Chemical Determination of Dieldrin in Crop Materials

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Two analytical methods have been developed for the determination of dieldrin residues at concentrations as low as 0.1 p.p.m. in agricultural crops. Both methods involve the separation of dieldrin from the plant material by extraction and chromatography. The dieldrin thus separated may be determined by analysis for chlorine or, preferably, by a spectrophotometric method. The latter method involves reduction of the epoxide group in dieldrin to an olefinic group, followed by conversion of the olefin to a colored product through reaction with phenyl azide. Both methods have been found to be capable of determining dieldrin residues of 0.1 p.p.m. or more in a wide variety of crops. The spectrophotometric method has proved to be more specific and sensitive.

THE INSECTICIDAL COMPOUND dieldrin (I), 1,2,3,4,10,10-hexachloro-6,7-epoxy-1,4,4a,5,6,7,8,8a - octahydro - 1,4-endo-exo-5,8-dimethanonaphthalene, is the product of epoxidation of the olefinic insecticide, aldrin (*4*). Analytical data on residues of dieldrin down to 0.1 p.p.m. in treated agricultural crops were desired. Although bioassay methods, such as those of Sun and Sun (*7*), have been useful in obtaining such data; chemical analytical methods were desired because of their greater specificity and their applicability in laboratories lacking bioassay facilities.

To satisfy this need, two chemical methods patterned after those adopted for aldrin residues have been developed. The first method, based on determination of chlorine in the isolated insecticide, involves chromatographic separation of dieldrin from naturally occurring (plant) halogen compounds and analysis by the combustion–chloride ion titration procedure of Agazzi, Peters, and Brooks (*1*). The second method also utilizes the chromatographic separation, but in this case the separated dieldrin is converted to partially dechlorinated aldrin (Compound III), which can then be determined by the modified phenyl azide–spectrophotometric method for aldrin (*5*). The latter method is more sensitive and specific for dieldrin.

Davidow and Laug (*2*) and Gunther, Kolbezen, and Blinn (*3*) have recently reported in a preliminary way on other spectrophotometric approaches for determining dieldrin, but did not give results for residues on crops.

Apparatus

The apparatus described in the method for aldrin (*5*), except the filter apparatus, is required in addition to that described below. Rubber, including rubber-base stopcock lubricant, should be excluded from all apparatus, as contact of sample or reagents with rubber causes serious interference in the spectrophotometric method through formation of bluish colors. A silicone lubricant is satisfactory for stopcocks.

Conversion tube, as shown in Figure 1.

Extract concentration flask, similar to the evaporation flask (*5*) but of 1-liter capacity and having an inner 19/38 standard-taper joint.

Filtering-washing apparatus, as shown in Figure 2.

Oil bath, as shown in Figure 1.

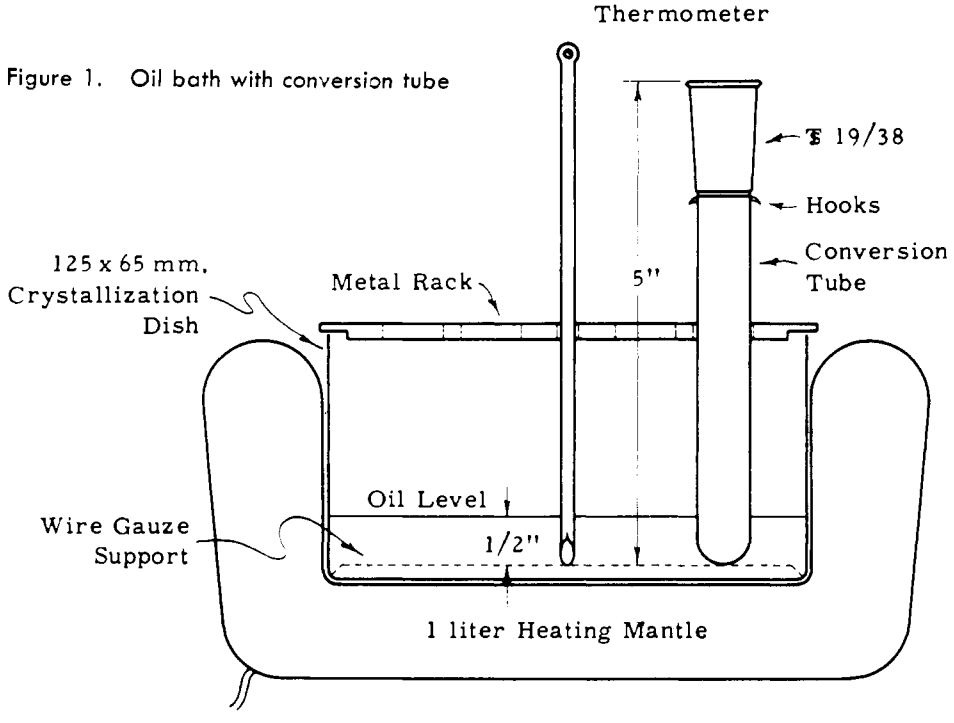
Reagents

The following reagents are required in addition to the extraction solvent and phenyl azide reagent used in the aldrin method (*5*).

Acid Mixture. Add 10 ml. of 48% C.P. hydrobromic acid to a glass-stoppered, 50-ml. Erlenmeyer flask, cool in an ice bath, and slowly add 20 ml. of reagent grade acetic anhydride. Stopper the flask and allow to stand at least 30 minutes at room temperature before use.

Magnesia Adsorbent Mixture. Thoroughly mix 2 parts by weight of magnesia with 1 part by weight of Celite 545 (a diatomaceous earth filter aid manufactured by the Johns-Manville Co.). Magnesia manufactured by the Westvaco Chemical Division, Food Machinery and Chemical Corp., Newark, Calif., and designated as "adsorptive powdered magnesia No. 2642" was

Figure 1. Oil bath with conversion tube



found to be the only satisfactory commercial magnesia of a number examined. Keep the magnesia in tightly closed containers to protect it from moisture and carbon dioxide. The loss on ignition at 1000° C. should be less than 10% and the loss on heating at 110° C. for 4 hours should be less than 2% but not less than 0.1%.

Asbestos. Medium fiber, acid-washed.

Diazotized 2,4-Dinitroaniline. Add 1.5 ± 0.05 grams of 2,4-dinitroaniline, Eastman white label material which has been recrystallized from aqueous ethyl alcohol (*8*), to 30 ± 0.5 ml. of concentrated sulfuric acid (97%) and warm to 40° C. to dissolve. Cool the solution in a salt and ice bath and slowly sift in 0.7 ± 0.01 gram of finely powdered sodium nitrite while stirring with a motor-driven stirrer. When all the solid has dissolved (approximately 3 hours), add 40 ± 0.5 ml. of 85% phosphoric acid at a rate sufficiently slow to keep the temperature below 20° C. Discontinue the stirring, remove the solution from the salt-ice bath, and allow to stand at room temperature for at least 2 hours before using. Discard the solution after 3 weeks or if it darkens to a deep orange color.

Dieldrin Standard Solutions. Use dieldrin of at least 99.5% purity (obtainable from Shell Chemical Corp., Denver, Colo.), dissolved in purified hydrocarbon solvent to make solutions containing 0, 5, 10, 20, 30, and 40 γ per ml.

Purified Hydrocarbon Solvent. Distill a C_6-petroleum fraction (Skellysolve B, produced by Skelly Oil Co. and available from chemical supply houses has been found satisfactory), using an all-glass apparatus having a short column and spray trap to prevent entrainment. Discard a 10% forecut and leave 15% bottoms. The residue from evaporation of 500 ml. of purified solvent should analyze less than 4 γ of apparent dieldrin by either method (omitting chromatography).

Sodium chloride solution, 20% aqueous.

Zinc dust, reagent grade.

Procedure

Preparation of Extract. Wash, macerate, and extract the crop as in the aldrin method (*5*). If glycerides are present (as in peanuts or cottonseed oil), remove them from an amount of extract representing 100 grams of crop as directed (*5*), and evaporate to a volume of 100 ± 5 ml. on a steam bath. If glycerides are not present, take a portion of extract representing 200 to 300 grams of crop for chlorine analysis or 100 to 200 grams for spectrophotometric analysis and evaporate to a volume between 75 and 100 ml. When the solution has cooled, dilute to 100 ± 5 ml. with purified hydrocarbon solvent.

Chromatographic Separation

Calibration of Adsorbent Mixture. Attach a 500-ml. round-bottomed flask with a 24/40 standard-taper joint to the bottom of the chromatographic column and apply a vacuum of approximately 200 mm. of mercury to the side arm. Place a small pad of cotton on the plate in the column. Weigh 50 ± 0.5 grams of the adsorbent mixture, and while tapping the column to ensure uniform packing, add the adsorbent mixture and lightly press the surface of the adsorbent using a flat-ended wooden rod. Add a top layer of 3 cm. of anhydrous sodium sulfate. Add 100 ml. of purified hydrocarbon solvent and draw down to the top of the sodium sulfate layer at a rate of approximately 100 ml. per 30 minutes.

Pour into the reservoir of the column a solution of dieldrin in the 100 ml. of concentrated, glyceride-free extract. Use 200 γ of dieldrin for the spectrophotometric method or 1000 γ for the chlorine method. Complete the transfer and wash down the sides of the reservoir with a few milliliters of purified hydrocarbon solvent as a stream from a wash bottle. Draw down the solution as before. Wash down the reservoir and draw the washing into the column. Add and draw down 100 ml. of purified hydrocarbon solvent. Release the vacuum over a period of not less than 5 seconds and replace the flask with a clean, dry 250-ml. round-bottomed flask. Discard the effluent.

Add and draw down 50 ml. of purified hydrocarbon solvent. Release the vacuum, remove the flask from the column, and designate as fraction 1. Repeat this operation 16 additional times, numbering the fractions consecutively from 2 to 17. Analyze each effluent fraction for dieldrin content.

Assuming that fractions assaying less than 0.125 absorbance (1-cm. light path) or 15 γ of apparent dieldrin (by chlorine method) are free of dieldrin, calculate V_1 and V_2 as follows:

$$V_1, \text{ml.} = 50\ F$$

$$V_2, \text{ml.} = 50\ (L - F + 3)$$

where F = number of the first fraction which contains dieldrin (F should be at least 2); L = number of the last fraction which contains dieldrin; V_1 = a volume of developer which does not elute appreciable dieldrin from the column; and V_2 = milliliters of additional developer necessary to elute all the dieldrin from the column. It includes developer corresponding to effluent fractions $F - 1$ and $L + 1$ to provide a safety factor in the event of minor displacements of chromatograms.

Separation of Dieldrin from Sample. Prepare a chromatographic column as for calibration of adsorbent mixture and wash with 100 ml. of purified hydrocarbon solvent. Quantitatively transfer the 100 ml. of concentrated extract obtained in the preparation of extract to the reservoir of the column, using a few milliliters of purified hydrocarbon solvent to complete the transfer. Apply a light vacuum (ca. 200 mm. of mercury) to the column and allow the solution to pass through the column until the liquid level drops just below the top of the sodium sulfate. Wash down the sides of the reservoir with a few milliliters of purified hydrocarbon solvent and draw the liquid level just below the top of the sodium sulfate. Repeat the washing and then add the volume of purified hydrocarbon solvent, V_1. When this has been drawn just below the top of the sodium sulfate, slowly release the vacuum and replace the flask with a dry flask. Add the volume of solvent, V_2, draw it into the column, and collect the effluent.

Determination of Dieldrin by Chlorine Method

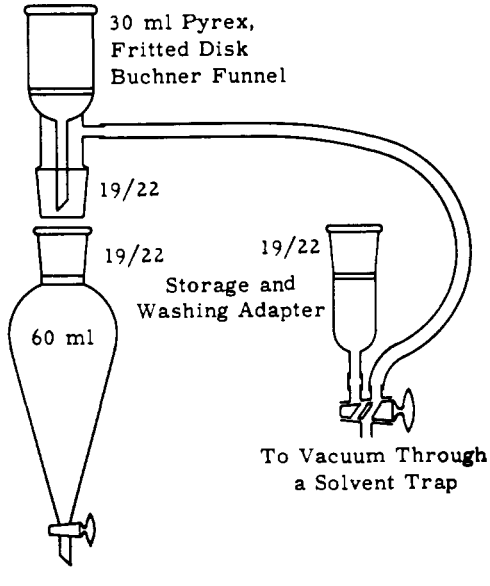
Analyze the fraction V_2 for chlorine content, using the procedure described in the aldrin method (*5*).

Determination of Dieldrin by Spectrophotometric Method

Preparation of Calibration Curve. Prepare a graph showing the relationship between dieldrin content and absorbance as follows:

Pipet triplicate 1-ml. aliquots of the dieldrin standard solutions containing 0, 5, 10, 20, 30, and 40 γ per milliliter into separate conversion tubes and evaporate the solvent by blowing with a gentle stream of dry air just until no solvent is visible. Pipet 1 ml. of the acid mixture into the tube. Heat in the oil bath for 30 minutes. Maintain the bath temperature (115° to 120° C. at sea level) to cause the reagents to reflux to within 1 or 2 inches of the top

Figure 2. Filtering-washing apparatus



of the tube. Remove the tube from the bath, and, after 5 minutes, add 0.4 ± 0.1 gram of zinc dust to each tube through a funnel to keep the dust away from the ground joint. Heat again in the oil bath for 15 minutes, remove, and rinse the outer surface with purified hydrocarbon solvent to remove adherent oil. Wash the mouth and inside of the tube with 5 ml. of purified hydrocarbon solvent. Add 5 ml. of aqueous sodium chloride solution to the tube, stopper, and shake it vigorously for 30 seconds. Transfer the liquids into the filter (see Figure 2), rinse the tube with a stream of purified hydrocarbon solvent, and filter. Wash the reaction tube with 20 ml. of the sodium chloride solution and filter. Break up any lumps of zinc in the tube or filter with a stirring rod and rinse the tube and filter with 20 ml. of purified hydrocarbon solvent. Stopper the funnel, and shake it vigorously. Withdraw and discard the lower aqueous phase. Rinse the sides of the separatory funnel with water and withdraw and discard the water. Quantitatively transfer the hydrocarbon solution out of the top of the funnel into an evaporation flask fitted with a low-actinic reaction tube, using purified hydrocarbon solvent.

Evaporate the solvent, treat with phenyl azide, and form the colored solution as in determination of aldrin in sample (*5*). Filter the colored solution through a 30-ml. fine-porosity sintered-glass Büchner funnel having a layer of asbestos which has been washed with ethyl alcohol and dried of all solvent by drawing air through the funnel.

Transfer the filtrate to an absorption cell and measure the absorbance of the solution relative to distilled water at 515 mμ using the spectrophotometer. Subtract the average absorbance of the zero dieldrin standards from the absorbance of each of the other standards. Plot the net absorbances as ordinates against the micrograms of dieldrin as abscissas and draw the straight line which best fits the points. The average absorbance of the zero dieldrin standards should not exceed 0.090 and the slope of the curve should be approximately 0.012 absorbance per microgram of dieldrin, when measurements are made using a cell with a 1-cm. light path.

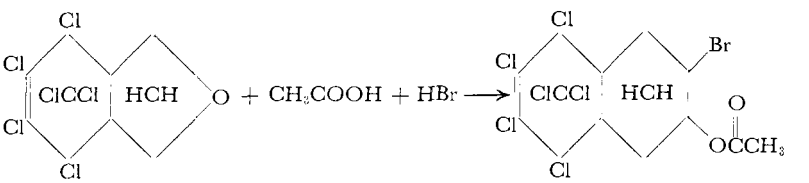
Determination of Dieldrin in Sample. Transfer the effluent containing the dieldrin (from V_2 in the separation of dieldrin from sample) to an extract concentration flask fitted with a conversion tube. Insert the distillation trap and evaporate the solvent on a steam bath until only a few milliliters of solvent remain. Remove the tube and evaporate the remaining solvent using a gentle stream of dry air.

Pipet 1 ml. of the acid mixture into the tube and continue as in the preparation of calibration curve.

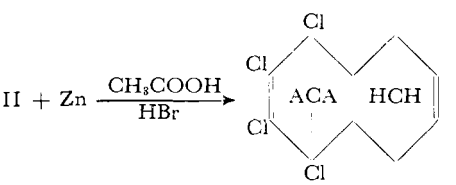
Make triplicate blank determinations by using 500-ml. portions of purified hydrocarbon solvent in lieu of the effluent from the chromatographic column and following the procedures described above. Correct the absorbance of the sample by subtracting the average absorbance of the blanks. From the calibration curve determine the weight of dieldrin corresponding to the net absorbance and calculate as parts per million in the original sample of crop material.

Development of Reduction–Phenyl Azide Procedure

Chemistry of Conversion Procedure The principal reactions involved in the conversion procedure of the method are:



Dieldrin (I) 6-Acetoxy-7-bromo-6,7-dihydroaldrin (II)



A = H or Cl
Partially dechlorinated aldrin (III)

The dieldrin reacts with a mixture prepared from 2 parts of acetic anhydride and 1 part of 48% hydrobromic acid to open the oxirane ring and form 6-acetoxy-7-bromo-6,7-dihydroaldrin (II) which is then reduced to partially dechlorinated aldrin (III) by adding zinc dust and refluxing. Approximately 2 chlorine atoms are removed from the methylene bridge (*6*) during the reduction. When III was analyzed by the phenyl azide–spectrophotometric method, the absorbance maximum was at 515 mμ, which is characteristic of the aldrin dye (*5*). Consequently, the reactions of converted dieldrin are presumed to be similar to those of aldrin.

Conditions for Conversion To be useful analytically, it was necessary that the procedure for converting pure dieldrin to an olefinic substance give reproducible, high yields and produce negligible amounts of contaminants from reagents. Additionally, it was desired that any olefin initially present be inactivated to prevent its interfering as converted dieldrin. To find conditions which achieved these objectives, investigation was made of the effect of reagent composition, reaction temperatures, and times in the conversion step. Tests were made with solutions of dieldrin in 500 ml. of purified hydrocarbon solvent. With these samples, maximum reproducible recoveries and low reagent blanks were obtained when the following conditions were used:

A reagent consisting of 1 to 5 volumes of acetic anhydride or acetic acid to one volume of 48% hydrobromic acid.

Bath temperature (115° to 120° C.) which caused the reagent to reflux to within 1 or 2 inches of the top of the conversion tube and

Times of 10 to 30 minutes for the ring-opening and reduction steps.

These conditions were tested for inactivating a reactive olefinic substance. Aldrin, which reacts quantitatively with phenyl azide, was studied, as is shown in Table I. More of the aldrin was rendered unreactive toward phenyl azide when the reagent was made from 2 volumes of acetic anhydride and 1 volume of 48% hydrobromic acid and times of the ring-opening and reduction steps were 30 and 15 minutes, respectively. The inactivation of aldrin may be explained as the addition of hydrobromic acid to the unsubstituted olefinic group, for zinc treatment would not reduce the resulting compound to an olefin.

Condition of Sample. Recovery of dieldrin in the presence of 1 ml. of deliberately unremoved hydrocarbon solvent was found to be low by as much as 50%. Therefore, solvents are removed prior to the ring opening step by blowing

Table I. Effect of Conversion Conditions on Interference of 3 Mg. of Aldrin[a]

Acid Ratio, Volume			Time of Ring Opening Reaction, Min.	Interference (Calcd. as Dieldrin), %
48% HBr	Glac. AcOH	Ac_2O		
1	..	2	30	1.6, 1.5
1	5	..	30	2.3, 3.8, 5.2
1	1	..	10	1.8, 9.0
1	..	2	10	4.2, 4.6
..	1[b]	..	10	9.2, 9.2
1	5	..	10	>10, >10

[a] 15 minutes used for reduction step.
[b] Saturated with hydrogen bromide.

with air. However, blowing must be controlled as blowing for 5 minutes, after all solvent was observed to be removed, caused losses up to 20% of the dieldrin.

The effect of a nonvolatile diluent was tested by analyzing samples of 25 mg. of paraffin wax and 0 to 40 γ of dieldrin. The dieldrin-free wax samples gave apparent dieldrin values of 4 to 6 γ while recoveries of added dieldrin were accurate to within 4 γ of dieldrin. This interference and variation could be tolerated in determination of 0.1 p.p.m. of dieldrin in 100-gram crop samples.

Conditions for Determination of Converted Dieldrin The phenyl azide–spectrophotometric method for aldrin (*5*), except for two minor changes, was employed for determining the converted dieldrin. Heating the phenyldihydrotriazole with ethyl alcohol before adding the hydrochloric acid and diazonium salt was found to be necessary in all cases. Turbidities occasionally encountered in the color solutions could not always be eliminated when filter sticks (*5*) were used, but were successfully eliminated with fritted-disk Büchner funnels coated with asbestos.

Results with Pure Dieldrin The constants for a typical calibration curve for the range of 0 to 40 γ of dieldrin are: slope, 0.012 absorbance per microgram; absorptivity, 120 liters per gram cm.; average blank, 0.07 absorbance; standard deviation 1.5 γ. The standard deviation was calculated from a straight line determined by the method of least squares. Essentially identical curves were obtained by three different operators.

Table II. Analysis of Westvaco Magnesia No. 2642

Determination	Lot A	Lot B	Lot C
Loss of ignition, 1000° C., wt. %	11.1	12.1	9.1
Basicity calcd. as MgO, wt. %	85.2	84.7	90.1
Available carbon dioxide, calcd. as CO_3^{--}, wt. %	0.65	...	0.97
Loss on heating, 120° C., wt. %	0.6	0.13	0.7
Specific surface area, sq. m./g. (nitrogen)	146	185	164
Pore volume, ml./g.	0.250	0.270	0.335

Table III. Application of Spectrophotometric Method to Crops

Crop	Dieldrin, P.P.M. Added	Determined	Recovery[a]
Cabbage	0.00	−0.01, 0.01, 0.02	
	0.05	0.02, 0.04, 0.05	0.01, 0.03, 0.04
	0.10	0.11, 0.12, 0.14	0.10, 0.11, 0.13
Cantaloupe, edible portion	0.00	0.00, 0.01, 0.02	
	0.10	0.06, 0.10, 0.12	0.05, 0.09, 0.11
Green beans	0.00	0.02, 0.04, 0.04	
	0.05	0.07, 0.07, 0.07	0.04, 0.04, 0.04
	0.10	0.12, 0.13, 0.13	0.09, 0.10, 0.10
Onions	0.00	0.00, 0.00, 0.00	
	0.05	0.03, 0.04, 0.04	0.03, 0.04, 0.04
	0.10	0.05, 0.08, 0.09	0.05, 0.08, 0.09
Peanuts	0.00	0.04, 0.04, 0.05	
	0.10	0.12, 0.12, 0.12	0.08, 0.08, 0.08

[a] Corrected for apparent dieldrin of dieldrin-free crop.

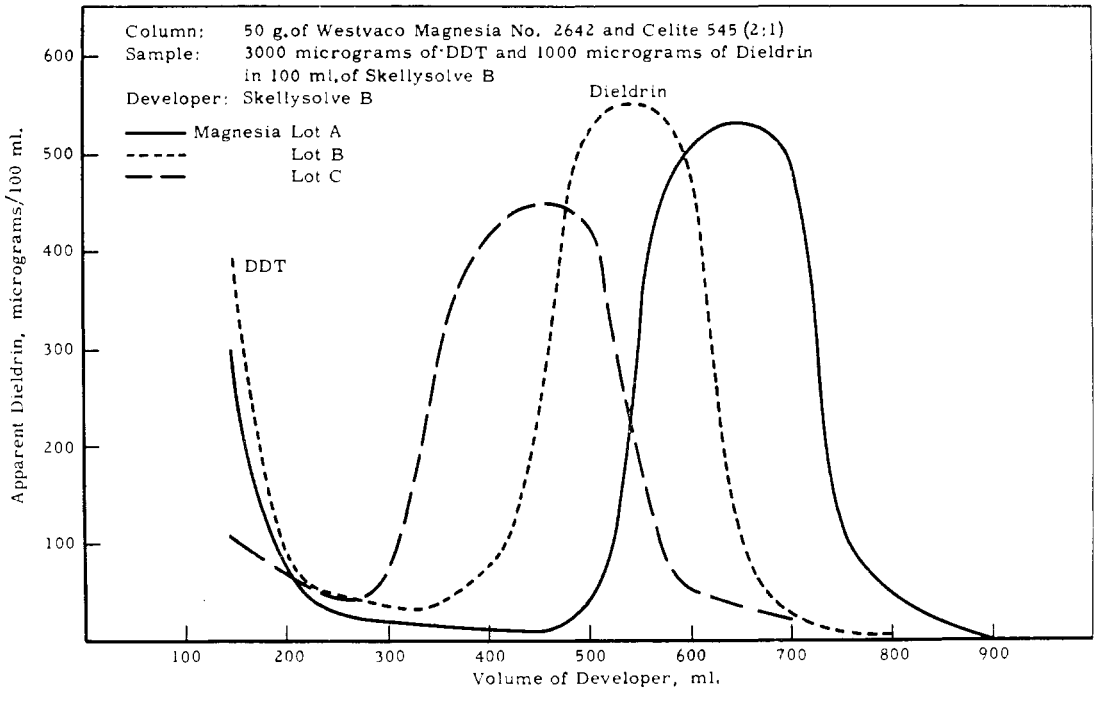
Development of Chromatographic Procedure

Certain crop extractives interfered in the determination of dieldrin. For example, interference was caused by pigments in the spectrophotometric method and by naturally occurring halogen-containing materials in the chlorine method. Chlorine-containing insecticides, such as DDT, interfered in the chlorine method; however, they did not interfere seriously in the spectrophotometric method.

Separation of most crop extractives from dieldrin prior to determination was therefore necessary. Before development of the spectrophotometric method, it was desired that dieldrin be separated from DDT, so that crops containing up to 10 p.p.m. of DDT could be analyzed by the chlorine method. For making the required separations, adsorption chromatography alone or in combination with saponification was satisfactory.

Adsorbent Westvaco magnesia No. 2642 was the only satisfactory commercial adsorbent found which gave the separations needed. However, this commercial adsorbent varies from lot to lot (see Table II). The adsorption characteristics of different lots of magnesia were tested by chromatographing solutions of dieldrin and DDT over columns of three different lots of adsorbent. As is shown in Figure 3, the volume of de-

Figure 3. Elution curves for dieldrin and DDT through magnesia-Celite mixtures



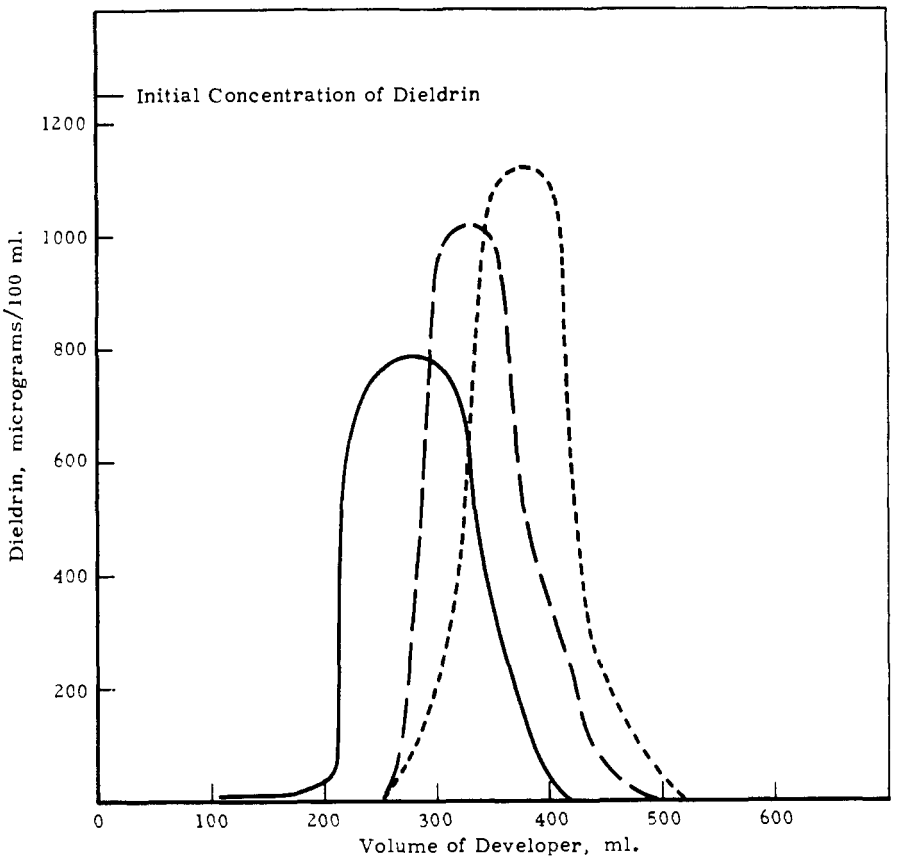


Figure 4. Elution curves for dieldrin in crop extracts

Column. 40 grams of magnesia adsorbent mixtures (lot C)
Sample. 1250γ of dieldrin
— — Extract of 600 grams of cantaloupe
—— Extract of 300 grams of orange peel or 125 grams of alfalfa meal
– – – Skellysolve B
Developer. Skellysolve B

veloper added before dieldrin appeared in the effluent varied from 250 to 500 ml. Approximately 500 ml. of additional developer was needed with each column to elute all the insecticide. DDT was eluted more readily than the dieldrin and with absorbent lot A, only 0.3% of the DDT was in the dieldrin effluent. No simple correlation of the behavior of the insecticides with the analyses of the absorbent (Table II) was apparent.

Plant Extractives. On chromatography of extracts of glyceride-free crops over a magnesia column the pigments and most of the waxes were retained by the adsorbent. Terpenes and certain colorless waxes preceded the dieldrin in the effluent. Part of the naturally occurring chlorine, equivalent to 0.04 to 0.06 p.p.m. in the original crop, preceded the dieldrin. The materials accompanying dieldrin were colorless waxes amounting to from 5 to 25 mg. with the various crops used. The effect of plant extractives on the elution behavior of dieldrin on chromatography over magnesia was tested using solutions of 1-mg. amounts of dieldrin in extracts of orange peel, cantaloupe, and alfalfa meal and in hydrocarbon solvent alone. The effect of the plant materials was to advance both the leading and trailing edges of the chromatograms, as is shown in Figure 4. The dieldrin elution curve with extract from 125 grams of alfalfa meal was similar to that for orange peel extract. However, with extract from 250 grams of alfalfa meal, most of the dieldrin was in the effluent from the first 100 ml. of developer. The column failed to separate dieldrin from glycerides; therefore, saponification is used to separate glycerides from dieldrin.

Applicability to Determination of Residues in Crop Extracts

Spectrophotometric Method Insecticide - free extracts of a leafy crop, a root crop, a green vegetable, a fruit, and a crop of high glyceride content and corresponding extracts containing added dieldrin were analyzed by the method. The values for the insecticide-free samples were 0.05 p.p.m. or less, calculated as apparent dieldrin in the original crop (see Table III). Recovery of added dieldrin was accurate to within 0.03 p.p.m. calculated as concentration in the original crop.

Comparable results have been obtained on the following crops at the Agricultural Research Division, Shell Development Co., Denver, Colo.: alfalfa, apples, beets, carrots, cauliflower, corn, corn ensilage, cranberries, figs, grapes, lettuce, milk, oats, orange pulp, peaches, peas, potatoes, radishes, rice, rutabagas, spinach, squash, sweet potatoes, tobacco, tomatoes, turnips, and wheat.

Chlorine Method Insecticide-free extracts of a forage crop, citrus, two fruits, two root crops, and a vegetable oil and corresponding extracts containing added dieldrin were analyzed. Results obtained in some of these tests are listed in Table IV and show that values of 0.09 p.p.m. or less apparent dieldrin in the original crop were obtained with the pesticide-free materials. The recovery of added dieldrin generally was within 0.05 p.p.m. of the amount added, expressed as concentration in the original crop.

Table IV. Application of Combustion-Chlorine Method to Crops

Crop	Dieldrin, P.P.M. Added	Determined	Recovery[a]
Beets, table	0.00	0.01, 0.02, 0.03	
	0.10	0.08, 0.09, 0.10	0.06, 0.07, 0.08
	0.33	0.25, 0.26, 0.30	0.23, 0.24, 0.28
Clover	0.00	0.01, 0.04	
	0.10	0.08, 0.08	0.05, 0.05
	0.33	0.27, 0.29	0.24, 0.26
Cottonseed oil	0.00	0.06, 0.08, 0.08	
	0.10	0.12, 0.15, 0.15	0.05, 0.08, 0.08
	0.33	0.32, 0.36, 0.38	0.25, 0.29, 0.31
Lemon peel	0.00	0.09, 0.09, 0.09	
	0.10	0.19, 0.19	0.10, 0.10
	0.33	0.44, 0.45	0.35, 0.36
Peaches[b]	0.00	0.01, 0.01, 0.02	
	0.10	0.10, 0.10, 0.10	0.08, 0.08, 0.08
	0.33	0.29, 0.34, 0.37	0.27, 0.32, 0.35

[a] Corrected for apparent dieldrin in dieldrin-free sample.
[b] Extract contained DDT equivalent to 10 p.p.m. in original peaches.

Table V. Interference of Various Materials in Determination of Dieldrin

Material	Apparent Dieldrin, Wt. %: Reduction–Phenyl Azide Method omitting chromatography; column A	with chromatography; column B	Chlorine method with chromatography; column C
Aldrin, 99.5%	1.5, 1.6	(<0.1)[a]	0.4
ASP-47, redistilled	0.0, 0.0	...	...
γ-Benzene hexachloride, recrystallized	0.1, 0.1	(<0.1)[a]	0.2
Chlordan, technical	2.8, 4.5	0.4, 0.5	4
DDD, recrystallized	...	...	<1
DDT	0.1, 0.1	(<0.1)[a]	<0.1
Endrin, 99%	0.1, 0.2	(<0.1)[a]	11
Heptachlor, recrystallized	0.2, 0.8	(<0.1)[a]	<1
Isodrin, 99%	...	...	<1
Methoxychlor, 90% technical	0.5, 0.6	(0.2)[a]	27
Octacide 264, technical	>10, >10	0.1, 0.1	...
Parathion, 96.5%	0.1, 0.1	...	...
Toxaphene, technical	9.3, 9.3[b]	1.6, 1.8	11

[a] Value estimated from extent of interference (column A) and extent of separation (column C).

[b] Interference presumably due to formation of double bonds during reduction, since Toxaphene gives only 0.1% interference by aldrin method (*5*).

Specificity of the Methods

A number of chlorine-containing materials were analyzed by the chlorine method (Table V). As halogen-free materials would not be determined by the method, they were not tested. While the method was shown to be somewhat specific, owing to the separations achieved by chromatography, four of the insecticides tested interfered from 4 to 27%.

Initial tests were made by the spectrophotometric method omitting chromatography. All but four of the materials tested interfered to less than 1% as apparent dieldrin (Table V). Further tests by the method including chromatography were made using three of the materials which interfered to more than 1%. The materials interfered from 0.1 to 1.8% in these tests. Comparison of the data in Table V shows that the spectrophotometric method was more specific than the chlorine method.

Acknowledgment

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RODENTICIDES

Comparative Toxicities of Warfarin and Some 2-Acyl-1,3-indandiones in Rats

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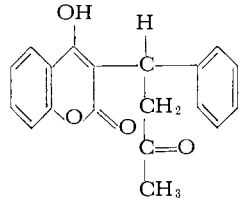
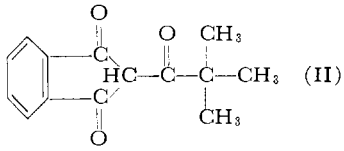
THE DEVELOPMENT and use of anticoagulant rodenticides within recent years has provided an efficient countermeasure to the ever-increasing rat population. The compound 3-(α-acetonylbenzyl)-4-hydroxycoumarin (I) was first described and tested by Link and his coworkers in 1944 (*7*). This material, now known as warfarin (after Wisconsin Alumni Research Foundation), is used widely as a rodenticide by military and civilian agencies (*3, 9*).



(I)

Warfarin is incorporated into a bait in low concentrations, and the bait is applied by the multiple-dose technique (*2*). Usually, an initial dilution in the form of a 0.5% powder with cornstarch is made. From this stock material, final dilutions of 0.025% warfarin are prepared with corn meal, rice meal, or other grain material. Because of the low concentration of warfarin in the final bait used, there is little danger of poisoning to other animals and man due to accidental single ingestion of the bait. Rats and mice feed for many days on the baits before becoming fatally poisoned. It is this "built-in" protective feature that makes the anticoagulant rodenticides so valuable.

In 1942, Kilgore (*5*) discovered that certain 2-acyl-1,3-indandiones were insecticidal in nature. Later, Kabat (*4*) described the anticoagulant properties of these compounds and also their acute toxicities. Of the compounds that he studied, the most effective material from the standpoint of anticoagulant and rodenticidal action was 2-pivalyl-1,3-indandione (II), commonly called Pival.



(II)

The water-soluble sodium salt of this compound, Pivalyn, is equally effective. Field tests of this material by the Branch of Predator and Rodent Control, Fish and Wildlife Service, soon disclosed that