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METABOLIC STUDIES OF
2,2',3',3',4,4',5,5'-HEXACHLOROBIPHENYL
IN GUINEA PIGS

by

MARGARET ANN PEARSON

A Thesis Submitted in Partial
Fulfillment of the Requirements for the Degree
Master of Science, Major in Chemistry,
South Dakota State University

1975
METABOLIC STUDIES OF 
2,2',3',3',4',5,5'-HEXACHLOROBIPHENYL 
IN GUINEA PIGS 

This thesis is approved as a creditable and independent investigation by a candidate for the degree, Master of Science, and is acceptable for meeting the thesis requirements for this degree. Acceptance of this thesis does not imply that the conclusions reached by the candidate are necessarily the conclusions of the major department.

Main Advisor  Date

Thesis Advisor  Date

Head, Chemistry Department  Date
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... Margy
IN DEDICATION

To Ms. Marcella Ann Havrevold. May you have the opportunity to pursue your special goal in life, the friends to guide you ever so gently and the fortitude to overcome all obstacles to achieve this goal.

Remembering the words of E. Hubbard, "'Fences were made for those who cannot fly.'"
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INTRODUCTION

Objective

This study was carried out to investigate the metabolism of 2,2',4,4',5,5'-hexachlorobiphenyl (Cl₆-PCB). This isomer is a major constituent in commercial polychlorinated biphenyl (PCB) mixtures containing more than 50% weight-to-weight (w/w) chlorine. To investigate this isomer, we obtained it in ³⁶Cl labeled and non-labeled forms. We developed procedures to isolate and purify both forms of the hexachlorobiphenyl for further study. The ³⁶Cl labeled biphenyl was then introduced into test animals for metabolic studies.

Historical

PCB's are composed of biphenyl rings substituted with one to ten chlorine atoms. They are numbered as indicated in Figure 1.

![Figure 1](image)

FIGURE 1

PCB's were first synthesized in 1881² and have been in commercial use for the past forty years,³ though commercial
production of PCB's in the U.S. today is limited to Monsanto
Chemical Co., St. Louis, Mo. under the trade name of Aroclor.

Since PCB's vary in physical form from oily liquids to
crystalline solids (depending upon the number and placement of
chlorines on the biphenyl rings), they are used in a wide range
of applications based on their physical properties. These uses
include: heat exchangers for electrical equipment; plasticizers
for paints; protective coatings for asphalts, wood, metal, and
concrete; microscope immersion oils; printing inks, waxes, and
adhesives; and as vapor suppressants for insecticide formula-
tion. Interest in the use of PCB's as an insecticide has
increased, since it has been shown that the toxicity of organo-
phosphorus insecticides to houseflies is increased due to the
addition of PCB's; and that some PCB mixtures have insecticidal
properties.

Theoretically, there are some 210 possible isomers of PCB's,
although there are only 102 probable combinations. Aroclor
1254, one of Monsanto's commercial preparations, has been shown
to contain more than fifty components when analyzed by gas
chromatography (GC).

PCB's are highly resistant to biodegradation in the
environment; and since increasingly large amounts of PCB's have
been entering the atmosphere from industrial sources, concern
has been expressed over the possibility of build-up in some food
chains. A recent study by Lucille Stickel has summarized the biological data on PCB's in animals other than man.\(^6\) This review has shown that PCB's are widespread in the environment and that they exhibit a high degree of toxicity for many bird species. The biological effects of PCB's on the environment have been further reviewed by David Peakall.\(^9\) He extended this review to include the effects on human health. Though PCB's have been shown to be toxic, very little is known about the specific toxicological aspects of the PCB mixtures or of their metabolism.\(^{10-14}\) In order to investigate the metabolic aspect of PCB's and to understand their elimination processes, it is necessary to obtain a specific isomer in pure form and labeled so that its metabolites can be traced through a biological model.

Syntheses of PCB's have been extensively discussed in the literature.\(^{15-22}\) These procedures have invariably led to a mixture of products, with individual PCB's isolated using re-crystallization and thin-layer chromatography (TLC) techniques. However, these techniques are not appropriate for the isolation of a pure PCB in large quantities. The techniques do not offer enough resolution to quantitatively separate isomers of similar chemical properties.

To synthesize the correctly tagged isomer, \(2,2',{^{364,364,5,5'}}\)-hexachlorobiphenyl (\(*\text{Cl}_6\)-PCB), procedures were utilized by
Dr. Worman which produced the desired isomer as the major component of a mixture of chemically similar PCB's. Separation of this mixture using re-crystallization or TLC was deemed unsuitable for isolation of the *Cl₆-PCB in pure form and in large enough quantities for further study. One analytical technique which offered enough resolution to separate the isomers was gas chromatography. Preparative GC has long been used to isolate experimental quantities of compounds from complex mixtures, if detection and collection procedures can be worked out. Development of a procedure which would utilize the separative power of GC for compounds of this type appeared to be the technique of choice, but none of the published procedures lent themselves to isolation of the PCB's. It was, therefore, decided that a GC procedure using thermal conductivity (TCD) detection with appropriate collection techniques would be developed. Once the appropriate isomer was isolated in sufficient quantities, in vivo metabolic studies could be carried out.

In metabolism studies it is necessary to know that the starting material is chemically pure and that the isotope used in tagging can be attributed to a specific compound. The technique used for metabolism studies in our laboratory involves introducing a pure labeled compound into the test animal and then chromatographically isolating the compound and its products.
The isolated peaks are then assayed for radioactivity. It is imperative that all of the radioactive label be attributable to the compound being studied and that as few as possible contaminants, which are chemically similar to the compound, be present in a metabolic starting material.

Previous workers have investigated the metabolism of the Cl₆-PCB in trout, pigeons, and rats. They observed no identifiable metabolites in these species, and due to undefined purity of the starting material and lack of labeling they were unable to identify any low-level metabolites. In order to study the low-level turnover of this hexachlorobiphenyl compound, a ³⁶Cl labeled isomer was prepared and procedures to isolate and characterize it were developed.

**Experimental Design**

In order to establish the presence of and identity of low-level metabolites of *Cl₆-PCB* the following studies were carried out. The experimental procedure used was based on the identification of extraneous peaks which are found during GC analysis and the determination of which peaks exhibit radioactivity. To carry this out it was necessary that all of the initial labeling be attributable to the *Cl₆-PCB*, and the samples be chemically pure.

In order to prepare a standard for quantitative analyses the Cl₆-PCB was separated, isolated, and characterized. The PCB's
were separated from general reaction materials by florisil-column chromatographic procedures as adapted by the Pesticide Laboratory, South Dakota State University. The Cl₆-PCB was then isolated from the other isomers of the mixture via GC equipped with a thermal conductivity detector (TCD/GC). Throughout the purification procedure, the sample was characterized by GC electron-capture procedures (ECD/GC), mass spectrometry, and sample weight. The radioactive hexachlorobiphenyl was purified in a similar manner including characterization via scintillation counting procedures.

The guinea pig (Cavia procellus) was chosen as the test animal due to its availability and a size that allowed high dosage. The amount of *Cl₆-PCB which was obtained dictated that only two test animals and one control animal would be used. Due to the small number of test animals, no statistical inferences would be made. The cages were cleaned and decontaminated. The animals were prepared and subcutaneously injected with the *Cl₆-PCB/corn oil mixture and remained on the study for ten days. During this period, the urine and feces were collected daily and stored. At the end of the ten-day study, the guinea pigs were sacrificed and necropsied. At the time of necropsy live weights of the animals as well as weights of vital organs were recorded. The organs chosen for analysis were the livers plus gall bladders, whole body, urine, and feces. The brains were saved for future
investigations. The four samples were extracted and analyzed for possible metabolites.
MATERIALS AND EQUIPMENT

Chemicals

All chemicals were used as received unless otherwise described.

Unpurified 2,2',4,4',5,5'-hexachlorobiphenyl (Cl₆-PCB) and 2,2',3,3',4,4',5,5'-hexachlorobiphenyl (*Cl₆-PCB) were synthesized by Dr. James J. Worman, Department of Chemistry, South Dakota State University. They were isolated, separated and purified as described in this thesis.

Nitromethane and α-naphthalene were obtained from Eastman Organic Chemicals, Rochester, N.Y.

Scintillation grade 2, 5-diphenyloxazol (PPO) and 1,4-bis-2-(5-phenyloxazole) -benzene (POPOP) were obtained from Packard Instrument Company, Downers Grove, Ill.

Dioxane was obtained from Fisher Scientific Co., Fair Lawn, N.J.

Scintillation grade toluene and nanograde hexanes, petroleum ether, benzene, acetone and diethyl ether were purchased from Mallinckrodt Chemical Works, St. Louis, Mo.

Aroclor 1260 was obtained from Monsanto Chemical Company, St. Louis, Mo.

Chromatography Supplies

Florisil, 60/100 mesh, was obtained from Fisher Scientific
Company, Fair Lawn, N.J.

Four types of gas/liquid chromatography column packings were used: 3.8% SE-30 on 80/100 mesh selenized Diatopost, 15% QF-1 on 60/80 mesh Chromosorb W (H/P A.W. DMCS), and 10% DC-200 on 80/100 mesh Chromosorb W (H/P A.W. DMCS) were obtained pre-coated from Varian Aerograph, Walnut Creek, Calif., while 3% OV-1 on 60/80 mesh Gas Chrom Q was obtained from Applied Sciences Laboratory, State College, Pa.

Uniplate thin-layer chromatography (TLC) plates of Silica gel G, 1 mm thick, were purchased preconditioned from Analtech, Inc., Newark, Del.

Kieselguhr G was obtained from Merck Chemical Co.

The TLC plates were developed in 11''x11-1/2''x3-3/4'' Pyrex TLC chambers purchased from Brinkman Instruments, Westbury, N.Y.

The chromatograms were viewed in a Chromato-Vue, Model CC-20, chamber from Ultra-Violet Products, Inc., San Gabriel, Calif. The long-wave UV light setting was used.

Test Animals

Three male guinea pigs were randomly selected from the closed colony at Veterinary Diagnostic and Research Center, South Dakota State University.

Apparatus

The flash evaporator used was a Buchler, Model PF-9, PGN
instrument manufactured by Buchler Instruments, Fort Lee, N.J. It was attached to a water aspirator and the water bath was operated at 30° C.

The centrifuge used for this study was purchased from International Equipment Co., Needham Heights, Mass. It was operated at a dial setting of 30 at room temperature.

A Sorvall Omni Mixer by Ivan Sorvall, Inc., Norwalk, Conn. was used for grinding and extracting the liver samples, feces samples, and whole body subsamples at room temperature at a speed setting of 3, unless otherwise stated. The frozen whole bodies were ground three times in a hand meat grinder prior to sampling.

The three 11"x8"x9" stainless steel cages were made by Acme Metal Products, Chicago, Ill. They were mounted on wooden standards modified with 1/4" mesh galvanized screen for fecal collection and aluminum drip trays for urine collection as shown in the photograph below (Picture 1).
Instrumentation

Gas chromatographs (GC's) used for quantitative measurements during these studies include: a Varian Aerograph, Model 200, connected to a Texas Instrument recorder, Model Servo/Riter-II, 1 mv; and a Varian Aerograph HY-FI, Model 600-D, connected to a Sargent recorder, Model SR, 1 mv. Each GC was equipped with an electron-capture detector cell with an 8 mc 63Ni source. Column, injector, and detector temperatures were 200° C, 210° C, and 280° C, respectively. The ranges were set at 10 and the attenuators were set at 1. Each contained a 1/8" O.D. x 6' borosilicate glass column packed with a 1:1 mixture of 15% QF-1 and 10% DC-200 silicone on 80/100 mesh chromosorb W (H/P A.W. DMCS). Each was operated isothermally with a flow rate of 40 ml/min. of nitrogen carrier gas. The Model 200 gas chromatograph was used for quantitative measurements of all samples during the isolation, separation, and purification of the non-radioactive and radioactive hexachlorobiphenyls. The Model 600-D gas chromatograph was used for all quantitative measurements of the biological samples.

Preparatory studies were performed on a Varian Aerograph gas chromatograph, Model 90-P, equipped with a Model S-R, 1 mv, Sargent recorder; a thermal-conductivity detector (TCD); and a Packard fraction collector, Model 852. A 1/4'' O.D. x 6' stain-
less steel column packed with 3.8% SE-30 on 80/100 mesh selenized Diatopost was used. It was operated isothermally at 200°C with a flow rate of 70 ml/min. of helium carrier gas. The detector was maintained at a current setting of 175 milli-amps and a temperature of 260°C. The injector was heated to a temperature of 220°C. The fraction collector was maintained at 250°C. The fraction collector tubes were filled with florisil which had been previously washed seven times with an equal volume of nanograde hexane. This rinsing was done to extract any PCB's which might be present in the florisil. The collector was housed in a bell-jar, which was evacuated via a water aspirator to prevent aerial radioactive contamination.

Mass spectrometry analyses were performed on a Finnigan peak identifier, GC/MS, Model 3000, equipped with a Gohlke separator. A 1/8" I.D. by 5' borosilicate glass column was packed with 3% OV-1 on 60/80 mesh Gas Chrom Q with a helium carrier gas flow of 40 ml/min. Temperature for the column, injector, and accessory were 200°C, 210°C, and 210°C, respectively. The ionization potential was 70 ev.

The scintillation counter used was a Packard Tri-Carb, Model CP-15, and the window amplification was optimized at 1.4 on 10% for 36Cl. All samples were read on the red channel. Two cocktails were used: (1) a scintillation fluid (SF) cocktail which was prepared by dissolving 100 mg POPOP and 3 mg PPO in 1 l. toluene,
and (2) a dioxane cocktail (DC) which was prepared by dissolving 7 g PPO, 0.3 g POPOP, and 100 g α-naphthalene in 1 l. dioxane. Each sample was placed in a scintillation vial and concentrated to almost dryness. Fifteen ml cocktail were then added and the sample was then counted for 10 min. Scintillation fluid was used for all samples previously extracted with hexanes and DC was used for all samples which had been extracted with ethanol or ether.

**Purification and Isolation**

Samples of Cl₆-PCB and *Cl₆-PCB were obtained from Dr. Worman in crude form and weighed 2.8543 g and 2.6037 g, respectively. The synthesis procedure used allowed the possibility of inorganic as well as organic contaminants, so purification was accomplished in stages.

The crude Cl₆-PCB and *Cl₆-PCB were first characterized by analyzing a 1 mg/ml hexane solution of each PCB sample by ECD/GC on the Varion Aerograph GC, Model 200, under the conditions stated earlier in this thesis. Several electron-capturing substances were detected at the 1 mg/ml level. The crude tagged *Cl₆-PCB was also analyzed by counting 1 mg of compound in 15 ml of SF in the scintillation counter under the previously described conditions.

In order to separate PCB's from the inorganic impurities, the florisil chromatographic procedure in use at the Pesticide Laboratory, South Dakota State University was adopted. A 0.1 g sample of crude *Cl₆-PCB was placed on a 40 g, 3% deactivated
florisil column which had been prewashed with 200 ml hexane. The material was eluted with hexane and collected in seven 100 ml fractions. Each fraction was evaporated to 10 ml on the flash evaporator and quantitatively transferred to glass-stoppered centrifuge tubes. Samples of 1 ml were taken from each of the seven fractions and placed in the scintillation vials, to which 15 ml of SF was then added, and the mixture was counted in the Packard Tri-Carb under the conditions described previously. The majority of the radioactivity (98%) was collected in the first three fractions. These three fractions were pooled and then reduced to 15 ml. Three concentrations of solution from each of the florisil-cleaned Cl₆-PCB and *Cl₆-PCB in hexane were prepared: 1 mg/ml, 0.01 mg/ml, and 0.0001 mg/ml. Each of the solutions was injected into the ECD/GC for qualitative and quantitative analyses. The above procedure was then adopted for routine clean-up of the Cl₆-PCB and *Cl₆-PCB using 0.1 g sample, 40 g florisil, and 300 ml hexane for each elution.

At this stage the Cl₆-PCB and *Cl₆-PCB were isolated from the impurities still present after florisil cleaning.

Separation of the Cl₆-PCB via a TLC method described by earlier workers was attempted using 0.25 mm thick plates prepared in the following manner. A homogeneous slurry was obtained by mixing 25 g of Kieselguhr G with 60 ml H₂O. The slurry was then transferred to a Desaga applicator, and five Pyrex glass plates
(20 cm x 20 cm) were then coated with a 0.25 mm thick layer of the mixture. The plates were air dried overnight, then heated at 105° C for one hour, and were cooled to room temperature. They were then placed in a flat tray filled with 8% paraffin oil, volume to volume (v/v) in petroleum ether. The plates were soaked for 2 min. and then air dried for 24 hours.

A plate was spotted four times with three different amounts (2.2, 4.4, 6.6, and 4.4 mg) of florisil-cleaned *C<sub>16</sub>-PCB. This was done to check the capacity of the TCL plates. The plate was developed to 14 cm according to the procedure by R.H. deVos and E.W. Peet. 24

Each spotting was scraped into eight sections: Section I being ±1 cm of the spotting line; Sections II-VI being the next five 2 cm sections; and Sections VII and VIII being the next two 1.5 cm sections. Each section was then eluted with 15 ml 1:1 petroleum ether/diethyl ether (v/v).

Procedures to isolate individual PCB isomers via ECD/GC were expanded to include isolation and collection of components in a florisil-cleaned PCB mixture utilizing a TCD/GC connected to a fraction collector.

The C<sub>16</sub>-PCB and *C<sub>16</sub>-PCB isomers were isolated using TCD/GC previously described in this thesis. A 50 mg/ml solution of the florisil-cleaned C<sub>16</sub>-PCB was prepared. It was injected in 50 ul quantities. Each injection was collected in three major fractions:
(1) one prior to the *Cl₆-PCB peak, (2) one which included only the *Cl₆-PCB, and (3) one which followed the *Cl₆-PCB peak. Each fraction was collected in one cotton-plugged collector tube packed with an adsorbent which had been previously washed seven times with hexane. The two adsorbents tested were Chromosorb W and florisil. Florisil was deemed superior and was, therefore, chosen for all later isolations. Several tubes (six or seven) of each fraction number were emptied into a flask. The adsorbent, plugs, and tubes were then eluted with 50 ml benzene. The fractions were subsampled for scintillation counting. The isolated isomers were characterized by weight.

Three concentrations of the TCD/GC cleaned Cl₆-PCB and *Cl₆-PCB in hexane were prepared (1 mg/ml, 0.01 mg/ml, and 0.001 mg/ml). Each of the solutions was injected into the ECD/GC for qualitative and quantitative analysis. The major components were characterized by GC/MS analysis. The TCD/GC cleaned Cl₆-PCB was used for preparation of quantitative standards, while the TCD/GC cleaned *Cl₆-PCB was used for in vivo metabolic studies. Activity measurements were performed on the *Cl₆-PCB by placing 1 ml of the 1 mg/ml solution in a scintillation vial, bringing the solution to near dryness, reconstituting the sample in 15 ml SF, and counting it in the scintillation counter. The number of disintegrations per minute (dpm's) and specific activity in uCi/m M were calculated.
Due to the amount of Cl₆-PCB available, a mixture of Aroclor 1260 was prepared to test relative toxicity of PCB. A 50 mg/ml solution of Aroclor 1260 in benzene was prepared. One ml corn oil was added to 6.58 ml of this solution. The benzene was then evaporated under nitrogen to a volume of 1 ml. This corn oil mixture then was injected into a 658 g guinea pig at a dose level of 500 mg PCB/kg body weight. The animal was housed in a cage for a period of five days, and then was sacrificed and necropsied. No analyses were performed.

Three male guinea pigs, approximately six weeks old and weighing between 500 and 1,000 g, were randomly selected from the breeding colony at the Veterinary Research and Diagnostic Laboratory, South Dakota State University. They were weighed and prepared for injection by first clipping hair from the area on their backs between their shoulders. The area was then sterilized with ethanol.

The TCD/GC cleaned *Cl₆-PCB was prepared for injection by dissolving 610 mg in 10 ml benzene. Two ml corn oil were then added to the solution and then the benzene was evaporated off under nitrogen. Each of the two guinea pigs received a subcutaneous injection of this mixture. They received 442 and 448 mg *Cl₆-PCB/kg body weight. The third guinea pig received a subcutaneous injection of 1 ml corn oil from which 5 ml benzene had been evaporated off under nitrogen. Each animal was swabbed
with a Kimwipe saturated with SF. The swab was counted in 15 ml SF to determine if the *Cl₆-PCB* depot was retained in the tissue.

The three guinea pigs were housed in three cages which have been previously described. They were fed and watered *ad libitum* throughout the ten-day, *in vivo* study. During this period, the urine and feces were collected daily. The samples were placed in the freezer for later analyses.

At the end of the study the three guinea pigs were weighed. They were then sacrificed by cervical dislocation and necropsied by Dr. George Ruth, Veterinary Research and Diagnostic Laboratory, South Dakota State University. The following organs from each animal were weighed during necropsy: brain, liver plus gall bladder, heart, kidneys, and spleen. The brain and liver plus gall bladder from each animal were saved and frozen for later analyses. The rest of the organs were returned to their respective carcasses for whole body analyses. The whole bodies were also frozen for later analyses.

**Tissue Extractions**

The four samples chosen for extraction were: liver plus gall bladder, whole body, pooled feces, and pooled urine.

**Liver Plus Gall Bladder Extractions**

The liver and gall bladder samples were combined and ground after thawing in a Sorvall Omni Mixer. Each of the three samples
were subsampled. One-third control animal and one-half of each of the livers and gall bladders from the injected guinea pigs were used for analyses. Each subsample was extracted twice with 50 ml hexane. The extracts from each subsample were decanted, combined, centrifuged, and decanted again. The three extracts were injected into the ECD/GC and GC/MS, then each was reduced to a volume of 1 ml on a flash evaporator. The 1 ml extracts were placed on a 40 g florisil column as described earlier in this thesis. Each sample was eluted with 300 ml hexane, and each elution was injected into the ECD/GC and GC/MS. Each florisil column was cut into four parts, and each part was then extracted with 100 ml hexane. These extracts were then injected into the ECD/GC and GC/MS at this concentration.

**Whole Body Extractions**

Each whole body was ground in a hand meat grinder, while partially frozen. Each sample was pressed through the grinder three times to accomplish mixing. The whole body was then subsampled into 30-60 g portions. Each portion was extracted twice with 100 ml hexane for approximately 5 min. in a Sorvall Omni Mixer. The two extracts were decanted, combined, centrifuged and decanted again. The decantates were injected into the ECD/GC and GC/MS at this concentration. Each sample was then evaporated to volume of 15 ml. The samples were then reinjected into the ECD/GC at this concentration. In order to remove the
unwanted lipids, 50 ul of this solution was injected into the TCD/GC as described previously. This separation procedure failed and was abandoned.

Feces Extractions

The ten-day samples from each guinea pig were combined, ground in a Sorvall Omni Mixer and subsampled. One-third of the control animal's feces and one-half of the feces from the injected guinea pigs were extracted three times with 100 ml hexane. The extracts were combined, injected into the ECD/GC and GC/MS as before.

Urine Extractions

The procedure used to analyze the urine sample was modified from one reported by Hutzinger and workers. The urine samples for the ten day study were thawed and pooled, centrifuged, and decanted into 1,000 ml round-bottomed flasks. Each sample was then hydrolyzed with an equal volume of 8N H₂SO₄ for one hour. The samples were then extracted three times with 200 ml of diethyl ether. The extracts were combined for each animal sample and evaporated to a volume of 15 ml. These extracts were then analyzed by ECD/GC and GC/MS.

It was observed that one of the samples from an injected guinea pig contained pentachlorophenol. In order to analyze the compound for radioactivity, the urine sample was streaked on a 1 mm thick Uniplate TCL plate along with a standard of penta-
chlorophenol and Cl₆-PCB. A separate plate was spotted with *Cl₆-PCB, and then each plate was developed, as reported by Hutzinger, et al., with a solution of 2.5:1 (v/v) hexane/acetone to 15 cm. The standard spots of pentachlorophenol and Cl₆-PCB were sprayed with a 10% (w/w) solution of Rhodamin B in ethanol. Spots appeared at 3 1/2 cm and 10 1/2 cm above the spotting line, respectively. Both plates were then sectioned as follows: Section I being 1 cm below to 2 cm above the spotting line; Section II being the next 2 cm; Sections III and IV being the next two 3 cm sections; Section V being the next 2 cm, and Section VI being the last 3 cm. Each section was eluted with 15 ml of 1:1 (v/v) diethyl ether/petroleum ether. One ml of each section was counted for radioactivity, and all sections were also analyzed by GC/MS.

Comparative analyses between the biological samples and the *Cl₆-PCB standard solution were performed. Differences between them as indicated on the GC and GC/MS patterns were attributed to metabolic changes of the *Cl₆-PCB.
RESULTS AND DISCUSSION

This work was part of an ongoing research effort to understand the metabolism and elimination of polychlorinated biphenyls (PCB's) in animal species. As discussed in the introduction, commercially used PCB preparations are complex mixtures with varying numbers of PCB isomers present. It has been suggested in several studies that the various isomers of PCB will not metabolize at the same rate, and Hutzinger has further suggested that the rate of metabolism may be dependent on the number of chlorines substituted on the phenyl rings, with metabolism decreasing as number of chlorines increase. Since excretion does appear to be dependent on the number of chlorines, if any isomer is likely to build up in the food chain it will be the highly chlorinated ones.

We chose to investigate the PCB isomer 2,2',4,4',5,5'-hexachlorobiphenyl (Cl₆-PCB) because it was the most abundant component of the commercial PCB preparation Aroclor 1260 and, being a hexachloro isomer, it would be a likely candidate for environmental buildup. Since this material is being introduced into the environment in relatively large amounts, it is of interest to know if any metabolic mechanism exists either as a pathway for elimination from the food chain or possibly as a pathway to convert the PCB to other substances toxic to the host
system. Previous workers\textsuperscript{11} were unable to identify any metabolites of the Cl\textsubscript{6}-PCB, but their procedures did not utilize radio-labeled materials and their starting material was of insufficient purity to investigate low-level metabolic products by direct chemical analysis.

In this study we attempted to overcome the limitations that the starting material imposed on the previous workers, thereby allowing us to investigate for possible trace metabolites. To accomplish this, it was necessary to obtain the Cl\textsubscript{6}-PCB as pure as possible in labeled and unlabeled form. The labeled material was obtained from Dr. J. Worman (Chemistry Department, SDSU) with \textsuperscript{36}Cl substituted in the 4 and 4' positions. The 4 and 4' positions were chosen because previous workers\textsuperscript{10} had indicated that in lower chlorinated PCB's, hydroxylation took place preferentially in these positions; and if the radio-label is to be of any aid in identifying the metabolite, it must be entirely removed from the parent compound during the reaction or be retained as part of the metabolite. The 4 and 4' positions were also chosen to provide labeling on both phenyl rings of the PCB to increase the chances of the label being part of a potential metabolic product. The synthesis procedure used by Dr. Worman, the Ullman reaction,\textsuperscript{18} produced the \textsuperscript{3}Cl\textsubscript{6}-PCB with a quite high specific activity which increased the possibility for identification of a metabolite containing the label. The drawback to the
material furnished by Dr. Worman was that the sample was not pure enough for use. The desired PCB was the major component of the mixture, but other PCB's and reaction by-products were also present. Thus, before any metabolism studies could be undertaken, procedures to isolate and purify the Cl₆-PCB had to be developed.

The work reported in this thesis involves the development of a suitable process/procedure to isolate and purify specific PCB isomers, the isolation and purification of 2,2',4,4',5,5'-hexachlorobiphenyl in sufficient quantities for metabolism studies, and initial metabolism studies in guinea pigs.

Isolation and Purification of PCB Isomers

In a metabolism study, it is important for the compound being studied to be pure with respect to the detection techniques being used before the compound is introduced into a test animal. Two powerful detection techniques used to study metabolites are ECD/GC analysis and scintillation counting, using an appropriately radio-labeled material. If ECD/GC analysis is being used to detect metabolites, the technique will be seriously compromised by a large number of impurities sensitive to EC detection. The impurities may either interfere with the identification of the metabolite or be mistaken for metabolic products. Thus, it is imperative that the sample be as pure as possible with regard to EC detection, and as well-characterized chemically as possible. The procedure to obtain materials for
metabolism studies should, therefore, produce a sample that has a minimum of components that are sensitive to EC detection or that will interfere with the detector.

If radio-labeled materials are being used, it is imperative that all of the label be attributed to the compound being studied or, at a minimum, that the number and identity of impurities containing the label be known. If labeled impurities are present, they may be mistaken for metabolic products and lead to incorrect results. Both the specific activity of the sample and the correct isomeric form of the material should also be known.

PCB mixtures have been separated and purified previously by florisil column chromatography and by thin-layer chromatography. These procedures were studied for possible use, but were found not to produce samples with the desired purity. The results of these studies are as follows:

A. **Florisil Column Chromatography**

The procedure of Greichus, et al. discussed in the Experimental section was used. It was found that the technique did separate the PCB's from the majority of organic impurities and essentially all inorganic materials present in the crude material. The technique was also successful in removing the PCB's from most other halogenated materials, but it did not offer enough resolution to separate the various PCB isomers.
Thus, the technique was adequate for isolation and cleanup of PCB's as a class, but not for isolation of specific isomers.

B. Thin-Layer Chromatography (TLC)

Mixtures of PCB's have been separated by a reversed-phase partition TLC procedure. This procedure was investigated as a possible method for further purification of the PCB isomers from contaminants in the florisil-cleaned mixtures and to be used to separate a specific PCB isomer from the mixture. A TLC plate was prepared as described earlier in this thesis. Three different amounts were spotted on the plate to evaluate the loading capacity of each plate. Following development, the bands were eluted and analyzed by scintillation counting for radioactive trailing. This means of detection indicated lack of resolution and trailing of the radioactivity at low concentration (2.2 mg) as tabulated in Table 1. The TLC method was excluded as a viable procedure because of the lack of resolution at low concentration levels of PCB's.

One analysis technique which offered the necessary resolution to separate and isolate the desired PCB isomer was gas chromatography. No preparative technique using GC to isolate PCB isomers was available.
TABLE I

TRAILING EXPERIMENT OF *C16-PCB ON TLC PLATE

<table>
<thead>
<tr>
<th>Sample Size</th>
<th>Radioactivity (% of Applied)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2.2 mg</td>
</tr>
<tr>
<td>Section I</td>
<td>6.61</td>
</tr>
<tr>
<td>Section II</td>
<td>52.99</td>
</tr>
<tr>
<td>Section III</td>
<td>28.91</td>
</tr>
<tr>
<td>Section IV</td>
<td>10.00</td>
</tr>
<tr>
<td>Section V</td>
<td>0.80</td>
</tr>
<tr>
<td>Section VI</td>
<td>0.21</td>
</tr>
<tr>
<td>Section VII</td>
<td>0.25</td>
</tr>
<tr>
<td>Section VIII</td>
<td>0.21</td>
</tr>
</tbody>
</table>
in the literature, so a program was undertaken to develop a procedure to isolate specific PCB isomers in large enough quantities and with sufficient purity for metabolism studies. A procedure was successfully developed and is outlined below.

The sample is first purified on a florisil column using the procedure of Greichus, et al.\textsuperscript{23} to separate the PCB's from other halogenated materials, inorganics and other contaminants. The sample is then analyzed using ECD/GC procedures to assay its purity and identify the isomer to be isolated. If labeled compounds are being purified, the florisil elutants are also assayed for radioactivity, and the GC peak attributed to the desired isomer is collected and assayed for radioactivity to insure labeling.

To separate and collect the desired isomer, a gas chromatograph equipped with a thermal-conductivity detector (TCD/GC) is used. The TCD is not as sensitive as an ECD, but has a much greater sample capacity (it will not overload as easily as will an ECD); and since the isolation procedure involves relatively large sample quantities, the sensitivity is not necessary. The temperature, carrier flow rate, and column conditions are adjusted to provide the necessary separation, i.e. the
desired isomer peak separated from all other peaks by at least one minute. (The specific equipment setup is discussed in the Materials and Equipment section of this thesis).

The GC is equipped with an effluent splitter which feeds into a collection-tube holder. Collection tubes (discussed in the Experimental section) are then rotated into the splitter stream as the desired peak is eluted. The collection can either be timed, based on the retention time of the desired isomer, which would allow the collection procedure to be automated; or be manually rotated by observing the recorder signal. Several solid supports are available for use in the collection tubes, and the choice should be made with respect to the sample being collected. For the support to be acceptable for collection, it must adsorb the volatilized material with high efficiency and then release the material upon elution with an appropriate solvent. Two such solid supports were tested for use with PCB's: Chromosorb W and florisil. Several injections of the $\text{Cl}_6$-PCB were collected in fractions on each of the two adsorbents.

When analyzed by ECD/GC, the amount of $\text{Cl}_6$-PCB, which was collected on and eluted from Chromosorb W, represented
84% of the material which was collected on and eluted from florisil. Therefore, florisil was chosen for the collection of PCB's in all further analyses; but it should be pointed out that the choice of collection-tube packing is a parameter that must be tailored to each class of compound collected.

Samples are then separately injected in relatively large quantities (50 ul) and each of the desired peaks are collected. After the crude sample has been passed through the GC in this fashion, all of the collection tubes for each peak collected are pooled and extracted with solvent. The samples are then reconstituted and analyzed by ECD/GC. If impurities still remain, the entire process is repeated using GC column conditions which provide a greater peak separation. The purity of samples collected this way is dependent upon the insertion of the collection tube at the right time and the removal of it before any additional materials are eluted from the column. Also, the efficiency of recovery is greatly dependent on correct timing in tube placement.

This procedure, when correctly used, can produce purified materials in quantities large enough for metabolism and other studies. It offers the possibility
of automation which would allow large quantities of materials to be purified and collected with a relatively low labor input, and can be used to isolate several isomers at the same time. Also, by adjusting column conditions to produce greater resolution, the possibility of greater purity exists. However, it should be remembered that to get greater resolution generally increases analysis time---so a tradeoff between purity and analysis time does exist---but the researcher is allowed the decision. It is not dictated by the procedure.

Possible drawbacks of the procedure mainly involve the choice of materials. If the GC column or conditions will not resolve the sample, if the collection-tube packing will not return the effluent, if the sample cannot be recovered from the packing material, or if the collection apparatus will not allow the collection tubes to be inserted and removed at the correct time, the procedure will not produce good yields of pure compounds.

The procedure was found to be very good when used to isolate specific PCB's as described in the Experimental portion of this thesis. The technique was used with good results to isolate the PCB isomer
Cl₆-PCB from its reaction mixture as is described in the next section of the discussion, and it is anticipated that similar results could be obtained in isolation of other PCB isomers.

**Isolation, Purification, and Characterization of 2,2',4,4',5,5'-Hexachlorobiphenyl**

The TCD/GC procedure discussed above was used to purify experimental quantities of Cl₆-PCB and ³⁶Cl-tagged Cl₆-PCB (designated by *Cl₆-PCB) for metabolism studies from preparations obtained from Dr. Worman. The results of the purification procedure can be seen in Tables II and III. The area response is calculated as a function of peak base width times peak height. The relative retention time (rrt) is calculated on the defined basis of p,p'-DDE being equal to 100.

Samples of *Cl₆-PCB were used to test the efficiency of elutions. This was accomplished for the column chromatography procedure by placing the crude, labeled material on the florisil column and collecting 100 ml fractions of the eluate. After analyzing a portion of each fraction in the scintillation counter, it was determined that 88% of the radioactivity recovered was collected in the first three fractions. Extraction and analysis of various parts of the florisil column indicated that a majority of unrecovered radioactivity was contained in the top quarter of the florisil column. It was decided that collection
of 300 mls of eluate would maximize isomer recovery. Using this procedure, 0.1 g of sample was separated for each 40 g of florisil column. Each florisil column was used only once due to the chemically radioactive contamination. Rapid separation of the PCB's from the reaction mixture was obtained and the procedure was adopted for cleanup of the two Cl₆-PCB samples obtained from Dr. Worman.

Following the florisil column cleanup, the Cl₆-PCB and *Cl₆-PCB samples were analyzed by ECD/GC. The chromatograms indicated that each sample still contained several electron-capturing substances at a 1 mg/ml concentration. The area response and the relative percentages were tabulated corresponding to each peak's relative retention time (rrt). (See Tables II and III).

Although the Cl₆-PCB and *Cl₆-PCB accounted for 99% of the area response on the chromatograms, further purification was necessary to remove the unwanted electron-capturing substances which were possibly radioactive. Removal of these contaminants was necessary to allow low-level metabolites to be observed through the use of ECD/GC and scintillation counting as previously discussed. The florisil-cleaned samples were then purified using the TCD/GC technique.

The conditions of the TCD/GC, described in the Experimental section, allowed separation and detection of the three major components of each of the two isomer mixtures: rrt's = 83, 123,
TABLE II

PURIFICATION OF THE Cl₆-PCB

<table>
<thead>
<tr>
<th>rrt¹</th>
<th>Florisil Cleaned</th>
<th>TCD/GC Cleaned</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Area Response</td>
<td>% Area</td>
</tr>
<tr>
<td>148</td>
<td>4,200,00</td>
<td>99.64</td>
</tr>
<tr>
<td>83</td>
<td>13,000</td>
<td>0.31</td>
</tr>
<tr>
<td>123</td>
<td>243</td>
<td>0.01</td>
</tr>
<tr>
<td>130</td>
<td>633</td>
<td>0.02</td>
</tr>
<tr>
<td>55</td>
<td>201</td>
<td>0.01</td>
</tr>
<tr>
<td>204</td>
<td>563</td>
<td>--</td>
</tr>
<tr>
<td>266</td>
<td>31</td>
<td>-0-</td>
</tr>
<tr>
<td>288</td>
<td>394</td>
<td>0.01</td>
</tr>
</tbody>
</table>

¹Based on rrt of p,p'-DDE = 100.
### TABLE III

**PURIFICATION OF THE *Cl₆-PCB**

<table>
<thead>
<tr>
<th>rrt</th>
<th>Florisil Cleaned</th>
<th>TCD/GC Cleaned</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Area Response</td>
<td>% Area</td>
</tr>
<tr>
<td>148</td>
<td>3,150,000</td>
<td>99.92</td>
</tr>
<tr>
<td>83</td>
<td>1,125</td>
<td>0.04</td>
</tr>
<tr>
<td>123</td>
<td>788</td>
<td>0.02</td>
</tr>
<tr>
<td>130</td>
<td>135</td>
<td>-0-</td>
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<tr>
<td>55</td>
<td>47</td>
<td>-0-</td>
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<tr>
<td>70</td>
<td>16</td>
<td>-0-</td>
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<tr>
<td>191</td>
<td>90</td>
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<tr>
<td>204</td>
<td>214</td>
<td>0.01</td>
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<tr>
<td>288</td>
<td>194</td>
<td>0.01</td>
</tr>
<tr>
<td>109</td>
<td>14</td>
<td>-0-</td>
</tr>
<tr>
<td>266</td>
<td>61</td>
<td>-0-</td>
</tr>
</tbody>
</table>

*Based on rrt of p,p'-DDE = 100*
and 148 (according to the ECD/GC). The lower detectable limit was approximately 0.5 ug under these conditions. Each injection/collection cycle took 48 minutes. The Cl₆-PCB fraction was collected using a set of three collection tubes each placed in the eluant stream for approximately 15, 5, and 28 minutes respectively. The first fraction was collected prior to the isomer peak, the second collected during the peak elution, and the third following the peak. To maximize purification collection, the second fraction was started well into the hexachlorobiphenyl peak, and the third before the peak curve had returned to the baseline. Immediately after Fraction two was collected, solvent was injected on the column to elute or drive off any possible remaining Cl₆-PCB. As with the florisil column procedure, crude *Cl₆-PCB samples were injected and the procedure was monitored for radioactivity.

The fractions were recovered and pooled for scintillation counting. Scintillation counting analysis indicated that 75% of the injected radioactivity was recovered in the three fractions collected. Of the collected radioactive material, 60% appeared in Fraction 2. The 25% loss of radioactivity may be attributed to removal of impurities and loss of tagged PCB before Fraction 2 and after Fraction 2, as well as that lost to inefficient collection by the collection-packing material. Scintillation counting also showed this procedure excluded trailing of
radioactivity from one injection to the next. Loss of material was justified in order to increase the purity of the hexachlorobiphenyl mixture. The non-labeled and labeled mixtures were separated and collected according to this procedure.

Following TCD/GC separation and collection, the samples were analyzed by ECD/GC. ECD/GC analysis indicated that 92 mg Cl₆-PCB and 623 mg *Cl₆-PCB were recovered from the reaction mixtures following TCD/GC separation and collection. This represented 59.90% and 42.62% of the injected materials, respectively. The Cl₆-PCB sample now contained four ECD/GC detectable components, while the *Cl₆-PCB contained nine ECD/GC detectable components. The peaks of the chromatograms were triangulated and the area responses were tabulated corresponding to each peak's rrt on Tables I and II. This method of purification served to eliminate the majority of the contaminants in the hexachlorobiphenyl mixtures.

No new electron-capturing contaminants were detected indicating that the procedure did not introduce any new contaminants. The remaining contaminants showed a decreased relative area response in all cases as shown in Tables II and III. Thus, it can be expected that the purity could be increased if desired, but at the expense of increased sample loss. Each of the TCD/GC-cleaned samples were analyzed by GC/MS (see Table IV).

Three components in the Cl₆-PCB mixture and four components
TABLE IV

MASS SPECTRAL DATA OF TCD/GC CLEANED Cl₆-PCB
AND *Cl₆-PCB MIXTURES

| Component (rrt)
| Parent Ion (m/e) | No. of Cl's |
|-----------------|-------------|-------------|
| Cl₆-PCB Mixture |             |             |
| 83              | 324         | 5           |
| 123             | 230         | None        |
| 148             | 358         | 6           |
| *Cl₆-PCB Mixture|             |             |
| 83              | 324         | 5           |
| 123             | 230         | None        |
| 130             | 358         | 6           |
| 148             | 358         | 6           |

'Based on rrt of p,p'-DDE = 100.
of the *Cl₆-PCB mixture were characterized as described by
Greichus, et al.²³ Each component was checked for homogeneity by
mass spectroscopy analysis at the beginning and the end of its
peak. The mass spectral data indicated that components in the
Cl₆-PCB mixture corresponding to the rrt's of 83, 123, and 148
had molecular weights of 324, 230, and 358, respectively. The
same results were observed in the *Cl₆-PCB mixture. A further
component with rrt of 130 was found to have a molecular weight
of 358. The molecular ion clusters at the parent ion indicated
5, 0, 6, and 6 as the number of chlorine atoms present in the
components of rrt's 83, 123, 130, and 148, respectively.
Comparative analyses of the molecular weight and number of
chlorines present in each of the molecules indicate that the
component with rrt 83 is a pentachlorobiphenyl and the components
with rrt's of 130 and 148 are hexachlorobiphenyls. A possible
structure for the component with rrt 123 was not determined. The
mass spectrum of component of rrt 148 compared directly to that
which is given in the literature for 2,2',4,4',5,5'-hexachloro-
 biphenyl.²⁶

The purified, untagged hexachlorobiphenyl was used to prepare
a standard solution for quantitative measurements.

The TCD/GC cleaned *Cl₆-PCB was analyzed in the scintillation
counter. The specific activity was calculated to be 3,600 dpm/mg
*Cl₆-PCB or 5.86 X 10⁻¹ uCi/m Mole *Cl₆-PCB. This value was
4.84 \times 10^2 \text{ times the specific activity of } ^{\ast}\text{Cl}_6\text{-PCB which was synthesized and reported by Hutzinger, et al.}^{16} \text{ The greater specific activity observed in our samples resulted from both the method of labeling the } ^{\ast}\text{Cl}_6\text{-PCB in the reaction synthesis and improvements in the procedure used to purify the PCB mixtures.}

These procedures produced sufficient quantities of pure, well-characterized } ^{\ast}\text{Cl}_6\text{-PCB isomer tagged in the 4 and 4'} \text{ positions with } ^{36}\text{Cl to initiate a metabolism study. In addition, the specific activity of the samples was in a range that would allow a good possibility of low-level metabolites retaining enough label to be identified with scintillation counting procedures.}

**Metabolism of } 2,2',4,4',5,5'\text{-Hexachlorobiphenyl in the Guinea Pig}^{**}

As discussed earlier, previous investigations had not identified any metabolic products in their studies of Cl$_6$-PCB, so it was anticipated that if any of the Cl$_6$-PCB was metabolized, it would be in low quantities. It was, therefore, decided that this study should focus on identifying possible trace or low-level products and not to attempt an accurate quantitation of peaks if found. The purification procedure had yielded a 0.623 g sample of } ^{\ast}\text{Cl}_6\text{-PCB which was well documented as to purity and had a high specific activity. To increase the changes of identification of metabolites, one-half of the available material was injected in two of the three animals used. The large sample size essentially swamped the animal and precluded obtaining accurate data on
elimination or metabolism rates, but the increased sensitivity with regard to trace metabolites was deemed worth it. Each animal was given half of the PCB sample in one dose and held for ten days before sacrifice and analysis to allow possible slow metabolic pathways to act on the PCB.

The animal model chosen for the study was the guinea pig. Guinea pigs were readily available from the SDSU animal colony. They were of sufficient size to handle the samples administered; and since this model had not been previously used in Cl₆-PCB metabolism studies, it offered the opportunity to check species variation by comparison of the results with previous studies.

The animals were obtained and housed as described previously. The toxicity of polychlorinated biphenyls in guinea pigs was not available. Due to the limited amount of Cl₆-PCB available, a mixture of the PCB Aroclor 1260 was chosen to test the relative toxicity. To do this, a solution of Aroclor 1260 in corn oil was prepared and subcutaneously injected into a guinea pig at the dose level of 500 mg PCB/kg body weight. The animal survived a five-day test period. It was then decided that introduction of the *Cl₆-PCB at a level of 500 mg PCB/kg would be attempted.

A subcutaneous injection was given to get as much as possible of the *Cl₆-PCB in the animal systematically. It was decided that oral ingestion of the material would result in too great a sample loss through excretion. Corn oil was used as a
depot to increase absorption of the PCB from the injection site, and hopefully increase the potential for metabolism by the body's organs.

After ten days, the three animals---two exposed to \*Cl\textsubscript{6}-PCB and the control---were sacrificed and analyzed as described in the Experimental procedure. Analysis of the liver, whole body, feces, and urine for the two exposed animals indicated that no major conversion of the \*Cl\textsubscript{6}-PCB had occurred. The samples selected for analyses were chosen because of their major role in body functions: the liver because it is a major organ of detoxification, the whole body for its role in storage of the starting material and any possible metabolites, and the feces and urine for elimination of these compounds. The lack of major metabolism is similar to that reported by Nutzinger, \textit{et al.}\textsuperscript{11} but unlike the Nutzinger study, we observed \textit{three} possible low-level metabolites in the exposed animals and their excreta. One of the extracted compounds was identified as pentachlorophenol and was found to have a radioactive label, indicating that it may be a product of \*Cl\textsubscript{6}-PCB breakdown. No pentachlorophenol was present in the sample injected into the animal in detectable amounts. The other two peaks were not positively identified; and due to sample interference have not as yet been assayed for radioactivity. These peaks were not present in the control animal or in the injected sample.
The following is a summary of the isolation and attempts to identify the three possible metabolic materials:

A. Pentachlorophenol

The urine samples were extracted using Hutzinger's procedure. The extract was injected into the ECD/GC, but the sample components plated out in the detector and prohibited analysis. The sample was analyzed using GC/MS techniques. A peak with a fragmentation pattern corresponding to pentachlorophenol was observed in the urine of one exposed animal, but not in the urine of the control animal or the other injected animal. Using the thinlayer chromatography procedure of Hutzinger, (described earlier) separation of the pentachlorophenol was achieved from the \(^{13}Cl_6\)-PCB present in the sample.

The pentachlorophenol band as analyzed by GC/MS, was recovered and found to be radioactive. When a sample of the \(^{13}Cl_6\)-PCB injection mixture was analyzed in a similar fashion, no radioactivity was found in the pentachlorophenol band position. Thus, the radioactivity cannot be attributed to \(^{13}Cl_6\)-PCB trailing.

Several possibilities for the occurrence of pentachlorophenol are considered. First, the \(^{13}Cl_6\)-PCB may have been metabolized by some unknown pathway to produce \(^{36}Cl\)-pentachlorophenol. This would include the
breakdown of the biphenyl moiety followed by the addition of chlorine atoms and a hydroxyl group to the phenyl ring producing the pentachlorophenol. In this case, the compound would be labelled with $^{36}\text{Cl}$ and would be considered a direct metabolite of $^\ast\text{Cl}_6$-PCB.

Secondly, pentachlorophenol is known to be a common wood preservative. The guinea pigs were housed on wood chips containing pentachlorophenol prior to these studies. During that period one guinea pig may have ingested some of the chips and excreted pentachlorophenol in the urine sample. The presence of radioactivity in this sample may be explained by radioisotope exchange of the chlorine atom(s) from the $^\ast\text{Cl}_6$-PCB to the pentachlorophenol.

Thirdly, pentachlorophenol may have been one of the lower level contaminants of the $^\ast\text{Cl}_6$-PCB injection mixture. This may have been produced as a reaction byproduct or as a result of radiolysis. The radiolysis may have included splitting of the biphenyl moiety allowing free radical reactions involving the additions of chlorine atoms and a hydroxyl group. By injecting $\sim310$ mg of the hexachlorobiphenyl mixture into one of the guinea pigs and extracting its urine, this compound may have been concentrated to a detectable level.
However, this does not explain the absence of pentachlorophenol in the other injected animal. Again, the presence of radioactivity may be explained by radioisotope exchange.

Fourthly, another possible metabolite, which was radioactive may have been contained in the pentachlorophenol sample. It may have been undetectable in low concentration by the ECD/GC and GC/MS under the conditions employed.

Following further studies, explanation of the presence of pentachlorophenol may be elucidated.

B. Peaks with rrt's of 53 and 93 Relative to p,p'-DDE

When liver plus gall bladder, the whole body, and the feces were extracted with hexane and the extract analyzed on the ECD/GC, three peaks were observed in each of these samples. These peaks had rrt's of 53, 93, and 148, respectively. The peak at 148 was identified as \(^{\oplus}Cl_6\)-PCB and constituted the major portion of the samples, while the combined area of peaks 53 and 93 were less than one percent of the 148 peak.

The hexane extraction of one liver sample was chromatographed on florisil and an ECD/GC analysis of the eluant showed only one peak at rrt 148. The florisil column was cut into four sections and extracted. The
peak with rrt 53 was recovered in the third section from the top by further extraction with hexane. The peak at rrt 93 was not recovered from the column with the procedures used. The hexane extract of the florisil column section which contained the peak 53 was counted and was found to be non-radioactive. No mass spectrum was obtained at this concentration. Attempts were made to isolate the peaks at 53 and 93 in the whole body samples by using the TCD/GC procedure, but the sample background interfered with the TCD (lipids appeared to plate out on the detector) and the attempt was abandoned. Attempts to carry out mass spectroscopic analysis on the peaks also failed. The rrt's could not be reproduced exactly on the GC/MS instrument used; and due to background interference, the two peaks could not be identified in the samples analyzed.

The two peaks in question were not observed in the urine samples. However, since the urine samples were not extracted directly with the hexane, as were the other samples, the extraction procedure used may have precluded observation of these materials. Thus, their presence or absence in the sample cannot be verified. All further attempts to characterize these peaks failed. Definite structural assignment of the peaks must
await further studies.
SUMMARY

In summary, this study developed a reliable and useful procedure to isolate selected isomers of PCB's in highly purified form. This procedure is an improvement over previous techniques, both in terms of product quality and labor needed to isolate a given amount of sample.

The metabolism studies carried out indicate that the 2,2', 4,4',5,5'-hexachlorobiphenyl may be metabolized in trace quantities in the guinea pig. The three products observed indicate that at these low levels there may be more than one metabolic pathway. Since two of the products were characterized with regard to radioactive label, it appears that metabolism pathways involving both the loss and retention of chlorine in the 4 and 4' positions are possible. The positive identification of the pentachlorophenol product containing a radioactive label suggests the possibility of a metabolic mechanism involving cleavage of the biphenyl moiety, though other pathways are possible.

This study indicates that further work is needed on this system to characterize the two peaks at rrt 53 and 93 in order to identify the metabolic pathway(s) that produce these products. This work and subsequent studies may lead to a better understanding of the elimination process of PCB's in a living organism.


