



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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Abstract:

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 insecticides 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.

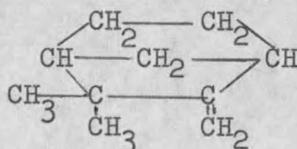
Furthermore it becomes of critical importance to study the problem of the safety of such an insecticide from the point of view of animal husbandry and, indirectly, of human consumption. When a substance, highly effective in destroying, for example, grasshoppers is applied to the foliage of vegetation 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, modifications, even complete revisions had to be applied.

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 wetttable powder and dust formulations.¹

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 follow¹.

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

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 plot¹. Care was taken to include all

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.

Preparation of sub-samples

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 hours¹.

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

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 origin¹. 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 recorded², and transferred to a one liter flask. By use of a steam bath³ 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 funnel⁴. 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

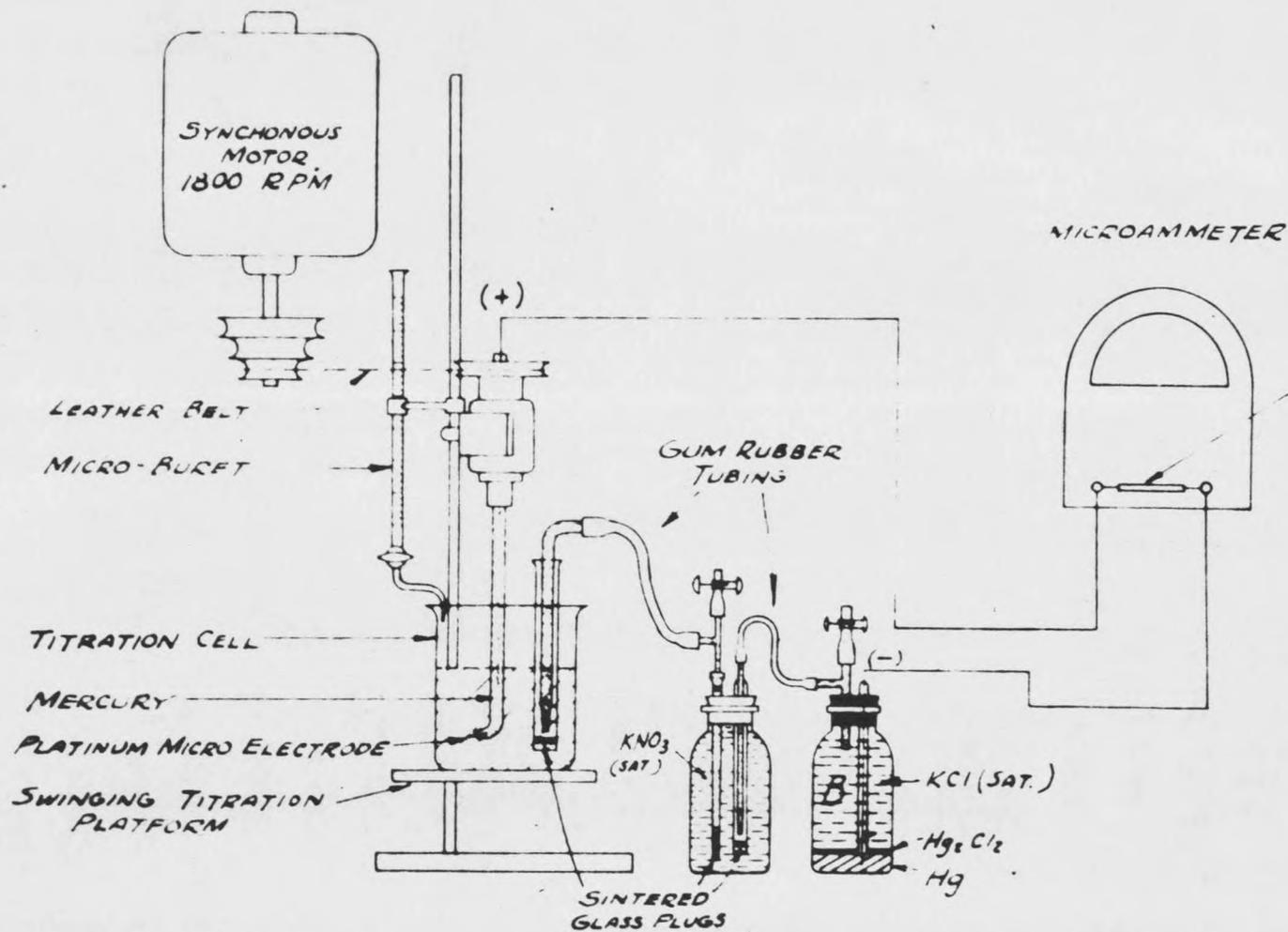
The chlorides which are now in titratable form are determined either amperometrically or by the Volhard method. The former is preferred where the amounts of inorganic chlorides are low and where the color of the solution may interfere with the Volhard. In addition, where large numbers of titrations must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

The essential features of the amperometric apparatus are shown in Figure 2¹. 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

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 Koit-hoff (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

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.

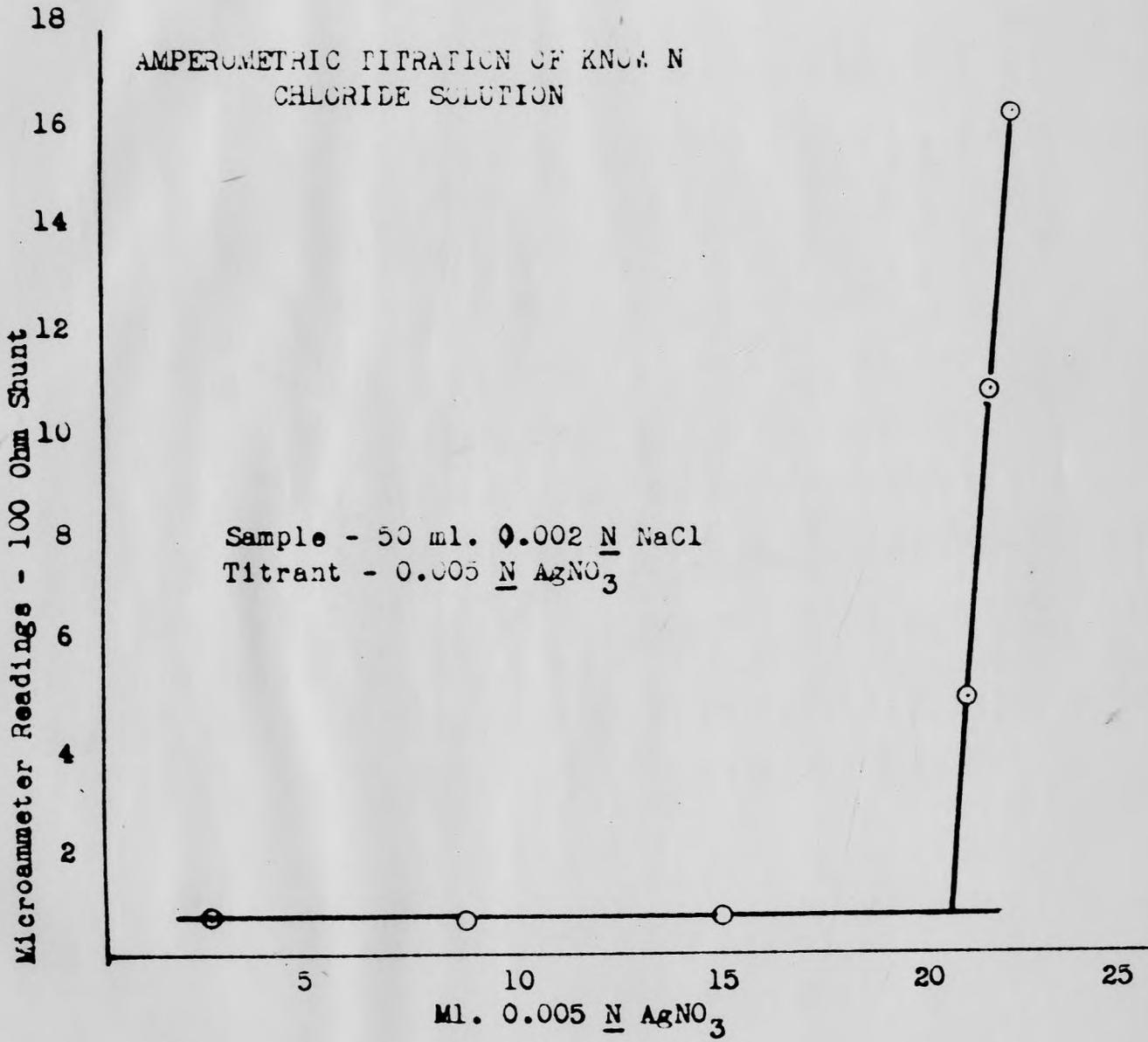


Figure 3

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 N x .03546 x a

where:

$$N = \text{normality of } \text{AgNO}_3$$

$$.03546 = \text{equiv. wt. of Cl} \div 1000$$

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

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

where:

$$.68 = \text{percent chlorine in Toxaphene}$$

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

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

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

$$453 = \text{gms per pound}$$

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

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.

