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INSECTICIDE RESIDUES

Determination of Heptachlor and Heptachlor Epoxide in Soil

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An improved method is described which makes possible the determination of 0.01 p.p.m. of heptachlor and heptachlor epoxide in soil with satisfactory accuracy. Soil from plots treated with 0.25, 0.50, 1, and 2 pounds per acre of heptachlor was analyzed 3 and $5^1/_2$ months after treatment. A second group of samples was taken after 8 months from an area which was treated with two different granular formulations. A third group of samples was taken in a treated area where biological control was incomplete, in an attempt to correlate chemical and biological data. The initial loss of insecticide from the soil appears to be rapid, and is followed by a much slower loss and conversion of a portion of heptachlor to heptachlor epoxide.

RECENT WORK in this laboratory demonstrated a need for a method to determine quantitatively heptachlor and heptachlor epoxide residues as low as 0.01 p.p.m. in soil. Since this is below the limits of present published methods (*2*, *4*, *10*), a study was undertaken to find a more sensitive procedure.

There were two possible approaches to the problem. The first would involve finding a reagent capable of yielding a more intense color than does the Polen-Silverman reagent (*7*) for both heptachlor and its epoxide. Davidow's (*1*) and Radomski's (*8*) work was in this direction, but did not attain the necessary sensitivity. The second approach would make use of a much larger soil sample with suitable elimination of the interfering substances. The latter method was selected for this study.

Table I. Insecticide Residues[a] of Four Different Dosages of Heptachlor after 3 and $5^1/_2$ Months; Series I

Lb./	3 Months[b]			3 Months[c]			$5^1/_2$ Months[c]		
Acre	Hept.	Epox.	Total	Hept.	Epox.	Total	Hept.	Epox.	Total
$^1/_4$	<0.1	0.2	0.2	<0.01	0.16	0.16	<0.01	0.17	0.17
	<0.1	0.2	0.2	<0.01	0.12	0.12	0.01	0.17	0.17
Av.	<0.1	0.2	0.2	<0.01	0.14	0.14	0.01	0.17	0.17
$^1/_2$	<0.1	0.2	0.2	<0.01	0.24	0.24	0.01	0.26	0.26
	0.1	0.2	0.2	<0.01	0.12	0.12	0.01	0.27	0.28
Av.	0.1	0.2	0.2	<0.01	0.18	0.18	0.01	0.26	0.27
1	0.1	0.6	0.7	0.04	0.62	0.66	0.04	0.67	0.71
	0.1	0.4	0.5	0.05	0.33	0.38	0.03	0.33	0.37
Av.	0.1	0.5	0.6	0.05	0.48	0.52	0.03	0.50	0.54
2	0.2	0.8	1.0	0.20	0.78	0.98	0.07	0.86	0.75
	0.3	0.9	1.2	0.28	0.86	1.14	0.08	1.06	1.14
Av.	0.3	0.8	1.1	0.24	0.82	1.06	0.08	0.96	1.04

[a] All values represent p.p.m. in the top inch of soil.
[b] Data from use of 50-gram soil samples without acid wash.
[c] Data from use of 250-gram soil samples with acid wash.

Experimental

Series I. Ten plots measuring 200 × 400 feet were set up in a large pecan grove. Two of these were not treated and were used as check plots throughout the experiment. The others were treated in duplicate with 0.25, 0.50, and 1 pound of heptachlor per acre, using 10 pounds of 2.5, 5, and 10% heptachlor granules, respectively, and with 2 pounds of actual heptachlor per acre, using 20 pounds of granules which contained 10% of heptachlor. The granules were applied by a Buffalo turbine mounted on the back of a jeep. The plots were treated on May 25, 1959, and sampled on August 29 and on November 2, 1959, 3 and $5^1/_2$ months, respectively, after treatment. Results are given in Table I.

Series II. An airfield of approximately 500 acres was divided into two treatment areas of similar size. Five 1-acre plots were selected throughout each treatment area, from which the soil samples were taken. Before treatment, blank samples were taken from these plots for use on the recovery curve. On one treatment area, a granular formulation containing 10% oil and 10% heptachlor was applied at 10 pounds per acre. On the other treatment area, granules which were formulated with no oil were applied at the same dosage. After 8 months, the five plots of each area were sampled and the residues were determined.

Series III. Samples were also taken from plots of another location which was treated with 10% oil-containing granules at the same time and dosage as in Series II. At the time of sampling, active ant mounds were found in the treated area. These mounds appeared to be in rows parallel to the line of flight taken by the aircraft while applying the insecticide. Samples were taken along

these rows of ant mounds to see if it could be demonstrated that skips in application could be chemically detected. Results are shown in Table II.

Reagents

Heptachlor standard solution containing 10 γ per ml. of pentane made from analytical grade heptachlor (Velsicol Chemical Corp., 300 East Grand Ave., Chicago 11, Ill.).

Heptachlor epoxide standard solution containing 10 γ per ml. of pentane made from analytical reference grade heptachlor epoxide (Velsicol Chemical Corp.).

Polen-Silverman reagent (*7*), prepared at least one month in advance, using the method suggested by Ordas *et al.* (*5*).

Benzene–isopropyl alcohol mixture, containing 4 parts of benzene to 1 part of isopropyl alcohol.

Pentane, pure grade (Phillips Petroleum Co.), further purified by the method suggested by Polen (*6*). This method involves passing 10 liters of pentane through a 5-liter percolator containing 500 grams of 60/100-mesh activated Florex (Floridin Co.) between two plugs of glass wool. The first and last 500 ml. are discarded and the middle fraction is collected and stored in glass containers. The Florex was previously activated for 48 hours at 135° C.

Carbon black, technical lamp black, Monsanto.

Fuming sulfuric acid, 7%, made by adding 1 part of concentrated sulfuric acid to 1 part of 15% fuming sulfuric acid. Caution: exothermic reaction.

Special Equipment

Extraction tumbler suitable to rotate concentrically eight 1-gallon paint cans held in a horizontal position.

Snyder distilling column, three-bulb, borosilicate glass, with 24/40 ground-glass joints.

Chromatographic columns of the type described previously (*9*), with the exception that a coarse-porosity sintered-glass disk was necessary.

Constant temperature oil bath (100° C.) equipped with rack to hold at least eight test tubes.

Centrifuge tubes, borosilicate glass, 15-ml., graduated in 0.1 ml., which were used as reaction tubes.

Bausch & Lomb Spectronic 20 or an equivalent spectrophotometer which is suitable for reading wave lengths of 567 and 410 mμ.

One-half-gallon fruit jars, equipped with standard snap lids and rings.

Sampling Procedure

Fifty cores were taken diagonally across each plot, using a 2-inch diameter auger set for a 2-inch depth. This method was substituted for random sampling to obtain a more representative sample. Since the plots were treated in strips, small overlaps and skips that could occur would be included in the sample. Random sampling would not give consistently representative samples with this type of treatment without resorting to a much greater number of cores with resultant handling problems.

As only a 1-inch core was needed, every core was measured and cut with a knife exactly 1 inch below the ground level. The lower part of the core was discarded, except random samples which were saved in separate containers. Two sources of error made it necessary to take deeper cores than needed. First, the grass formed a pad of inconsistent thickness, and second, the grass roots made it impossible to break the core at the required depth. These two errors combined could result in as much as a 50% error, using an auger set for a 1-inch depth. Taking cores of greater length and cutting them overcame these difficulties. The cores were then placed in 25-pound lard cans for storage until extraction.

The mixing procedure was accomplished by manually grating the moist cores through $^1/_4$-inch wire mesh, making sure to strip the soil from the grass and roots as completely as possible. The grass and roots were discarded and the soil was collected in 25-pound lard cans. It was mixed further by hand-stirring and by sieving through wire mesh a second time.

Analytical Procedure

The moisture content was determined by drying a weighed amount of soil on the steam bath and reweighing. For analysis, an unheated sample of 500 grams (based on the dry weight of the soil) was placed in a 0.5-gallon jar and 1000 ml. of a 3 to 1 mixture of pentane and isopropyl alcohol was added. The jars were tightly sealed and placed on the extraction tumbler for 4 hours. After removal from the tumbler, they were kept sealed until analysis was begun.

Five hundred milliliters of the extract was filtered into a 1000-ml. Erlenmeyer flask, which was then fitted with a Snyder column and the extract evaporated on the steam bath to about 250 ml. It was then transferred quantitatively to a 500-ml. separatory funnel, where it was washed twice with 200-ml. portions of distilled water to remove the isopropyl alcohol. Any emulsions which formed were broken by the addition of small amounts of a saturated sodium bicarbonate solution. The pentane layer was then dried, in a 500-ml. Erlenmeyer flask, over anhydrous sodium sulfate.

It was then poured into a clean, dry 500-ml. separatory funnel, and the volume increased to 300 ml. with pentane. Thirty-five milliliters of 7% fuming sulfuric acid was added slowly and the separatory funnel was shaken moderately for 1 minute. After separation, the sulfuric acid layer was drawn off. Another 35-ml. portion of fuming sulfuric acid was added, and the mixing process was repeated. Vigorous shaking causes emulsions which are difficult to break. After the second sulfuric acid wash there may be a small amount of emulsion left in the separatory funnel. This may be broken with the careful addition of water, one drop at a time, making sure to allow for the dissipation of heat between drops. Caution: Never attempt this addition of water if more than 1 inch of emulsion is present in the bottom of the separatory funnel.

When the emulsion was completely broken and the sulfuric acid layer was discarded, the pentane layer was washed with two separate 150-ml. portions of distilled water. After discarding the water, the pentane layer was dried over anhydrous sodium sulfate in a 500-ml. Erlenmeyer flask. The dried sample was then decanted into another 500-ml. Erlenmeyer flask, and the sodium sulfate was washed with pentane several times.

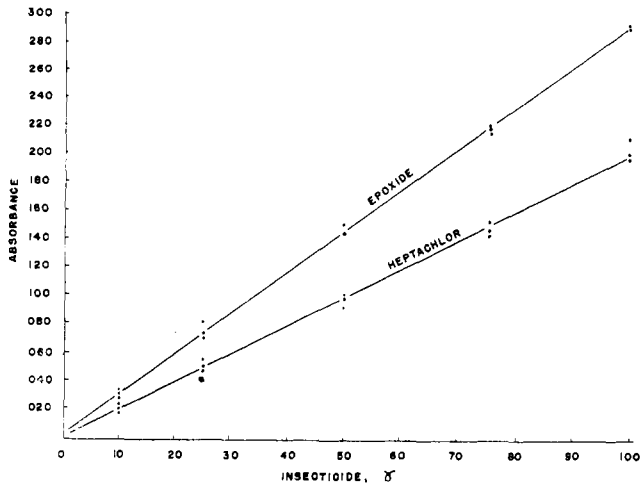
Table II. Determination of Heptachlor and Heptachlor Epoxide in Soil from Series of Field Plots[a]

Plot No.	Heptachlor, P.P.M.	Epoxide, P.P.M.	Total,[b] P.P.M.	% of Original
	Series II (without oil)			
1	0.25	0.15	0.40	13
2	0.13	0.14	0.27	9
3	0.11	<0.1	0.16	4
4	0.18	0.15	0.33	11
5	0.54	0.23	0.77	26
			Av.	12
	Series II (with oil)			
1	0.32	0.20	0.52	17
2	0.18	<0.1	0.23	8
3	0.17	<0.1	0.22	8
4	0.13	0.18	0.31	10
5	0.13	0.13	0.26	9
			Av.	10
	Series III (with oil)			
1	<0.1	0.40	0.45	15
2	0.10	0.36	0.46	15
3	0.10	0.16	0.26	9
4	0.22	0.28	0.50	17
			Av.	14
	Series III (skips in application)			
1	0.10	0.18	0.28	9
2	<0.1	<0.1	0.10	2
3	0.11	<0.1	0.16	4
			Av.	5
	Checks[c]			
1	0.12	0.92	1.04	95
2	0.14	1.00	1.14	104
3	0.50	0.47	0.97	97
4	0.48	0.53	1.01	101
5	0.17	0.25	0.42	105
6	0.22	0.23	0.45	112
			Av.	102

[a] 50-gram soil samples were used for analysis with the sulfuric acid wash eliminated, giving a lower limit of 0.1 p.p.m.

[b] In calculation of total p.p.m., the figure <0.1 was given an arbitrary value of 0.05 p.p.m.

[c] Following amounts of insecticide added to blank soil for checks: checks 1 and 2, 0.10 p.p.m., heptachlor and 1.00 p.p.m. heptachlor epoxide; 3 and 4, 0.50 p.p.m. heptachlor and 0.50 p.p.m. heptachlor epoxide; 5 and 6, 0.20 p.p.m. heptachlor and 0.20 p.p.m. heptachlor epoxide.



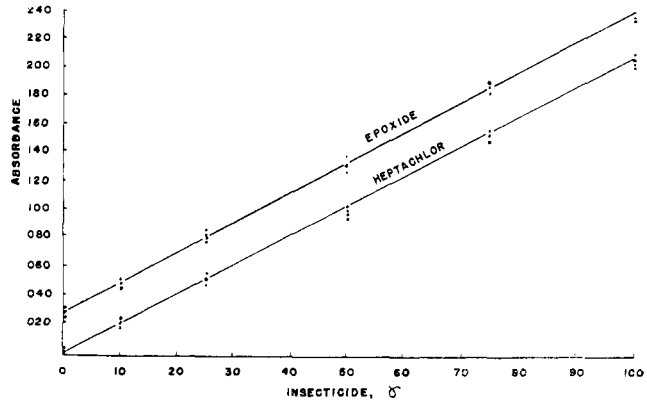


Figure 1. Standard calibration curve for heptachlor and heptachlor epoxide

The sample was then evaporated to approximately 20 ml. on the steam bath by using a Snyder column.

When the residues of both heptachlor and its epoxide are above 0.2 p.p.m., the fuming sulfuric acid wash may be eliminated from the procedure by using a 50-gram soil sample weight (100 ml.). If the acid wash is eliminated, the sample should be washed with water three times to remove all alcohol prior to introduction into the chromatographic column. Table I shows a comparison between samples run with and without the acid wash. The results in Table II were obtained without the acid wash.

The chromatographic columns were filled, using dry materials. A 1/4-inch layer of sodium sulfate next to the sintered-glass disk stopped the disk from becoming clogged with Florisil. Ten grams of Florisil (60/100-mesh, Floridin Co.) was poured into the column and gently tapped to smooth the top of the powder. Ten grams of 15 to 2 Florisil-carbon mixture was added next, with the same tapping procedure. On top of this was added another 5 grams of Florisil. A 1/2-inch layer of sodium sulfate was added to protect the column from any moisture or mixing action of the added solvents.

The column was washed with 100 ml. of 5% ether in pentane, followed by 100 ml. of pentane. The sample was then added and washed into the column with two 20-ml. portions of 2% benzene in pentane. As soon as the sample was added, the collection of the heptachlor fraction in a 500-ml. Erlenmeyer flask was begun. When the liquid level approached the top of the sodium sulfate layer, 100 ml. more of 2% benzene in pentane was added to the column and collected as part of the heptachlor fraction.

When the column again approached dryness, 225 ml. of 5% ether in pentane was added to elute the heptachlor epoxide. The flasks were changed immediately after this addition, and the heptachlor epoxide fraction was collected.

Both the heptachlor and heptachlor epoxide fractions were evaporated, using Snyder columns, to approximately 5 ml. on the steam bath. It is extremely important that the samples do not go to dryness on the steam bath, as the insecticide is lost quickly at this temperature. The samples were quantitatively transferred to graduated 15-ml. borosilicate glass centrifuge tubes, using pentane as the washing solvent. The volumes were then reduced to 0.5 ml. by evaporation in a 40° C. water bath using a gentle flow of dry air. One milliliter of Polen-Silverman reagent was added and the tubes were placed in a 100° C. constant-temperature bath for exactly 15 minutes. Then the tubes were removed and cooled under the tap to room temperature, with care not to allow water inside the tubes.

The volume was increased to 6 ml. with the benzene–isopropyl alcohol mixture. The heptachlor was read at the wave length of 567 mμ while the epoxide was read at 410 mμ. The readings were then compared with the recovery curves which were previously prepared by using blank soil samples containing known amounts of insecticide.

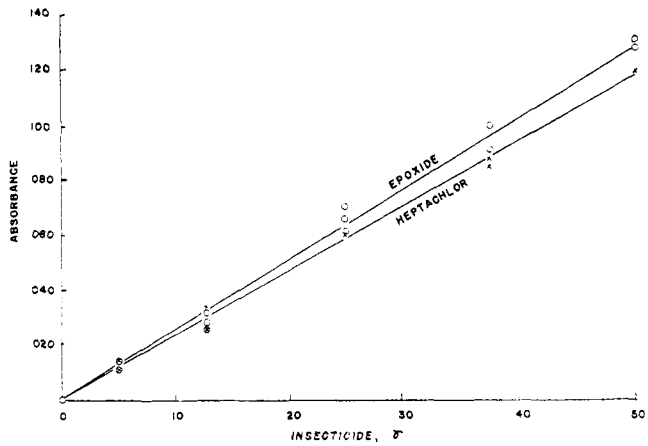


Figure 2. Recovery of heptachlor and heptachlor epoxide in 50 grams of soil

Figure 3. (Left). Recovery curves of heptachlor and heptachlor epoxide in 250 grams of soil

Calibration and Recovery Curves

A calibration curve was prepared by placing 1.0, 2.5, 5.0, 7.5, and 10.0 ml. of standard solutions of heptachlor and heptachlor epoxide in the graduated centrifuge tubes. They were evaporated and the color was developed exactly as the procedure described. The absorbance was then plotted against concentration (Figure 1).

A recovery curve was prepared by adding known amounts of the insecticides to the soil blanks. The samples were carried through the entire procedure described above. The amount of insecticide added, in micrograms, was plotted against the absorbance (Figures 2 and 3). Addition of known amounts of the insecticides to soil extract gave results identical with those obtained when the insecticides were added prior to extraction. Several types of soil were analyzed and all gave consistent values on blank determinations.

Unless a method of analysis can be provided which will completely eliminate color due to the reagents and the untreated soil, the use of a standard with accompanying standard curves as presented in this paper becomes of major importance. The purpose in preparing

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.

Acknowledgment

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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,