COLUMN CHROMATOGRAPHIC SEPARATION OF POLYCHLORINATED BIPHENYLS FROM CHLORINATED HYDROCARBON PESTICIDES, AND SUBSEQUENT GAS CHROMATOGRAPHIC QUANTITATION IN TERMS OF DERIVATIVES

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Summary. A novel liquid chromatographic technique, based on charcoal, has been developed for the quantitative separation of PCB's from organo chlorine pesticides.

A gas chromatographic method for the identification and quantitation of PCB's in terms of a well-defined derivative has also been developed. It is based on exhaustive chlorination of PCB's to decachlorobiphenyl. The catalytic dechlorination of PCB's to bicyclohexyl is also discussed.

Polychlorinated biphenyls (PCB's) are universal physiologically active pollutants (1,2,3). The analytical (and bio-) chemistry of these materials is poorly developed (2). Although identification of these compounds can be achieved by GC/MS techniques, the quantitation problem has not yet been solved. The analytical difficulties have been reviewed (4,5). This paper describes a column chromatographic technique for the separation of PCB's from organochlorine pesticides and their subsequent quantitation in terms of well-defined derivatives - bicyclohexyl and decachlorobiphenyl.

This system of analysis is in routine operation in our laboratory. It has been applied to a variety of samples, mainly from the aqueous environment. For example, PCB's (roughly of the Aroclor 1254 variety) have been isolated and quantitated in samples from ducks and seagulls, porpoise blubber, sewage sediments and bottom fauna in general. A water sample from Lake Michigan, at the inlet to a water purification plant supplying a major city was found to contain 275 ppt PCB's (determined as decachlorobiphenyl but expressed in terms of Aroclor 1254).

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Separation of PCB's from DDT Group of Pesticides

For the quantitative determination of PCB's by gas chromatography, it is necessary to separate them from other chlorinated pesticides. More or less successful column chromatographic separation procedures have been published: Reynolds-Florisil (4), Holden and Marsden-alumina and silica gel (6), Armour and Burke - silicagel and Celite (7). For proper separations, the adsorbents have to be partially deactivated with water. In our hands, these techniques have not been too successful. In more general terms, our past experience in other areas of column chromatography has indicated that it is extremely difficult to reproduce the activity of the adsorbents from day to day and from one laboratory to the other. Recently, Zitko (5) discussed these and other difficulties in some detail. In particular, he indicated that the commercial pesticide-grade hexane from various suppliers was contaminated with benzene. The separation of Aroclor 1254 from organochlorine pesticides on silicic acid was affected markedly by the addition of small (5-20 mls/l) quantities of benzene. In addition, he pointed out that several batches of commercial silica gel were contaminated with PCB's of the Aroclor 1254 type. Although the PCB's could be removed from the contaminated silica by washing with acetone, the treatment irreversibly changed the chromatographic properties of the adsorbent, and the required activities could not be restored.

In view of these difficulties, we developed a separation technique based on activated charcoal. It was based on the observation that PCB's once adsorbed onto charcoal could not be recovered by hot chloroform extraction (8). We discovered, however, that they could be removed quantitatively with cold benzene. By trial and error, it was found that the DDT group of pesticides and a number of others* could be eluted from the charcoal column with an ether-acetone mixture, while the PCB's may later be recovered with benzene. The choice of charcoal is critical. Of the several commercially available materials, only one is found to be suitable - Fisher cocoanut charcoal for "decolorizing". There is some contribution of EC materials from the adsorbent. These can be removed effectively by Florisil cleanup of the eluates. Oily or fatty extracts of up to about 100 mg of oil do not change the separations markedly. After the charcoal separations, the oils are also removed by Florisil cleanup.

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*Lindane, Aldrin, Dieldrin, Endrin, Heptachlor and Heptachlorepoxide

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It should be noted that the standard eluotropic solvent series used in chromatographic separations is reversed on charcoal (9). Thus, benzene is the strongest solvent in the series and n-hexane is next to it. Consequently, the sample should be added in the minimum amount of acetone or 25% acetone in ethyl ether. Wet packing of the column gives more reliable results.

One particular run gave the following recoveries: (using 90 mls of 25% acetone in diethyl ether followed by 60 mls of benzene).

	Per cent
p,p'-DDE	91
p,p'-DDD	92
p,p'-DDT	92
O,p'-DDT	94
PCB-Aroclor 1254	90

The recoveries are quite consistent. The PCB's are recovered with very little alteration to overall GC peak profile. Under our experimental conditions, about 1.1 g of charcoal, the system can handle about 5 ug PCB's and about 0.5 ug each of the DDT's.

Identification and Quantitation of PCB's in Terms of Derivatives

The identification and quantitation of PCB's by gas chromatography is a difficult matter. Although a certain amount of information can be obtained by the trial and error matching of chromatographic traces with those of commercial PCB's and measuring of prominent peak heights or total areas, the quantitative results are very uncertain.

In particular, it is difficult to determine PCB residues in biological samples. Thus, for example, Mulhern et al. found that GLC analyses of Japanese quail and eggs from mallards fed Aroclor 1254 gave peak profiles that did not match those in Aroclor 1254 (10). In view of this, it would be desirable to base the quantitation of PCB's on a single well-defined derivative. Our experimental work has indicated that PCB's can be converted quantitatively into bicyclohexyl or to decachlorobiphenyl. Outlines of our techniques have been disclosed (11, 12).

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Catalytic Dechlorination of PCB's

Under suitable conditions, the PCB's can be dechlorinated quantitatively with hydrogen over Pt or Pd catalysts. The reaction product is a single compound - bicyclohexyl. Our experimental procedure followed essentially the guidelines established by Thompson et al, in the early sixties (13). The major reason for following Thompson's procedure was the discovery that fairly large, about 2.5 mls, quantities of hexane were necessary to recover the reaction product quantitatively from the reactor. Also, occasionally, with low-activity catalysts, the conversion was not complete. In this case, the products could be recycled. A somewhat different approach was taken by Gunther et al., who used carbon-skeleton chromatography (14). The conversions were however not quantitative. Depending on the conditions, the PCB's were dechlorinated to cyclohexylbenzene, biphenyl and bicyclohexyl.

During the early stages of our work, this technique was used extensively for the identification and quantitation of PCB's. At the 10 ug levels, the conversions were good, better than 95%. The conversions were somewhat better with the palladium rather than platinum catalysts. There were occasional problems with the reproducibility of catalyst activities. The main disadvantage of this procedure, however, was that the hydrocarbon had to be determined with GC/FID instrumentation at a fairly low level of sensitivity. When it was realized that PCB's could be perchlorinated on a preparative scale to another well-defined derivative - decachlorobiphenyl that could be determined with GC/EC instrumentation (15, 16), attempts were made to adapt this macro reaction to analytical problems.

Perchlorination of PCB's

The treatment of PCB's with antimony pentachloride under elevated temperatures and anhydrous conditions gives very good yields of decachlorobiphenyl. On the preparative scale (0.5 g), the yields are better than 90%, while on the analytical scale, 1 ug, the yields are somewhat lower, but consistent at 85 ± 5%. The latter procedure includes a Florisil cleanup for GC/EC determinations.

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The preparative reaction is best carried out in a Teflon-lined screw-cap vial. The temperature and time do not appear to be critical. Treatment for about 5 hours at 170°C will produce a complete conversion. For analytical preparation, it was found necessary to carry out the conversion in sealed glass tubes. On occasion, the tubes were left overnight at the reaction temperature. No by-products were formed. The hydrolysis of the unreacted antimony pentachloride was carried out with 20% hydrochloric acid in order to suppress the precipitation of antimony oxychloride and to avoid possible entrainment of product. The gas chromatographic behaviour of decachlorobiphenyl is elementary. Since no separation from other materials is involved, the determination can be carried out with a short column. In our laboratory, the analysis is carried out with a 2 ft. x 1/8" stainless steel column. With the column temperature at 215°C, the retention time for decachlorobiphenyl is about 9 minutes (Figure 1). The Aroclor 1254 is eluted in about 3 minutes. The decachlorobiphenyl peak is well removed from those of common chlorinated pesticides and those of Aroclor 1254 and Aroclor 1260. This was confirmed recently by Zitko et al. (17). The relative retention time (six foot column, SE-30) in terms of p,p'-DDE=1, for decachlorobiphenyl was 8.20, while for two octachlorobiphenyls they were about 2.5. It was also noted that the relative detector response in terms of p,p'-DDE=1 for decachlorobiphenyl was 1.6.

EXPERIMENTAL

LC Separation of PCB's from DDT Group Pesticides

Materials and Reagents

Column - 6 mm ID x 140 mm Pyrex glass tube with a 25 ml reservoir attached.

Charcoal - Fisher No. 5-690 charcoal, activated, 50-200 mesh, (Fisher Scientific Co., no substitutes have been found). The absorbent is boiled with acetone on a steambath. It is then cooled, and the solvent is removed by suction. This procedure is repeated. The filter cake in the funnel is washed with some cold acetone. The charcoal is air dried and stored at 135°C.

Ottawa Sand - acid washed.

Glass Wool - solvent extracted.

Eluting Solvent A - 25% acetone in ethyl ether (anhydrous)

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Eluting Solvent B - benzene

Procedure

The tube is plugged with a small wad of solvent-washed glass wool. About 25 mm of acid-washed Ottawa sand is then added; this amount of sand is necessary to retain the fines from the charcoal. A solvent (acetone) slurry of the adsorbent is then added until the column of the charcoal is 90 mm long (1.1 gm dry weight). The sample is injected directly onto the top of the column. The DDT group pesticides are eluted with 90 mls of Solvent A and the PCB's with 60 mls of Solvent B. Heavily contaminated eluates might require cleanup on Florisil. For testing the column efficiency, the following loadings are suitable:

PCB - Aroclor 1254	- 1 ug
p,p'-DDE	- 0.5 ug
p,p'-DDD	- 1.0 ug
o,p'-DDT	- 1.0 ug
p,p'-DDT	- 1.0 ug

Catalytic Dechlorination of PCB's

Catalyst

5% by weight Pt or Pd as metal on 60-80 mesh porous glass beads (Applied Science Laboratories, State College, Pa.). An aqueous solution of chloroplatinic acid or palladium chloride is evaporated gently in contact with the glass beads. The catalyst is reduced in situ.

Reactor and Procedure

12 x 0.25 inch stainless steel tubing fitted with a modified Swagelok tee; one arm of the tee is fitted with a silicon rubber septum, the other one is inlet for hydrogen gas. The other end of the reactor is fitted with a 2 ft. length of 1/8 inch Teflon spaghetti tubing. The coiled tubing is cooled in ice water. The products are collected in a cooled 15 ml centrifuge tube. The reactor is kept at 180°C. The PCB is injected in hexane. After a few minutes, 5-6 o.5 ml quantities of hexane are injected in order to purge the reaction products. The conversion is about 95% at the 10 ug level with on-catalyst injection.

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Perchlorination of PCB

Macro Synthesis

A 0.5 g quantity of PCB (Aroclor 1254) was reacted with 3 mls of $SbCl_5$ in a Teflon capped glass tube. After 8 hours at 150°C the tube was cooled, opened and the contents treated with 20% hydrochloric acid (to prevent the precipitation of antimony oxychloride). The aqueous phase was extracted with warm benzene. The extract was shaken with aqueous bicarbonate solution, water and finally dried with anhydrous sodium sulphate. Evaporation gave a 95% yield of crude decachlorobiphenyl. The material was crystallized from a 50:50 mixture of benzene and pet. ether, M.P.304°C.

The white crystalline material gave one peak on GC. IR spectrum was obtained with Perkin-Elmer 221 spectrophotometer (Figure 2). It was sublimed in vacuum for elemental analysis.

MW-498.6 $C_{12}Cl_{10}$

	Calc.	Found
C	28.88	28.99
Cl	71.12	70.78

The molecular weight of the material was obtained by mass spectrometry.

Micro Synthesis

The PCB, one microgram or less, is treated with 0.2 mls of $SbCl_5$ in a sealed glass tube.

The PCB in a small quantity of a suitable solvent is transferred into a Carius tube, 8 mm OD, 6 mm ID, 160 mm long. The solvent is removed by gentle vacuum - thin Teflon tubing inserted into the glass tube. A quantity of 0.2 mls of $SbCl_5$ is added and the tube is sealed. After a minimum of three hours at 160-170°C, the tube is opened and the contents are worked up according to instructions given above for macro scale. With careful attention to the details, the recovery is about 85 ± 5%.

Gas Chromatographic Conditions for the Determination of:

1. Bicyclohexyl

   Instrument - Varian 1500 FI Detector

   Column - 8 ft. x 1/8" stainless steel, 10% DC-710 on Chromosorb W

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Temperature - 1. Column 90°C for 2.5 minutes then programmed at 10°C per minute

2. Injector 220°C

3. Detector 220°C

Gas Flow - Air 200 mls/min.
Hydrogen 20 mls/min.
Nitrogen 25 mls/min.

Range - 1

Attenuation - x8

Recorder - 1 mv full scale

Under these conditions, an injection of 50 ng of bicyclohexyl gives a chart response of 45 units. The retention time is 9.0 minutes.

2. Decachlorobiphenyl

Instrument - Varian 1500, Tritium Detector

Column - 2 ft. x 1/8" stainless steel 5% Se-30 on Chromosorb W

Temperature - 1. Column 215°C

2. Injector 220°C

3. Detector 215°C

Gas Flow - Nitrogen at 50 mls/min.

Range - 1

Attenuation - x8

Recorder - 1 mv full scale

The peak is Gaussian in shape. The retention time is approximately 9 minutes (Figure 1).

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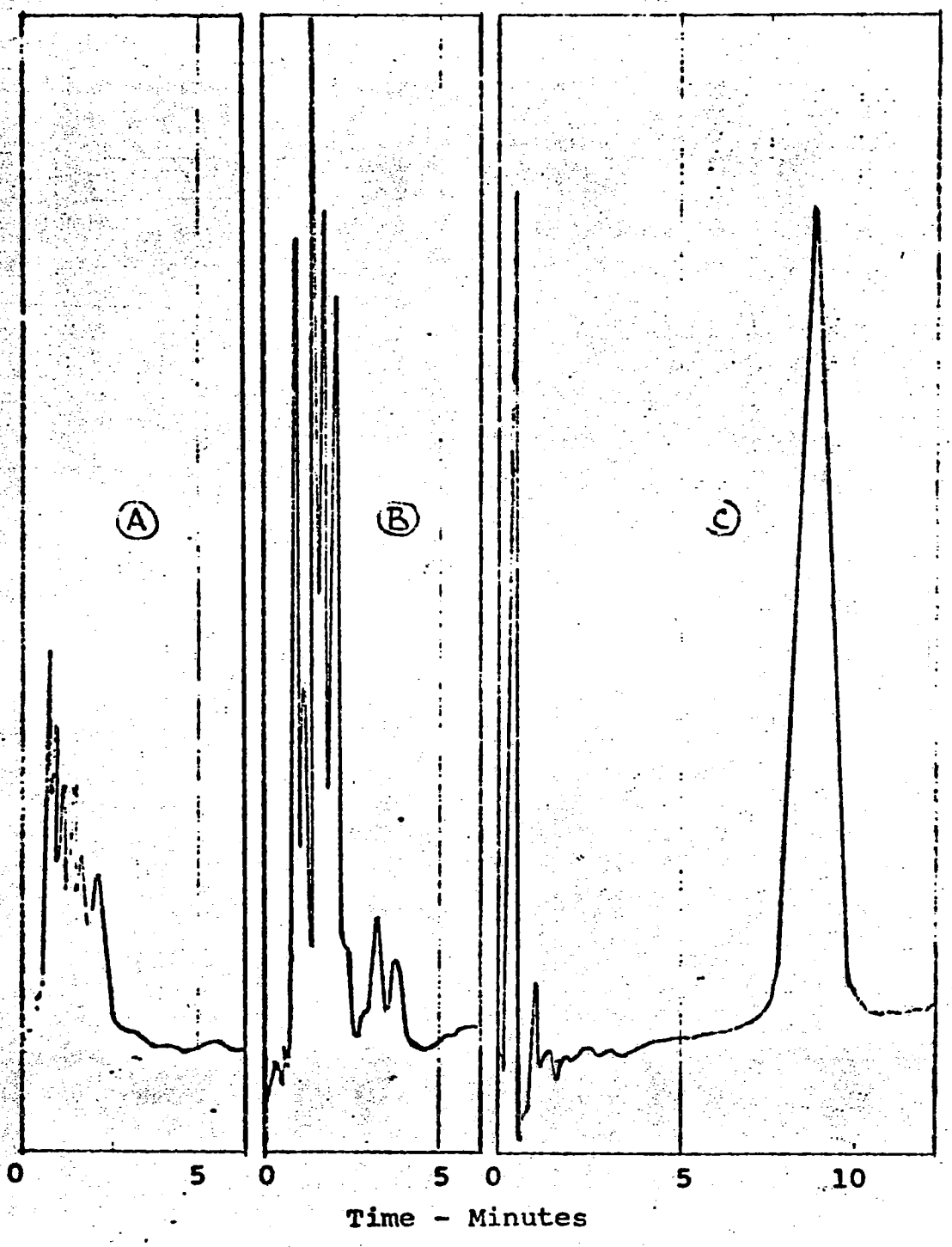
FIGURE 1.

GC/EC CHROMATOGRAM OF A GULL TISSUE SAMPLE.



A - Aroclor 1254

B - Fraction from charcoal column

C - Fraction after chlorination and Florisil clean-up.

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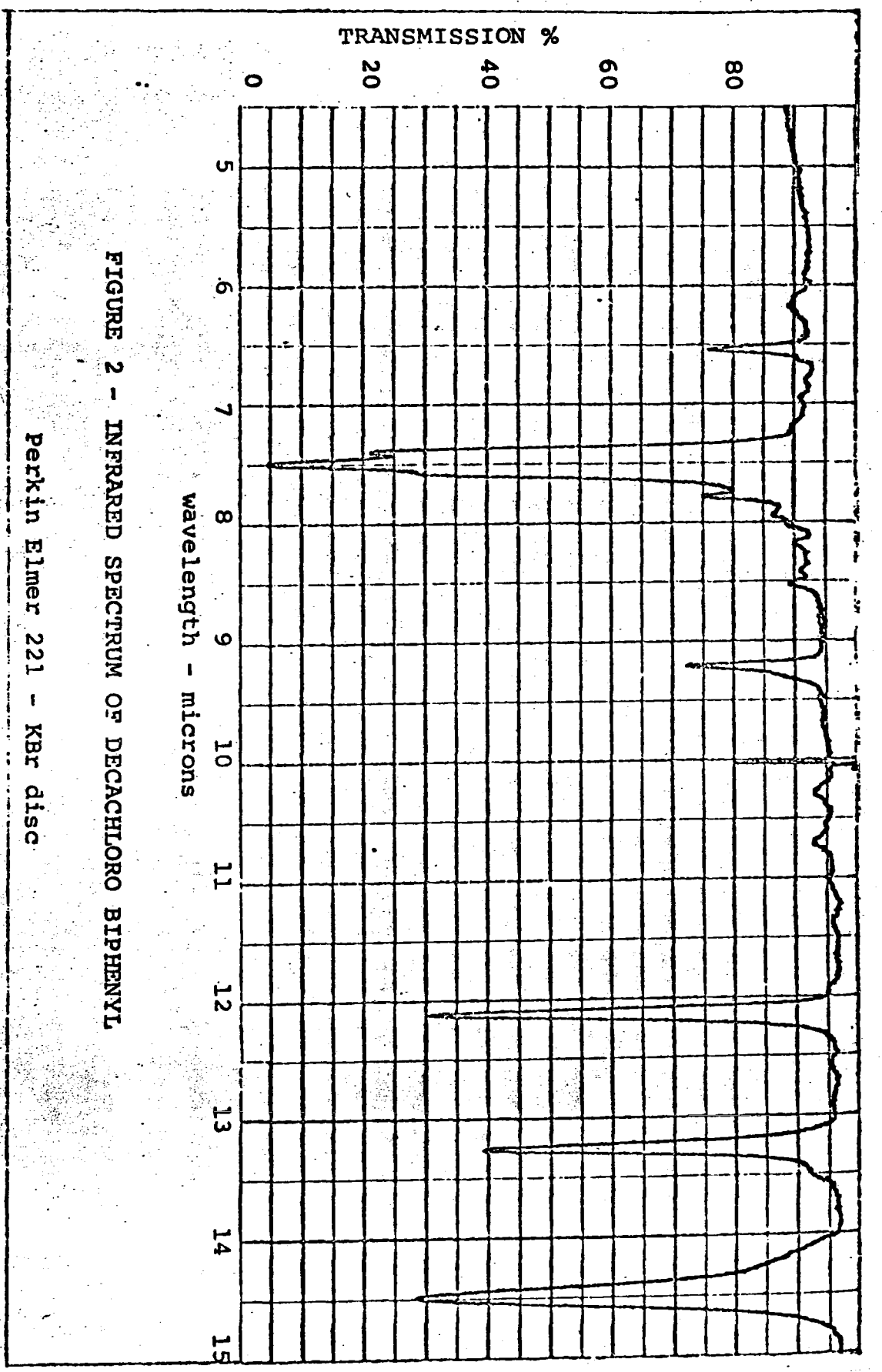

FIGURE 2 - INFRARED SPECTRUM OF DECACHLORO BIPHENYL

Perkin Elmer 221 - KBr disc

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