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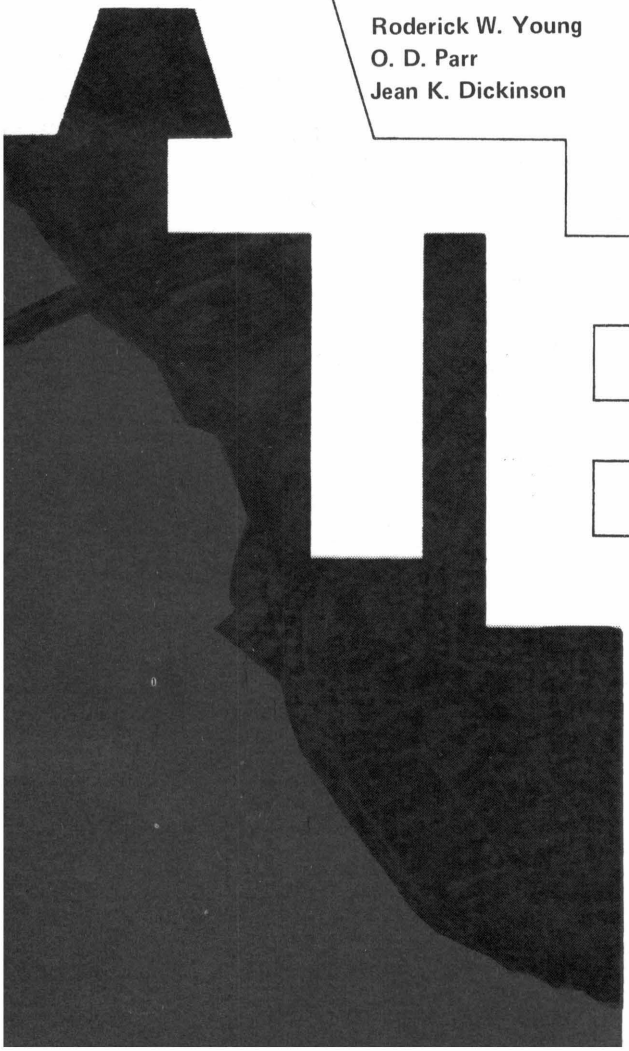

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ABSTRACT

This research evaluated various methods that have been advanced for separation of polychlorobiphenyls from chlorinated pesticides, to see which were effective in detecting and removing these residues often present in the aquatic environment. Preliminary investigations also were made of a new approach, the silylation of carbamates. Finally, a gas chromatographic (GLC) procedure for chlorophacinone (CPN) was developed by brominating the parent compound.

INTRODUCTION

General increases in the recycling of water from industrial usage and in drainage from areas treated with pesticides, particularly such organic chlorides as PCB's, DDT, and Endrin, have caused deleterious effects to the environment and criticism from the environmentalists and the general public. The findings of Jensen [1966; Jensen et al., 1969] and Widmark [1967] identified the presence of polychlorinated biphenyls in the Swedish environment, indicating that the problem we face is due to industrial as well as pesticide residues. This complex problem appears even in the laboratory, where the presence of PCB's interferes with identification of the two types of chlorinated residues found in the environment. And surely the problem can only increase with introduction of new industrial products (such as brominated compounds), organic phosphates, carbamates, and other types of pesticides for the purpose of complementing or replacing the chlorinated compounds.

The research described here represents only a beginning in the development of new methods of improvements of existing methods to insure proper identification of these chemicals being introduced into the environment. To be most helpful, these detection methods must be capable of identifying the deleterious residue at very low levels—before they become a problem to the environment. With the constant addition of chemicals to the environment, the continuous movements of water, and the never-ending exchange of materials between the air and the soil, it is apparent that all possible precautions must be taken to protect our natural resources.

METHODS

This investigation variously evaluated, improved, or developed methods which enhance our ability to correctly determine the residues present, preferably in the nanogram range of detection. Methods that were evaluated included those designed to separate polychlorinated biphenyls (PCB's) from the chlorinated pesticides, using only Arochlor/1242 and 1254 (PCB's) which represent the greatest interference with DDT and its analogs. These methods were developed by Armour and Burke [1970], Reynolds [1961], Snyder and Reinert [1971] and Karasz, Schermerhorn, More and Pollman [1972] to eliminate chromatographic interference between the two groups of chlorinated residues.

Improvements of existing methods for the detection of mixed carbamates also were studied, but findings are not sufficiently complete for publication.

A gas chromatographic (GLC) procedure for chlorophacinone (CPN) was completed satisfactorily by brominating the parent compound (CPN).

RESULTS AND DISCUSSION

The methods for extracting many residues that may be found in, or in the proximity of, aquatic environments are under investigation for evaluation, improvement, or development. These include the polychlorinated biphenyls (PCB's), various chlorinated pesticides, carbamates, and chlorophacinone (CPN). These compounds represent only a few of the residues that enter into the interferences encountered during analysis.

Due to the continued use and persistence of these residues, coupled with the constant introduction of additional new industrial chemicals and pesticides, we find ourselves in a state of transition from an almost totally chlorinated hydrocarbon residue environmental situation to one which includes carbamates and organic phosphates as well.

At present, there still is no clear understanding of the impact or the effects of this mixture of PCB's with chlorinated pesticides in our environment. Neither is it known whether mixtures of chlorinated pesticides and PCB's are present in residue samples received in the various laboratories throughout the nation. In a number of cases, intereferences between PCB's and chlorinated pesticides have been observed, and several methods for their attempted separation have been developed. However, as is indicated in annual reports from residue laboratories in California, Texas, New York, Florida, Kentucky and Mississippi, there is a lack of agreement on the usefulness of these methods.

By graphing the overlays of chlorinated pesticides and PCB charts, this investigation demonstrates that Aroclor 1232 (Chart 1), Aroclor 1242 (Chart 2), and Aroclor 1248 (Chart 3) do not interfere with DDT, its analogs, or other chlorinated hydrocarbons, while Aroclor 1254 (Chart 4) and Aroclor 1260 (Chart 5) do interfere with practically all of them. Also, recoveries for Aroclors 1232 and 1242 are low (30 percent or less), as these contain low chloride levels and are highly volatile. Aroclors 1254 and 1260 demonstrate much higher recovery levels (90 percent or greater), since they contain higher chloride levels resulting in a compound that is less volatile.

As shown in Table 1, each method requires the use of two solvent systems in attempting to accomplish separation. The main DDT groups under study are the pp'DDT's. The Karasz

method [Karasz et al., 1972] converts the DDT's to DDE by treating the sample with hot alcoholic KOH, but in so doing completely destroys lindane and some of the other chlorinated residues present. Although the interfering residue peaks are removed from the sample, such a method does not answer the problem of detecting what specific residues may be present. In the methods under investigation, with the exception of Karasz's method, the PCB's are found in both fractions. The DDT analog DDE was found both in fractions of the Armour and Burke [1970] and the Snyder and Reinert [1969] methods, but appeared only in the first fraction with the other methods. The Reynolds [1969] and Mills [1961] methods have DDD in both fractions, but it appears only in the second fraction when using Armour and Burke [1970] and Snyder and Reinert [1969] methods. The Mills [1961] method retains 98 percent of the DDT in the first fraction but DDT is found only in the second fraction of the other methods.

Development of a method for analyzing samples containing one or more carbamate pesticides is under study. This study is employing a combination of improvements in existing methods and is developing a derivatized compound by using silylation methods.

The initial extraction is accomplished by using dichloromethane rather than chloroform, as was used by Butler and McDonough [1968]. Samples are cleaned up with coagulating solutions and then extracted from coagulating solutions using dichloromethane. Hence, the only major change from the extraction and clean up of the original carbamate method is the substitution of dichloromethane for chloroform.

The silylation of carbamates represents a new development in analysis. Two approaches were used. In the first study, 0.4 ml trimethylsilyl imidazole (TMCS) and 0.4 ml acetonitrile were added to the test tube containing the dry sample. The sample then was stoppered tightly and heated in an oil bath at 125° C for 15 minutes or at 60° C for three hours. Following this, the sample was cooled, taken up to volume with benzene, shaken, and the benzene layer injected into the GLC.

In the second study, a column silylation of the sample was accomplished by injecting 2 μl of a mixture consisting of 75 per-

cent trimethyl-chlorosilane (TMCS) and 25 percent bis(trimethylsilyl)trifluoroacetamide (BSTFA, Supelco) directly onto the column. Within a half hour, the sample from the clean-up procedure was injected onto the GLC column. Use of this silylation procedure resulted in more reproducable chromatograms.

Three different column packings were utilized in this study: (a) 20 percent SE-30 on 60-80 mesh Supelcoport; (b) 1.5 percent OV-17/1.95 percent OV-210 on Chromosorb W-HP, and (c) 1.5 percent SP-2250/1.95 percent SP-2401 on 100-120 mesh Supelcon SW-DMCS. All were packed in ¼-inch glass columns to prevent any reaction between the sample and metal columns. The optimum temperatures for these columns is shown in Table 2 and ranges from 185° to 195° C. A nitrogen flow rate of 120 ml per minute gave the sharpest peaks.

Additional studies needed to develop this approach further would include determination of the various carbamates present in samples and augment separation of these residues.

The lack of sensitivity experienced by Chempar Company [1970] with the spectrophotometric method for the analysis of chlorophacinone (CPN), which was a minimum detection level of 100 mg, does not lend itself to the lower ppb limits of detection sought by monitoring agents. This requires the development of more sensitive procedures. Derivatizing CPN by bromination allows the use of GLC procedures for its analysis. The GLC electron capture detector provides the sensitivity and specificity needed to determine ppb levels (0.02 ppm) of CPN. The extensive extraction and cleanup procedures provided by the Chempar Company spectrophotometric method are ample for the preparation of the samples for the GLC procedure and are outlined in the following procedure used for this analysis.

Apparatus

(a) *Gas Chromatograph*—The Tracor Model MT 220 is equipped with a ^{63}Ni electron capture (EC) detector operating at a voltage output of 30 D.C. A 6′ x 4″ id U-shaped glass column is packed with 1.5 percent OV -17 $+$ 1.95 percent QF_1, on 100-120 mesh Supelcoport (Supelco, Inc., Bellefonte, Pennsylvania). Operating temperatures are: Column, 180°; inlet, 220°; EC detector, 300° C; Purified nitrogen carrier gas is used

at flow rate of 80 ml/min, and GLC sensitivity settings are Range 10 and Attenuation 8. The column was conditioned for 24 hours at 220° C (N_2 flow rate 50 ml/min.) connected only at the injection port. Following the initial heating period, the temperature is reduced to 180° C and the column is connected to the detector. The column is saturated with several 10 μl injections of the standard over a 48-hour period before injection of samples. When the column is not in use for several days, at least four 10 μl injections of the standard are injected to saturate the column before samples are run. The glass wool is replaced in the column inlet once a week to protect the column from deterioration caused by incomplete clean-up of samples.

(b) *Recorder*—Westronics MT-11; 1 MV chart speed, one-half inch per minute for recording the GLC chromatograms.

(c) *Shaker*—Burrell wrist—action shaker

(d) *Flash Evaporator*—California Laboratory Equipment Company (1165 67th Street, Oakland, Calif. 94608).

Reagents

(a) *Solvents*—Methanol and ethyl acetate, nanograde (Mallinckrodt), absolute ethanol (U. S. Industrial Chemical Company); acetone, analytical reagent grade (Fisher Scientific Company), redistilled in glass; and hexane, reagent grade, 55 gallon drum (Phillips Petroleum Company), redistilled over metallic sodium in glass.

(b) *Aluminum Oxide*—Merck 1097.

(c) 5 *percent Bromine, Iodine Glacial Acetic Acid Solution*—glacial acetic acid saturated with iodine is mixed with 5 percent (by volume) liquid bromine.

(d) *Sodium Hydroxide*—50 percent aqueous solution (J. T. Baker Chemical Company)

(e) *Sodium Sulfate Solution*—2 percent sodium sulfate aqueous solution (Mallinckrodt, anhydrous).

(f) *Sulfuric Acid*—concentrated (J. T. Baker Chemical Company).

(g) *Chlorophacinone (CPN)*—Standard: 2(1-(p-chlorophenyl) -1-phenyl-acetyl-1, 3-indandione).
The spectrophotometric method requires 20 mg of CPN dissolved in 50 ml of of acetone and made up to 100 ml with distilled water (0.2 mg/ml).

The gas chromatographic method requires 50 mg of CPN made up to 50 ml with acetone (1 mg/ml), Solution A, and Solution B, 1.25 ml of Solution A diluted to 25 ml with acetone (0.05 mg/ml). (CPN is obtained from Chempar Chemical Company, Inc., 200 Madison Avenue, New York, N. Y., and is a product of Lipha, Lyon, France).

Extraction Procedure

Accurately weigh 25 g of chopped plant material or 50 g of soil (screened through a number 20 mesh sieve), and transfer to a 500 ml glass-stoppered Erlenmeyer flask. Add 100 ml of ethyl acetate and shake for 30 minutes on a wrist action shaker (Burrell). Decant the solvent containing the CPN off the sample, filter through a cotton pad, and collect in a 600 ml beaker. Repeat the extraction approximately six time, using only 50 ml of ethyl acetate per extraction, until the extract is colorless. Evaporate the sample on a flash evaporator to approximately 3 ml. Make up to 5 ml with ethyl acetate and save for sample cleanup.

Sample Cleanup

Transfer the 5 ml sample from the extraction step onto an aluminum oxide column (2 cm x 20 cm glass) equipped with a Teflon stopcock. (The chromatographic aluminum oxide cleanup column is prepared by placing a cotton plug in the bottom of the column, closing the stopcock, adding approximately 50 ml of ethyl acetate to the column, and pouring 15 g (for a soil sample) or 20 g (for plant-tissue sample) of dry aluminum oxides into the column. Open the stopcock; as the solvent drains slowly from the column, the packing settles in column. Just before the column drains almost to dryness, rinse the sample beaker with two 5 ml portions of ethyl acetate onto the column.

Interfering compounds, including the pigments, are removed from the sample in the following manner:

Add, separately, five 25 ml portions of ethyl acetate; one 15 ml portion of absolute alcohol containing 20 percent ethyl acetate; two 10 ml portions of absolute alcohol; one 10 ml portion of methanol containing 20 percent absolute alcohol; two 10 ml portions of methanol, and one 10 ml portion of acetone containing 20 percent methanol and two 10 ml portions of acetone (the last solvent added eluates the pigments). All of the eluates from the column are discarded at this point. The chlorophacinone is eluted from the column with two 2 ml portions of 50 percent V/V aqueous acetone into a beaker. Evaporate the sample under a stream of air until there is no noticeable odor of acetone.

Derivatization of the Sample for GLC

Following the evaporation of the acetone from the aqueous acetone solution, transfer the sample to a 100 ml volumetric flask with 50 ml of acetone, add 10 ml of hexane, make up to volume with 2 percent Na_2SO_4, and shake vigorously for 1 minute. Pipette 5 ml of the hexane (upper) layer off the sample, transfer to a 10 ml volumetric flask, and evaporate to dryness with a gentle stream of air. Add 1 ml of glacial acetic acid, 5 drops of concentrated H_2SO_4, and 0.2 ml of 5 percent (by volume) bromine solution (acetic acid saturated with iodine crystals plus 5 percent liquid bromine) to the 10 ml volumetric sample flask, and mix thoroughly.

Insert a 14.5 cm straight condensing column with a ground glass 10/30 joint into the 10 ml sample flask and immerse to a depth of 1 cm into a 130° C constant—temperature oil bath for one hour. Remove the flask from the oil bath, rinse down the condensor column with approximately 5 ml of distilled water, and remove from the flask. Cool the flask in a cold water (ice floating) bath to room temperature. Add 3 ml of 50 percent NaOH while swirling the flask to mix the solution. Add 1 ml of hexane, make up to volume with distilled water, and shake vigorously for 1 minutes. Transfer one-half ml of the hexane layer to a 5 ml glass-stoppered centrifuge tube.

Make the sample up to the desired volume, usually 1 or 2 ml (depending upon the concentration of the CPN), and inject onto the gas chromatography column using the conditions described earlier for the operation of the GLC. Under the GLC conditions

described earlier for CPN, the retention time was 5.75 minutes. A standard and reagent blank must be run to determine the retention time for new GLC columns. Each GLC column prepared has slightly different operating parameters causing slight changes in the retention time.

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TABLES AND CHARTS

TABLE 1

Separation Methods of PCB's from DDT and Its Analogs (in ppm)

Methods	PCB's Fraction		pp'DDE Fraction		pp'DDE Fraction		pp'DDE Fraction	
	1	2	1	2	1	2	1	2
Armour and Burke (1970)	88	12	91	9	0	100	0	100
Reynolds (1969)	72	28	100	0	20	80	0	100
Mills (1961)	85	15	100	0	90	10	98	2
Snyder and Reinert (1971)	85	15	50	50	0	100	0	100
Karasz (New York) (1972)	100	0	100	0	0	0	0	0

TABLE 2

Optimum Temperature for Three GLC Columns

Column	Column Temperature
20%SE-30 on 60-80 mesh supelcoport	185° C
1.5% SP-2250/1.95% SP-2401 on 100/120 mesh Supelcon AW-DMCS	190° C
1.5% OV-17/1.95% OV-210 on Chromosorb W-HP	195° C

CHART 1

GLC Comparison of Aroclor 1232 (PCB) with Clorinated Pesticides

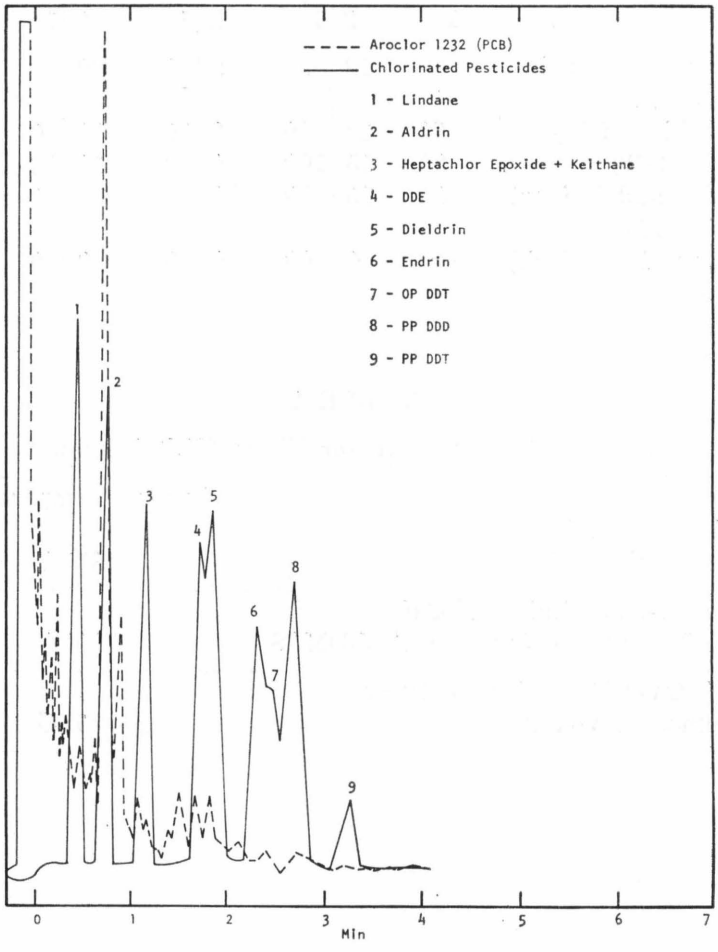


CHART 2

GLC Comparison of Aroclor 1242 (PCB) with Clorinated Pesticides

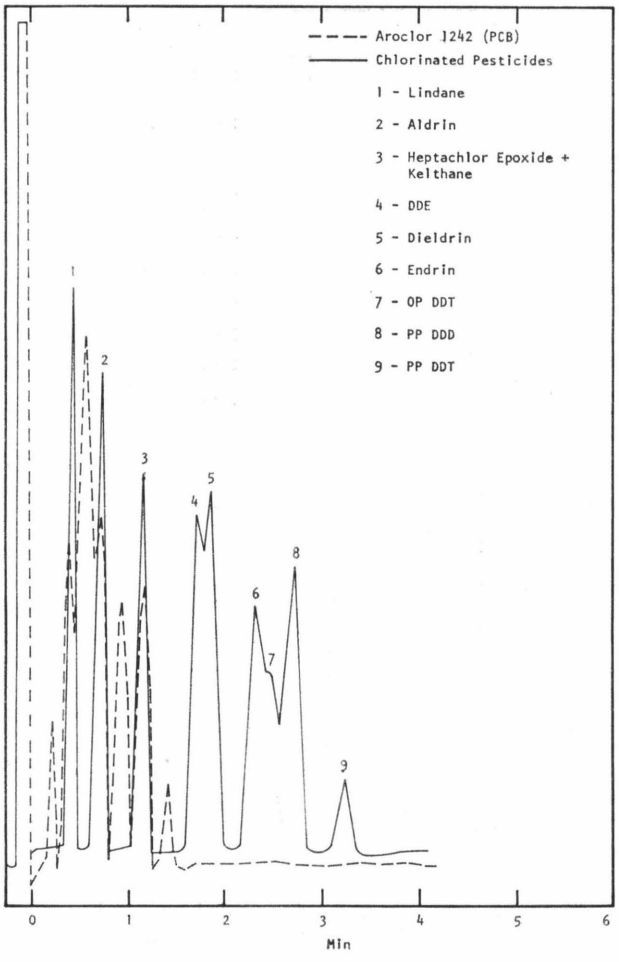


CHART 3

GLC Comparison of Aroclor 1248 (PCB) with Clorinated Pesticides

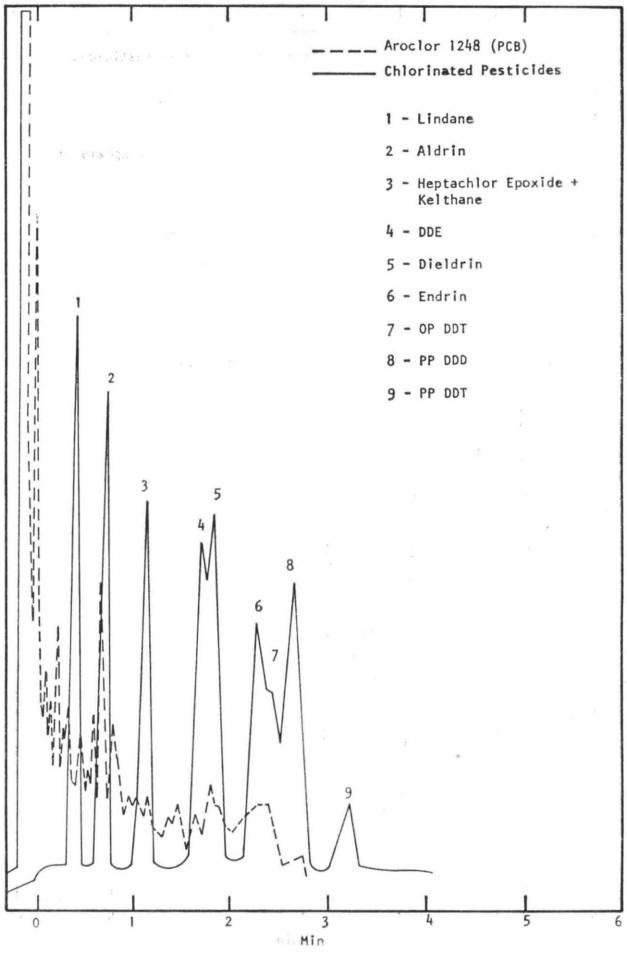


CHART 4

GLC Comparison of Aroclor 1254 (PCB) with Clorinated Pesticides

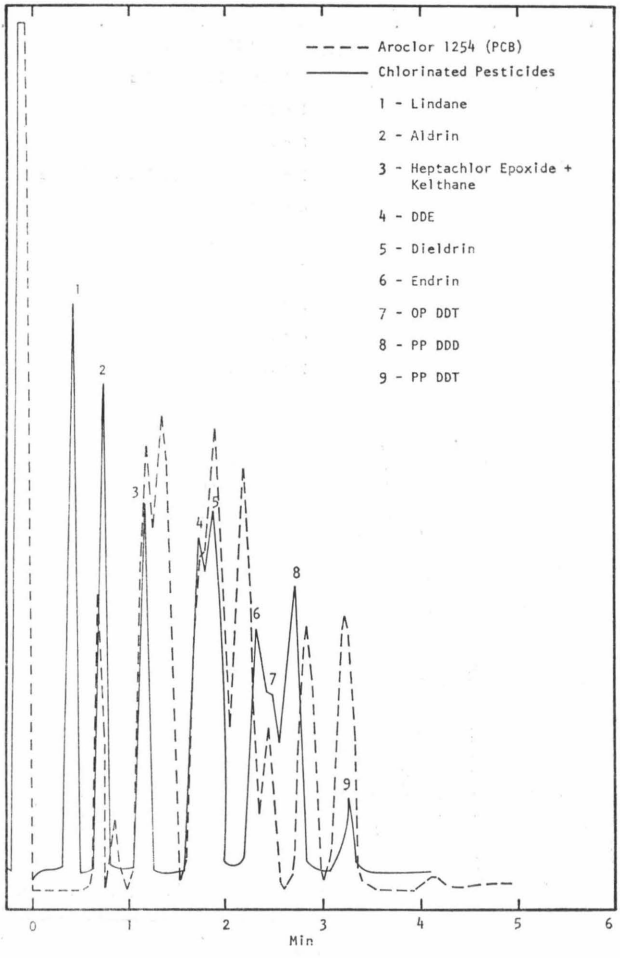
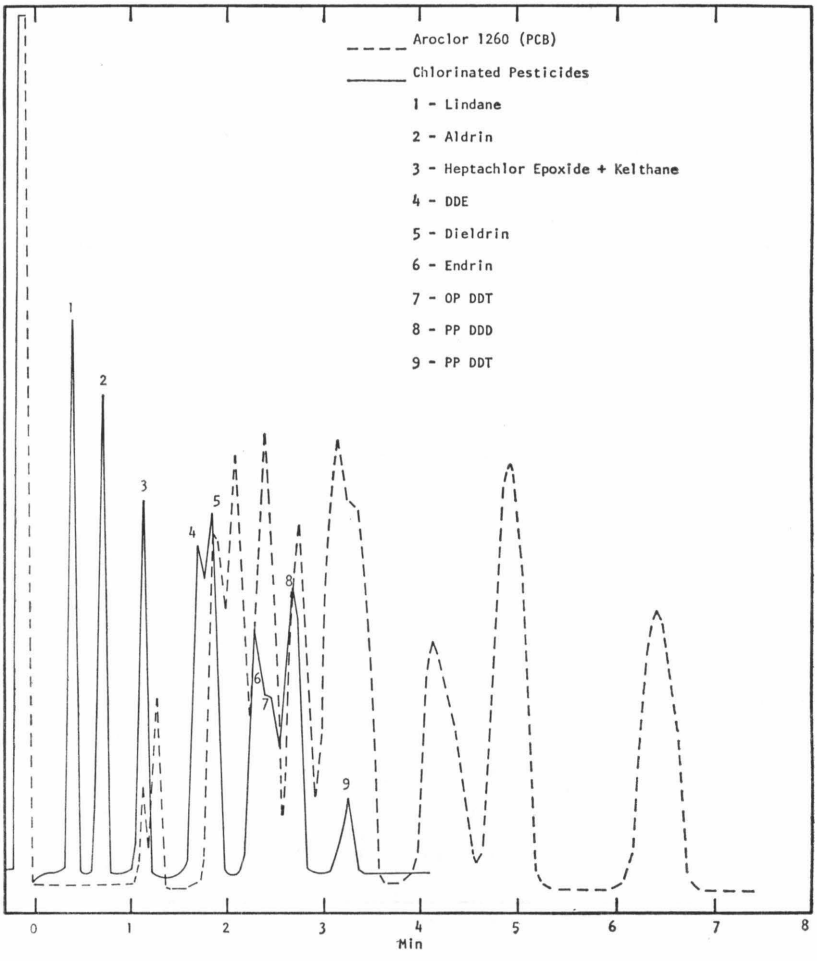


CHART 5

GLC Comparison of Aroclor 1260 (PCB) with Clorinated Pesticides



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