa).

Sample No.	29 Mar. 50	31 Mar. 50	3 Apr. 50	5 Apr. 50	Average
Field sample #1	30.1	31.2	17.9	35.2	29
Field sample #2	60.8	40.4	44.4	58.2	51
Field sample #3	56.7	30.7	49.0	55.9	48
Average					43
Dehydrated sample #1	5.4	4.2	4.1	6.2	5
Dehydrated sample #2	6.4	6.0	7.1	9.1	7
Dehydrated sample #3	8.8	5.7	5.8	6.7	8
Dehydrated sample #4	7.6	4.9	5.3	9.1	7
Average					7

shown in Tables 20 to 23. The regression lines for the toxaphene standard are shown in Fig. 4. A summary of the ppm of toxaphene present in each sample is shown in Table 24. These data show that the alfalfa dehydrating process decreased the toxaphene content of the alfalfa from 188 ppm before dehydration to 64 ppm after dehydration.

A comparison of the effect of alfalfa dehydration on the residues of aldrin, chlordane, parathion and toxaphene are shown in Table 25.

Table 20. Data for toxaphene residue tests on April 10, 1950.

A. Determination of the regression curve from dosage-mortality data obtained by using various dosages of the standard benzene solution.

Dosage of standard solution	Mortality		Calculated regression curve values
(X) micrograms	%	(Y) Probits	$\hat{Y}$
24	10	3.72	3.66
36	18	4.08	4.01
48	24	4.29	4.37
60	34	4.59	4.72
72	50	5.00	5.07
84	72	5.58	5.42

B. Regression equation:[1] $\hat{Y} = 0.029X + 2.956$

C. Data

	% Mortality	Probits $\hat{Y}$	Micrograms of insecticide as read from the standard curve X
2.5 ml Field sample #1	46	4.90	66
2.5 ml Field sample #2	46	4.90	66
3.0 ml Field sample #3	48	4.95	68
3.0 ml Field sample #4	14	3.92	33
2.5 ml Dehydrated sample #1	56	5.15	75
2.0 ml Dehydrated sample #2	58	5.20	77
2.5 ml Dehydrated sample #3	18	4.08	38
2.5 ml Dehydrated sample #4	36	4.64	57

Micrograms per gram dry weight of alfalfa

Field sample #1	202.3	Dehydrated sample #1	69.0
Field sample #2	202.3	Dehydrated sample #2	88.5
Field sample #3	173.7	Dehydrated sample #3	34.9
Field sample #4	84.3	Dehydrated sample #4	52.4
Total	662.6	Total	244.8
Average	160.6	Average	61.2

[1] Calculated by method of least squares.

Table 21. Data for toxaphene residue tests on April 11, 1950.

A. Determination of the regression curve from dosage-mortality data obtained by using various dosages of the standard benzene solution.

Dosage of standard solution	Mortality		Calculated regression curve values
(X) micrograms	%	(Y) Probits	$\hat{Y}$
24	6	3.44	3.63
36	18	4.08	3.96
48	28	4.42	4.30
60	36	4.64	4.63
72	52	5.05	4.96
84	56	5.15	5.30

B. Regression equation:[1] $\hat{Y} = 0.028X + 2.962$

C. Data

	% Mortality	Probits $\hat{Y}$	Micrograms of insecticide as read from the standard curve X
2.5 ml Field sample #1	26	4.36	50
2.5 ml Field sample #2	56	5.15	79
3.0 ml Field sample #3	46	4.90	69
4.0 ml Field sample #4	46	4.90	69
2.5 ml Dehydrated sample #1	62	5.30	84
2.5 ml Dehydrated sample #2	60	5.25	81
3.0 ml Dehydrated sample #3	48	4.95	71
2.5 ml Dehydrated sample #4	28	4.42	53

Micrograms per gram dry weight of alfalfa

Field sample #1	153.3	Dehydrated sample #1	77.2
Field sample #2	242.1	Dehydrated sample #2	74.5
Field sample #3	176.2	Dehydrated sample #3	54.4
Field sample #4	132.2	Dehydrated sample #4	48.7
Total	703.8	Total	254.8
Average	175.9	Average	63.7

[1] Calculated by method of least squares.

Table 22. Data for toxaphene residue tests on April 12, 1950.

A. Determination of the regression curve from dosage-mortality data obtained by using various dosages of the standard benzene solution.

Dosage of standard solution	Mortality		Calculated regression curve values
(X) micrograms	%	(Y) Probits	$\hat{Y}$
24	6	3.44	3.46
36	10	3.72	3.85
48	30	4.48	4.24
60	38	4.69	4.64
72	44	4.85	5.03
84	68	5.47	5.42

B. Regression equation:[1] $\hat{Y} = 0.033X + 2.676$

C. Data

	% Mortality	Probits	Micrograms of insecticide as read from the standard curve
		$\hat{Y}$	X
3.0 ml Field sample #1	48	4.95	70
2.5 ml Field sample #2	78	5.81	96
3.0 ml Field sample #3	50	5.00	71
4.0 ml Field sample #4	80	6.08	105
2.5 ml Dehydrated sample #1	70	5.52	87
2.5 ml Dehydrated sample #2	82	5.88	89
3.0 ml Dehydrated sample #3	68	5.47	86
3.0 ml Dehydrated sample #4	61	5.28	80

Micrograms per gram dry weight of alfalfa

Field sample #1	178.8	Dehydrated sample #1	80.0
Field sample #2	294.2	Dehydrated sample #2	81.8
Field sample #3	181.3	Dehydrated sample #3	65.9
Field sample #4	201.1	Dehydrated sample #4	61.3
Total	855.4	Total	289.0
Average	213.8	Average	72.2

[1] Calculated by method of least squares

Table 23. Data for toxaphene residue tests on April 13, 1950.

A. Determination of the regression curve from dosage-mortality data obtained by using various dosages of the standard benzene solution.

Dosage of standard solution	Mortality		Calculated regression curve values
(X) micrograms	%	(Y) Probits	$\hat{Y}$
24	4	3.25	3.38
36	14	3.92	3.79
48	26	4.36	4.20
60	32	4.53	4.61
72	44	4.85	5.01
84	70	5.52	5.42

B. Regression equation:[1] $\hat{Y} = 0.034X + 2.569$

C. Data

	% Mortality	Probits	Micrograms of insecticide as read from the standard curve
		$\hat{Y}$	X
3.0 ml Field sample #1	40	4.75	65
2.5 ml Field sample #2	54	5.10	75
3.0 ml Field sample #3	60	5.25	79
4.0 ml Field sample #4	80	5.84	97
2.5 ml Dehydrated sample #1	50	5.00	72
2.5 ml Dehydrated sample #2	36	4.64	61
3.0 ml Dehydrated sample #3	44	4.85	67
3.0 ml Dehydrated sample #4	68	5.47	86

Micrograms per gram dry weight of alfalfa

Field sample #1	166.0	Dehydrated sample #1	66.2
Field sample #2	229.9	Dehydrated sample #2	56.1
Field sample #3	201.8	Dehydrated sample #3	51.3
Field sample #4	185.8	Dehydrated sample #4	65.9
Total	783.5	Total	239.5
Average	195.9	Average	59.9

[1] Calculated by method of least squares.

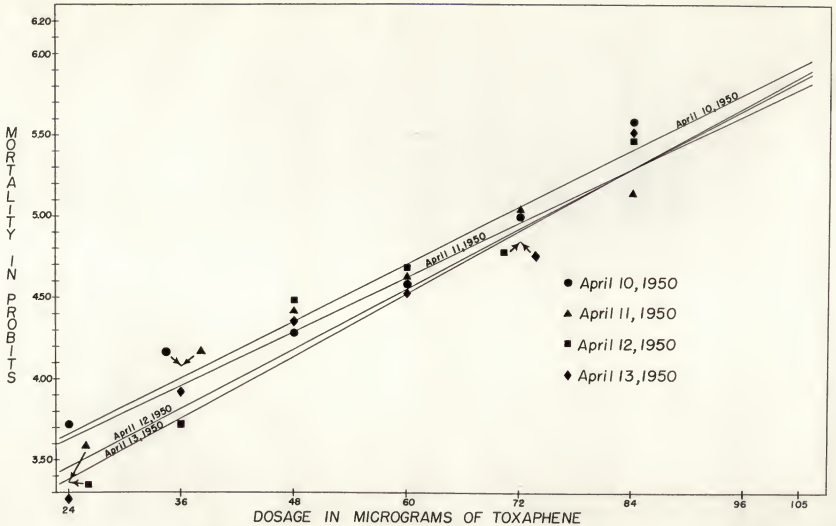


Fig. 4. Regression of mortality-probits on dosage of toxaphene - used as standards.

Table 24. A summary of the ppm of toxaphene present in each sample (dry weight of alfalfa).

Sample No.	10 Apr. 50	11 Apr. 50	12 Apr. 50	13 Apr. 50	Average
Field sample #1	202.3	153.3	178.8	166.0	175
Field sample #2	202.3	242.1	294.2	229.9	242
Field sample #3	173.7	176.2	181.3	201.8	183
Field sample #4	84.3	132.2	201.1	185.8	151
Average					188
Dehydrated sample #1	69.0	77.2	80.0	66.2	73
Dehydrated sample #2	88.5	74.5	81.8	56.1	75
Dehydrated sample #3	34.9	54.4	65.9	51.3	53
Dehydrated sample #4	52.4	48.7	61.3	65.9	57
Average					64

Table 25. A summary of the effect of alfalfa dehydration upon residues of aldrin, chlordane, parathion, and toxaphene.

Insecticide	Formulation	Rate of Application of Insecticide	Recovery of Insecticide		Insecticide Residues		Decrease of Insecticide in Dehydrated Sample
					Field Samples	Dehydrated Samples	
		lb/A	lb/A	%	p.p.m.	p.p.m.	%
Aldrin	25% E.C.*	0.5	0.09	18	45	14	69
Chlordane	74% E.C.	1.5	0.47	31	233	45	81
Parathion	15% W.P.**	0.5	0.09	18	43	7	84
Toxaphene	65% E.C.	2.25	0.38	17	188	64	66

* Emulsifiable concentrate

** Wettable powder

DISCUSSION

To the writer's knowledge, this is the first work with the effect of alfalfa dehydration on insecticide residues. To compare the data from one test with the data of another test is difficult. Different rates of application, different formulations and different methods of application are usually employed. Each has its effect on the residues deposited. In the case of alfalfa, the size of the alfalfa plants at the time of application of the insecticide and at the time the samples are taken are additional factors to increase variation. In a gross way the percentage deposition and the percentage recovery of this experiment compare favorably with the work of Hoskins (1949), Ginsburg et al. (1949, 1950) and Laakso and Johnson (1949). In reviewing these papers, only the toxaphene data can be compared more or less directly with the results obtained in this study. Laakso and Johnson found that a water emulsion of toxaphene (laboratory mixed) applied at the rate of two pounds per acre and sampled 24 hours later gave a residue of 0.42 to 0.50 pound per acre. In this experiment 0.38 pound of toxaphene per acre was recovered.

The work done with the degradation or dissipation of these insecticides by Hoskins, Ginsburg et al. and Laakso and Johnson show that under normal dehydration procedures the insecticide residues of parathion and toxaphene would be much less than the residues used in this experiment. In the case

of parathion, it is highly probable no residue would be left under normal dehydration procedure for if a week has passed after the application of parathion only a few parts per million would be on the alfalfa before dehydration.

Further work will be necessary to determine the amount of residue present after dehydration when normal insect control procedures are practiced.

SUMMARY

In this experiment, a biological assay method for determining the residues of aldrin, chlordane, parathion and toxaphene on alfalfa was developed. The results of over four hundred individual biological assay tests show that residues of aldrin, chlordane, parathion and toxaphene are greatly reduced by the alfalfa dehydration process.

Aldrin was applied to alfalfa as a 25 percent emulsifiable concentrate at the rate of 0.5 pound per acre. The alfalfa was cut and dehydrated immediately. Biological assays showed a decrease of 69 percent in the residue of aldrin due to the alfalfa dehydration process. An average of 45 ppm of aldrin was found on the alfalfa before dehydration and an average of 14 ppm of aldrin was found after dehydration.

Chlordane was applied to alfalfa as a 74 percent emulsifiable concentration at the rate of 1.5 pounds per acre. The alfalfa was cut and dehydrated immediately. Biological assays showed an 81 percent decrease in the chlordane residue due to

the dehydrating process. An average of 233 ppm of chlordane was found before dehydration and an average of 45 ppm of chlordane was found after dehydration.

Parathion was applied to alfalfa as a 15 percent wettable powder at the rate of 0.5 pound per acre. The alfalfa was cut and dehydrated immediately. Biological assays showed a decrease of 84 percent in the parathion residues due to the alfalfa dehydration process. An average of 43 ppm of parathion was found before dehydration and an average of 7 ppm was found after dehydration.

Toxaphene was applied to alfalfa as a 65 percent emulsifiable concentrate at the rate of 2.25 pounds per acre. The alfalfa was cut and dehydrated immediately. Biological assays showed a decrease of 66 percent in the residues of toxaphene due to the alfalfa dehydration process. An average of 188 ppm of toxaphene was found before dehydration and an average of 64 ppm of toxaphene was found after dehydration.

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