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SEPARATION AND IDENTIFICATION OF DDT ANALOGS IN THE PRESENCE OF POLYCHLORINATED BIPHENYL COMPOUNDS BY TWO-DIMENSIONAL THIN-LAYER CHROMATOGRAPHY

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SUMMARY

A two-dimensional thin-layer chromatographic method has been developed to separate *p,p'*-DDE and other DDT analogs from polychlorinated biphenyl compounds. Separation results from the different migration patterns and migration distances, relative to *p,p'*-DDE, that mixtures of polychlorinated biphenyl compounds exhibit under the two sets of conditions selected. A discussion of results and certain precautionary measures are included.

INTRODUCTION

In recent years, several authors have reported the presence of polychlorinated biphenyls in fish and fish-eating birds[1–7]. These residues can interfere with the quantitative and qualitative determination of DDT and its alteration products (DDT analogs) when many of the multi-residue methods for chlorinated pesticides are used.

Several approaches have been taken in an attempt to solve this problem. REYNOLDS[1] has reported the separation of several pesticides by Florisil column chromatography; however, *p,p'*-DDE is eluted with the polychlorinated biphenyl compounds (PCB's). ARMOUR AND BURKE[2] have developed a procedure for separating the DDT analogs and PCB's with a silicic acid column. The identification of these residues as PCB's by combined gas chromatography–mass spectrometry (GLC–MS) has been reported[3]; more recently BAGLEY *et al.*[4] identified PCB's by using a preliminary thin-layer chromatographic (TLC) separation followed by GLC–MS. A GLC–MS system combined with a previous isolation step would be the preferred method; however, the cost of instrumentation is prohibitive to most residue laboratories.

This paper describes a TLC method that can be used to supplement present GLC and TLC methods in the identification and separation of the DDT analogs from the PCB's.

During the confirmation of the DDT analogs found in samples of fish taken from the Great Lakes by the usual TLC method employed in this laboratory[8], we observed that the spots (suspected PCB residues) interfering with the *p,p'*-DDE spot exhibited different migration patterns with the two mobile solvents, *n*-heptane alone and

n-heptane–acetone (98:2). Two-dimensional TLC was employed, using MN-Kieselgel G-HR as the adsorbent and silver nitrate incorporated into the adsorbent layer as the chromogenic agent. *p,p'*-DDE was separated from the PCB residues under the conditions described later in this paper.

The PCB mixtures used in this study were the Aroclors manufactured by Monsanto Chemical Company[9]. Ten of the Aroclors were examined by this method to determine their TLC patterns with respect to the pattern of the DDT analogs, particularly *p,p'*-DDE. Aroclors 1254 and 1260 are the most important since their GLC retention times are similar to those of compounds found in several fish samples analyzed in this laboratory. These compounds elute through several types of GLC columns to give peaks in the same general region as the DDT analogs[1,2,5,7,8] and therefore might interfere with quantitation.

EXPERIMENTAL

Reagents

MN-Kieselgel G-HR, "adjusted for aflatoxin separation", manufactured by Machery, Nagel & Co., distributed by Brinkmann Instruments Co., Cantiague Road, Westbury, N.Y. 11590, was used. The silver nitrate was reagent grade. *n*-Heptane, acetone and 2,2,4-trimethylpentane, "distilled in glass", were purchased from Burdick and Jackson Laboratories, Inc., Muskegon, Mich. 49442.

Pesticide standards kelthane, *o,p'*-DDT, *p,p'*-DDT, *p,p'*-TDE and *p,p'*-DDE were obtained from U.S. Food and Drug Administration, Division of Pesticide Chemistry and Toxicology, Residue Chemistry Branch, Reference Standards Section, Washington, D.C. 20204. A solution was prepared to contain 40 ng each per μl in 2,2,4-trimethylpentane and was labeled "DDT analog standard mixture".

The Aroclors were provided by the Monsanto Chemical Company, Organic Chemicals Division, 800 N. Lindbergh Blvd., St. Louis, Missouri. Solutions were prepared to contain 200 ng/μl.

Apparatus

Glass plates for TLC, 20 × 20 cm.

TLC spreader capable of applying an adsorbent layer 250 μ thick on the specified plates.

Glass developing chamber with top. Two tanks are required.

Sandwich Chamber Kit, Camag* No. 25-210, or cardboard spacers 1.5 mm thick, 20 × 20 cm, Camag No. 25-213, and appropriate spring clips. Available from Camag Inc., 11830 West Ripley Avenue, Milwaukee, Wisc. 53226.

Irradiation cabinet equipped with 4 General Electric G15T8 Germicidal UV lamps.

Preparation of TLC plates

Prepare a slurry consisting of 30 g of MN-Kieselgel G-HR and 50 ml of 0.2 % silver nitrate, freshly prepared in water, and shake for at least 30 sec to insure proper mixing. The slurry is sufficient to spread five plates at a thickness of 250 μ. Dry the

* Reference to specific brand names does not imply endorsement of such brands by the Food and Drug Administration.

plates at 80° for 20 min and store in a desiccator cabinet. *Note*: Wash the spreader immediately after use to prevent the silver nitrate from reacting with the metal spreader.

Preparation of developing chambers

Prepare the developing chamber for the first dimension by adding 30 ml of *n*-heptane to an unlined glass chromatographic tank and cover with glass top.

Prepare the tank for the second dimension by pouring 200 ml of *n*-heptane–acetone (98:2) into an unlined glass tank, covering the tank with a glass cover plate and allowing the tank to equilibrate for at least 1 h before the plate is inserted for the second dimension development. After the solvent mixture has equilibrated for at least 1 h the tank can be used for several hours thereafter to develop more plates.

Procedure for first dimension

Scrape adsorbent layer from the top and both sides of the plate approximately 1 cm from the edge (Fig. 1). Apply an amount of sample equivalent to 80 ng of *p,p'*-DDE, as determined by GLC, on the spot identified as No. 1 in area 1 of Fig. 1, at a point 3 cm from the bottom edge and 3 cm from the left edge of the plate. As many as five spots can be applied on the spotting line in area 2; however, no spot should be farther than 6 cm from the right edge of the plate. Suggested spots for area 2 (spots 2, 3, and 4) are the DDT analog standard mixture (2 μl), the sample, and about 200 ng each of the Aroclors 1254 and 1260. Score a solvent front line at least 2 mm wide across the plate, 10 cm above the spotting line.

Assemble the sandwich chamber as directed by the manufacturer, using a glass cover plate and a 1.5 mm cardboard spacer. Place the assembled sandwich chamber in a glass chromatographic tank containing 30 ml of *n*-heptane. Place the top on the tank and allow the solvent to migrate to the 10 cm line. Development time is approximately 15–20 min.

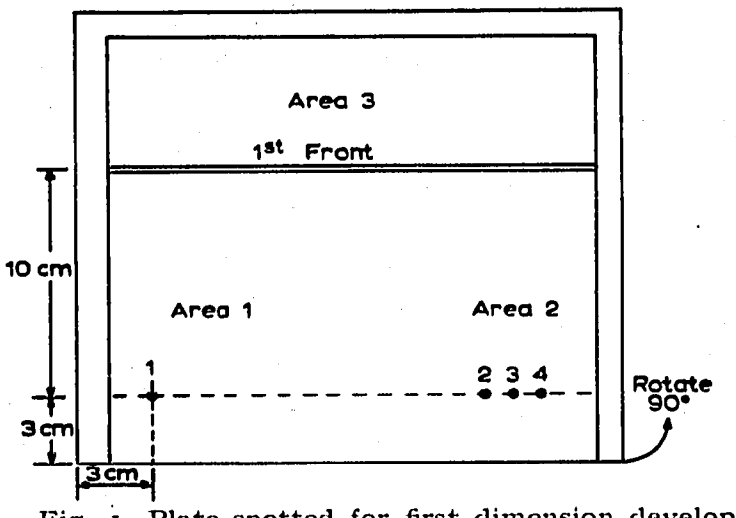

Fig. 1. Plate spotted for first dimension development to be performed in sandwich chamber. Mobile solvent: *n*-heptane.

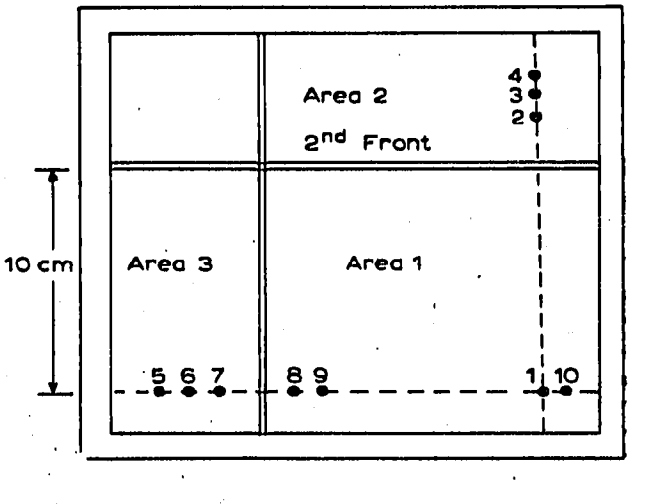

Fig. 2. Plate spotted for second dimension development to be performed in partially saturated tank (see text). Mobile solvent: *n*-heptane–acetone (98:2). Locations of spots after first dimension development are not indicated since spots are not visible at this point.

Procedure for second dimension

Disassemble the sandwich chamber and air-dry the chromatogram 5–10 min. Rotate the plate 90° counter-clockwise and score another solvent front line (2 mm wide) across the plate 10 cm above the point of sample application (Fig. 2). Scrape off the adsorbent layer approximately 1 cm from the right edge of the plate. Spot area 3 with the same solutions used for spotting area 2 for the first dimension development.

To reveal any change in the slope of the solvent front during the second development, additional spots of standards should be placed at positions 8, 9 and 10 in area 1 (Fig. 2); examples are shown in Figs. 3–13. Place the plate in the chamber prepared for the second dimension development and allow the solvent to rise to the 10 cm front.

After the second development, air-dry the plate and then place it in the irradiation cabinet 1 in. below the UV lamps and irradiate it for about 2 min. Remove the plate, invert it, and hold it over an open steam bath for a few seconds, allowing the steam to play over the adsorbent layer. Irradiate again for 2–5 min. All chlorinated compounds become visible as either black or gray spots. The intensity of the spots is enhanced by repeated short steam treatments and irradiations. Long irradiation periods under the UV lamps will darken the adsorbent layer of the plate.

RESULTS AND DISCUSSION

If the humidity in the laboratory is too high, the compounds will migrate too far up the plate during the second dimension development and the procedure will not provide an effective separation. This problem cannot be eliminated by drying the plate in the oven after it has been spotted, since the pesticides and PCB's will react with the silver nitrate in the adsorbent layer and the reaction products do not chromatograph like the parent compounds.

Since the second dimension development is affected by high humidity to a greater extent than the first, an attempt was made to perform the second dimension development in a commercially available constant humidity chamber, but this was not successful. The design of the constant humidity chamber was such that the second dimension had to be developed in a sandwich chamber. Thus, since both first and

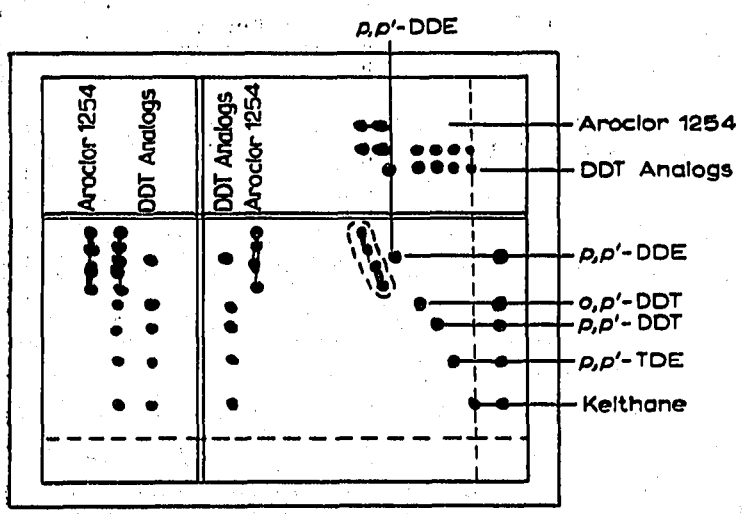

Fig. 3. Separation of Aroclor 1254 from the DDT analogs.

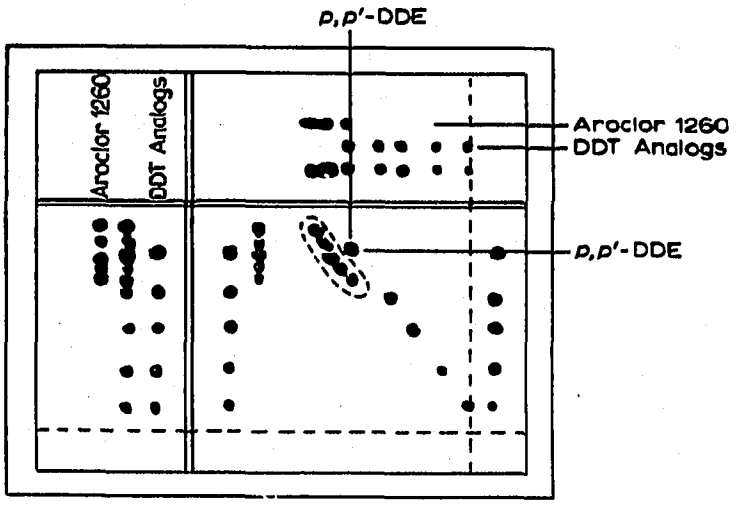

Fig. 4. Separation of Aroclor 1260 from the DDT analogs.

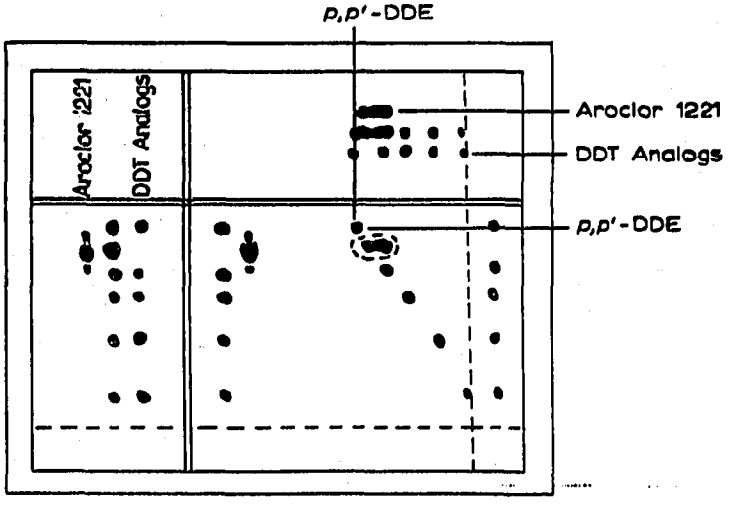

Fig. 5. Separation of Aroclor 1221 from the DDT analogs.

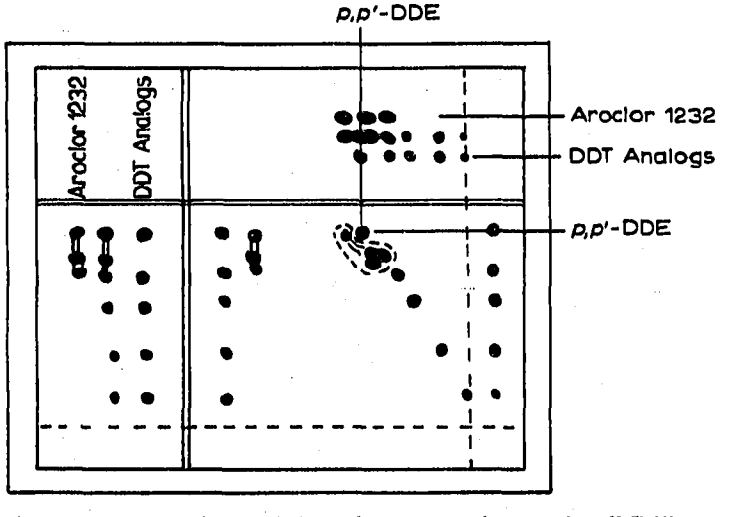

Fig. 6. Separation of Aroclor 1232 from the DDT analogs.

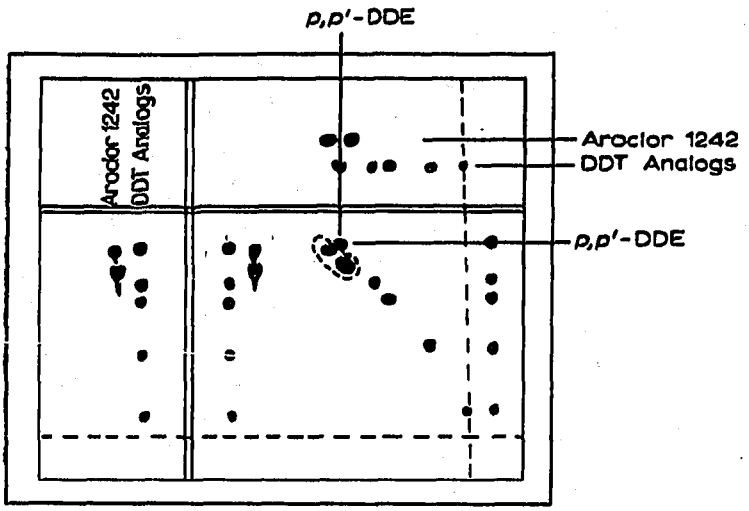

Fig. 7. Separation of Aroclor 1242 from the DDT analogs.

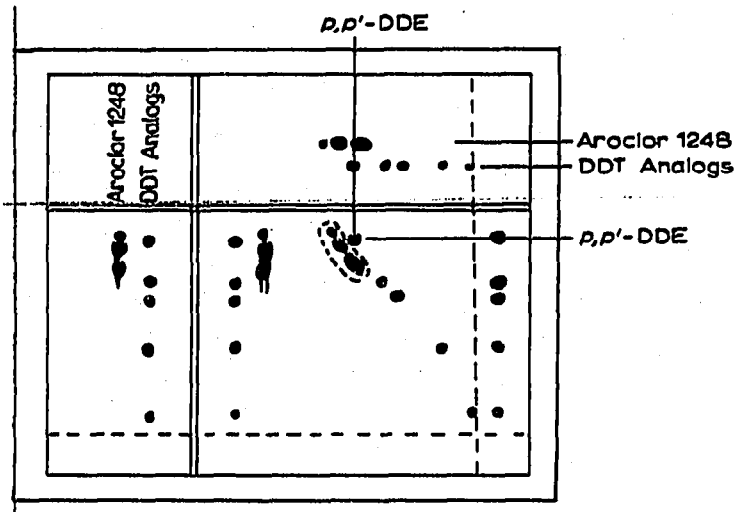

Fig. 8. Separation of Aroclor 1248 from the DDT analogs.

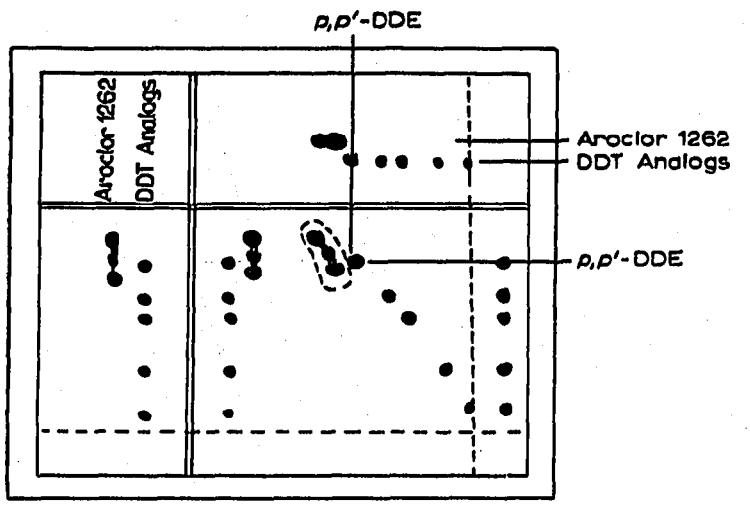

Fig. 9. Separation of Aroclor 1262 from the DDT analogs.

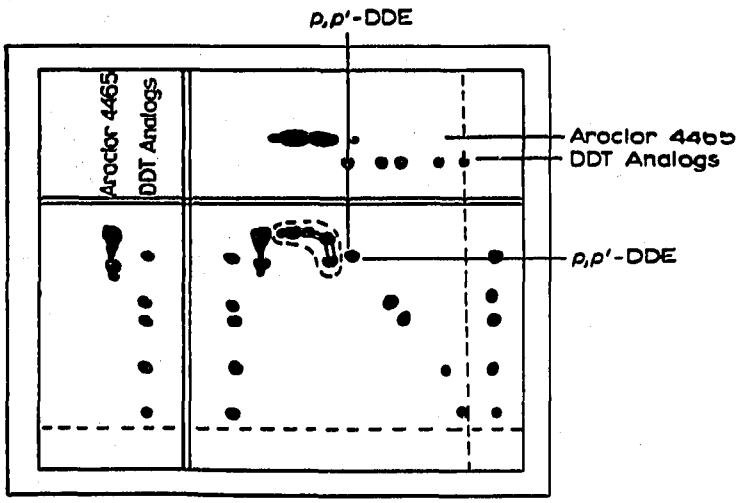

Fig. 10. Separation of Aroclor 4465 from the DDT analogs.

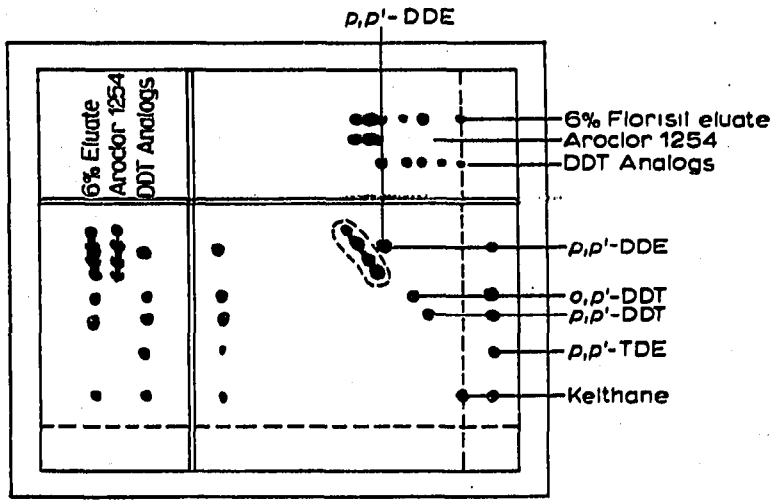

Fig. 11. 6% Florisil eluate from fish sample containing PCB's, kelthane, *p,p'*-DDT, *o,p'*-DDT and *p,p'*-DDE.

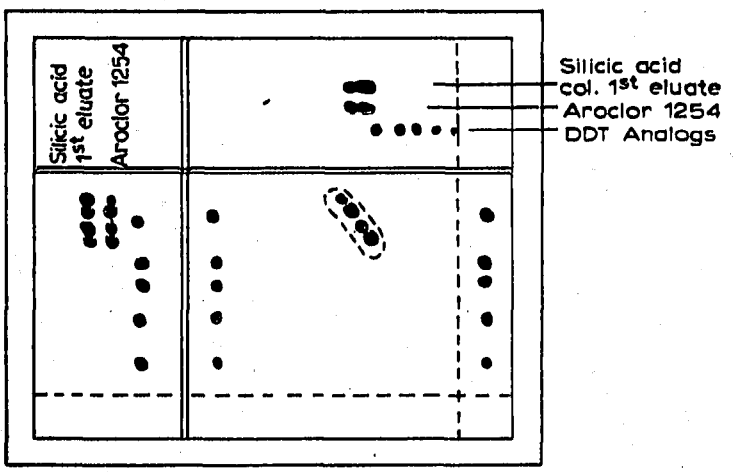

Fig. 12. PCB's isolated from fish sample by the silicic acid column separation of ARMOUR AND BURKE.

second dimensions were developed in sandwich chambers, the migrations were too similar to effect the desired separation. No adequate solution to the humidity problem can be offered at this time.

Figs. 3–10 are examples of the two-dimensional thin-layer chromatograms obtained by this procedure. The sample spot in each example (spot No. 1, Fig. 1) was a mixture of five DDT analogs and one Aroclor, which is identified by its number. The Aroclor pattern on each plate is outlined with a dotted line for easy reference.

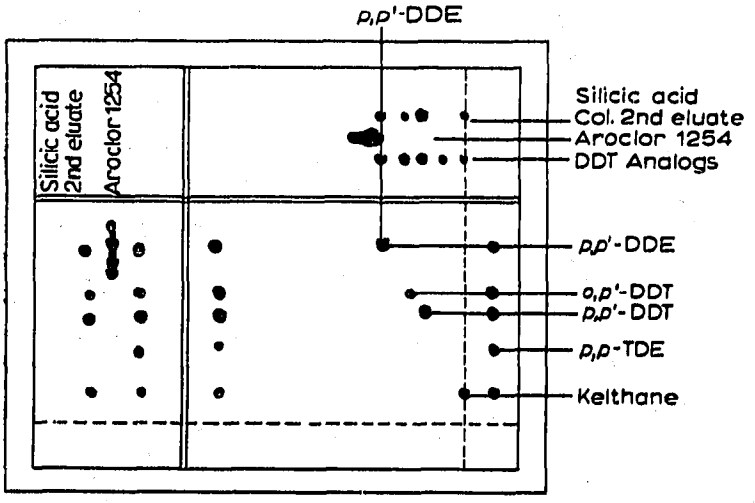

Fig. 13. Kelthane, *p,p'*-DDT, *o,p'*-DDT and *p,p'*-DDE isolated from PCB's in fish sample by the silicic acid column separation of ARMOUR AND BURKE.

Aroclors 1254 and 1260 are the two of greatest interest in pesticide work because their GLC peaks elute in the same general region as the DDT analogs. They also chromatograph on many TLC systems at approximately the same R_F value as *p,p'*-DDE.

This two-dimensional procedure has been successfully applied to fish samples containing PCB's. The PCB's were separated from the DDT analogs using the silicic acid column of ARMOUR AND BURKE[2]. The 6 % Florisil eluate and the two silicic acid column eluates were spotted and chromatographed by the two-dimensional procedure described here; Figs. 11–13 are drawings of the chromatograms. Fig. 11 represents the chromatogram of the 6 % Florisil eluate before the silicic acid separation, and Fig. 12 represents the first eluate from the silicic acid column, showing the PCB's in the sample. Fig. 13 represents the second eluate from the silicic acid column and shows the DDT analogs in the sample. These chromatograms show that the PCB's and the DDT analogs, which are eluted together from the Florisil column, can be separated. The first eluate from the silicic acid column exhibited a series of spots similar to those of Aroclors 1254 and 1260. The second eluate shows kelthane, *p,p'*-DDT, *o,p'*-DDT and *p,p'*-DDE, with no other spots visible.

Aroclors 5442 and 5460 tend to streak and are not satisfactorily separated from the DDT analogs by this procedure; therefore, example drawings of the two-dimensional thin-layer chromatograms of these Aroclors are not included.

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