a standard curve, using soil extract from untreated soil to which are added known amounts of heptachlor and epoxide, is to provide a reliable standard to which unknown readings may be compared. Consequently, in Figure 2, if the blanks from the soil were subtracted from the epoxide curve, the curve would go through the origin.

With the use of this technique an epoxide reading (Figure 2) must be greater than 0.030, with the soil used in this study, to be indicative of the presence of any epoxide at all. Values above 0.030 can be read directly as epoxide. The precision of this procedure is greater at higher epoxide levels, but this does not detract from its utility, as this limitation is characteristic of many methods. In Figure 3 the soil blank has been subtracted from the epoxide curve.

Discussion

The sampling procedure employed in these tests represents the best of several methods which were tried. At first, 3-inch-long cores were taken for analysis (*3*), but as the insecticide was found only in the top inch, it was diluted by a factor of 3, making the lower limit of the test too high to be of maximum value. Analysis of the second inch showed no trace of heptachlor or epoxide on any of the plots tested.

The mixing procedure was checked by extracting three samples from the same group of cores. The results compared within 5% of each other. Subsequent work on other soil experiments has verified the ability to obtain a uniform sample by this method. It is important that the soil be extracted while still moist. An experiment on loss of insecticide from stored soil samples showed as high as 25% loss in 10 days when the soil was allowed to dry. There is a negligible loss when the soil is stored in tightly sealed cans.

Certain factors in the operation of the columns were found critical. If the column is not packed too tightly, it will flow approximately 5 ml. per minute. This rate gives a clean separation in a relatively short time. Not more than four columns should be attempted at one time, as each column must be watched constantly to keep it from going dry.

The adsorbance of the Florisil does not appear to be uniform from one batch to another. For this reason, it is necessary to run preliminary tests to determine the exact polarity of solutions needed to elute the insecticide from the column for every new batch of Florisil.

An expedient in the development of color is the use of graduated centrifuge tubes as reaction tubes. These tubes allow for the adjustment of the final volume, so that the color concentration is within the limits of the curve. If it is necessary to use other than a 6-ml. total volume, the micrograms found should be multiplied by the fraction $X/6$, where X = milliliters of final volume.

Conclusions

The lower limit of the method is approximately 2.5 γ; the largest amount of soil which could be analyzed was 250 grams. The lowest concentration of insecticide in soil which could be accurately detected was 0.01 p.p.m.

The over-all results of the analyses shown in Table I are in agreement with the theory that a rapid loss of insecticide occurs immediately following application. They also show that once the insecticide becomes "fixed" in the soil there is very little loss, even in warm weather.

Table II shows no significant difference between residues left by two formulations. The check plots of series III show some disagreement with those in series II, perhaps caused by a more dense grass cover on series III plots.

In series III there was 64% less insecticide on the suspected skips than on the average of the treated plots. This shows that the described method is sufficiently sensitive to give correlation with biological data.

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INSECTICIDE RESIDUES

The Fate of Heptachlor in the Soil Following Granular Application to the Surface

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Only in recent years has any real concern been shown about the mechanisms of loss of insecticides from the soil. There is apparently a relatively rapid loss of insecticidal action in soil treated with some chlorinated hydrocarbons. The term "breakdown" has been used as an all-inclusive explanation of this loss of insecticidal action. Perhaps using such a term for a little understood phenomenon has delayed study by creating an impression of fact for what was really guesswork.

In the current program for eradication of the imported fire ant, *Solenopsis saevissima richteri*, from 21,000,000 acres in nine southern states, the problem of the residual effectiveness of heptachlor when applied to soil is of considerable importance. Heptachlor is the most effective insecticide tested so far against the imported fire ant. Lofgren and Stringer (*4*) reported that heptachlor, on 24-hour exposure, has an LD_{50} for the imported fire ant of 0.04 p.p.m. in the soil, heptachlor epoxide, a metabolic product, has an LD_{50} of 0.015 p.p.m., while that for an alternate insecticide, dieldrin, was 0.045 p.p.m. Since these laboratory studies are so much at variance with recommended application rates (originally 2 pounds of heptachlor per acre, giving 6 p.p.m. in the top inch of soil, were recommended), a study was undertaken to determine what happened to heptachlor after application to the soil.

Gannon and Bigger (*2*) have shown that in soil application, a portion of the applied heptachlor is converted to heptachlor epoxide. Since they did not give details of their method of sampling,

The all-inclusive term "breakdown" has been used to account for loss of effectiveness of insecticide residues following soil treatment. This study was set up to determine the factors involved in loss of heptachlor residue and possible means of controlling this loss. Much of the loss can be accounted for by vaporization of the insecticide. A number of additives may be used to lower loss by vaporization. An increase in soil residues results from the use of some of the additives used.

Table I. Analysis of Soil for Heptachlor and Heptachlor Epoxide at Various Intervals, Following Granular Application to the Surface[a,b]

When Sampled	Insecticide Found, P.P.M. Heptachlor	Heptachlor epoxide	Total	Insecticide Remaining, %
1 week, 5–24–59	1.4	<0.1	1.4	24
1 month, 7–18–59	0.6	0.2	0.8	14
3 months, 9–15–59	0.2	0.4	0.6	10
6 months, 1–14–60	0.2	0.5	0.7	12

[a] 5.76 p.p.m. of heptachlor actually applied to top inch of soil. All concentrations are based on parts per million in top inch of soil.
[b] Date of application June 17 to 18, 1959.

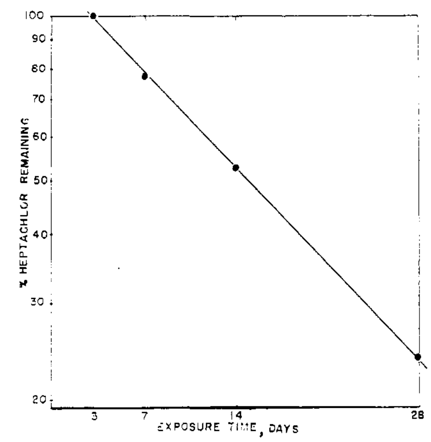

Figure 1. Volatilization of heptachlor from 10% heptachlor granules

Exposed at 70° to 100° F. on August 25, 1959

Table II. Insecticide[a] Remaining Following Application[b] of Heptachlor Granules

Solvent, %	Insecticide Remaining, % 1 week	1 month	3 months	6 months
5	24	12	15	14
10	24	14	10	12
20	24	16	11	12

[a] Heptachlor plus heptachlor epoxide.
[b] Applied June 17 and 18, 1959.

it is not possible to determine their total recovery of insecticide, but it appears to be less than 10% of that applied, if they sampled the top 3 inches of the treated soil.

Young and Rawlins (*7*) made a study of persistence of heptachlor in the soil, when the insecticide was incorporated in the soil, and reported a loss of 74% from field test plots in 21 months. On surface application of heptachlor emulsion, they reported losses as high as 92% in the first week. They checked this work with bioassay and obtained good agreement between specific chemical methods and bioassay, although they did not determine the heptachlor epoxide.

In the author's first experiment, heptachlor was applied at a nominal rate of 2 pounds per acre [20 pounds of 10% of government specification (*1*) granules] to test plots. At intervals of 1 week and 1, 3, and 6 months, soil samples were taken from the treated plots and analyzed for heptachlor and heptachlor epoxide by the method of Murphy and Barthel (*5*). Results of this test are given in Table I.

The difference between the 3- and 6-month samples reflects the usual analytical variation. The data show that there is an initial rapid loss of heptachlor, followed by a long period in which there

Table III. Percentage of Insecticide[a] Remaining in the Soil Following Granular Application[b] of Vegetable and Animal Fat Formulations with Heptachlor

Additive	Insecticide Remaining, % 1 week	1 month	3 months	6 months
Corn oil	32	12	12	15
Peanut oil	38	21	14	12
Neat's foot oil	32	16	18	18
Tallow	29	12	13	16

[a] Heptachlor plus heptachlor epoxide.
[b] Applied June 17 and 18, 1959, at the nominal rate of 1.25 pounds per acre.

is little loss of insecticide and conversion to heptachlor epoxide of a portion of that remaining. The concentration of epoxide was almost constant after 3 months in this test.

When such a rapid loss of insecticide following granular application was noted, a study was made to determine the rate of loss of heptachlor from intact granules. For this study, 10-gram samples of 10% heptachlor granules were spread out over shallow pans and exposed to air under a metal roof, which protected them from rain, yet permitted free circulation of air. A recording thermometer indicated a variation in temperature between 70° and 100° F. The duration of the test was 28 days with two pans being taken for duplicate analyses at 3, 7, 14, and 28 days. The granules were weighed immediately before analysis, and the loss in weight corresponded to the loss of oil solvent plus insecticide. The data are plotted in Figure 1. The loss was apparently by volatilization which commenced as soon as the oil solvent used in the formulation had evaporated.

The loss from intact granules can largely be attributed to volatilization,

Table IV. Effect of Additives on Insecticide[a] Remaining in Soil Following Granular Application of Heptachlor

Additive	Insecticide Remaining, % 1 week	1 month	3 months	6 months
Aroclor	44	32	29	34
Rosin	28	30	22	21
Heavy aromatic naphtha	24	14	10	12

[a] Heptachlor plus heptachlor epoxide.

and since it seemed to follow the loss of the oil solvent, an experiment was set up to determine the effect of various percentages of this oil on the loss of heptachlor, when the granules were applied to the soil. For this study, granules were formulated containing 5, 10, and 20% heavy aromatic solvent (alkylated naphthalenes) and 10% heptachlor on the usual Attapulgus clay granules. The nominal application rate was 12.5 pounds of these 10% granules per acre. The concentration in the top inch of soil in parts per million of heptachlor was calculated from the weight actually applied per square foot. After analysis, the percentage loss of insecticide could be determined.

In Table II, data are presented showing percentage of heptachlor plus heptachlor epoxide remaining in the soil following application of granules formulated with various amounts of oil on Attapulgus clay. The percentage of oil did not affect the loss of insecticide. This may mean that the oil evaporates so rapidly that it has little effect on the long-term residue. In this study, and others, no heptachlor or heptachlor epoxide has been detected in the second inch of soil and leaching has been ruled out as a factor in the loss of heptachlor.

A second experiment was set up to determine the effect of some nonvolatile vegetable and animal oils on the residual life of heptachlor in the soil. It was thought that the presence of oils with low vapor pressures would tend to suppress the vapor pressure of the heptachlor. The materials used and results obtained are listed in Table III. In this work four vegetable and animal oils were substituted for mineral oil and formulated with heptachlor on Attapulgus clay at the rate of 10% heptachlor, 10% oil, and the balance clay and deactivator. Application and calculations were carried out as previously described.

The use of vegetable and animal oils gave a slightly greater residue of insecticide in the soil than that of mineral oils, but not as great as might be anticipated from the difference in vapor pressures. The apparent rapid loss of insecticide from the nonvolatile oil might be explained by considering that there is a partitional separation of the heptachlor from the oil on the soil surface followed by normal loss of insecticide by volatilization.

It was of interest to compare some nonvolatile solids as additives for the heptachlor formulation since these would keep partitional separation at a minimum. For this preliminary study, rosin and Aroclor 5460, a chlorinated biphenyl were used. Hornstein and Sullivan (*3*) had previously reported prolonged residual effects with lindane from the use of Aroclor 5460. Formulation of the additives and heptachlor with clay was carried out in the usual manner, except that a cosolvent, methylene chloride, was used. After formulation, the methylene chloride was allowed to evaporate. The heptachlor and solid additives each amounted to 10% of the finished formulation.

The retention percentages of insecticide, using these solid additives as compared with normal heavy aromatic naphtha solvent, are given in Table IV. The solid additives retained about twice the amount of insecticide on the soil as did the normal heavy aromatic naphtha.

Throughout the analytical studies one consistent and striking behavior of the residues was noted. The heptachlor epoxide content seldom exceeded 10% of the amount of heptachlor applied. This was also observed (*6*) in an earlier study involving the dosage rates from 0.125 to 2 pounds per acre.

In Table V are listed the heptachlor, the heptachlor epoxide, and the total concentration in parts per million remaining at the end of 6 months, as well as the parts per million of heptachlor applied to test plots using formulations with various additives. The original application was made on June 18 to 19, 1959. Samples were taken for analyses on January 14, 1960.

Table V. Heptachlor and Heptachlor Epoxide Content of Plots Treated with Granular Heptachlor after 6 Months

Additive	*Heptachlor Applied in Top Inch, P.P.M.*	*Insecticide Remaining, P.P.M.* Hept.	Hept. epox.	Total
Aroclor	4.05	1.03	0.36	1.39
Rosin	4.35	0.53	0.40	0.93
Neat's foot oil	4.98	0.49	0.39	0.88
Corn oil	4.05	0.22	0.39	0.61
Tallow	3.48	0.18	0.37	0.55
Peant oil	4.26	0.11	0.42	0.53
Heavy aromatic naphtha	5.76	0.18	0.50	0.68

The data in Table V show a 10-fold variation in heptachlor content without significant difference in heptachlor epoxide in the treated soil. This may indicate that the epoxide formed is proportional to the amount of heptachlor applied, while the amount of heptachlor remaining is dependent on many factors that may influence loss by volatilization.

One important factor affecting loss by volatilization is ground cover. To check this, two series of plots were examined. Both series were in Bahia pasture treated with 0.25, 0.50, 1, and 2 pounds of heptachlor per acre in duplicate plots of 1 acre each. Both series were sampled and analyzed after $4^{1}/_{2}$ months. The first series, located on the Moran pasture, was covered with short grass at the time of application. The other series on the Ritchie pasture was long grass in a pecan grove and provided good ground cover. Another difference in these plots, which was thought inconsequential at the time, was that the Moran plots were treated with 20- to 60-mesh clay granules, while the Ritchie plots were treated with 16- to 30-mesh clay granules. The finer mesh on the Moran plots may have favored more rapid loss because of the greater surface area of the granules. Rainfall and weather factors were similar.

In Table VI are data showing the heptachlor and heptachlor epoxide content of soil from these two series of plots. These data were further evidence that factors favoring volatilization of the heptachlor (poor ground cover, fine mesh granules) coincide with rapid disappearance of the insecticide from the soil. In the Ritchie series, the highest conversion to heptachlor epoxide was observed of any field test conducted so far in this work. The maximum conversion was from 15 to 20% of the amount of heptachlor applied.

Table VI. Analysis[a] of Soil from the Moran-Ritchie[b] Tests Following Granular Application of Heptachlor

Application, Lb./Acre	*Residue After $4^{1}/_{2}$ Months, P.P.M.* Moran Hept.	Moran Hept. epox.	Ritchie Hept.	Ritchie Hept. epox.
0.25	<0.01	0.03	<0.01	0.17
0.50	<0.01	0.02	<0.01	0.26
1	<0.01	0.04	0.03	0.50
2	0.01	0.08	0.08	0.96

[a] For comparison purposes 0.25 lb./acre of heptachlor application is 0.75 p.p.m. of heptachlor in top inch; 0.50 lb. is 1.5 p.p.m.; 1 lb. is 3 p.p.m.; and 2 lb. are 6 p.p.m. All values are based on samples from top inch.

[b] Moran plots treated on April 15, 1959; sampled on August 28, 1959. Ritchie plots treated on May 25, 1959; sampled on November 2, 1959.

An attempt was made to study the possibility of decomposition of heptachlor as a factor in its loss from soil. At the low concentrations studied, no soils could be found with a sufficiently low blank to permit the use of the total chloride method. The many total chloride analyses run in conjunction with the specific method indicated no apparent accumulation of decomposition products of heptachlor in the soil.

In this study, the major loss of heptachlor was concluded to be by volatilization. The other significant loss was by conversion of heptachlor to heptachlor epoxide. This was found to reach a maximum of 20% of the amount applied. Losses due to decomposition and leaching were too small to be detected by the methods of analysis used.

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