INSECTICIDE DETERMINATION

Colorimetric Determination of Dieldrin and Its Application to Animal Fat

CIPRIANO CUETO, Jr.[1]
Communicable Disease Center, Bureau of State Services, Public Health Service, U. S. Department of Health, Education, and Welfare, Phoenix, Ariz.

This method for determining microgram quantities of dieldrin is based on the color complex formed between dieldrin and diphenylamine in the presence of zinc chloride. The absorption at 650 mμ by color reaction products in an acetic acid solution follows Beer's law. The method is general for most chlorinated insecticides, but specificity is gained through alkaline hydrolysis and chromatography.

THE EXTENSIVE USE of insecticide commonly known as dieldrin or HEOD (1,2,3,4,10,10-hexachloro-6,7-epoxy-1,4,4*a*,5,6,7,8,8*a*-octahydro-1,4-*endo*-*exo*-5,8-dimethanonaphthalene) has created the need for a simple, sensitive method of analysis adaptable to routine determinations. Such a method is of prime importance in the study of the toxicity and hazards of dieldrin to humans, and might also find use in the determination of residues on food products and in the analysis of formulations.

An evaluation was made of the existing methods reported in technical literature for the colorimetric determination of microgram quantities of dieldrin. Bioassay and total organic chlorine techniques were not considered because of the lack of specificity. The only thoroughly described colorimetric procedure is that of O'Donnell, Johnson, and Weiss (*9*), which is long and tedious and subject to unduly high and variable blank values.

Davidow and Laug (*2*) reported a method involving the reaction of dieldrin with anhydrous hydrobromide in dioxane. The method is rather long, and the requirement of anhydrous hydrobromide limits its convenient use as a routine method.

Gunther, Kolbezen, and Blinn (*5*), and more recently Johnson (*6*), have reported on color reactions for the detection of dieldrin. These techniques have not as yet been expanded into quantitative methods of analysis. The color reaction of Gunther *et al.* appears to give inconsistent results, and that of Johnson lacks the desired sensitivity.

In 1945, Jones (*7*) and Lemley (*8*) reported on a color reaction of DDT with zinc chloride, phenol, and diphenylamine. Graupner has developed a method for toxaphene (*4*) based on the blue-green color reaction of this insecticide with diphenylamine and zinc chloride. On investigating the possibility that this color reaction under certain conditions is a more general one (*3*), it was noted that dieldrin gave a purple color of an intensity suitable for colorimetric determination.

The method described in this paper involves saponification, extraction, chromatography, and color development. In a final volume of 1.0 ml. of solution and using a semimicro absorption cell having a 10-mm. light path, a sensitivity of 5 γ with about 7% variation can be obtained. The method has been applied to the determination of dieldrin added to animal fat. Although all the possible variations have not been studied, it is believed that the following description will satisfy the immediate need for a sensitive, simple, routine method of determining dieldrin and will facilitate the expansion of the method to other biological materials and to metabolic and residue studies.

Apparatus

Beckman spectrophotometer, Model B, with red-sensitive phototube.

Aluminum heating block containing holes 22.5 mm. in diameter and 50 mm. deep. The block should be maintained at 205° ± 5° C.

Glass gas-drying unit charged with indicating Drierite.

Reagents

Diethyl ether, reagent grade. Ether containing peroxides is unsatisfactory and should be purified and redistilled before using. To test for peroxides, shake a small portion of the ether with an aqueous potassium iodide solution. The release of free iodine, as determined by the starch test, indicates the presence of peroxides. These are decomposed by washing the ether with a ferrous sulfate or sodium bisulfite solution until the potassium iodide test is negative. The ether is then washed with water, dried with sodium sulfate, and redistilled.

Alumina, adsorption grade, 80 to 200 mesh. Do not activate.

Diphenylamine solution, 0.25%. Dissolve 250 mg. of diphenylamine, recrystallized at least twice from hexane, in 100 ml. of redistilled *n*-hexane.

Zinc chloride reagent, 0.25%. Dissolve 250 mg. of zinc chloride in purified, redistilled ether. A slight suspension is formed which precipitates on standing. Resuspend before use. Do not filter through paper. This reagent is unstable and must be prepared each day.

Solvent for colored reaction products, 10% by volume of C.P. acetic anhydride in C.P. glacial acetic acid.

Dieldrin standard solutions. Dieldrin (at least 99.5% pure) is dissolved in purified redistilled *n*-hexane to give solutions containing 1, 10, and 20 γ per ml.

Procedure

Preparation of Standard Curves. Standard curves are prepared in three ranges of concentrations, depending upon the required sensitivity. In the 1- to 10-γ range, the colored products are dissolved in 1.0 ml. of solvent, and the absorbance is measured in a microabsorption cell having a 50-mm. light path. In the 5- to 50-γ range, the colored products are dissolved in 1.0 ml. of solvent, and a semimicrocell is used having an 0.8-ml. capacity and a light path of 10 mm. At the higher concentrations, 20 to 150 γ, a total volume of 3.0 ml. of solvent is used. The absorbance is measured in cells having a 10-mm. light path and a 3-ml. capacity.

Appropriate aliquots of the standard solution of dieldrin to give at least four points on each curve are pipetted into test tubes (22 × 175 mm.). The samples are evaporated just to dryness in a water bath at 40° C. with the aid of a

[1] Present address, Technical Development Laboratories, P. O. Box 769, Savannah, Ga.

gentle stream of dry, clean air filtered through a laboratory glass gas-drying unit. Two milliliters of the diphenylamine and zinc chloride reagents are added and the samples are again evaporated to complete dryness under the same conditions as before. A colorless, dry residual film should appear in each tube. The samples then are placed in the aluminum heating block at 205° ± 5° C. for exactly 3.0 minutes. The tubes are removed and cooled in running tap water. The colored reaction products are dissolved in either 1.0 or 3.0 ml. of the 10% acetic anhydride in glacial acetic acid solvent, depending upon the range of the curve being studied. The absorbance of the samples is read at 650 mμ within 10 minutes after the addition of the solvent; water or the acid solvent itself is used as reference. A reagent blank is carried through with the standards under exactly the same conditions. Plotting micrograms of dieldrin *vs.* absorbance on rectangular coordinate paper should give straight-line curves.

Analysis of Animal Fat. SAPONIFICATION AND EXTRACTION. To a weighed amount of animal fat (1.0 gram) in a 300-ml. ⅔ ground-glass-joint Erlenmeyer flask, 15 ml. of 95% ethanol (redistilled) and 3 ml. of 50% aqueous potassium hydroxide are added. The sample is refluxed gently for at least 1 hour or until a homogeneous solution is obtained. It is then removed from the source of heat and cooled, and 10 ml. of water and 50 ml. of redistilled *n*-hexane are added. The sample is shaken vigorously and the phases are allowed to separate. This operation is repeated three times, and then the lower phase is aspirated off. The organic solvent extract is washed once with 50 ml. of 1*N* hydrochloric acid, once with 25 ml. of water, and once with 25 ml. of 20% sodium chloride, the aqueous layer being aspirated off each time. The extract then is dried with sodium sulfate and a 40-ml. aliquot pipetted into a test tube. The sample is evaporated with a gentle stream of air in the 40° C. water bath to a volume of about 5 ml.

CHROMATOGRAPHY. The chromatographic columns are prepared by adding small amounts of dry adsorption alumina and tapping the tubes (10 × 33 mm.) gently to assure packing. This is repeated until a column of adsorbent 10 cm. in height is obtained. A plug of glass wool confines the alumina.

The *n*-hexane extract of the saponified fat is transferred to the column with the aid of a few milliliters of the 10% diethyl ether in hexane eluent. When the extract is completely taken up into the alumina, the column is eluted with 10% ether in hexane, and three 40-ml. samples of the eluate are collected in test tubes (22 × 175 mm.). The samples are evaporated just to dryness in a water bath at 40° C. with the aid of a stream of dry clean air. Two milliliters each of the diphenylamine and zinc chloride reagents are added, and the analysis is completed as for the preparation of the standard curves. A column blank is run through both the chromatographic and color reaction procedures. Its absorbance at 650 mμ is used as a correction for the absorbance value of the fat sample.

In the determination of unknown quantities of dieldrin, the amount of the colored reaction product formed after fusion offers a visual aid in determining whether this residue should be dissolved in 1.0 or 3.0 ml. of acetic acid solvent. This then establishes the absorption cells and the standard curve to be used. Alternatively the fusion products are dissolved in 1.0 ml. of the acid solvent. Then, if the color intensity of this solution warrants it, further appropriate dilutions are made.

It is important to run a blank analysis through the entire procedure on a sample of the same amount and type of material as that being analyzed. Further, a sample of the standard solution of dieldrin should be run through the chromatographic procedure to determine the quantitative recovery of dieldrin in the three eluates.

Results and Discussion

Standard Curves. Acetone, methanol, and ether were used as solvents for the two color reagents, diphenylamine and zinc chloride. The three solvents appeared to be satisfactory, but further work revealed that acetone and methanol gave higher reagent blank values than ether. This difference became significant when micro absorption cells were used. Care must be taken that the ether is pure and does not contain peroxides. These oxidation products cause high blank values and, depending upon their concentrations, may even cause extraneous color in the fused residue.

In the early work on this method, evaporation of the ether solution of the two color reagents, prior to fusion, occasionally gave a residue containing visible amounts of moisture. Furthermore, if a few milliliters of *n*-hexane were mixed with the ether solutions, the evaporation proceeded satisfactorily to complete dryness, and a colorless residual film was obtained at the bottom of the test tube. This same satisfactory effect was consistently achieved by preparing the diphenylamine reagent in *n*-hexane. The reagent blank values were not altered.

Varying the concentration of the color reagents between 0.25 and 1.0% did not increase the sensitivity of the method but increased the blank values. The effect of varying the reaction temperature on the absorbance of the color developed by dieldrin is shown in Table I. Reaction time was maintained constant at 3.0 minutes. The data show an increase in color intensity and in blank values with an increase in temperature. Varying the reaction time from 1 to 10 minutes and maintaining the temperature at 205° C. resulted in essentially the same effect—increased blank values and color intensity with some decomposition at the maximum time. These results indicate that the optimum time and temperature for the reaction of dieldrin with diphenylamine are the same as those reported for toxaphene (*4*)—e.g., 3 minutes at 205° C.

Table I. Effect of Reaction Temperature on Intensity of Color Developed by Dieldrin with Reference to Water

(Time of reaction, 3.0 minutes. Volume of colored solutions, 3.0 ml.)

Temp., ° C.	Absorbance at 650 Mμ: Reagent blanks	Absorbance at 650 Mμ: Dieldrin, 50 γ
125	0.003	0.007
160	0.014	0.124
205	0.020	0.337
225	0.093	0.420
250	0.170	Decomposed

Table II. Effect of Reaction Product Solvent on Intensity of Color Developed by 100 γ of Dieldrin

Solvent	Absorbance at 650 Mμ
Acetone	0.304
Methyl ethyl ketone	0.565
Methanol	0.158
Ethanol, 95%	0.160
2-Propanol	0.168
Ethyl acetate	0.200
Benzene	Insoluble
Glacial acetic acid	0.520
Acetic anhydride–acetic acid mixtures	
1:10	0.624
2:10	0.638
5:10	0.616

The effect of various solvents on the color intensity and thus the sensitivity of the method was studied using a reaction time of 3 minutes at 205° C. The representative data given in Table II indicate that, of the solvents studied, the acetic anhydride–acetic acid mixtures permitted the maximum development of color.

The color produced in all of the solvents studied faded rather rapidly. In the acetic anhydride–acetic acid solvent, the loss of color intensity is 15 to 20% in the first hour. It is therefore of prime importance that the absorbance of the samples be determined within a specified time. When the absorbance was measured at random, but within 10 minutes of the addition of the acid solvent, the variation between several standard curves prepared was not greater

Table III. Variables in Standardization of Colorimetric Determination of Dieldrin in Three Concentration Ranges

Variables	Range, γ 1–10	5–50	20–150
Volume of colored solution, ml.	1.0	1.0	3.0
Light path of cells, mm.	50	10	10
Maximum absorption, mμ	650	650	650
Reagent blank at λ_{max} abs.	0.100	0.020	0.010
Absorptivity	0.0869	0.0191	0.00641
Variation, %	7	7	5
Sensitivity, γ	1.0	5.0	15

Table IV. Recovery from Rabbit Fat of Dieldrin Added at Various Concentrations

Dieldrin, P.P.M. Added	Found	Recovery, %
20	18	90.0
40	39	97.5
80	75	93.8
100	94	94.0

Table V. Recovery of 100 γ of Dieldrin Added to 1 Gram of Animal Fat

Source of Fat	Dieldrin per Eluate, 40-Ml. Fractions, % 1	2	3	Total Recovery, %
Human A[a]	75	16	0	91
Human B[b]	68	24	0	92
Monkey	90	0	0	90
Rabbit	91	0	0	91
Rat	83	14	0	97
Dieldrin[c]	55	37	0	92

[a] Obtained in 1958.
[b] Obtained before advent of chlorinated insecticides.
[c] Standard dieldrin, no fat, subjected to complete analysis.

than 7%. This is satisfactory for most colorimetric determinations. Representative data pertinent to the standardization of the method are given in Table III.

Analysis of Animal Fat. SAPONIFICATION AND EXTRACTION. Davidow (*1*) has described a convenient technique for the isolation of the insecticide DDT from fat using a sulfuric acid–impregnated Celite column. However, this effective cleanup method is not applicable to dieldrin because the compound, unlike DDT, is not stable to the action of concentrated mineral acids but is stable to that of alkali. The latter property was utilized by O'Donnell *et al.* (*9*) in the isolation of dieldrin from glycerides.

In the present study, 1-gram samples of rat, rabbit, monkey, and human fat were saponified directly. Analysis of materials other than fat may require extraction prior to saponification. During the initial extraction of the saponified mixture, emulsion formation is avoided by adding only a minimum amount of water. No emulsions were formed in any of the samples analyzed when the alcohol content was reduced to 50 to 60% by the addition of water.

In the evaporation procedures, if a stream of air is used to aid in the complete removal of the solvent, the samples must be maintained at or below 40° C. to prevent loss of insecticide. If air is not used, hexane distillation temperatures may be utilized to concentrate extracts or eluates without loss of dieldrin. In any event, the evaporation should never be prolonged.

CHROMATOGRAPHY. O'Donnell *et al.* (*9*) described a magnesium oxide–Celite chromatographic column used in the isolation of dieldrin from extracts of saponified glycerides. This column is effective but requires vacuum or pressure to produce a flow of the eluents and necessitates collection of large volumes of eluate. A less time-consuming technique was desired.

After several adsorbents had been evaluated, a simple chromatographic procedure was developed capable of isolating dieldrin from an extract of saponified fat. Columns 10 mm. in diameter and packed 10 cm. high with adsorption grade alumina retained the impurities of an extract of 1 gram of saponified animal fat and permitted the quantitative recovery of dieldrin in about 80 ml. of eluate. A flow rate of 2 ml. per minute was used. If larger or different types of samples are to be analyzed, the column should be calibrated for its efficiency in cleanup. Column blank absorbance values were 0.015 to 0.030, using an absorption cell of 10-mm. light path and a 3.0-ml. final solution volume. Under the same conditions, control fat samples in general gave absorbance values of 0.020 to 0.050.

Table IV shows the recovery of known amounts of dieldrin added to rabbit fat. The eluates were collected in three 40-ml. fractions and analyzed individually and the results were totaled.

The recovery of 100 γ of dieldrin from 1 gram of different types of fat is shown in Table V. A fourth fraction of eluate, not shown in the table, was also collected and subjected to the dieldrin analysis. Although the added insecticide was recovered quantitatively in the first two fractions, the fourth fraction of eluates of some fat samples gave an extraneous violet color which faded rapidly. This was characteristic also of the human B fat sample, which was obtained from a patient before the advent of chlorinated

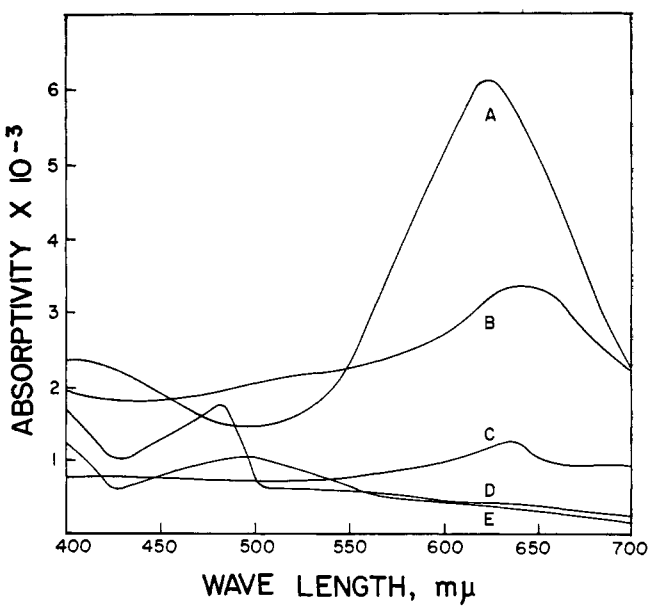

Figure 1. Spectra of chlorinated insecticides subjected to direct dieldrin colorimetric analysis

A. p,p'-DDD
B. Chlordan
C. Heptachlor
D. p,p'-DDT
E. o,p'-DDT

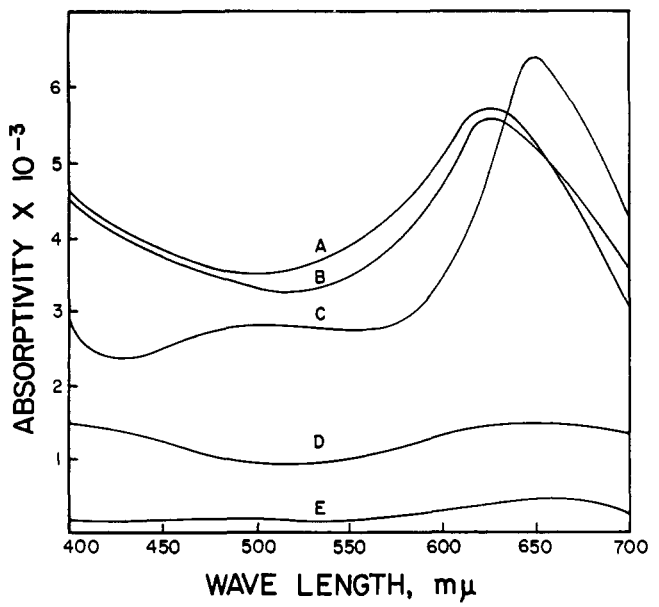

Figure 2. Spectra of chlorinated insecticides subjected to direct dieldrin colorimetric analysis

A. Strobane
B. Toxaphene
C. Dieldrin
D. Endrin
E. Aldrin

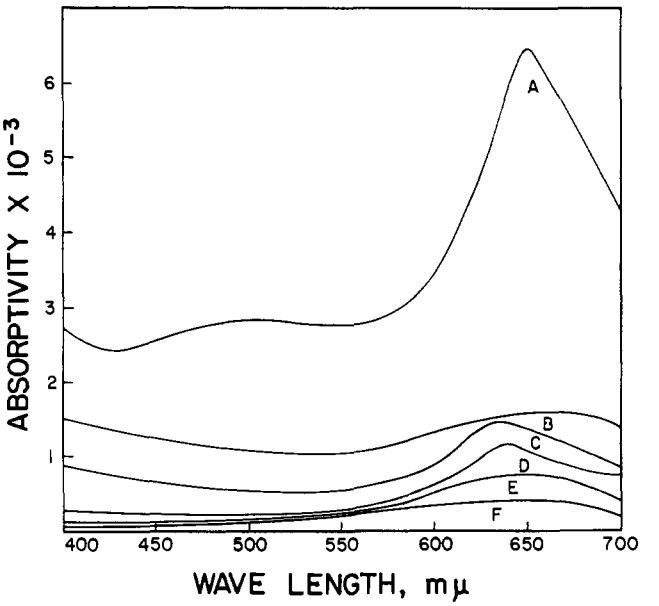

Figure 3. Spectra of chlorinated insecticides subjected to dieldrin colorimetric analysis after saponification and chromatography

A. Dieldrin
B. Endrin
C. Toxaphene
D. Strobane
E. Chlordan
F. Heptachlor

hydrocarbon insecticides. The findings indicate that continued elution of the chromatographic columns may result in recovery of a material of noninsecticidal origin. Therefore, adherence to the collection of eluates in fractions is desirable.

Reaction and Interference of Related Compounds. The nature of the color reaction is not thoroughly understood. Feigl (*3*) has reported that amines when melted with fluorescein chloride and anhydrous zinc chloride under conditions similar to those of this method produce a color complex. Fluorescein chloride is dehalogenated and the amine adds to the molecule. A similar reaction may be the basis of the colorimetric determination of dieldrin.

To determine the extent of this color reaction with related compounds, several chlorinated hydrocarbon insecticides were subjected directly to analysis. No saponification or chromatography was used.

Figures 1 and 2 give the spectra of the color complex formed with several insecticides and show that the wave length of maximum absorption and the intensity of color vary with most of the compounds. This may be due in part to variation of optimum conditions of color development for each material. For example, chlordan, when subjected to this analysis, gives an intense color without heating. Strobane also appears to be more reactive than toxaphene. DDE, DDA, dehydrochlorinated DDD, and lindane, not shown in the figures, gave no color reaction. From the spectra of some of the compounds certain possibilities are evident, such as the development of a simple method for the determination of DDD and its differentiation from DDT. This differentiation is not possible by the usual Schechter-Haller (*10*) procedure.

The general aspects of the reaction pose the problem of interference of related compounds on the determination of dieldrin. Some specificity is gained in the variations of color intensity and wave length of maximum absorption. To determine if further specificity could be gained through alkaline hydrolysis and chromatography, compounds shown in Figures 1 and 2 to offer maximum interference were subjected to the complete procedure described for the isolation of dieldrin from fat. Figure 3 shows both the spectra and the extent of interference of these insecticides after hydrolysis and chromatography. DDD on saponification is dehydrohalogenated to its ethylene derivative, which gives no characteristic color reaction. The instability of all of the compounds, except endrin, to alkaline hydrolysis is shown to be a means of limiting or of eliminating, by more intense saponification, their interferences in the dieldrin determination.

A confirmatory test for dieldrin was obtained by adding 0.2 ml. of concentrated sulfuric acid to the final colored solution. A characteristic color change occurred with the solution now having a maximum absorption at 540 mμ. The color is relatively stable but of low intensity. Oxidizing agents interfere with the test.

Acknowledgment

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Literature Cited

(1) Davidow, B., *J. Assoc. Offic. Agr. Chemists* **33,** 130–2 (1950).
(2) Davidow, B., Laug, E. P., *Federation Proc.* **12,** 314 (1953).
(3) Feigl, F., "Qualitative Analysis by Spot Tests," 3rd ed., p. 371, Elsevier, New York, 1946.
(4) Graupner, A. J., Hercules Powder Co., M 100-50.1, General Methods, Agr. Prod. Toxaphene Content, Jan. 24, 1958.
(5) Gunther, F. A., Kolbezen, M. J., Blinn, R. C., *J. Econ. Entomol.* **47,** 185 (1954).
(6) Johnson, D. P., *J. Assoc. Offic. Agr. Chemists* **39,** 490 (1956).
(7) Jones, W. L., *Australian Tech. Papers* **1037** (Australian Scientific Liaison Office, 1907 K St., N.W., Washington, D. C. (February 1945).
(8) Lemley, J. D., Mass. Inst. of Tech., M. R. No. **176,** Project A 11.1 (1945).
(9) O'Donnell, A. E., Johnson, H. W., Weiss, F. T., J. Agr. Food Chem. **3,** 757 (1955).
(10) Schechter, M. S., Soloway, S. B., Hayes, R. A., Haller, H. L., *Anal. Chem.* **17,** 704–9 (1945).

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