Toxaphene residues on alfalfa
by John W Laakso

A THESIS Submitted to the Graduate Committee in partial fulfillment of the requirements for the degree of Master of Science in Chemistry
Montana State University

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The residual properties of a chlorinated camphene insecticide, Toxa-phene, were investigated. Sampling and analysis of Toxaphene-treated alfalfa were conducted for the purpose of determining residuals on the foliage after field application. Analysis was based on the determination of organic chlorides, calculated on basis of pounds per acre and/or p.p.m. Toxaphene. Sampling and extraction methods were extensively studied.

The reproducibility of results obtained by the sodium-isopropanol conversion method were examined. Comparisons were made of initial deposition, percentages and subsequent residuals of various formulations of Toxaphene. Analyses were made of residues in baled alfalfa to determine Toxaphene loss during storage.

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ABSTRACT

The residual properties of a chlorinated camphene insecticide, Toxaphene, were investigated. Sampling and analysis of Toxaphene-treated alfalfa were conducted for the purpose of determining residuals on the foliage after field application. Analysis was based on the determination of organic chlorides, calculated on basis of pounds per acre and/or p.p.m. Toxaphene. Sampling and extraction methods were extensively studied. The reproducibility of results obtained by the sodium-isopropanol conversion method were examined. Comparisons were made of initial deposition percentages and subsequent residuals of various formulations of Toxaphene. Analyses were made of residues in baled alfalfa to determine Toxaphene loss during storage.

INTRODUCTION

One test that must be applied in the evaluation of any new insecticide is its ability to retain its efficacy as an insecticide for longer periods of time. Particularly is this true of insecticdes subject to the weathering effects of sun, wind and rain. In the development and commercial introduction of a new insecticide, as we have in this investigation, it becomes desirable for both manufacturer and consumer to know the stability of the product against the elements of the weather.

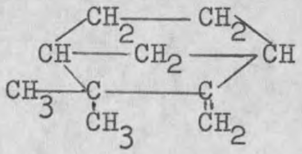
Furthermore it becomes of critical importance to study the problem of the safety of such an insecticide from the point of view of animal husbandry and, indirectly, of human consumption. When a substance, highly effective in destroying, for example, grasshoppers is applied to the foliage of vegetation such as alfalfa, and that alfalfa is fed to livestock it is of utmost importance to know what amounts of toxic material remain on such vegetation at the time of feeding.

For such reasons as these this investigation was undertaken. Insecticides and fungicides had been applied to vegetation such as fruit trees for a long time. The application of insecticides to foliage of vast acreages, however, is a comparatively new development in man's incessant battle against crop devouring insects. Because of the effective nature of this new insect control technique and because of the appearance of a new, highly toxic insecticide it was considered very worthwhile to make a study of its physical behavior.

BACKGROUND

In many of its aspects, the research undertaken had only very limited precedent. Such a problem as the mathematically reliable sampling of a large area, for instance, had to be worked out more or less by the trial and error method. Fortunately, quantitative methods for the determination of organic chlorides had been developed, particularly in DDT analysis (2). These methods were adaptable to the present problem.

The insecticide under study, Toxaphene, is a chlorinated camphene product.[1] The chemical configuration of the Toxaphene molecule is not definitely known. Camphene itself has the following structure:



The camphene has been chlorinated to the extent of 68% chlorine by weight. While it is not the purpose of this paper to prove the structure of Toxaphene, it is, nevertheless, probably that eight chlorine atoms have been added or substituted into the molecule in order to result in a composition of 68% chlorine. It is probable, of course, that drastic changes may have occurred in the nucleus such as degradation in the ring, in which case the number of chlorine atoms could vary considerably.

The literature has been useful from the point of view of a plan of attack in regard to certain difficulties that arose. In other cases no precedent existed. In every case, eliminations, additions, mofifications, even complete revisions had to be applied.

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[1] Developed by the Hercules Powder Co., and given the trade name, Toxaphene.

EXPERIMENTAL METHOD

Location of Test Plots

In order to provide for the widest possible variation in weather and growth conditions, test plots were selected in different sections of Montana; namely, Bozeman, Miles City, and Forsyth. All samples were sent to Montana State College at Bozeman for analysis. Insect counts were made at the outlying fields in order to correlate with residual Toxaphene. No such counts were made on the Bozeman plots since insect control was no problem there.

Weather Data

It was considered highly desirable to have an accurate weather record during the entire period of the investigation. Particularly important factors were: amount of and type of precipitation, percent of sunshine, and average temperatures.

Application Methods

The importance of careful technique and good judgment at this stage of the project can hardly be overestimated. Much of the reliability of the analysis depended on an uniform coverage over the particular plot. The problem resolved into one of using up the entire specified amount of insecticide so that there would be neither plot nor insecticide remaining. This goal was particularly hard to achieve with wettable powder and dust formulations.[1]

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[1] This phase of the program in charge of Dr. J. R. Parker, Entomologist in charge, Bureau of Entomology and Plant Quarantine, U. S. D. A.

An irrigated, 30 acre field of alfalfa was selected at Bozeman. Nine plots of varying acreage were marked off; sufficient area remained to provide for an untreated control area. Applications of Toxaphene follow[1].

Table I.

Plot No.	Formulation	Rate - lbs. Toxaphene/acre	No. of Applications
1	Water emulsion	1	1 (10-in. stage)
2	Water emulsion	2	1 (10-in. stage)
3	Water emulsion	4	1 (10-in. stage)
4	Water emulsion	1	2 (10-in. and prebloom stage)
5	Water emulsion	2	2 (10-in. and prebloom stage)
6	Water emulsion	4	2 (10-in. and prebloom stage)
7	Oil Solution	2	2 (10-in. and prebloom stage)
8	Wettable powder	2	2 (10-in. and prebloom stage)
9	Dust	2	2 (10-in. and prebloom stage)

The three formulations were prepared according to the following scheme:

Water emulsion - 1 lb. Toxaphene in 8 gallons emulsion

Oil solution - 1 lb. Toxaphene in 2 gallons distillate

Wettable powder - 25% Toxaphene

Dust - 10% Toxaphene

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[1] The author has limited this report principally to the second cutting, inasmuch as procedures used in first cutting testing were unsatisfactory and hence modified considerably.

To eliminate as much drift of the spray as possible, the sprayings were accomplished when wind velocity did not exceed 4 or 5 miles per hour. The early forenoon proved to be most calm. Dusting required the presence of dew on the foliage and was executed accordingly.

Application was accomplished by means of a power sprayer mounted on a truck platform. The material was propelled fanwise from the rear. Rate of application could therefore be controlled both by mechanical adjustment and the speed of the truck. Skill and judgment by the operators determined uniformity of application.

To stimulate actual insect control conditions two applications were made on plots 1-9 as indicated in Table I. The two applications were identical in formulation and rate and were spaced 12 days apart.

Sampling

Herein lay one of the most difficult and most important aspects of the entire study. Since residue values were to be determined on the weight-per-acre basis, it became necessary to conduct the sampling on an area basis since a weight basis would manifestly lead to errors due to that variable factor, growth of the plants.

The following scheme was used in the first series of tests with the understanding that modifications would be instituted as necessary: one acre areas were used for samplings. Where the plot contained several acres, a strip one acre in area was marked off and this same strip was used for all subsequent testing. Using a foot-square rigid frame all of the material in 10 square feet was collected from each plot[1]. Care was taken to include all

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[1] To provide a check on sampling error the east and west halves of each plot were tested separately. This procedure was followed throughout the study.

weeds such as dandelion leaves. A zig-zag course was followed through the strip and the frame cast out more or less at random to assure average thickness of growth. The collected material was thoroughly composited, air-dried and analyzed.

It was suspected from the beginning that the initially planned scheme would be inadequate. Such proved to be the case, as the results of the first series of analysis conclusively demonstrated. Evidently the plots could not be sprayed with sufficient uniformity to permit such superficial sampling. A revised scheme was, therefore, worked out and placed on trial.

A two square foot frame was constructed by means of which 80 sq. feet of alfalfa were taken from each acre; that is, 40 squares from each half of the plot. The revised sampling diagram shown in Figure 1 indicates the much greater thoroughness than was applied initially.

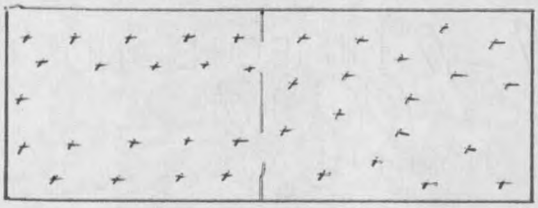

Figure 1.

Each small cross represents two square feet of area. The material from the east and west halves of each plot was handled separately through the entire analysis.

<u>Preparation of sub-samples</u>

The large amounts of material collected for each test sample necessitated extreme care in compositing. The following procedure was finally adopted:

After weighing, the green sample was separated into four approximately equal parts. Each part was then spread evenly in successive layers on a ten foot long table, taking care to keep the stalks horizontal to avoid the "brush heap" effect. The entire process was executed three times. A portion (approximately one-fourth) was selected at random, weighed and air dried for 72 hours[1].

Extraction of Toxaphene from the alfalfa

The problem of extraction proved to be troublesome. Several methods with considerable modifications were tried before a satisfactory procedure was found. The objective was to secure a complete removal of the Toxaphene with a single extraction the first time and yet not use samples so small as to unduly increase experimental error.

A weighed portion (between 120 and 160 grams) of the dried alfalfa was packed into a tin coated wide mouthed can of gallon capacity. 1500 ml. of thiophene-free benzene were added as the extractant. The containers were then fastened to a motor driven tumbling machine whose end-over-end action caused the extractant to flow completely through the material. Prolonged study of extraction time showed 90 minutes to be of sufficient duration. A slow rate of rotation was maintained to insure a more complete action of the benzene.

Chloride determination

In the determination of Toxaphene in benzene extracts, the chloro compounds present are quantitatively determined and these values recalculated

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1. Drying was accomplished in cheesecloth "hammocks". An electric fan furnished the necessary circulation.

on the basis of 68% chlorine in Toxaphene. The chlorine is split out by treatment with metallic sodium-iso-propyl alcohol as in the U. S. Department of Agriculture method for DDT (1) and the chlorine titrated amperometrically.

This method gives about 90% recovery of added Toxaphene, as determined during the investigation. Naturally, the method is not specific for Toxaphene but will include all chlorides of whatever origin[1]. It was therefore necessary to analyze samples of untreated material in conjunction with each set of samples collected and analyzed.

Conversion to inorganic chlorides

The benzene was poured through cotton into a graduated cylinder, the amount recorded[2], and transferred to a one liter flask. By use of a steam bath[3] and an air stream, the benzene was evaporated to 10-75 ml., and then transferred to a 250 ml. ground glass Erlenmeyer flask where evaporation was continued to 5-10 ml. but not to dryness. 25 ml. of isopropanol were added to the flask, followed by 2.5 grams of sodium cut into 1/8 inch cubes. After refluxing for 30 minutes using a ground glass joint condenser, the excess sodium is destroyed by adding 10 ml. of 50% aqueous isopropanol dropwise from a 50 ml. dropping funnel[4]. The mixture was boiled for an addi-

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[1] Possible sources: chlorides present in reagents and in the distilled water and mineral chlorides in the vegetation being tested.

[2] For convenience in calculations a 750 ml. aliquot was evaporated.

[3] An electric hot plate may be used but introduces a fire hazard as well as the possibility of disintegration of the chlorides in case of inadvertent evaporation to dryness.

[4] To preclude the possibility of accident in case the alcohol is added too rapidly it is well to place a safety shield between operator and flask at this point.

tional 10 minutes, removed from the hot plate, and the condenser washed down with 10-15 ml. of distilled water. When cool the solution was acidified with 1 : 1 HNO_3 to the phenolphthalein end point and 10 ml. added in excess. The mixture was again cooled, 10 ml. of 1% gelatin added and sufficient acetone to make a 50% acetone solution. The chlorides present were quantitatively determined using the amperometric titration method.

Titration apparatus

The chlorides which are now in titratable form are determined either amperometrically or by the Volhard method. The former is preferred where the amounts of inorganic chlorides are low and where the color of the solution may interfere with the Volhard. In addition, where large numbers of titrations must be performed the amperometric method presents the advantage of speed.

The essential features of the amperometric apparatus are shown in Figure 2[1]. The circuit consists of a saturated calomel electrode as a reference electrode connected to the electrolytic cell by means of Kcl and KNO_3 salt bridges in that order, a rotating microelectrode, and a microammeter to measure current change. The reference electrode is prepared from an 8 oz. wide mouth bottle fitted with a 2-hole rubber stopper. Sufficient mercury is placed in the bottle to cover the platinum contact unit held in place by the rubber stopper. A layer of calomel, Hg_2Cl_2, is connected to a saturated KNO_3 pool by means of a KCl bridge. The KNO_3 pool in turn makes contact with the electrolytic cell through a KNO_3 salt bridge. The sintered glass

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[1] Used by permission of Floyd Diephius, Hercules Powder Company.

AMPEROMETRIC TITRATION APPARATUS

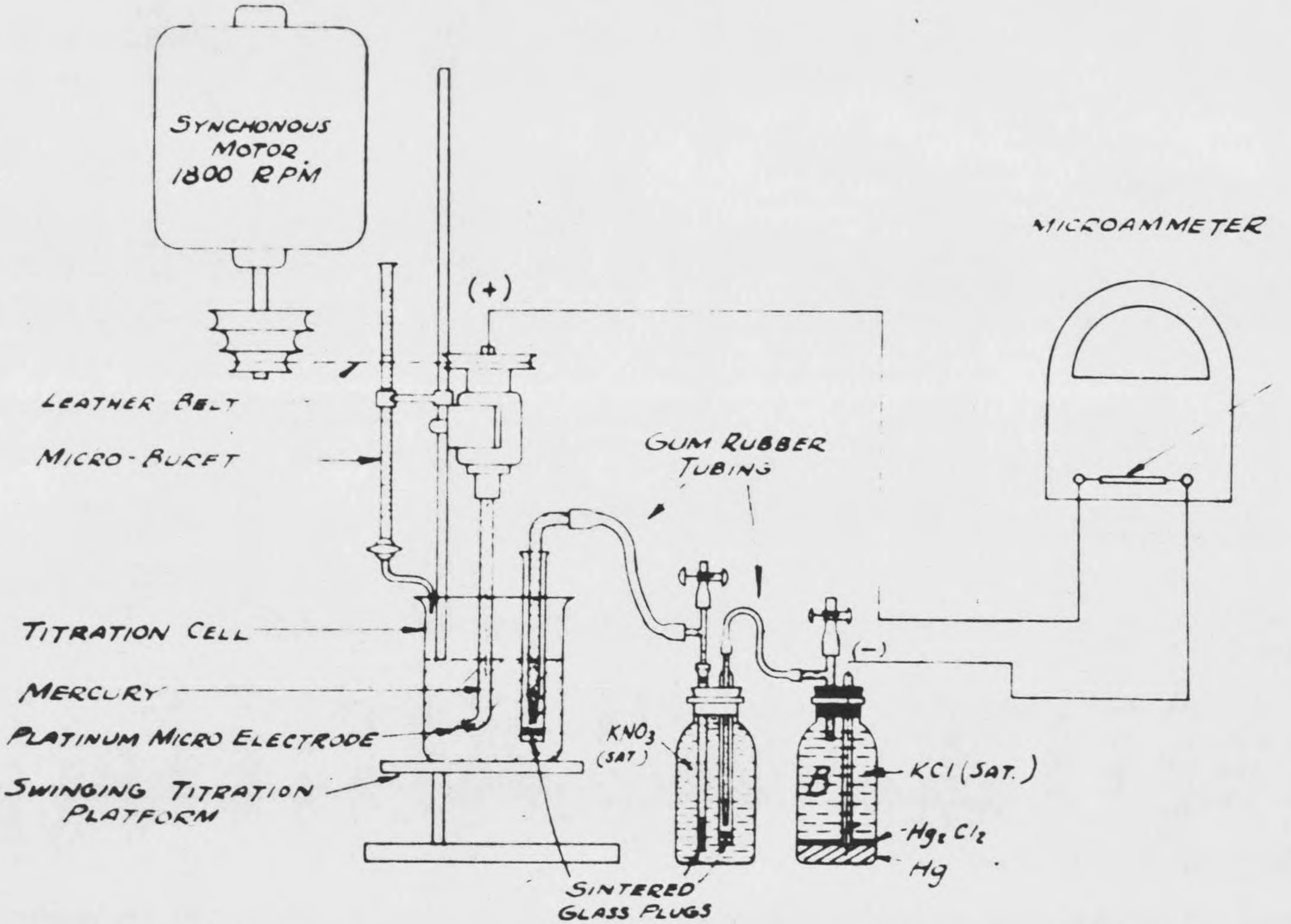

Figure 2

filter tubes shown in the drawing may be made according to Laitinen (5).

The rotating platinum microelectrode, introduced by Laitinen and Kolthoff (4), acts as a variable speed stirrer. Current readings are made on a Weston d. c. microammeter equipped with a variable shunt resistance to obtain the desired sensitivity.

Amperometric determination

In order to obtain good sensitivity, the volume of the solution to be titrated was kept at about 100 ml.[1]. After turning on the motor to rotate the platinum electrode, the system was allowed to come to equilibrium before the ammeter reading was recorded. This procedure was repeated after adding 1.0, 1.5, and 2.0 ml. of $AgNO_3$. Then sufficient $AgNO_3$ was added to obtain a large permanent deflection of the ammeter. At this point the motor was stopped and any deposition was removed from the electrode. The titration was then continued in small increments until 3 or 4 readings beyond the end point were obtained.

The ammeter readings were plotted against ml. of $AgNO_3$ added. The end point was determined by the intersection of the two straight lines connecting the points indicating current values before and after the equivalence point.

Theory of amperometric titration

The method just described has been studied in detail by Laitinen, et al (5). It is particularly simple when no potential need be applied from an

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[1] Strictly speaking, a correction factor is necessary for a linear relationship between volume of $AgNO_3$ and microamperes; the factor becomes negligible if the titrant is ten times more concentrated than the chloride.

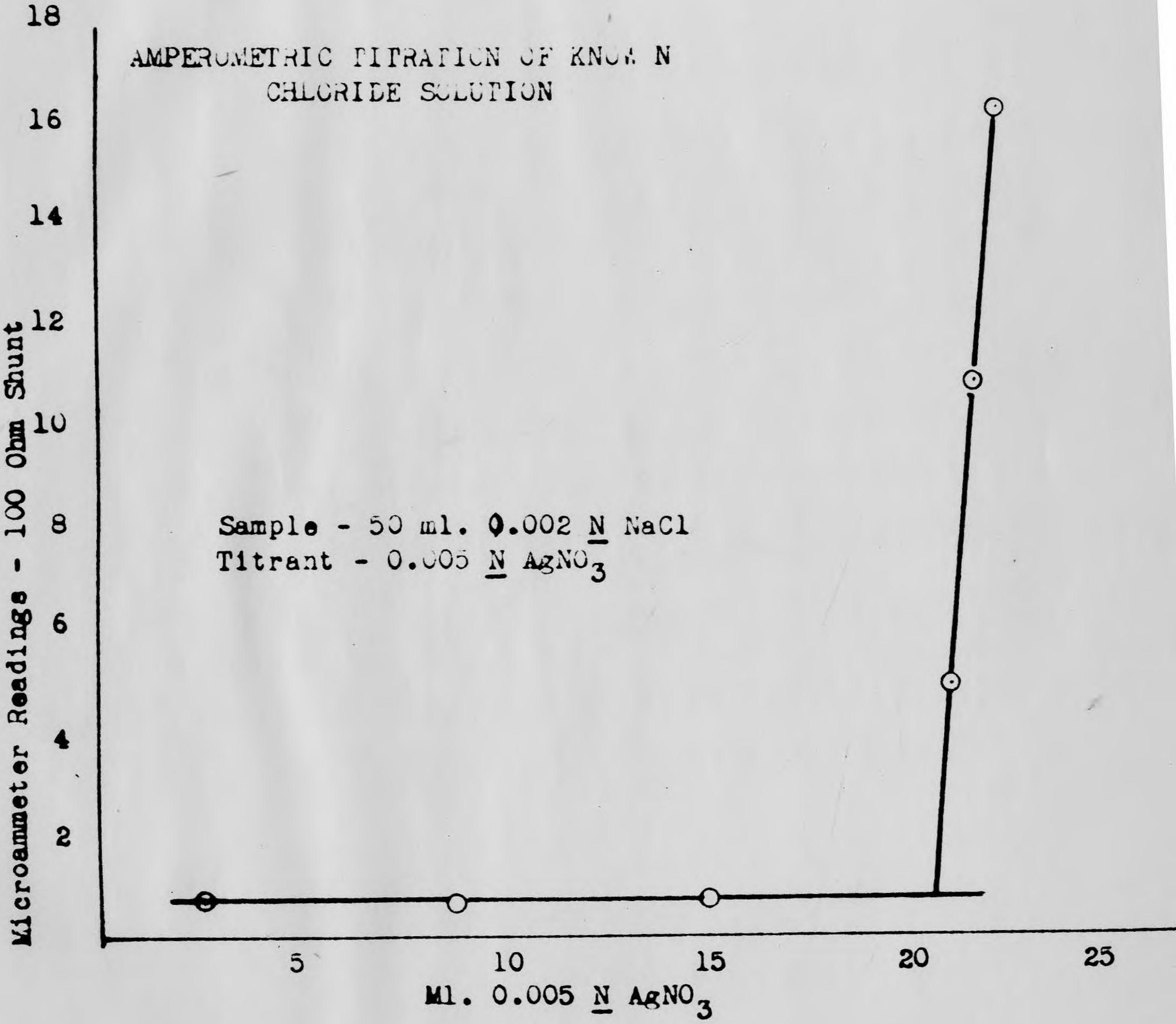
AMPEROMETRIC TITRATION OF KNOWN
CHLORIDE SOLUTION
Sample - 50 ml. 0.002 N NaCl
Titrant - 0.005 N AgNO3
Microammeter Readings - 100 Ohm Shunt
18
16
14
12
10
8
6
4
2
5
10
15
20
25
Ml. 0.005 N AgNO3

Figure 3

external source. The current read on the ammeter is the diffusion current in the substance titrated. The rate of diffusion and, therefore, the magnitude of the current is proportional to the diffusing substance in the bulk of the solution. The platinum electrode is made negative enough to plate out silver ions thus leaving a layer next to the electrode depleted of silver. By using a rotating electrode the thickness of the diffusion layer is decreased thereby increasing the sensitivity and reducing the time necessary to attain a steady diffusion state.

In this method of titration the current is independent of the E. M. F. of the electrode because of the extreme state of concentration polarization at that point. Since the concentration of the material undergoing electrode reaction is maintained at nearly zero, the current is limited by the supply of fresh material to the surface of the electrode by diffusion. It is evident that a titration curve of diffusion current against volume of reagent is in general a straight line (3).

Failure to add gelatin results in a large irregular current caused by depolarization of the electrode by AgCl particles. Addition of gelatin causes peptization and the gelatin coated particles are apparently no longer reducible at the cathode. The gelatin aids further in promoting the deposition of finely deposited silver on the cathode, and in so doing, makes for a more uniform diffusion current.

The presence of acetone has a pronounced effect on the accuracy of the results. In general the introduction of a medium in which AgCl is less soluble gives higher values and results in greater accuracy. However, the factor of error due to dilution begins to enter in when acetone percentages much above 50 are titrated. The writer therefore limited acetone addition to approximately 50 ml. since the samples averaged 50 ml. exclusive of acetone.

Calculations

1. Gm chloride = ml $AgNO_3$ x $\underline{N}$ x .03546 x a

   where:

   N = normality of $AgNO_3$

   .03546 = equiv. wt. of Cl ÷ 1000

   $$a = \frac{\text{total vol. of extractant}}{\text{ml. taken for analysis}}$$

2. $$\text{Gm Toxaphene} = \frac{\text{gms chloride}}{.68}$$

   where:

   68 = percent chlorine in Toxaphene

3. $$\text{Pounds Toxaphene per acre} = \frac{\text{gms Tox. in sample x b x c}}{453}$$

   where: $$b = \frac{\text{total wt. of green alfalfa collected}}{\text{wt. of subsample}}$$

   $$c = \frac{\text{sq. ft. per acre}}{\text{area collected}}$$

   453 = gms per pound

4. $$\text{P.P.M. Toxaphene} = \frac{\text{gms Toxaphene x } 10^6}{\text{wt. of sample}}$$

DETERMINATION OF TOXAPHENE IN ALFALFA BALES

A further objective of the research was to study the effect of storage on residue values in baled alfalfa. As a matter of conjecture, little decrease in Toxaphene content would be expected while in bales; however, experimental evidence was considered necessary.

Procedure

Bales were chosen at random from plots representative of the three methods of application and all rates of application. Sampling was performed according to the following scheme:

Plot 5 - - - - - - - - 3 bales
Plot 6 - - - - - - - - 3 bales
Plot 7 - - - - - - - - 2 bales
Plot 8 - - - - - - - - 2 bales
Plot 9 - - - - - - - - 2 bales

These bales were held apart to permit subsequent sampling. A wool coring apparatus was used to extract samples without necessitating opening of the bale. 600 to 800 gram samples were removed, composited and air dried for 48 hours. The material from each plot was analyzed separately in order to make comparison of successive analyses more reliable.

Extraction and analysis procedures were identical with those used in field sample tests. Results were calculated in p. p. m. Toxaphene. From a knowledge of tonnage of alfalfa procured, residues were computed on pounds-per-acre basis

RESULTS AND DISCUSSION

Toxaphene recovery study

It was of extreme importance to ascertain the percent of recovery of Toxaphene from treated vegetation. This factor was then employed in computation of actual residue values. For example, it was not known how effectively the sodium-isopropanol method converted organic to inorganic chlorides.

Accordingly, dried alfalfa was treated with a known volume of Toxaphene dissolved in benzene. The usual extraction and analytical procedures were followed and weight of Toxaphene calculatedd. Periodic repetitions of such analyses showed an average recovery of 90%.

Duplicate Analysis Study

This part of the investigation was carried out to determine whether compositing of the green samples was sufficiently thorough, and to provide a general over all check on analytical procedure. For this test duplicate analyses were made from the same sub-samples holding rigidly to prescribed procedures. Deviations were approximately 2% on low chloride values and less than 2% in samples higher in chloride content.

Spraying and Sampling

Spraying on plots 1, 2 and 3 was accomplished on June 30, 1947. Samples were collected 24 hours later and again one week later. Analysis of this material showed conclusively that the sampling scheme employed was inadequate. Application had not been sufficiently uniform to make ten square feet of material per acre a reliable sample. It is to be recalled that the emulsion was applied fan-wise over a 30 foot swath by use of a power sprayer. Manifestly, the center of each swath received a heavier dosage than the edges. Furthermore, drifting due to atmospheric currents and variation in the speed of the vehicle added to lack of uniformity of dosage.

Table II gives the residue values obtained from samplings I and II as compared to values from sampling III obtained by the revised scheme. All weights represent pounds of Toxaphene per acre.

Table II

FIRST CUTTING ANALYSES

		Test I (7/1/47) Lbs/acre Toxaphene	Test II (7/8/47) Lbs/acre Toxaphene	Test III (7/16/47) Lbs/acre Toxaphene
Plot 1	East	.71	3.14	1.57
	West	1.21	2.25	1.00
Plot 2	East	.65	.835	.28
	West	.05	.503	.27
Plot 3	East	.06	1.21	.43
	West	1.27	1.12	.48

Subsequent analyses demonstrated the reliability of the new method of collecting samples. Figure 1, page 9, illustrates the scheme in detail.

Percent Deposition

Analyses of alfalfa samples taken within 24 hours of the time of spraying were used to indicate the amount of Toxaphene actually deposited on the foliage. The difference between this and the actual rate of dosage may be presumed to have been lost by falling through the foliage to the ground or carried away by air currents. Table III shows the average percent deposition for each type of application.

Table III

PERCENT DEPOSITION

Application	Rate	% Deposition on Foliage
Water emulsion	1 to 4 lb./A	29
Wettable powder	2 lb./A	24
Oil solution	2 lb./A	14
Dust	1.5 to 2 lb./A	7

The average percent deposition of any one type of application was the same regardless of the level of application. For example, water emulsions sprayed at 1, 2 and 4 lbs. per acre yielded average depositions 27, 28 and 32% respectively. Individual values varied up to 10% (in the water emulsion plots) from the average. Regarding the 24% deposition for wettable powder, it is probable that the figure is not significantly different from the water emulsion value. Only a single plot received this type of treatment thus precluding the averaging of a wider range of values.

Decrease in Toxaphene residues due to weathering

The chief objective of this investigation was to determine the rate of loss of Toxaphene applied by water emulsion, wettable powder, oil and dust, and in all the different pound rates per acre. Table IV, summarizes the residue values on the nine Bozeman plots. This table includes all of the samplings on the second cutting, all values being calculated in terms of pounds of Toxaphene per acre. While analyses were actually carried out on two samples from each plot, the results in Table IV are the averages of the two values[1].

An examination of the tabulation shows that Toxaphene applied in the form of an oil solution had the best staying qualities; approximately 80% of the amount deposited still remained after 17 days. Water emulsion treatment at the one and two pound levels showed about a 50% loss in the first week after application and a very slow rate of loss thereafter. The 4 lb. per acre water emulsion treatment demonstrated a much slower rate of loss

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[1] The individual analysis shows an average deviation of 0.03 lb./acre.

Table IV

RESIDUAL TOXAPHENE ON BOZEMAN PLOTS (All values in lbs/acre)

Plot	First Application	1 Day	8 Days	18 Days	31 Days
1	4 lb./A.-Water Emulsion	1.89	1.56	1.03	0.70
2	1 lb./A.-Water Emulsion	0.37	0.21	0.13	0.10
3	2 lb./A.-Water Emulsion	0.50	0.23	0.20	0.18
4	1 lb./A.-Water Emulsion	0.19	0.07		
5	2 lb./A.-Water Emulsion	0.42	0.21		
6	4 lb./A.-Water Emulsion	0.65	0.39		
7	2 lb./A.-Oil Solution	0.26	0.25		
8	4 lb./A.-Wettable Powder	0.73	0.44		
9	2 lb./A.-Dust	0.12	0.08		

Plot	Second Application	1 Day	9 Days	17 Days
4	1 lb./A.-Water Emulsion	0.32	0.18	0.13
5	2 lb./A.-Water Emulsion	0.98	0.45	0.33
6	4 lb./A.-Water Emulsion	1.71	1.37	1.06
7	2 lb./A.-Oil Solution	0.55	0.49	0.44
8	4 lb./A.-Wettable Powder	1.06	0.75	0.43
9	2 lb./A.-Dust	0.19	0.12	0.11

than the one and two pound treatment, a 50% loss occurring only after 20 days. The wettable powder plots showed about the same rate of loss as the two pound water emulsion plots. Dust treated plot residues diminished somewhat less rapidly than either the water emulsion or the wettable powder residues.

Final samples on each plot were collected one or two days prior to harvesting. The amount of residual Toxaphene at that time varied with three factors (listed in the order of decreasing effect): rate of application, time elapsed since treatment and type of application. The dust plot was notable in that it showed only approximately one-fourth the residual values of the others.

Rate of Toxaphene Decrease in Plots outside Bozeman

Applications on plots located at Miles City and Forsyth were of similar nature as to those in Bozeman except that only dust and water emulsions were employed and only at the rate of 1.5 pounds per acre. Sampling schemes followed were patterned after the Bozeman system[1]; after compositing, a subsample was sent to Bozeman for testing. Only single samples were collected from each plot.

Table V summarizes the values of residual Toxaphene obtained from the said plots. Average rates of decrease tended to be somewhat greater than on the Bozeman plots receiving equivalent treatment. Treatments at Miles City and Forsyth were limited to single applications. Insect counts were

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[1] Samples on Miles City and Forsyth plots collected by D. K. Scharf and E. B. Hastings, Montana State College.

Table V

RESIDUAL TOXAPHENE - MILES CITY AND FORSYTH PLOTS

Plot	Application	18 Hrs.	6 Days	12 Days
Miles City 6	1.5 - Dust	0.08	0.03	0.02
7	1.5 - Water Emulsion	0.32	0.10	0.09

Plot	Application	2 Hrs.	3 Days	9 Days	12 Days
Miles City 10	1.5 - Dust	0.16	0.05	0.03	0.04
9	1.5 - Water Emulsion	1.00	0.27	0.14	0.16

Plot	Application	6 Hrs.	2 Days	7 Days	15 Days	18 Days
Forsyth 20	1.5 - Water Emulsion	0.56	0.47	0.22	0.10	0.07

(All of the above results are in pounds of Toxaphene per acre.)

taken periodically for correlation with residual determinations.[1].

Residual Toxaphene at Time of Cutting at the Bozeman Plots

TableVI is a compilation of the average amount of Toxaphene on each plot remaining on the foliage at the time of cutting. The values were obtained from samples collected one to two days previous to harvesting operations. The final column headed P. P. M. represents pounds of Toxaphene per million pounds of dry vegetation. Values may be conveniently compared with, for example, Toxaphene residuals obtained from baled alfalfa as tabulated on page 26. First cutting yields of hay amounted to approximately two tons per acre while second cutting tonnage was about one-half that figure. We find the following ratios of residues to p. p. m. (averages

Table VI

RESIDUAL TOXAPHENE AT TIME OF HARVESTING

Plot	Cutting	Time After Last Application	Residue Lb./A.	P.P.M.
2	1st	17 Days	0.28	48
3	1st	17 Days	0.46	83
1	1st	17 Days	1.29	257
2	2nd	31 Days	0.10	46
3	2nd	31 Days	0.18	78
1	2nd	31 Days	0.10	267
4	2nd	17 Days	0.13	56
5	2nd	17 Days	0.33	166
6	2nd	17 Days	1.06	565
7	2nd	17 Days	0.44	171
8	2nd	17 Days	0.43	141
9	2nd	17 Days	0.11	48

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1. Final figures on insect counts obtainable from D. K. Scharf, Assistant Entomologist, Montana Agricultural Experiment Station.

from all plots):

1st cutting . . . . 1 lb./A. equivalent to 185 p.p.m.

2nd cutting . . . . 1 lb./A. equivalent to 420 p.p.m.

Inasmuch as growth rate in the plots was variable, that is, thickness and height of the plants were not uniform, p.p.m. basis could not be used to compare the staying qualities of the various formulations and rates of application. Such values, however, are indicative of actual amounts of Toxaphene present in hay to be fed to livestock.

Residues in Baled Alfalfa

Table VII below summarizes Toxaphene residue values obtained from samples collected from bales of treated alfalfa. The results shown are averages of the individual analyses of the two and three bales per plot tested. It would have been impracticable to collect material from all bales procured from each plot, hence data obtained was to serve chiefly as indicative of effects of baling and storage. The column titled "Final Field Sampling" is inserted for comparison of final p.p.m. values found in growing vegetation with those found in bales.

Table VII

TOXAPHENE RESIDUES FOUND IN BALED ALFALFA

Plot	Final Field Analysis	November 21, 1947	January 2, 1948	February 26, 1948
5	165 p.p.m.	110 p.p.m.	70 p.p.m.	55 p.p.m.
6	560	320	320	274
7	170	140	95	115
8	140	50	20	55
9	45	25	5	30

Graphical Interpretation

In the following pages an attempt has been made to show graphically the facts presented in the foregoing tabulations. The objective has been to show at a glance such facts as; the average amount of Toxaphene deposited, average rate of loss with time for each type of application studied and residues in baled alfalfa.

Generally speaking, the data have been composited into two types of curves, those showing absolute values of residues in pounds of Toxaphene per acre, and those obtained by plotting per cent decrease in residues against elapsed time[1]. In preparation of the latter type of curve, the amount of residue found 24 hours after application was taken as 100% and subsequent amounts calculated as per cents of this initial value. The curves may be said to represent what can be predicted to occur on the average if alfalfa were treated with Toxaphene in the manner indicated.

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[1] Figures numbered 4, 5, 6, 10, 11, 12, 13, 14 reproduced by permission from curves prepared by Floyd Diephuis, Hercules Powder Company, who used the author's original data.

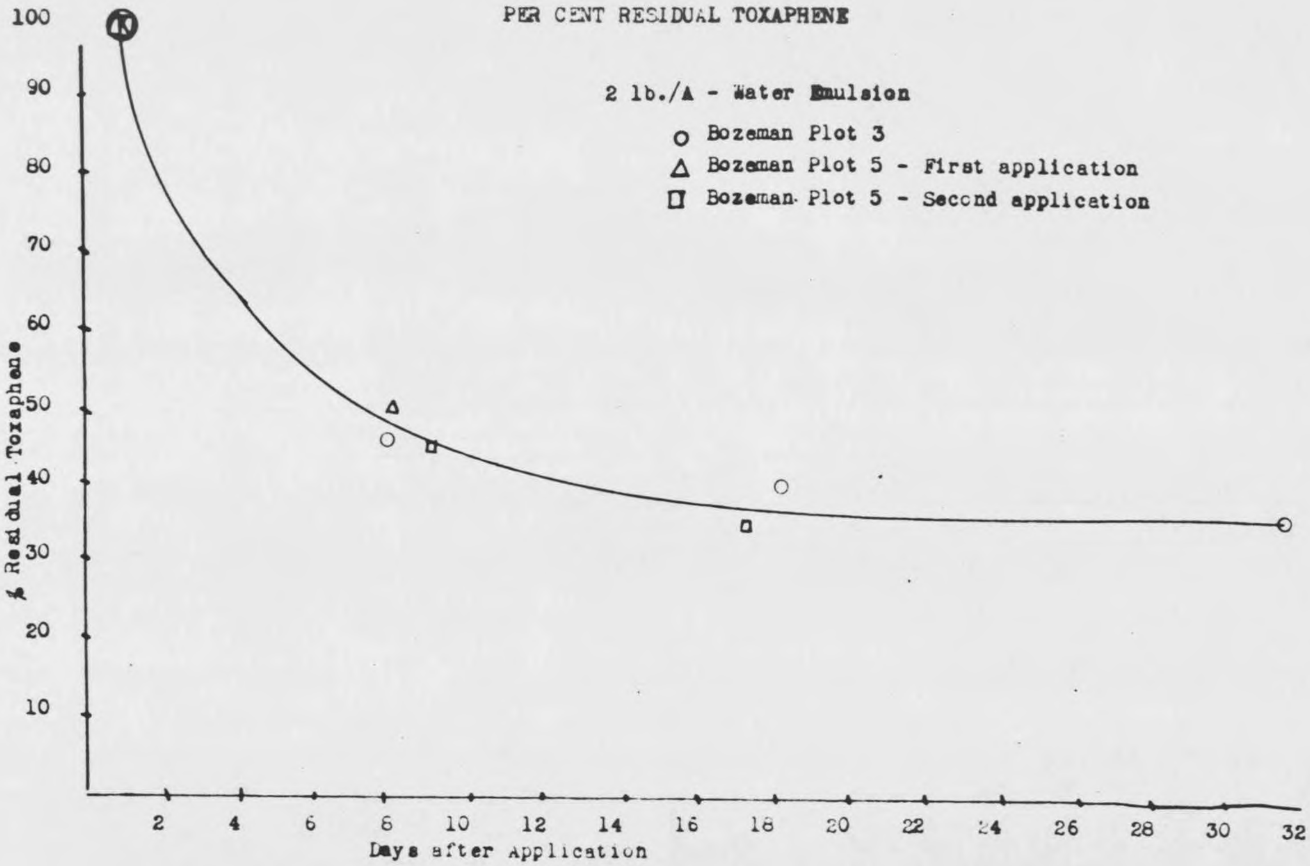


Figure 4

PER CENT RESIDUAL TOXAPHENE

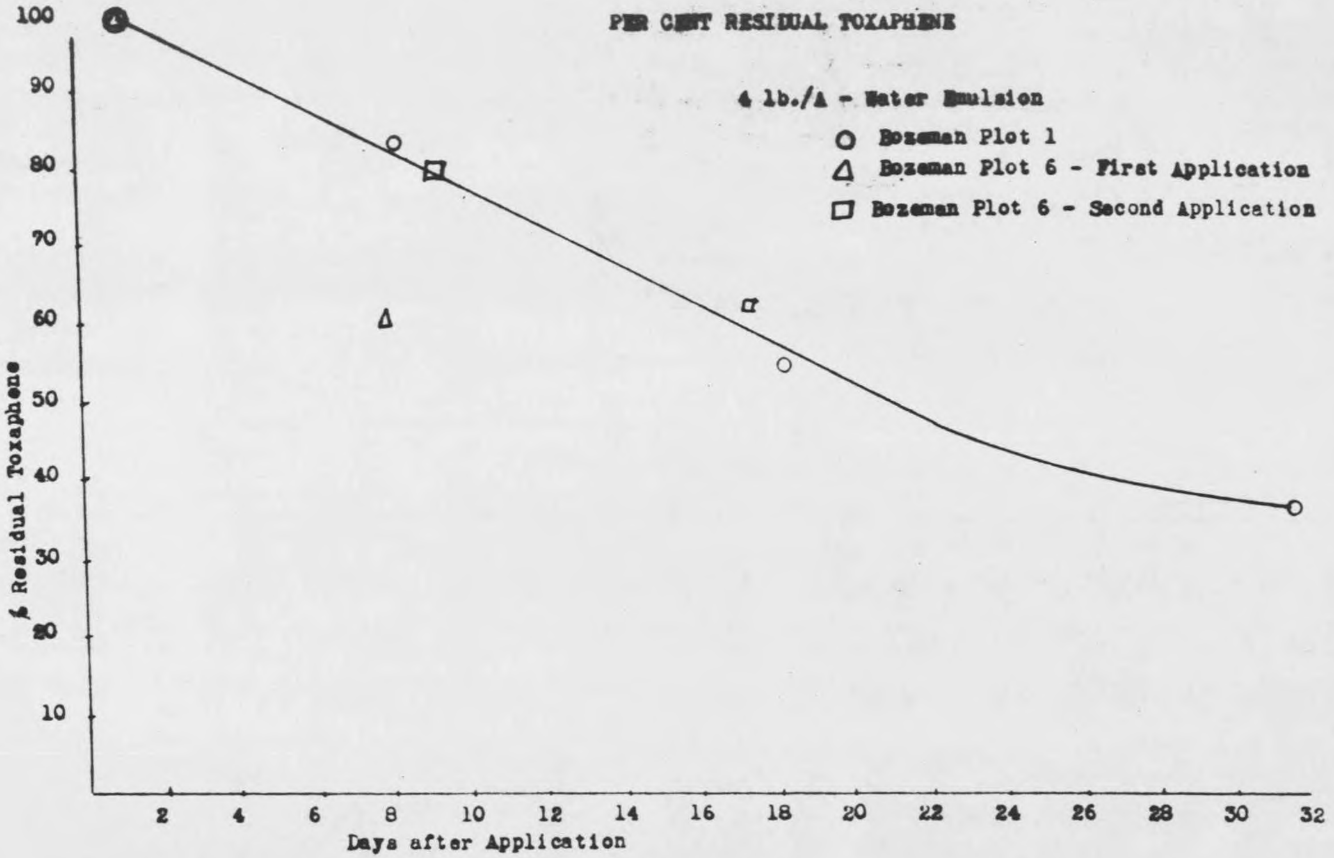
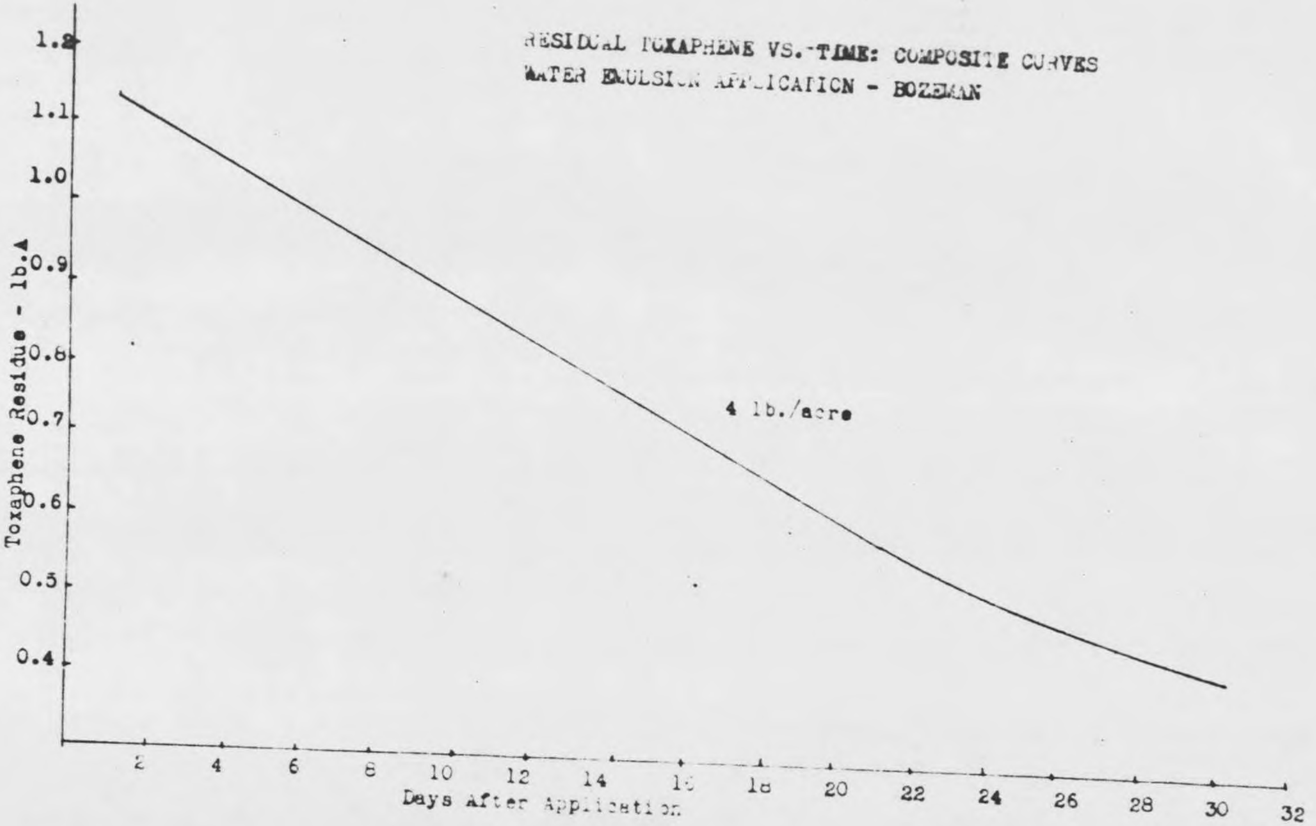

PER CENT RESIDUAL TOXAPHENE
4 lb./A - Water Emulsion
○ Bozeman Plot 1
△ Bozeman Plot 6 - First Application
☐ Bozeman Plot 6 - Second Application
% Residual Toxaphene
100
90
80
70
60
50
40
30
20
10
2
4
6
8
10
12
14
16
18
20
22
24
26
28
30
32
Days after Application

Figure 5

Figure 9


RESIDUAL TOXAPHENE VS. TIME: COMPOSITE CURVES
WATER EMULSION APPLICATION - BOZEMAN

RESIDUAL TOXAPHENE - TWO APPLICATIONS OF WATER EMULSION TO ALFALFA-
ORIGINAL DATA

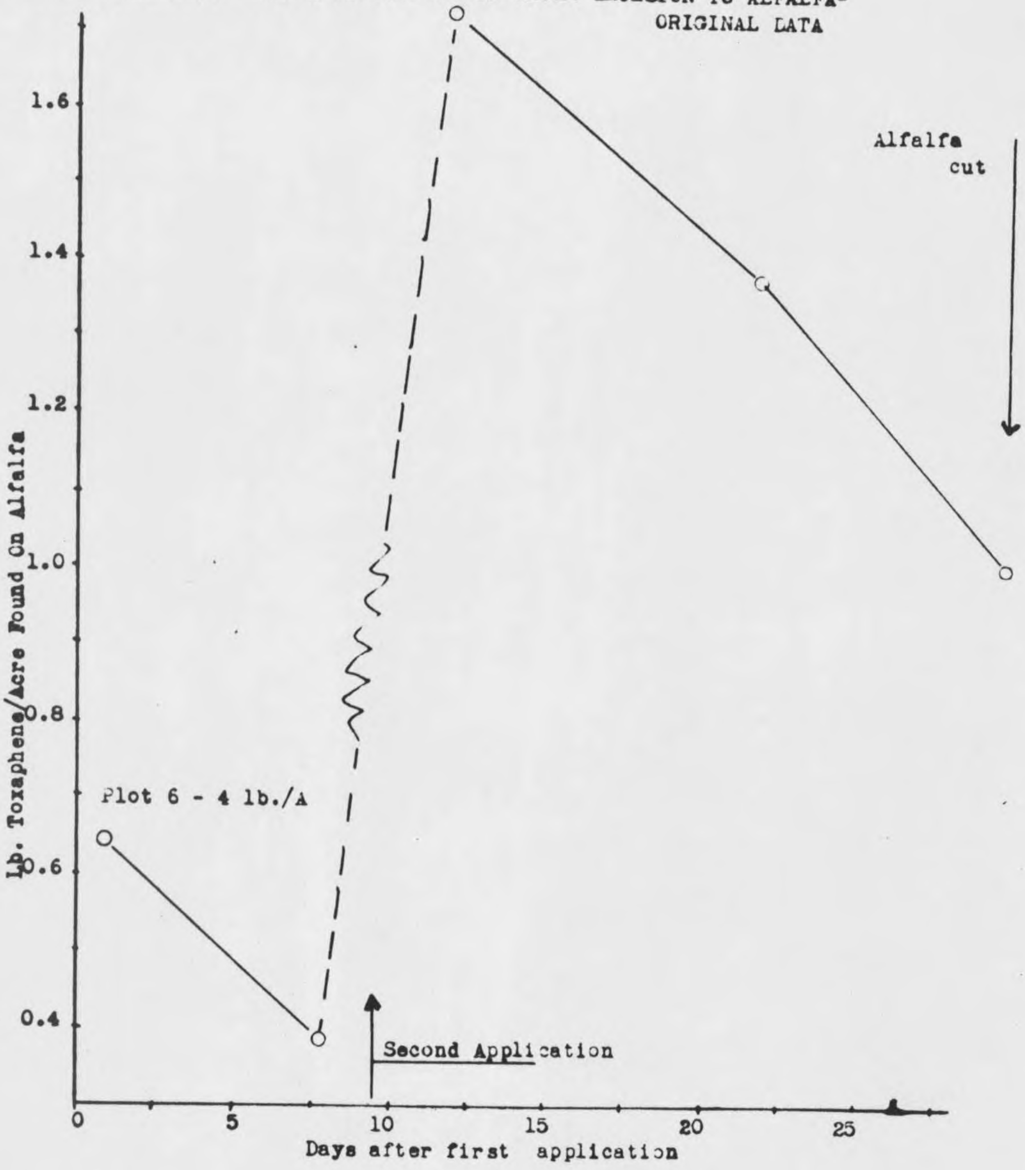


Figure 7

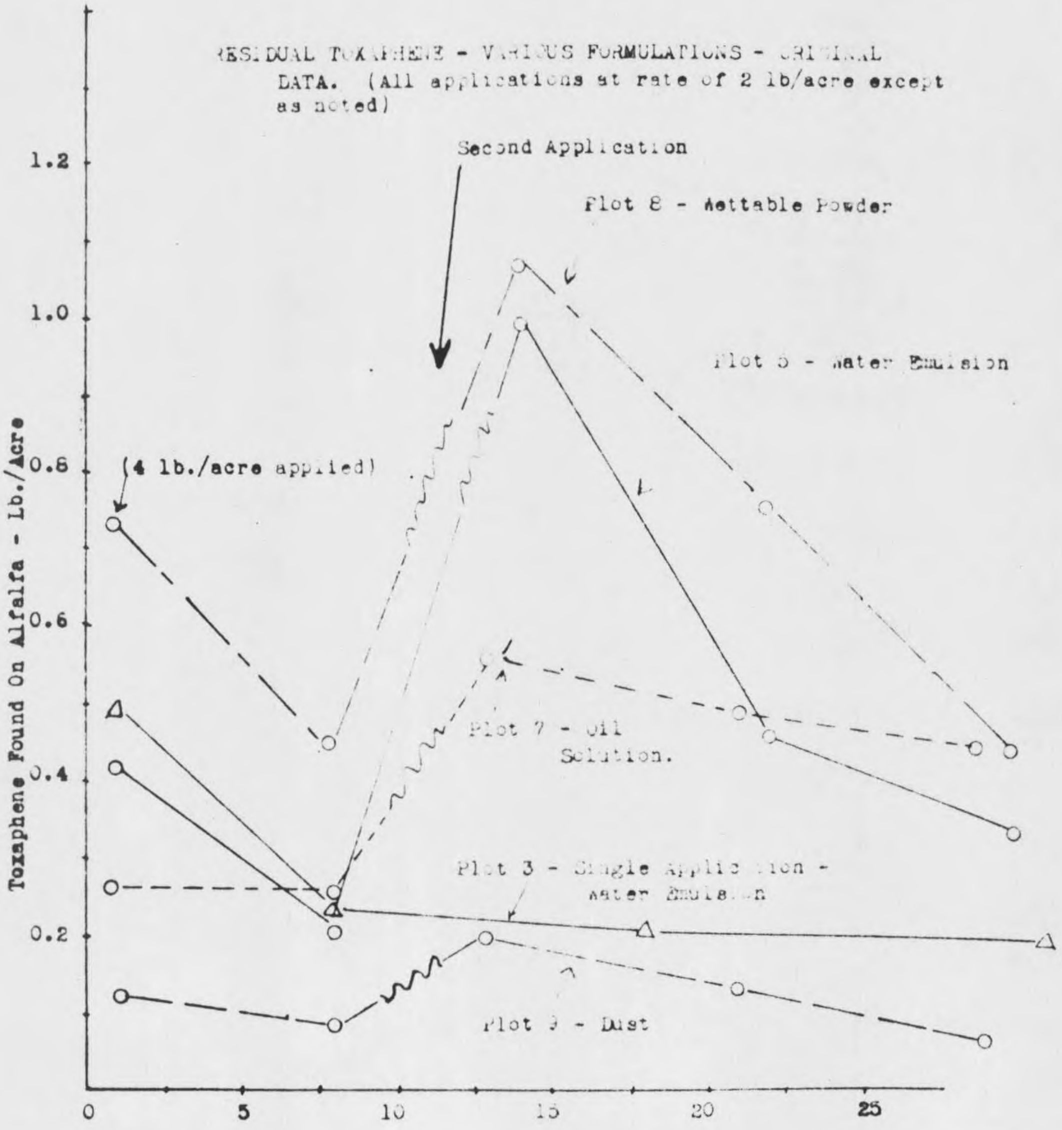

RESIDUAL TOXAPHENE - VARIOUS FORMULATIONS - ORIGINAL DATA. (All applications at rate of 2 lb/acre except as noted)
Second Application
Plot 8 - Wettable Powder
Plot 5 - Water Emulsion
(4 lb./acre applied)
Plot 7 - Oil Solution.
Plot 3 - Single Application - Water Emulsion
Plot 9 - Dust
Toxaphene Found On Alfalfa - Lb./Acre
1.2
1.0
0.8
0.6
0.4
0.2
0
5
10
15
20
25

Figure 8

RESIDUAL TOXAPHENE - TWO APPLICATIONS OF WATER EMULSION TO ALFALFA - ORIGINAL DATA

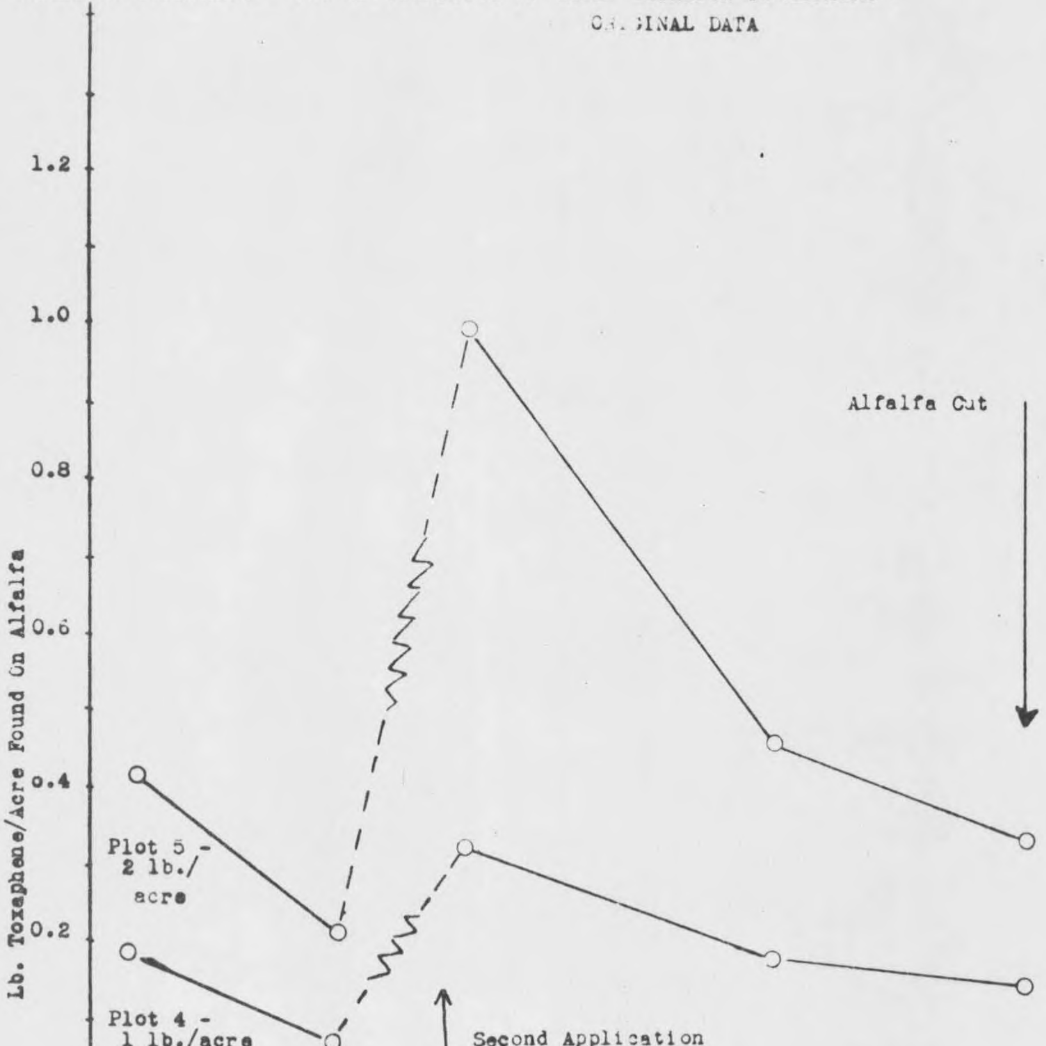
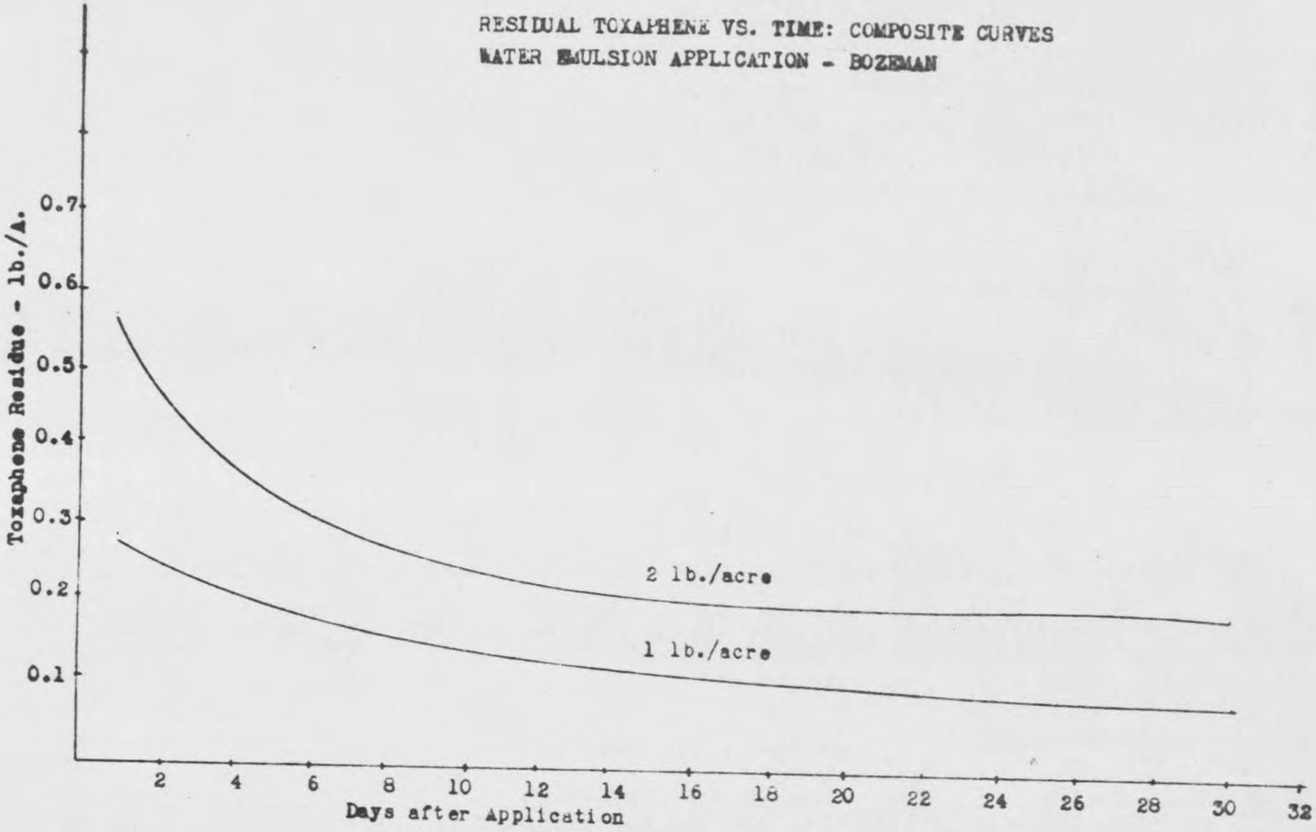


Figure 9



RESIDUAL TOXAPHENE VS. TIME: COMPOSITE CURVES
WATER EMULSION APPLICATION - BOZEMAN

Figure 10

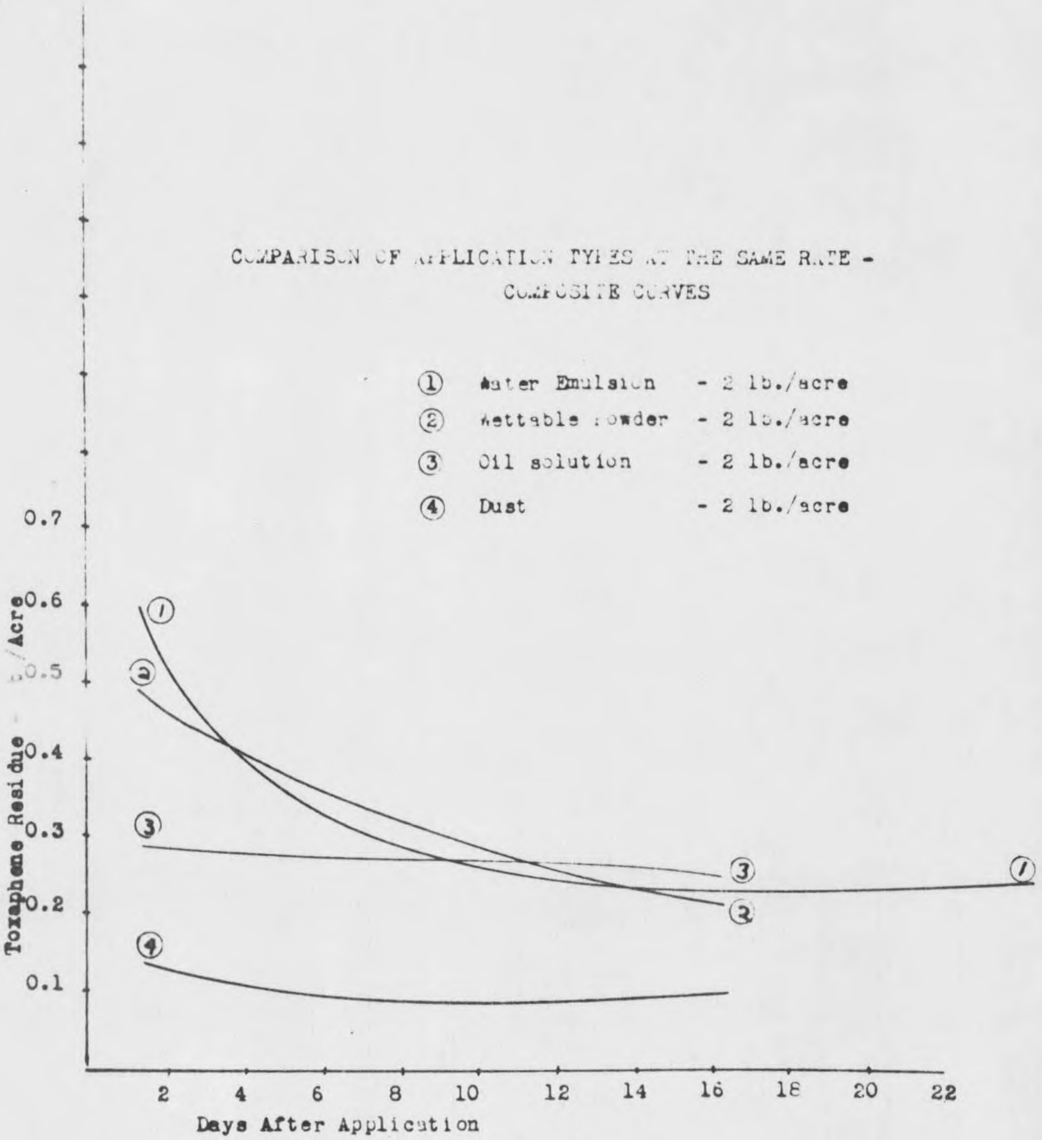

COMPARISON OF APPLICATION TYPES AT THE SAME RATE -
COMPOSITE CURVES
① Water Emulsion - 2 lb./acre
② Wettable Powder - 2 lb./acre
③ Oil solution - 2 lb./acre
④ Dust - 2 lb./acre
0.7
0.6
0.5
0.4
0.3
0.2
0.1
Toxaphene Residue - lb./Acre
①
②
③
④
③
①
②
2
4
6
8
10
12
14
16
18
20
22
Days After Application

Figure 11

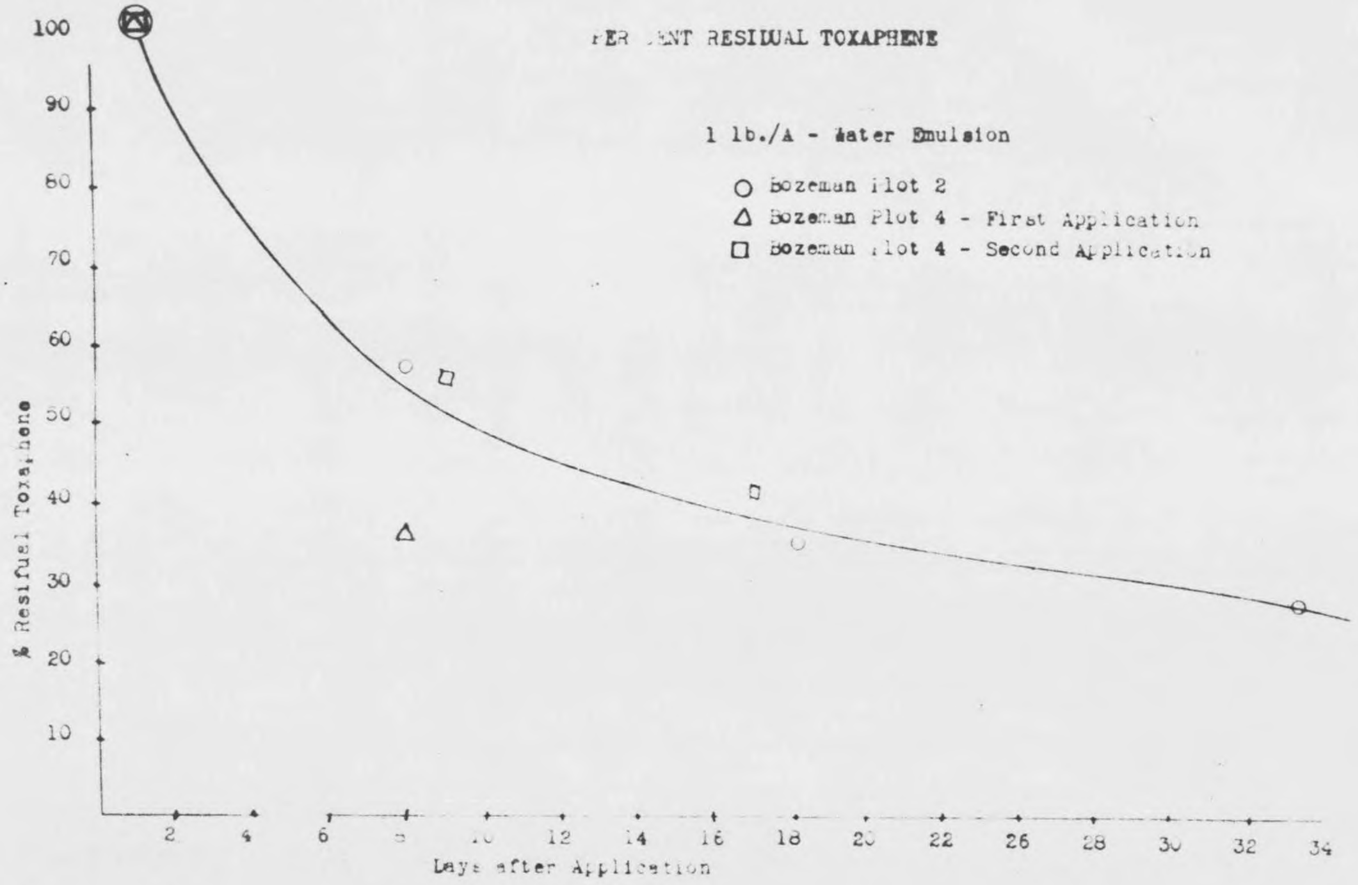

PER CENT RESIDUAL TOXAPHENE
1 lb./A - Water Emulsion
○ Bozeman Plot 2
△ Bozeman Plot 4 - First Application
□ Bozeman Plot 4 - Second Application
% Residual Toxaphene
100
90
80
70
60
50
40
30
20
10
2
4
6
8
10
12
14
16
18
20
22
26
28
30
32
34
Days after Application

Figure 12

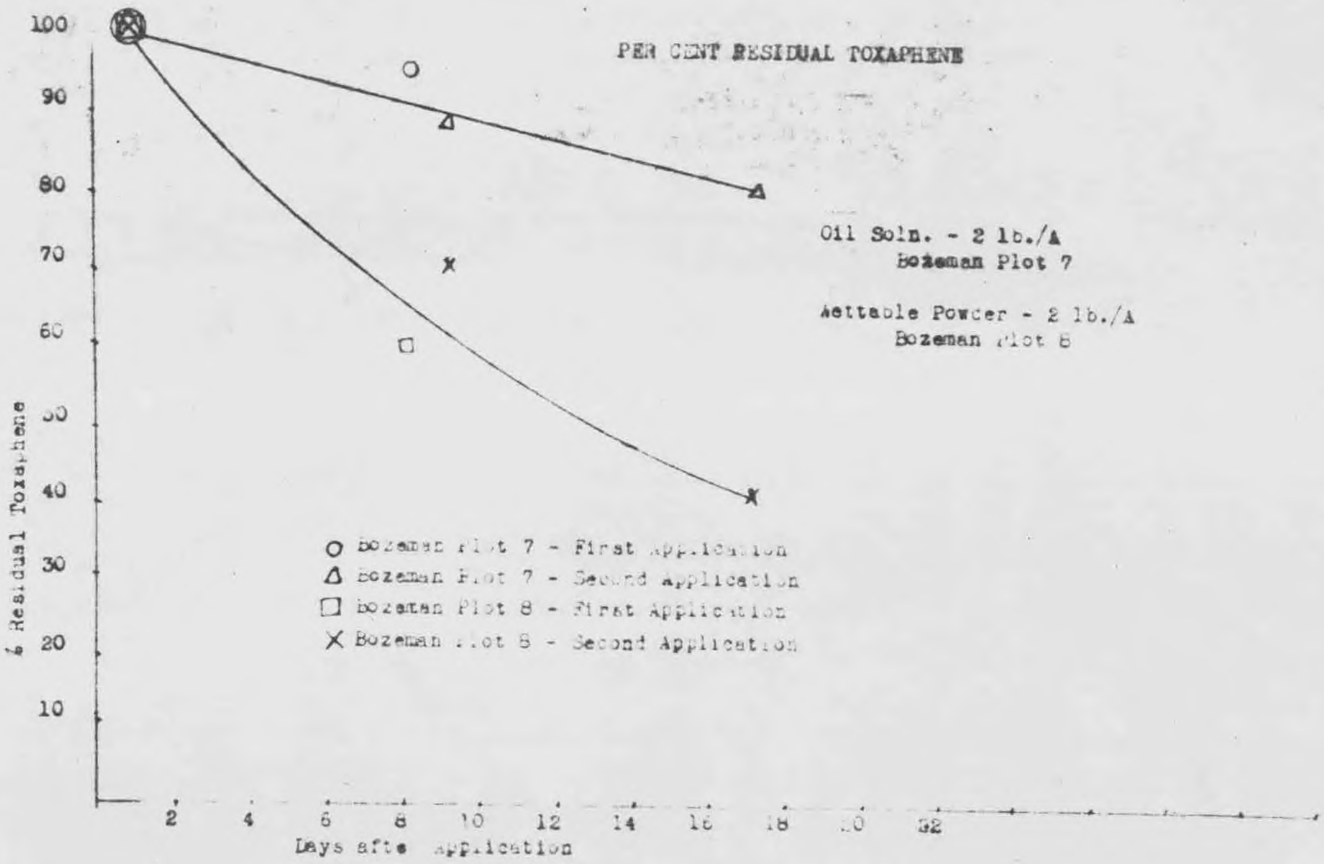

PER CENT RESIDUAL TOXAPHENE
Oil Soln. - 2 lb./A
Bozeman Plot 7
Wettable Powder - 2 lb./A
Bozeman Plot 8
% Residual Toxaphene
100
90
80
70
60
50
40
30
20
10
2
4
6
8
10
12
14
16
18
20
22
Days after application
○ Bozeman Plot 7 - First Application
△ Bozeman Plot 7 - Second Application
☐ Bozeman Plot 8 - First Application
✕ Bozeman Plot 8 - Second Application

Figure 13

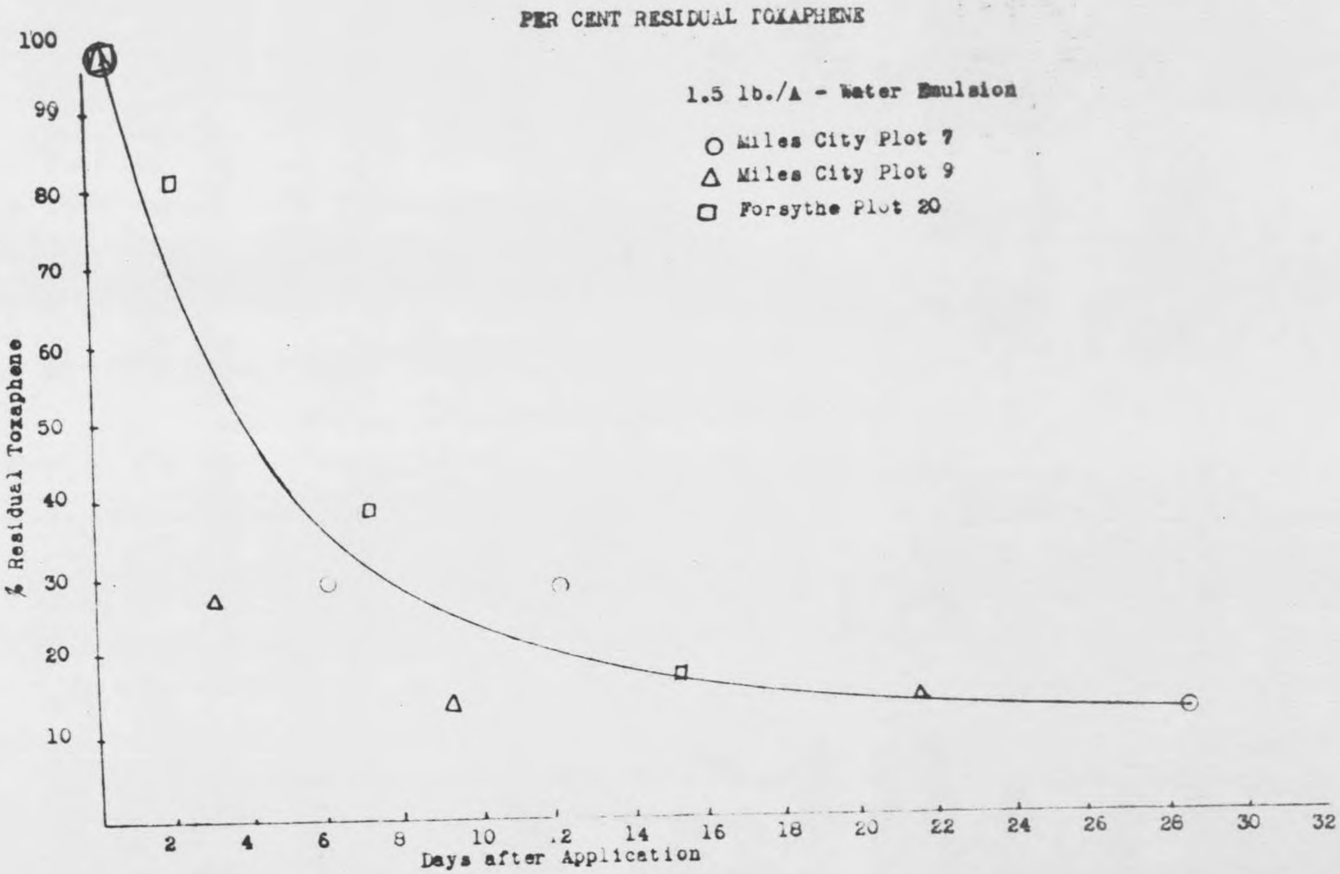
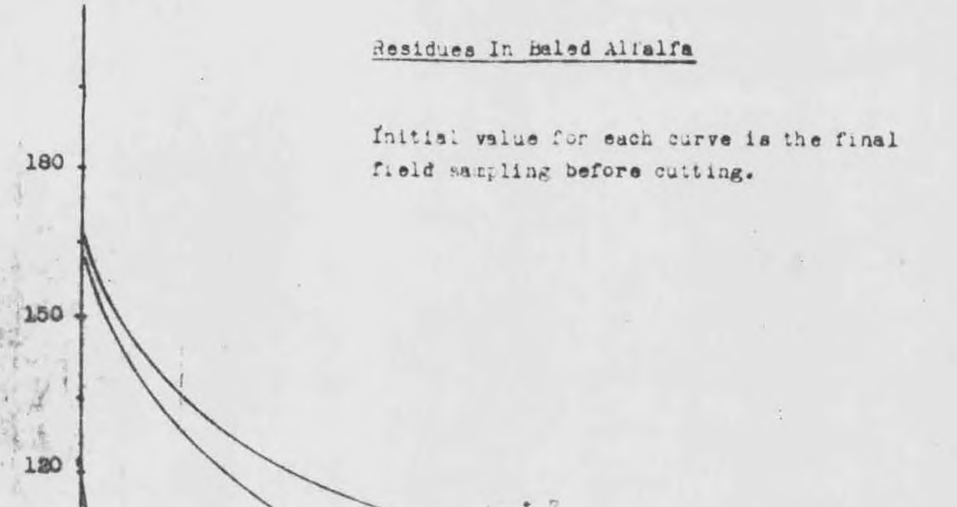

PER CENT RESIDUAL TOXAPHENE
1.5 lb./A - Water Emulsion
○ Miles City Plot 7
△ Miles City Plot 9
☐ Forsythe Plot 20
% Residual Toxaphene
100
90
80
70
60
50
40
30
20
10
2
4
6
8
10
12
14
16
18
20
22
24
26
28
30
32
Days after Application

Figure 14


Residues In Baled Alfalfa

Initial value for each curve is the final field sampling before cutting.

Figure 15

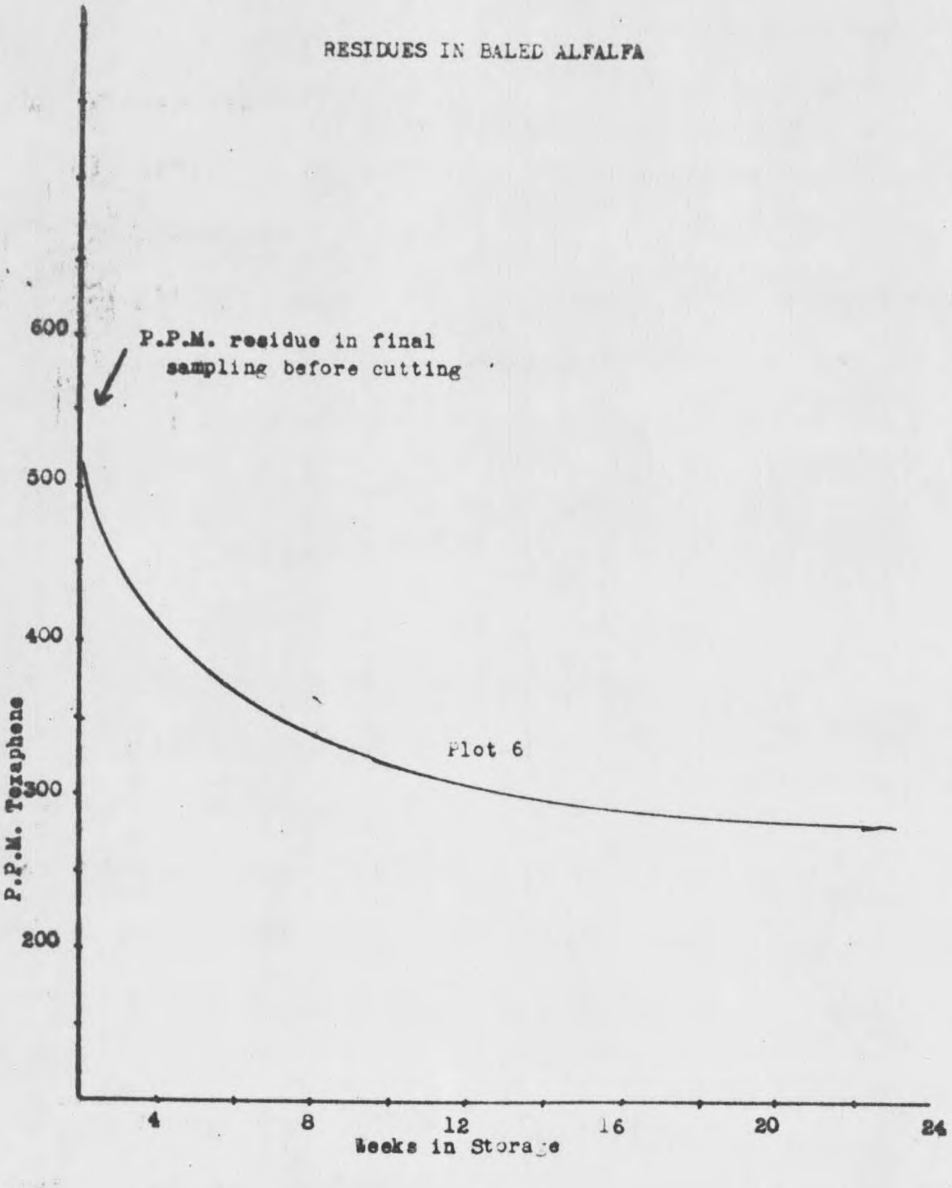

RESIDUES IN BALED ALFALFA
P.P.M. residue in final sampling before cutting
Plot 6
P.P.M. Toxaphene
600
500
400
300
200
4
8
12
16
20
24
Weeks in Storage

Figure 16

CONCLUSIONS

At this point a review of the objectives of this investigation might prove advantageous. Such objectives fall into the following classifications:

A. Primary Objectives

1. Study of rate of deposition of various Toxaphene formulations on foliage.
2. Study of comparative loss rates of the various formulations.
3. Study of influence of weather factors on rate of loss.
4. Determination of Toxaphene losses in baled alfalfa.

B. Secondary Objectives

1. To develop a reliable plan of sampling of large plots of growing vegetation.
2. Development of analytical methods suitable for the extraction and analysis of Toxaphene.

In the discussion of results in the preceding section the writer has already introduced certain of the more obvious conclusions. With reference to the objectives enumerated above, a more detailed discussion of interpretations and conclusions follows.

Deposition on Foliage

On the Bozeman plots first samples were taken 24 hours subsequent to application; at that time water emulsion deposition averaged 29%. On plots at Miles City and Forsyth, however, sprayed foliage was collected as early as two hours after application; this collection showed a 16% deposit of Toxaphene. At Forsyth a collection taken six hours after spraying showed approximately 40% deposition. While the data is not sufficient to be conclusive, indications are that a rapid breakdown occurs in the initial 24 hours.

Comparison of the four types of formulations shows definitely higher per cents of deposition in the case of water emulsion and wettable powder over oil solution and dust. Discounting the effects of atmospheric currents on the lighter oil spray, it is difficult to explain the low deposition of the oil formulation. One possibility may well be the effect of foliage wet with dew, since the spraying was accomplished during the morning hours. Certainly, there could have been some effect of shedding the oil droplets by wet alfalfa leaves and stems.

The case of the dust application, where deposition was only one-fourth that of water emulsion, can be reasonably well accounted for. Here prime requisites for successful application were still air and thoroughly wet foliage. Add to these the difficulties attached to uniform dusting at prescribed rates per acre and it becomes quite apparent why dust residues were low.

Finally, although wettable powder 24 hour residues do not appear significantly different from those of water emulsion, there is one factor worthy of comment. This is the matter of loss through handling. It was very noticeable, during the compositing process especially, that the powder was falling off the samples. This was quite unadvoidable and presumably the same kind of loss was constantly taking place on the plot itself due to wind agitation. Determination of such losses could conceivably be accomplished by means of soil sampling--analysis of the top several inches of the soil. Correlating such measurements with foliage residuals would be a long step toward discovering what actually happened to the material after it left the sprayer tank. Such an investigation presents a problem; one that, to this writer's knowledge, has rarely been attempted.

Rate of Decrease in Toxaphene Residue Values

A significant property of an insecticide is its resistance to weathering: i.e., its staying qualities. The problem of testing for such qualities comprised the major portion of this study. Here also is the reason for the four different formulations and the large variance in the rate of application.

It was expected that the various formulations would have varied resistance to weathering. Such indeed proved to be the case. The oil solution treated plot showed the least per cent decrease, a loss of only 20% in 17 days compared to a 50% loss in the first week in water emulsion plots. Wettable powder exhibited residual percentages similar to water emulsion while dust plots residues diminished more slowly.

Any factual data to explain these phenomena would require exhaustive research far beyond the scope of this paper. One can only conjecture as to varying effects of electromegatively charged leaf surfaces on oil films or emulsion films deposited on them. There are, no doubt, surface tension forces that allow a water droplet to remain on a leaf surface in globular form, while causing the lower tension oil droplet to spread and leave its suspended insecticide distributed evenly after evaporation of the oil. With regard to the dust form of application, one conclusion at least seems quite obvious; the rate of deposition is very low, but that amount resists weather elements as well or better than the water emulsion treatment.

Admittedly, that is only slight compensation for the serious drawback just mentioned.

One further observation regarding residue decrease ought to be recorded. The 2 pounds-per-acre and 4 pounds-per-acre water emulsion plots did not show the same loss rate, the 4 pound rate showing a much slower decrease. 50% losses did not occur until 20 days after application as compared to one week for the lower rate. This phenomenom could well call for further study; any efforts toward explanation at this time would be useless.

Influence of Weather Factors

Regular recording of weather data; temperature, precipitation and sky condition, was carried out during the entire study. The most significant fact arising from this observation was the lack of any discernible effect of weather changes on rate of Toxaphene decrease. No drastic changes occur in the curves representing per cent loss against time. While precipitation is quite limited in Montana during July, August and September, there was sufficient rain to justify the above conclusion. Limitations of time and facilities precluded the possibility of collecting samples immediately before and after a heavy rain. Such data would, of course, have been of more conclusive nature. On the other hand a uniform decrease in residue is a good indication of the resistance of Toxaphene to "washing" effects.

Toxaphene Residues in Baled Alfalfa

Admittedly, sampling of alfalfa in bales could not approach the thoroughness of field sampling. To do so would have required collecting samples from all of the bales of the plots to be tested. This was impossible since the hay was being fed. A limited number of bales from designated plots were, therefore, used; these bales being set aside for successive testing. Under these circumstances, it was expected that sampling deviations would be rather large and that data would have to be collected over longer periods of time before conclusions could be drawn. Interpretation of data obtained from three sets of samples taken four, nine and sixteen weeks after beginning of storage, leads to the following conclusion: no consistent decrease in residual values apparently occurs in alfalfa while in baled status. While certain bales showed definite losses, other remained unchanged, in some cases even showing increases. All variations may quite reasonably be due to variations within the bale itself.

There is, however, a very substantial drop in p.p.m. values between the last field sampling and the initial bale analysis. These losses averaged 70% for wettable powder; 45%, dust; 38%, water emulsion; and 27%, oil solution. Cursory samples of the field dried hay collected just previous to baling indicated definitely that these losses were incurred during cutting and drying operations. The action of a tedder, for example, may well account for the high rate of loss in the wettable powder plot where material was evidently quite loosely held.

Incidental Findings

Of secondary nature but none the less interesting and valuable are certain conclusions in relation to experimental procedure. One vexing problem, whose solution was fairly well achieved, was the question of what constitutes valid sampling procedure on large plots of growing vegetation. Satisfactory schemes in this study were arrived at by trial and error; since nonuniformity of application was the all important factor in determining collecting procedure, a statistical approach was not deemed workable. The initial scheme of collecting all of the material in 10 sq. feet per acre proved to be wholly inadequate. When this was increased to 80 sq. feet, results showed gratifying consistency. It must, therefore, be concluded that analyses of this nature ought to be preceded by preliminary sampling trials in order to ascertain adequate procedures. In this project, .2% of the plot area was collected at each sampling. That might be considered a minimum in many instances.

Finally, certain aspects of the chemical analysis itself are worthy of comment. Tests indicated recovery of Toxaphene approaching 90%. The sodium-isopropanol method is apparently effective up to that point in converting organic to inorganic chlorides. Toxaphene is quite stable toward heat; several inadvertent evaporations of benzene to dryness over a hot-plate showed no significant loss in values. The amperometric titration of chlorides provides a rapid and convenient means of chloride determination, no more than 5 minutes being required for a single titration.

SUMMARY

1. The investigation of Toxaphene residues on growing alfalfa was undertaken from the strictly practical point of view, no attempt being made to study its chemical behavior.
2. For sampling of large plots the material collected from 40 two square foot areas proved to be a valid sampling procedure.
3. Thiophene free benzene was found to be a suitable extractant.
4. Conversion of the organic to inorganic chlorides with sodium-isopropanol showed approximately 90% recovery of Toxaphene.
5. Amperometric titration of chlorides using a rotating platinum electrode is an effective, rapid means of chloride determination.
6. Comparison of residues on samples taken 24 hours after application showed highest per cent deposition in the case of water emulsion (29%); lowest in case of dust (7%).
7. Plots treated with oil solution had the lowest rate of decrease in residue values.
8. Weather, particularly precipitation, had no significant effects on rate of decrease.
9. Measurable losses of Toxaphene in treated alfalfa occurred during the cutting, drying and baling operations, particularly where wettable powder and dust formulations have been employed.
10. Only slight decreases in residues occur during storage of baled alfalfa from Toxaphene treated plots.

ACKNOWLEDGEMENT

The author takes this opportunity to express his appreciation to Dr. Leon H. Johnson who gave freely of his time for this study and whose counsel proved invaluable in surmounting the mamy obstacles; and furthermore, to Professor P. C. Gaines for his excellent advice during the course of this investigation.

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