

1. At most, a faint wine-purple color in the first beaker, indicating a trace of 2,4-D acid.

2. A deep wine-purple color in the second beaker, indicating most of the original 0.10 mg. of 2,4-D acid.

3. A pale wine-purple color in the third beaker, indicating only a small portion of the original 0.10 mg. of 2,4-D acid.

4. No wine-purple color noticeable in the fourth beaker, indicating the absence of 2,4-D acid.

The fourth 50-ml. portion of 7.0% methanol in chloroform is not run through the tube during a regular analysis. It is done here for testing the chromatographic separation to make sure that all of the 2,4-D acid is eluted with the first 150 ml. of 7.0% methanol in chloroform.

If the results do not show a good separation of the 2,4-D acid, the amount of methanol in the two solutions of chloroform should be adjusted.

### Discussion

Phosphotungstic acid is used to separate proteins when the 2,4-D acid is extracted with chloroform from the acidified aqueous solution.

The buffered extraction solution sepa-

rates the 2,4-D acid from some of the small amount of acidic material remaining with the 2,4-D acid after the chromatographic separation.

After the final separation of the 2,4-D acid, a very small amount of acidic material from the grain sample may be present, which will give an amber color when heated with chromotropic acid reagent. The stannous chloride in the dilute sulfuric acid bleaches the amber color, but under the conditions of the experiment it does not bleach the wine-purple color. Though the solution from an untreated grain sample obtained by analysis is not water-white, the per cent transmittancy is high and constant.

The color reaction of 2,4-D acid with chromotropic acid is not quite specific. Formaldehyde will produce the same wine-purple color (1). According to Freed (2), phenoxyacetic acid and its derivatives will give the same color, perhaps because formaldehyde is a decomposition product.

The procedure can probably be used for phenoxyacetic acid and many of its derivatives besides 2,4-D acid, although the solutions of methanol in chloroform may have to be modified to make a proper chromatographic separation of

the desired compound. The procedure as given has been used to determine 4-chloro-*o*-toloxyacetic acid (MCP acid).

Although this procedure was developed principally for the determination of 2,4-D acid in grain and seed, it can probably be used for other agricultural products. Fresh vegetables such as peas should be dried by suitable means and the analysis made on a meal of the dried sample.

It is possible to detect less than 0.05 p.p.m. of 2,4-D acid in samples by this analytical procedure. Interferences by other compounds were not experienced as, under the conditions of the procedure, the color test is specific for aryloxyacetic acids.

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## INSECTICIDE MIXTURE ANALYSIS

# Chromatographic Separation of Dichlorodiphenyltrichloroethane and Dichlorodiphenyldichloroethane Mixtures

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Previous attempts to analyze mixtures of dichlorodiphenyltrichloroethane with dichlorodiphenyldichloroethane had proved unsatisfactory because of the similarity of chemical reactions. The physical differences of the two indicated they might be separable by chromatography. Dichlorodiphenyltrichloroethane is separated from dichlorodiphenyldichloroethane upon a silicic acid chromatographic column with *n*-hexane saturated with nitromethane, as the solvent. Dyes D and C red No. 18 and violet No. 2 can be used as a visual means of determining the point of separation. The compounds are recovered quantitatively upon evaporation of the solvent. This is a rapid, simple, and accurate means of analyzing mixtures of these compounds. It eliminates the uncertainty found in attempts to analyze the two simultaneously.

BECAUSE OF THE SIMILARITY of dichlorodiphenyltrichloroethane to dichlorodiphenyldichloroethane, analysis of mixtures of the two compounds based upon a chemical reaction is not completely satisfactory. Reactions which are used to determine dichlorodiphenyltrichloroethane quantitatively cannot be used in the presence of dichlorodiphenyldichloroethane. Wichmann *et al.* (5) have reported methods of

determining dichlorodiphenyltrichloroethane or dichlorodiphenyldichloroethane quantitatively by analysis for total and mobile chlorine. Although the ratio of total to mobile chlorine in the two compounds is different, the procedures are not sufficiently accurate to enable an analysis of mixtures of the two compounds to be made.

The oxidation of the compounds to the ketones and subsequent spectro-

photometric determination of the 2,4-dinitrophenylhydrazones of the ketones (5) cannot be used because both compounds yield the same ketone upon oxidation.

Colorimetric determination of nitration products is unsatisfactory because of the color's fading as well as the uncertainty of the products of nitration.

Infrared analysis of the mixtures has been found to be unsatisfactory (4).

**Table I. Adsorbents and Solvents Studied**

Adsorbent	Solvent
Silicic acid	<i>n</i> -Hexane (c.p.) <sup>a</sup>
Silica gel	<i>n</i> -Hexane (commercial) <sup>a</sup>
Fuller's earth	Petroleum ether
Alumina	Diethyl ether
Magnesia	
Sodium sulfate with Celite	

<sup>a</sup> Both hexanes were saturated with nitromethane.

**Table II. Recovery of DDT and DDD by Evaporation of Solvent**

Sample No.	I	II	III
Mixture of DDD, DDT taken, gram	0.3511	0.3789	0.4211
Mixture of DDD, DDT recovered, gram	0.3507	0.3780	0.4202
Recovery of DDD, DDT, %	99.9	99.8	99.8

Many examples of chromatographic separations of dichlorodiphenyltrichloroethane from other compounds have been reported. Davidow (2) has separated dichlorodiphenyltrichloroethane from fats by chromatographic methods, where as Harris (3) has separated dichlorodiphenyltrichloroethane and hexachlorobenzene by these procedures. Beckman (7) has described the separation of Aldrin and Dieldrin from dichlorodiphenyltrichloroethane on a chromatographic column. The difference in the physical properties of dichlorodiphenyltrichloroethane and dichlorodiphenyldichloroethane should be such that they also can be separated upon a chromatographic column.

### Reagents

All adsorbents used were c.p. grade. The solvents were all Eastman Kodak white label except one sample of *n*-hexane (Skellysolve B), obtained from the Esso Co. The reagents were used without any further treatment. The dyes used to follow the separation were D and C red No. 18 and D and C Violet No. 2 obtained from the Pylam Products Co., Inc., New York, N. Y. Pure samples of dichlorodiphenyltrichloroethane and dichlorodiphenyldichloroethane were furnished by Lebanon Chemical Corp., Lebanon, Pa.

### Experimental

The mixture to be analyzed is dissolved in a suitable solvent and passed through a chromatographic column. The components in the mixture are separated upon the column and collected in different fractions of the solvent as they issue from the column. Upon evaporation of the solvent, the solid

residue is weighed to determine the amount of the component in the mixture.

The chromatographic column was a glass tube, 18-mm. inside diameter and 125 cm. in length. This tube was fused to a 10-mm. inside diameter glass tubing to give a constriction at the bottom. A piece of glass wool at this joint helped to support the adsorbent.

A gel of the adsorbent under study was prepared by adding 100 grams of the adsorbent to 300 ml. of the solvent and beating in a Waring Blender for about 1

mixture will not be effected on the column. The top of the column should be tamped to give an even surface so that the bands move evenly down the column. A rubber stopper fitted to the end of a glass rod works very well as a tamper.

The sample to be analyzed was dissolved in the solvent and placed in the chromatographic tube above the gel. The pressure was again increased to 100 mm. Weighed beakers were used to catch 5-ml. cuts of the solution as they issued from the column. As each cut was taken from the bottom of the column the same volume of solvent was added to the top. The total of 500 ml. of solvent was passed through the column. The beakers were placed upon a steam bath and the solvent was evaporated. The beakers containing the solid residue were reweighed to determine the weight of solid present.

### Results and Discussion

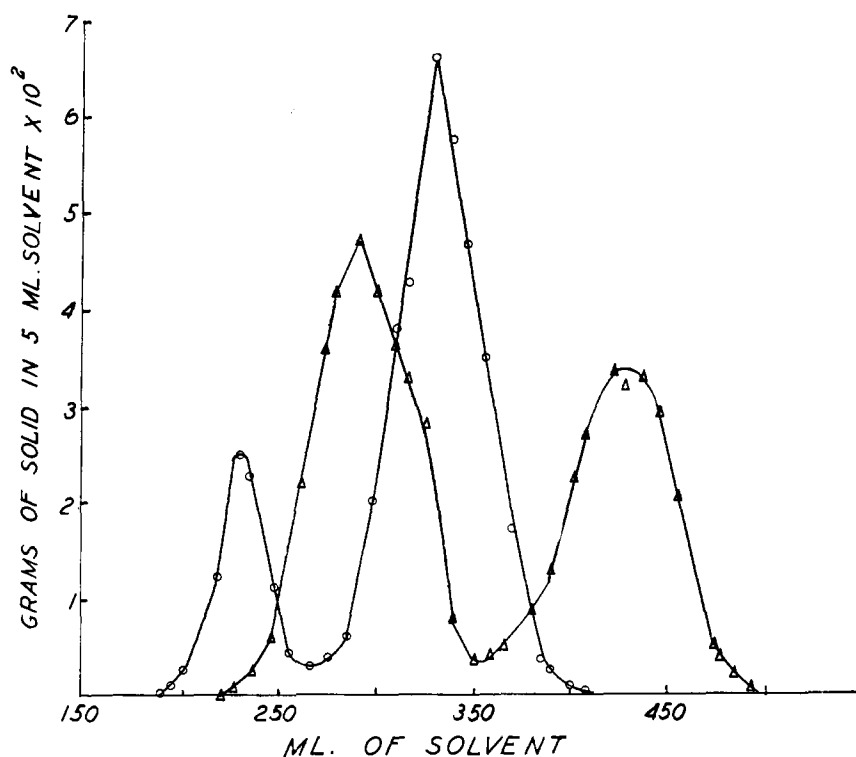
Various solvents and adsorbents were studied in this way to determine the best combination of solvent and adsorbent for separation. The systems studied are given in Table I.

The best separation was obtained by using silicic acid as the adsorbent and *n*-hexane as the solvent, the *n*-hexane being saturated with nitromethane. The Eastman Kodak white label *n*-hexane gave a slightly better separation than the commercial *n*-hexane but it was decided to use the commercial reagent because it is considerably cheaper.

Once the solvent-adsorbent system

minute. This gel was immediately placed in the chromatographic column. The top of the column was closed by a rubber stopper containing a tube leading to a tank of nitrogen and a manometer. A pressure of 100 mm. was applied to the gel in the column by opening the valve of the nitrogen tank. This caused the gel to pack firmly in the column.

The packing of the column is the critical procedure. Care must be taken so that the column packs evenly and that no channels exist or separation of the

**Figure 1. Separation of DDD-DDT mixtures**

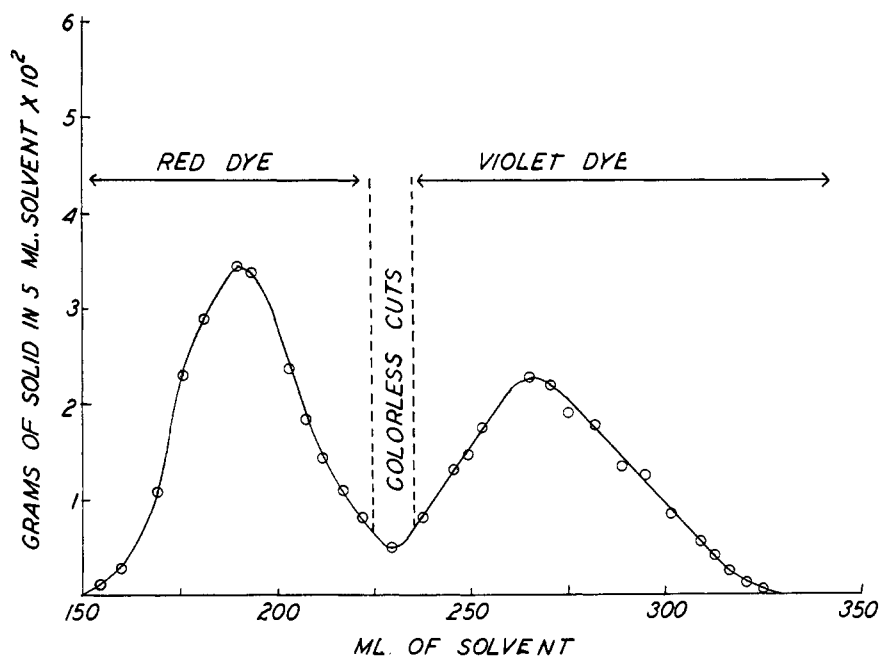


Figure 2. Separation of DDD-DDT mixtures with dyes added

had been chosen it was necessary to show that all of the solid dichlorodiphenyltrichloroethane or dichlorodiphenyldichloroethane could be recovered upon evaporation of a solvent. Samples of pure dichlorodiphenyltrichloroethane and dichlorodiphenyldichloroethane were weighed into beakers and dissolved in the solvent. The solutions were placed upon the steam bath and the solvent evaporated. When all of the solvent had evaporated the beakers were removed from the bath, cooled, and weighed. Results of these experiments are given in Table II. This table shows a very satisfactory recovery of the insecticides. Precaution must be taken that the solid residue does not remain on the steam bath after the solvent is completely evaporated for some of the solid will sublime.

To determine which component, dichlorodiphenyltrichloroethane or dichlorodiphenyldichloroethane, is eluted from the column first, different weights of pure dichlorodiphenyltrichloroethane and dichlorodiphenyldichloroethane were taken in a mixture and passed through a column. The samples were collected in the manner described. The separations obtained are shown in Figure 1 and results of the analysis of these mixtures are given in Table III. The first component through the column is the dichlorodiphenyltrichloroethane and the separation is such that unknown mixtures can be analyzed by this method.

Although this method can be used to analyze mixtures of the insecticides, it is long and tedious, and subject to many weighing errors. Two dyes, which are eluted at the same rate as the two compounds, make possible a visual separation of the two compounds and necessi-

tate the taking of only three or four cuts instead of the numerous cuts described. Harris (3) and Beckman (7) used two dyes, D and C red No. 18 and D and C violet No. 2, to determine the point of separation of the components in their mixtures. These dyes were tried with the mixture of dichlorodiphenyltrichloroethane and dichlorodiphenyldichloroethane. Solutions of known com-

Table III. Analysis of Synthetic Samples of DDT and DDD

Sample	I	II
Original DDT, gram	0.3057	0.0952
Original DDD, gram	0.3176	0.3052
DDT recovered, gram	0.3044	0.0947
DDD recovered, gram	0.3160	0.3036
DDT recovered, %	99.6	99.5
DDD recovered, %	99.5	99.5
Material balance, %	99.8 <sup>a</sup>	99.6 <sup>a</sup>

<sup>a</sup> Material balance is higher than per cent recovery because of some material recovered from original container.

Table IV. Analysis of Synthetic DDD-DDT Mixtures Using Dyes

Sample	I	II
Original DDT, gram	0.3248	0.2226
Original DDD, gram	0.2703	0.2122
DDT recovered, gram	0.3209	0.2218
DDD recovered, gram	0.2642	0.2066
DDT recovered, %	98.8	99.8
DDD recovered, %	97.7	98.0
Material balance, %	99.9 <sup>a</sup>	98.9 <sup>a</sup>
DDT original sample, %		
	54.6	51.4
DDT experimentally determined, %	54.8	52.0

<sup>a</sup> Material balance is higher than per cent recovery because of some material recovered from original container.

position were prepared, weighed quantities of the two dyes added, and the mixtures passed through a prepared column. In the first experiments the cuts were taken as in the experiments without added dyes to determine the position of the dye with respect to the insecticides as they issued from the column. Data of these experiments are given in Figure 2. In a second set of experiments four cuts only were taken: a preliminary cut preceding the red dye; a cut containing the red dye; a cut of colorless solvent between the two dyes; and a cut containing the violet dye. These dyes marked the separations of the insecticides. The cuts were evaporated and weighed. Results are given in Table IV. This table shows that these dyes can be used to determine the point of separation of the dichlorodiphenyltrichloroethane and dichlorodiphenyldichloroethane mixture. The four cuts taken in the second experiment with the dyes give a satisfactory analysis of mixtures of these two insecticides.

Unknown mixtures of dichlorodiphenyltrichloroethane with dichlorodiphenyldichloroethane were analyzed by this procedure. The results of these analyses agreed with the results obtained by other methods of analysis (4).

As a final test of the simplicity and precision of this technique, unknown mixtures of dichlorodiphenyltrichloroethane with dichlorodiphenyldichloroethane were given to students in an advanced quantitative analysis class. This was the first work that these students had done in chromatography. The results of these experiments were entirely satisfactory, agreeing within 1% of the correct results in almost all cases. The procedure gives good results when carried out by inexperienced personnel.

#### Acknowledgment

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