A P P E N D I X I

Polychlorinated Biphenyls:

Distribution and Storage in Body Fluids and Tissues of Sherman Rats

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Abstract

An analytical method for determination of PCB (Aroclor 1254(R)) in blood, urine, feces, and tissues of Sherman strain rats after prolonged dietary intake (100, 500, and 1000 ppm) is presented. The method involves homogenization and extraction with hexane, cleanup on a silica gel microcolumn and analysis by electron-capture gas-liquid chromatography. Recovery data are shown at 0.1, 5, 14, and 100 ppm. Mass spectroscopic characteristics of PCB in tissues are given. Distribution, storage, and excretion rates are presented during feeding and after discontinuance of PCB in the diet.

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In 1881, polychlorinated biphenyls (PCB's) were first described by Schmidt and Shultz.[1] Commercial production was begun in 1930.[2] These compounds have been and still are widely used as plasticizers and resins and in chlorinated rubber.[3,4] They are known to enhance the insecticidal properties of lindane[5], DDT, and dieldrin.[6] They may be used as stationary phases in gas-liquid chromatography.[7]

Their widespread use may have resulted in contamination of the ecosystem as suggested by many recent reports. Aroclor and DDT were both used during World War II. In 1948, total organic chlorine analysis provided data for the first determination of DDT storage in man.[8] Since this method has no specificity for DDT, the total organic chlorine content would not preclude the presence of other organic chlorine-containing moieties. In terms of halide specificity, the Dohrmann microcoulometric gas chromatographic method for detecting pesticides was well established by 1960[9], and electron-capture was in use by 1961.[10] Roburn[11] first observed extraneous peaks in 1965; he used a potentiometric mode of analysis for total chlorine and found disagreement between this method and total chlorine content calculated from gas chromatographic results in which Argon ionization and electron-capture were used for the analysis of tissues and eggs of wild birds. These samples showed significant amounts of unknown electron-capturing compounds. Roburn postulated that these were metabolic intermediates of one or more of the organochlorine pesticides. These differences could have been indicative of PCB contamination. It was not until 1966 that S. Jensen[12] using electron-capture and mass spectrometry was able to identify unknown peaks found in fish and a bird carcass as PCB's. Undoubtedly, the time lag between use and detection of PCB in the environment is a matter of accumulative concentration and/or instrumentation development.

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The presence of PCB's in environmental samples has been well established.[13,14] Acute and subacute feeding studies to determine toxicities and some residue levels have been done with rats, birds, and guinea pigs.[15,16,17,18,22] Tolerance studies have been done with fish and other forms of marine life.[19,21,22] Pathological changes in the liver and other organs have been shown in guinea pigs, rats, and rabbits at high concentrations.[22] Hydropericardial edema and growth depression proportional to dietary levels of the toxicant have been produced in the chicken.[23] PCB's are known to affect estrogen levels in birds which in turn reduces calcium reserves and results in thin eggshells. Chemical porphyria from oral administration has also been shown in chicks.[18]

This paper presents distribution, storage, and excretion rates of Aroclor in certain tissues, body fluids, and excreta of rats following 1) a single oral dose, 2) repeated dietary intake, and 3) after discontinuance of PCB in the diet.

Methods

Aroclor 1254(R) and 1260(R) were supplied by the Monsanto Chemical Company with respective lot numbers, AK-38 and AK-3. Dietary formulations were prepared according to the methods described previously by Gaines and Kimbrough.[24]

Experiment A: Aroclor 1254(R) and Aroclor 1260(R) were administered at 1600 mg/kg and 3200 mg/kg, respectively as a single oral dose, by stomach tube to three female, 173- to 183-day-old, white Sherman rats weighing 260-318 g. Four control rats, two in each group, were given peanut oil only. Twenty-four hours later the animals were sacrificed, and blood (oxalated), fat, muscle (abdominal), liver, kidney, lung, and brain were taken for analysis.

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Experiment B: Aroclor 1254(R) at 1000 ppm was fed for 98 days to 29- to 34-day-old Sherman strain male and female weanling rats, in groups

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of 10. Controls were fed only plain chow. Food consumption was measured at intervals as described previously by Kimbrough and Gaines.[42] All rats were weighed on a weekly basis from experiment initiation until time of sacrifice or death. The rats consumed an average of 72.7 mg/kg/Aroclor per day.. Blood (oxalated), fat, muscle (abdominal), liver, kidney, lung, and brain were taken from each rat at time of sacrifice. Tissues were preserved in 10% formalin and held for analysis.

Experiment C: Sherman strain weanling rats (61-to 66 days-old) were fed a diet of plain chow or chow fortified with Aroclor 1254(R) at a concentration of 100 ppm for 58 days. Fortified chow was discontinued, and samples were collected during the recovery period. Food consumption was measured at 2-day intervals for a period of 8 days; after 42 days food consumption was measured daily for 4 days. Surviving rats were weighed once a week. At various times during the experiment rats were individually housed in metabolism cages. Urine and feces were collected over a 16-hour period during which time plain chow was fed. Following excreta collections, rats were sacrificed. Blood (oxalated), fat, muscle (abdominal), liver, kidney, and brain were taken from each rat at time of sacrifice. Tissues, urine, and feces were frozen at time of collection. Whole blood was centrifuged and the plasma refrigerated. The outline of the experiment as aforementioned is summarized as follows: These animals (two on the diet and one control) were sacrificed on day 4, 9, 13, 17, 25, 34, 42, and 58. After discontinuance of PCB in the diet, two animals were sacrificed on day 8, 16, 24, and 71.

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Experiments D & E: Sherman strain weanlings 36- to 41 days-old) were fed Aroclor 1254(R) at dietary levels of 100 ppm or 500 ppm for 252 days. Body weights were determined initially and weekly thereafter for 121 days. On the basis of food consumption measurements, the average rates for intake of Aroclor 1254(R) were 6.8 mg/kg/day and 36.4 mg/kg/day for 100-ppm and 500-ppm dietary levels, respectively. Samples were collected in the same manner as Experiment C.

Sample Preparation

Polychlorinated biphenyls like DDT, are not water soluble but are, in fact, highly lipid soluble. Solvents for tissues and plasma extractions were selected for this property.

Plasma (at least 2 mls) was extracted by the method of Dale et al.(25) Later in the study, the Bryant-Thompson modification of this method was used.(26) No significant difference has been found between these methods in this laboratory with the exception that convenience favors the Bryant-Thompson modification.

Samples of fat (250 mg), liver, muscle, kidney, and lungs (500 mg each) were homogenized two times in 4 ml of hexane, and the extracts were combined.

Brain (500 mg) was homogenized two times in 4 ml of acetone, and the extracts were combined.(27)

Urine (mean volume of 10 ml) at pH ≤ 7 (using pHydrion paper) was diluted to a volume of 25 ml by the addition of water and 1 ml isopropanol. These samples were extracted in 125-ml separatory funnels three times with 10 ml hexane. The hexane extracts were combined, washed once with 3 ml of water, and dried with a minimum amount of anhydrous sodium sulfate.

Feces were desiccated over calcium chloride for 1 week. One gram was

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then pulverized in a mortar and pestle and extracted for 1 hour with 25 ml of acetone on an automatic shaker. The acetone extract was decanted and placed in a 50-ml centrifuge tube. Particulate matter in the extract was removed by centrifuging and/or decanting. The extracts were evaporated with a stream of dry nitrogen to a volume of 0.5 ml in a water bath (40°C). The acetone extracts were evaporated just to dryness and made to a volume of 0.5 ml in hexane. The samples were dried with anhydrous sodium sulfate and held for cleanup and analysis by electron capture gas-liquid chromatography.

Preparation of Silica Gel and Micro-Column

Silica gel[27] from Woelm (Activity grade 1) was activated at 130°C for 4 hours and desiccated over calcium chloride for 16 hours before deactivation at 3% (V/W) by the addition of water. The silica gel was allowed to equilibrate at least 2 hours before use. After comparing the weight of the silica gel before and after activation, it was found to be activity grade I as specified by the manufacturer and further activation was unnecessary; however, each new batch was routinely activated.

One gram of the deactivated silica gel was placed in a disposable pipet, which had been loosely plugged with silanized glass wool, and gently seated. Columns were pre-washed with 10 ml of hexane. Each sample was placed on the column in a volume of 0.5 ml. At least 1 ml of hexane was used to quantitatively transfer the sample to the head of the column. The column was eluted with 10 ml of a 1:1 benzene:hexane mixture.[27] Samples were chromatographed in sets of eight with duplicate column blanks. Aroclor 1254(R) standards were eluted simultaneously in order to ascertain the variance in silica gel activity and to detect any possible contamination from solvents, glassware, or silica gel. Samples from experiments A, C, D, and E were treated as above

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and analyzed by electron-capture gas-liquid chromatography under one of the following conditions using a MicroTek MT-220 gas chromatograph: pulse mode (using dual columns and dual detectors); cell voltage, 36.5 volts; pulse width, 5.5 μsec; pulse rate, 120 μsec; detector, H^3, parallel plate design, No. 1 205°C, No. 2 198°C; solumn, 6' x 1/8" O.D. U-shaped pyrex glass packed with 5% OV 210 on 80/100 mesh Chromosorb W, high performance, 170°C; inlet 238°C; carrier gas, argon-methane (95-5%), 50 psi; flow-through Detector No. 1, 50 cc/min; Detector No. 2, 60 cc/min. The DC mode was as follows: cell voltage, 15 volts; detector, H^3, parallel plate design, 210°C; Column, 6' x 1/4" O.D. U-shaped pyrex glass packed with 1.5% OV-17/1.95% QF-1 on 100/200 mesh Chromosorb W, HP, 200°C; inlet, 230°C; carrier gas, nitrogen, 40 psi; flow-through detector, 75 cc/min.

Samples from Experiment B were injected directly as hexane extracts and analyzed by electron-capture gas-liquid chromatography under the following conditions using a MicroTek MT 220 gas chromatograph: cell voltage, 40 volts DC; detector, Ni^{63}, 250°C; column, 6' x 1/8" O.D. U-shaped glass column packed with 1.5% OV-17/1.95% QF-1 on 100/200 mesh Chromosorb W, HP 200°C; inlet, 210°C; carrier gas, nitrogen, 40 psi; flow-through detector, 80 cc/min, 20 cc/min scavenger plus 60 cc/min carrier gas flow.

Evaluation of Methodology

The efficiency of the silica gel micro-column and the extraction techniques employed were evaluated by determining the recovery of Aroclor 1254(R) from *in vitro* fortification.

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Recovery studies were completed using the following methods for control tissues, plasma, and 48-hour urine and feces collections. The fortification level for each type of sample was the mean concentration, with the exception of fat, of Aroclor 1254(R) found for that sample in Experiment C; fat samples were fortified with 100 ppm.

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Tissues (fat, liver, kidney, brain, and muscle): Aroclor was added directly to the hexane extract prior to liquid chromatography.

Plasma: Aroclor was added directly to fresh plasma, thoroughly mixed using a mini-mixer, and allowed to equilibrate overnight under refrigeration. Samples were then treated as mentioned in methods.

Feces: Aroclor was added directly to pulverized dry feces and treated as mentioned in methods.

Urine (three slightly different approaches were used):

No. 1- Aroclor was added to 1 ml of isopropanol, then mixed with a solution of urine and water (10 ml + 15 ml) and analyzed as mentioned above; No 2- Aroclor was added to a hexane extract of 10 ml of urine which had been prepared as mentioned above; No. 3- Urine (5 ml) was added to a known quantity of Aroclor in a 15-ml centrifuge tube, mixed on a minimixer, and allowed to equilibrate at room temperature for 16 hours. Urine was extracted three times with 3 ml of hexane; the hexane extracts were combined and evaporated to 0.5 ml.

All samples were eluted from a silica gel micro-column. Recovery data for all samples are presented in Table 1. All in vitro recoveries, whether from liquid chromatography or a combination of extraction and liquid chromatography, are at an acceptable level with the exception of urine. Recoveries of DDT from human urine using liquid-liquid extraction techniques have been reported by Cueto & Biros[28] and Cranmer et al.[29] with recoveries of 72 and 96%, respectively. In both instances, their extracting solvent or combination of solvents was more polar than hexane. The recovery in approach No. 1 and possibly 3 might be attributed to the partition coefficient of hexane in an aqueous system. In order to evaluate this, reciprocal p-values were determined using

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the techniques of Bowman and Beroza.[30] Aroclor 1254(R) (1.787 µg) was then added to 5 ml of a 25:1 water:isopropanol mixture which had previously been allowed to equilibrate with hexane overnight. (A mixture of water + urine: alcohol in the same ratio, was used in Experiment C.) Five ml of hexare was added to this solution and the two immiscible phases mixed for 1 min using a mini-mixer. The layers were allowed to separate, and an aliquot of the hexane layer was analyzed by electron-capture. The average of triplicate analyses gave a reciprocal p-value of 0.764, or 76.4% of the Aroclor was partitioned into the hexane layer from the aqueous system. This value agrees favorably with approach No. 3 but does not account for the 17% difference in approach No. 1. Zitko[20] states that he found fractionation taking place when emulsions resulting from mixing Aroclor 1254(R) with water were broken by centrifugation. Gas chromatographic analysis of a hexane extract of the supernatant showed it to be richer in the lower chlorinated biphenyls than the original preparation. This varied with batches of Aroclor. When a peak-for-peak comparison was made, whether in vitro fortification or p-value studies were being considered, recovery was generally low for all constituents, and the average recovery was reported. Low recovery from the urine was probably due to the extracting solvent. The values reported were not corrected for in vitro recoveries.

Interference of DDT-PCB

The presence of PCB's, DDT, and DDT-like moieties in samples presents quite a problem both qualitatively and quantitatively during analysis. Initially, samples of liver and fat from control rats for each sampling period were analyzed for DDT and its metabolites; samples were taken from the oldest control rat in the study. These tissues usually showed measurable amounts of DDT, DDD, and DDE. The values for liver and fat, respectively, are as follows: p,p'-DDT, 0.046 and 1.366 ppm; p,p'-DDD, 0.036 and 0.546 ppm; p,p'-DDE, 0.016 and 1.098 ppm.

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Since preliminary evaluations showed liver and fat storage of PCB's to be a minimum of 10 to 100 times the above mentioned values, interference was considered to be nonexistent. However, three possible approaches to separation of PCB and DDT-like materials without any chemical degradation of the pesticides concerned were evaluated. Thin-layer chromatography was considered first. Using silica gel G and essentially the techniques as outlined by Walker and Beroza[31], both Aroclor 1254(R) and Aroclor 1260(R) in the presence of the chlorinated hydrocarbons, p,p'-DDE, p,p'-DDT, o,p'-DDE, o,p'-DDT, dieldrin, and heptachloride epoxide, moved with the solvent front. Using polar and non-polar mobile phases, the Rf values for PCB's and p,p'-DDE were identical and not resolved. This can possibly be used in confirmation and identification of Aroclors.

The two other approaches utilize column chromatography. Florisil was used, as proposed by Reynolds[32], in which PCB's are eluted from the column with hexane, while other chlorinated hydrocarbons are eluted with a diethyl-ether:hexane mixture. Reynolds evaluated Florisil with a mixed standard of Aroclor 1254 and a chlorinated hydrocarbon mixture containing lindane, heptachlor, aldrin, heptachlor epoxide, p,p'-DDE, dieldrin, p,p'-DDD, and p,p'-DDT. Aldrin, p,p'-DDE, and heptachlor are eluted in the PCB fraction. Although these pesticides were not separated, this approach might still be acceptable since aldrin and heptachlor are seldom found in environmental samples in their unepoxydated forms. However, when an attempt was made to reproduce the work of Reynolds using PR grade Florisil, a mixture of Aroclor 1254(R) plus the chlorinated hydrocarbons, lindane, heptachlor epoxide, dieldrin, p,p'-DDD, p,p'-DDE and p,p'-DDT, measurable quantities of p,p'-DDT, p,p'-DDD and lindane were found in Fraction 1, i.e., 66, 10, and 32% of the total, respectively.

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Reynolds does not mention the quality of the Florisil although he does mention how it was handled. The problem, however, seems not to be related to the grade of Florisil. Bevenue and Ogata[33] have presented data using both qualities of Florisil and were unable to reproduce the work of Reynolds. There is some disagreement between the fractioning pattern of Bevenue and Ogata[33] and the one observed in this work. This could probably be due to the quality and treatment of the Florisil. These problems suggest that the elution characteristics of Florisil should be evaluated before use for separating PCB and DDT.

Armour and Burke[34] used a column of silicic acid (treated) and Celite 545. The column is eluted first with petroleum ether for PCB's and then with a mixed solvent system of dichloromethane-acetonitrile-hexane (80:1:19) for the chlorinated hydrocarbons. This system has been evaluated using standards at levels below those that would be expected in environmental samples, i.e., 0.5 ppm Aroclor 1254(R) and Aroclor 1260(R) and 0.1 ppm γ- and β-isomer of BHC, heptachlor epoxide, o,p'-DDE, p,p'-DDE, dieldrin, p,p'-DDD, and p,p'-DDT Separation has been a problem with the p,p'-DDE; Fraction 1 contained 20% p,p'-DDE with the remaining 80% in Fraction 2. Although some recoveries were less than 85%, all other pesticides evaluated were only found in Fraction 2. According to Armour & Burke[34] the elution pattern can be altered by the water content of the silicic acid. P,p'-DDE was the first affected in the group of pesticides that were used to show these changes.

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In Vivo Recovery Study

An attempt was made to account for the total dosage of Aroclor using individual rats.

(1) Aroclor intake was calculated from average food consumption and diet fortification level.

(2) Fat tissue was assumed to constitute 10% of the average body weight of each rat.

(3) Whole blood volume was taken as 6.7 ml/100 g of body weight, and the hematocrit was then used to calculate total plasma volume.

(4) Total weight of each sample collection of feces and urine for a 16-hour period was used.

A maximum of 30% of the Aroclor intake was recovered under these conditions. As time on the diet increased, recoverability decreased; this could indicate that a great deal of Aroclor was being stored in tissues other than those analyzed and/or that the excretion of Aroclor would have been greater with time.

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Reagents and Apparatus

Silica gel	M. Woelm Activity Grade I treated as mentioned in methods. (Waters Associated, inc. Framingham, Mass. 01701).
Sodium sulfate	Baker and Adamson Reagent grade treated according to H.F. Enos. Perrine Primate Research Center, Perrine, Fla.
Water	De-ionized and double distilled. Extracted three times with n-hexane before use.
Hexane	Nanograde quality, no treatment.
Benzene	Nanograde quality - stored over Na^{o}.
Glasswool	Silanized glasswool from Analabs (North Haven, Conn.)
Micro-columns	Disposable capillary pipets, 5 3/4" length (Owens-Illinois, Toledo, Ohio).
Receivers	Glass stoppered 15 ml centrifuge tubes.
Homogenizer tubes	Thomas Scientific Apparatus (Phila. Pa.)
Tri-R Stirrer	Eberbach Corp. (Ann Arbor, Mich.)

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Results and Discussion

Routinely, this laboratory operates dual channel solid state electrometers at a sensitivity of 4×10^{-10} amps full scale which permits the detection of 10 to 30 picograms of the more common chlorinated hydrocarbons. There was a significant difference between the response characteristics of the Aroclors as compared to the chlorinated hydrocarbons. To achieve a representative gas chromatographic trace, at least 350 and 490 picograms of Aroclor 1254(R) and Aroclor 1260(R) were required. A gas chromatogram illustrating the difference between the electron-capture response characteristics of these compounds as compared to chlorinated hydrocarbons is shown in Fig. 1.

Experiment A

Data resulting from electron-capture analysis of plasma and tissues in Experiment A are presented in Table 2. Quantitation is an approximation. In all cases, the peak heights (in mm) of all PCB components were summed for the respective standard at a given picogram quantity injected. A response factor was determined for each standard in picogram/mm. For each sample, a summation was made of the total response (in mm), and the product of this times the response factor calculated to give picograms of PCB or PCB-derived material in the sample. When the gas chromatograms for the samples were compared with those of the Aroclor standard, there were no recurrences of the general Aroclor pattern. Some peaks had completely disappeared. This was also true for each of the succeeding studies.

Although the dosage level of Aroclor 1260(R) was twice that of Aroclor 1254(R), the mean storage levels after 24 hours were essentially the same. Considerable variation in the storage levels was found in each group. Differing absorption rates from the gut might account for the variation in levels

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found; variation was highest in the fat and lowest in the plasma. According to Hayes[35], acute dosages, such as the one given here, do not result in storage, but rather in a flooding of the organism.

Experiment B

Only surviving rats were analyzed in Experiment B. Five of the ten male rats died-one on the last day (day 98), and one each on days 85 and 56 and two on day 50. Eight of the ten female rats died-one each on days 89, 80, 47, 41, and 35, and three on day 55. Tucker and Crabtree[15] fed male albino rats 1000 ppm Aroclor 1254(R) and reported similar results for 0 of 6 rats including a 21% reduction in food consumption. All survivors in Experiment B appeared to be normal; however no increase in weight was observed in surviving males after 84 days of dietary intake and after 77 days for surviving females. Food consumption for dosed males and females was 66 and 64%, respectively, of consumption by controls,

Data from electron-capture analysis of tissues and plasma are presented in Table 3. The variation in the concentrations of PCB in this experiment, as in Experiment A, is least in the serum and brain and highest in the muscle and fat. No significant difference ($p>0.50$) has been found for storage of Aroclor 1254(R) by females as compared to males. Hayes[35] and Dale et al.[36] report that storage of DDT in female rats exceeds that of male rats when both are maintained on the same diet and that the enhancement in storage exceeds the greater food intake by females.

The absence of certain peaks in the Aroclor standard when compared to PCB-derived material in the samples became more dramatic in this as compared to Experiment A. Generally, the percentage of matching Aroclor peaks found in each tissue was as follows: plasma 81%, fat 74%,

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brain 66%, liver 64%, lung 63%, muscle 62%, and kidney 58%. These differences could be attributed to:

(1) selectivity in storage for each tissue

(2) enzyme activity resulting in some form of metabolism

(3) excretion favoring some isomeric forms as opposed to others.

Experiment C

The data from the analysis of tissue, plasma, and excrement obtained according to the schedule as outlined in Experiment C are graphically illustrated in Fig. 2 and 3. Apparently, there is a steady buildup of Aroclor in all tissues, while the excretion trend is quite erratic. The excretion trend may be due in part to the discontinuous collections; therefore, these results reflect only the excretion pattern at specific times. The residue levels observed in Experiment B created some doubt that levels observed after the 58-day dietary intake were representative of steady state values for Aroclor 1254(R). Hayes[35] states that DDT fed to rats at a constant rate is increasingly stored in their fat until it reaches a plateau, and on a diet containing 200 ppm or less this plateau is reached within 90 to 140 days. Dale et al.[36] found mean concentrations of DDT and DDE in the fat of male rats fed 200 ppm in the diet for 90 days to be 525 and 51 ppm, respectively, while male rats on the same diet for 140 days had mean concentrations of 506 and 42 ppm. A maximum concentration in liver, kidney, and brain is achieved within a few days for a dietary level of 1000 ppm.[35]

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Experiment D and E

Results of Experiment D showed that rats on the same dietary levels

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stored more Aroclor in their tissues after 240 days than at the end of 58 days. The 240-day group showed no significant difference in the quantity of the PCB-derived material found in the urine but showed significantly more PCB excreted in the feces.

Analysis of tissue, plasma, and excreta from Experiment E tends to indicate that Aroclor storage, like DDT, is directly related to the daily dosage. However, the points at which equilibrium storage is achieved are yet to be determined.

At dietary levels of 100, 400, and 800 ppm for 2 years[37], the mean values of DDT stored in the fat of rats at equilibrium were reported as 55,1028, and 4200 ppm, respectively. The concentrations of Aroclor 1254(R) found in the fat after 240 days at dietary levels of 100 and 500 ppm were 1101 and 10,021, respectively. Generally, the levels of DDT stored in the fat of male rats after 180 days are equivalent to levels after 2 years[37], although there is some reduction in DDT storage beyond 2 years. These levels in the fat illustrate the vast differences in storage at the steady states for these two compounds, and the vast differences in their relative toxicities and/or lipid solubilities. (The brain concentration, rather than fat concentration is a better indication of toxicity)[38].

DDT storage can be reduced if exposure is reduced or discontinued. Lang and Fitzhugh[37] found retention of 50-75% and 25% of DDT in the fat, 30 and 90 days after discontinuing diets containing 5 to 50 ppm. After 58 days on a dietary level of 100 ppm Aroclor and a recovery time

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of 71 days, 80% of the concentration in the fat remained. Elimination seems to parallel that of DDT.

These data indicated the concentration of Aroclor found at time of sacrifice to be in the following order: fat > liver ≥ feces > kidney > brain > plasma ≥ urine. The order of storage for tissues and plasma seems to parallel lipid content. Fecal excretion exceeds urine excretion with Aroclors as it does with DDT and other chlorinated hydrocarbons.

Although p,p'-DDT and p,p'-DDE were determined at the 0.01 ppm sensitivity level in liver from a control rat, the degree to which silica gel removes interfering moieties from tissue or excreta extracts cannot be determined at this time. Before and after elution from silica gel there usually was a marked difference in the appearance of the extracts depending on the weights of tissue and excreta used. (Law and Goerlitz [39] report the success of pigment removed with silica gel from water samples.)

Muscle and plasma were the least affected by liquid-solid chromatography and could have been analyzed very easily without this treatment. In fact, for the concentrations of Aroclor found, most of the samples could have been analyzed without any cleanup, taking into consideration the problems associated with column and detector contamination.

After elution patterns of Aroclor 1254(R) were established, 10 ml of benzene:hexane were not required for quantitative elution of PCB, in fact, only 4 ml were required. This saved time in subsequent analyses.

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The elution pattern of Aroclor 1254(R) was unchanged at the fortification levels mentioned in Table 1. The Aroclor 1254(R) standard that was chromatographed simultaneously with each batch of samples analyzed had a mean recovery of 104% with a Standard Error of ± 3.1 for 23 samples.

The fact that there was not a complete peak for peak match between stored and excreted PCB and the dietary standard is not new (Fig. 4). Whether this is evidence of metabolism is yet to be determined. These differences were successfully demonstrated by electron capture gas-liquid chromatography and gas chromatography-mass spectrometry in that there were changes in the relative abundances of some of the components of Aroclor 1254(R) in fat and the urine samples. This has not yet been demonstrated in any other tissue.

The mass spectra showed molecular ions at m/e 288, 323, 358, and 392 containing Cl_4, Cl_5, Cl_6, and Cl_7 isotopic clusters in the fat and 254, 288 324, and 358 in the urine. Two additional ions which were observed in the urine only, were m/e 304 and 405. These ions contained the Cl_4 isotopic cluster.

The presence of unaltered Aroclor in the feces may be indicative of lack of absorption; this was the only sample that showed no alteration, whatsoever, when compared to standard Aroclor 1254(R)

The neutral urinary excretion pattern was quite the contrary to feces. This may in part be due to a Solubility phenomena which Zitko[20] encountered when working with PCB and water. The situation of Zitko with either uring or alcohol-water mixtures under *in vitro* conditions was not reproduced. Under *in vivo* conditions, there very well may be selective solvation of certain constituents of Aroclor. Selected samples of urine were analyzed

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by gas chromatography using the electrolytic conductivity detector in the reductive mode. The presence of chloride ion was further substantiated. The retention times of these components compared favorably with peaks in the Aroclor standard.

The effects of cellular concentrations of PCB may be as indeterminate as effects of DDT. In contrast to DDT a pure compound was not used. Isolation of the Aroclor constituents in tissues and body fluids in pure form will be undertaken to determine which of these are responsible for the many effects resulting from chronic or acute exposure to Aroclor. These data will be reported separately.

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Conclusions

It is hoped that these data will elucidate some of the problems inherent in studying the polychlorinated biphenyls: and that some correlations can be found between the residue levels reported and the LD_{50} determinations including the pathological findings to be reported in a later publication by Kimbrough et al.[40] There are some basic generalizations that can be drawn from the data presented:

(1) Following dosage with Aroclor 1254(R) or 1260(R), whether acute or chronic, residue amounts can be detected in all body tissues, fluids, and excrement.

(2) At the same dosage level rats store more PCB's than DDT.

(3) No significant difference is apparent in the storage of PCB's by male or female rats when fed the same dietary levels.

(4) PCB's are stored primarily in adipose tissue.

(5) Since there was no recurrence of the general Aroclor pattern in the gas chromatograms or total ion current traces of the mass spectrometer between the Aroclor standards and components observed in the fat and urine, possible metabolism or differential absorption is suggested.

(6) As the chlorine content in the PCB molecule decreases, toxicity apparently increases; this suggests that some components in the mixture are less stable and more reactive, thus possibly producing some metabolite.

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Footnotes

The Monsanto Company manufactures PCB's chiefly in the United States under the trade name of Aroclor. They are manufactured in France by Prodelec, and in Germany by Bayer, with trade names, Phenochlor and Colphen, respectively.

These compounds are designated by numbers. The first two digits represent the molecular type: 12, chlorinated biphenyls; 25 and 44, blends of chlorinated biphenyls and chlorinated terphenyls (75% biphenyl and 60% biphenyl, respectively); 54, chlorinated terphenyls. The last two digits give the weight percent of chlorine. Thus, Aroclor 1254(R) and 1260(R), with which this paper is concerned, are chlorinated biphenyls containing 54% and 60% chlorine, respectively.

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TABLE 1

Recovery of In Vitro Fortification of Control Samples with Aroclor 1254

Tissue	Weight	Fortified (ppm) AR 1254	Percent Recovery *
Plasma	2.0	0.5	98.6
Fat	.250	100	82.2
Liver	.500	6.0	94.8
Muscle	.500	4.0	103.1
Brain	.500	3.0	100.4
Kidney	.500	2.0	100.6
Feces	1.000	14.0	99.8
Urine (Approach 1,2,3)		0.1	62.0
		0.1	114.0
		0.1	77.0

* Plasma, fat, liver, miscle, brain, kidney, feces - mean of 4 determinations, and urine $\bar{X}$ mean of duplicate determinations.

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TABLE 2

Storage of Aroclors 24-hours After Oral Ingestion by Stomach Tube

	Aroclor 1254(R) (dosage level - 1600 mg/kg)	Aroclor 1260(R) (dosage level - 3200 mg/kg)
Plasma		
Mean	24.03	15.79
SE	± 8.08	± 0.99
Brain		
Mean	138.00	145.17
SE	± 36.41	± 23.50
Fat		
Mean	1146.9	930.0
SE	± 574.5	± 426.6
Liver		
Mean	141.20	236.1
SE	± 47.80	± 116.3
Kidney		
Mean	274.03	328.5
SE	± 30.47	± 114.9
Lung		
Mean	65.91	105.17
SE	± 29.48	± 21.13
Muscle		
Mean	80.29	37.00
SE	± 67.58	± 22.37

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TABLE 3

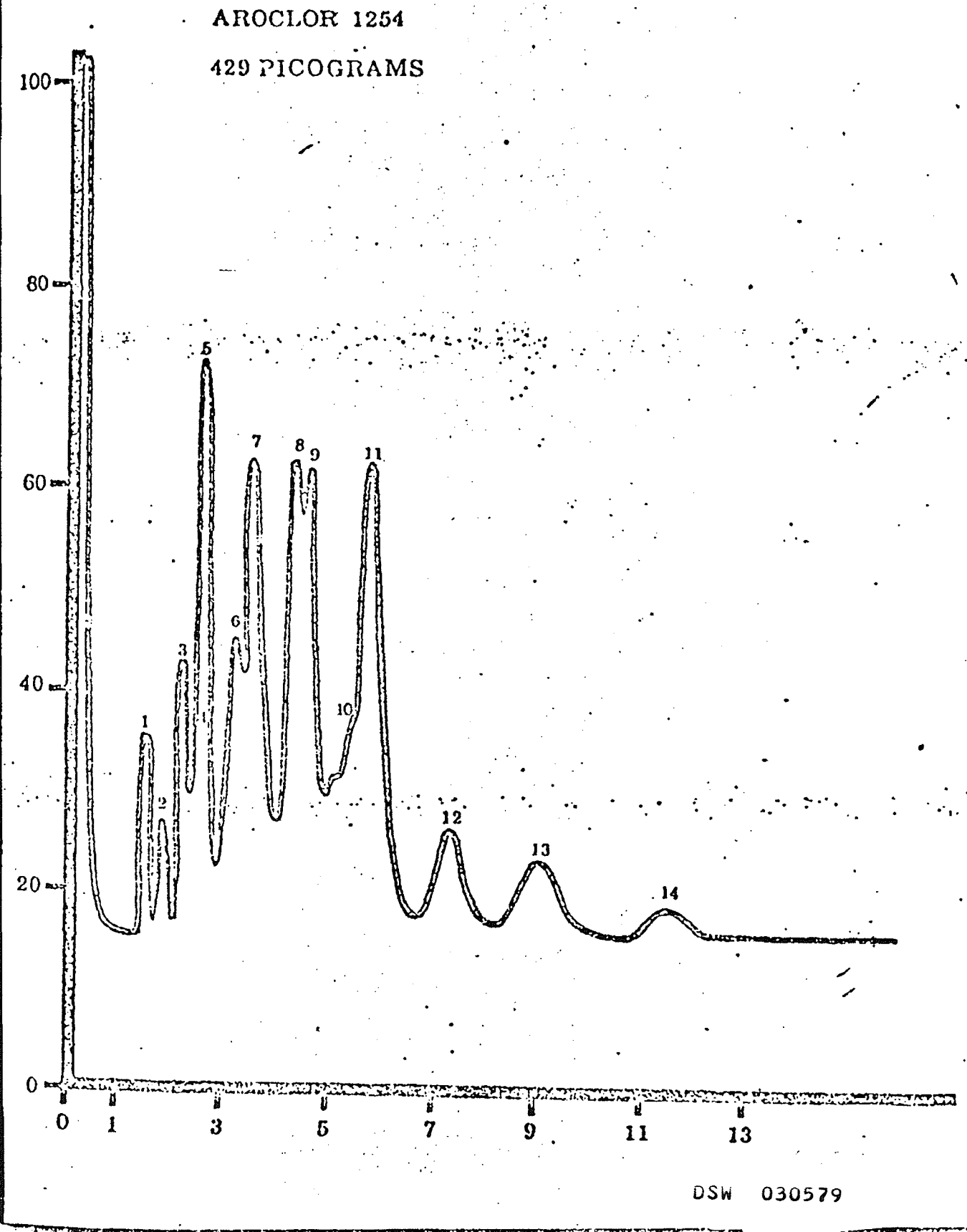
Distribution of PCB-Derived Material Following 98-Day Exposure to a Dietary Level of 1000 PPM Aroclor 1254(R)

	Males (ppm)	Females (ppm)	Males vs. Females
Plasma			
Mean	17	18	$p > 0.50$
SE ±	6	5	
Fat			
Mean	11278	8431	$p > 0.50$
SE ±	5742	579	
Muscle			
Mean	155	753	$p > 0.20$
SE ±	117	721	
Lung			
Mean	78	52	$p > 0.50$
SE ±	23	20	
Brain			
Mean	111	94	$p > 0.50$
SE ±	14	21	
Kidney			
Mean	56	54	$p > 0.50$
SE ±	9	24	
Liver			
Mean	155	210	$p > 0.20$
SE ±	30	13	

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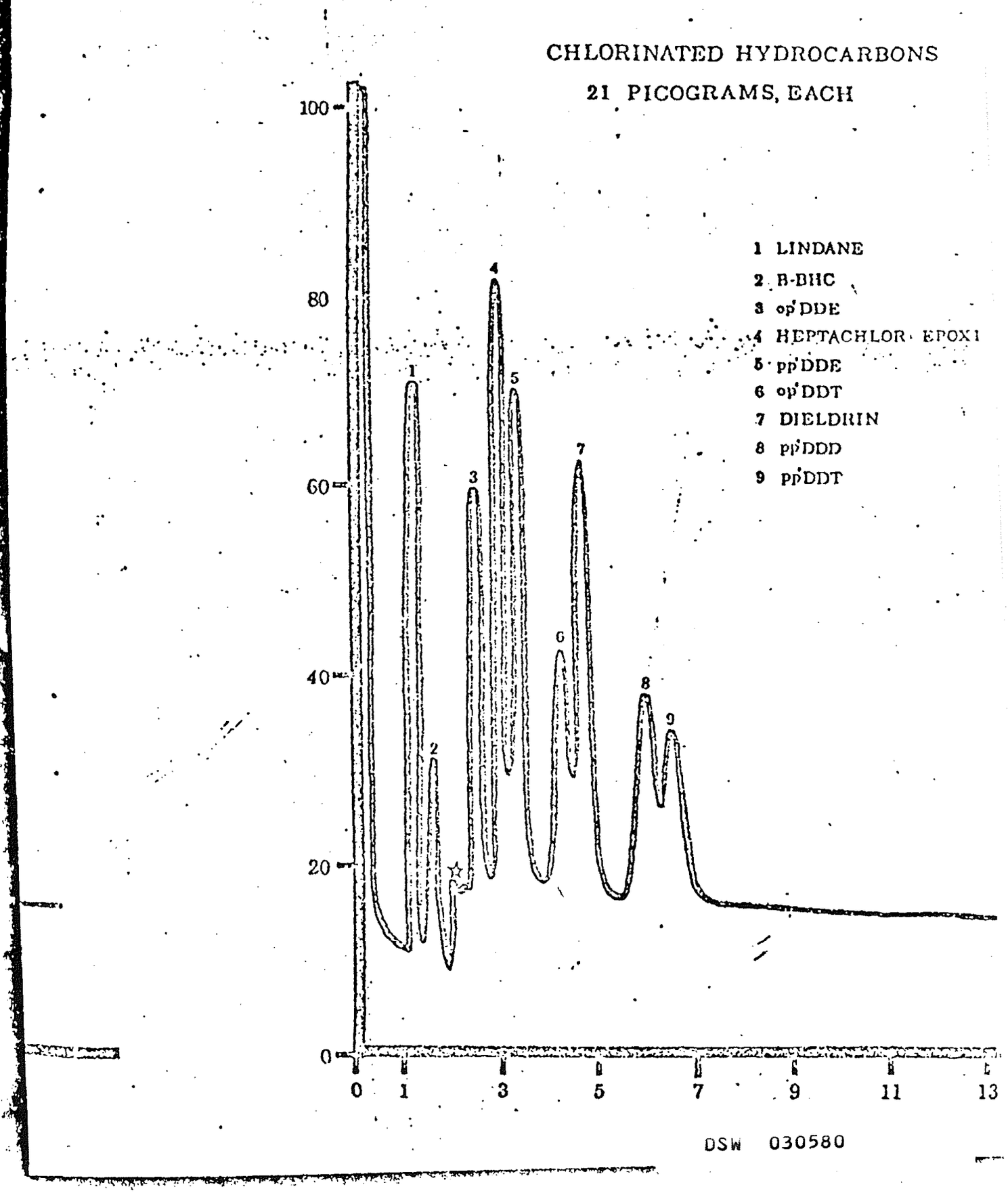
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Fig.1



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Fig. 1



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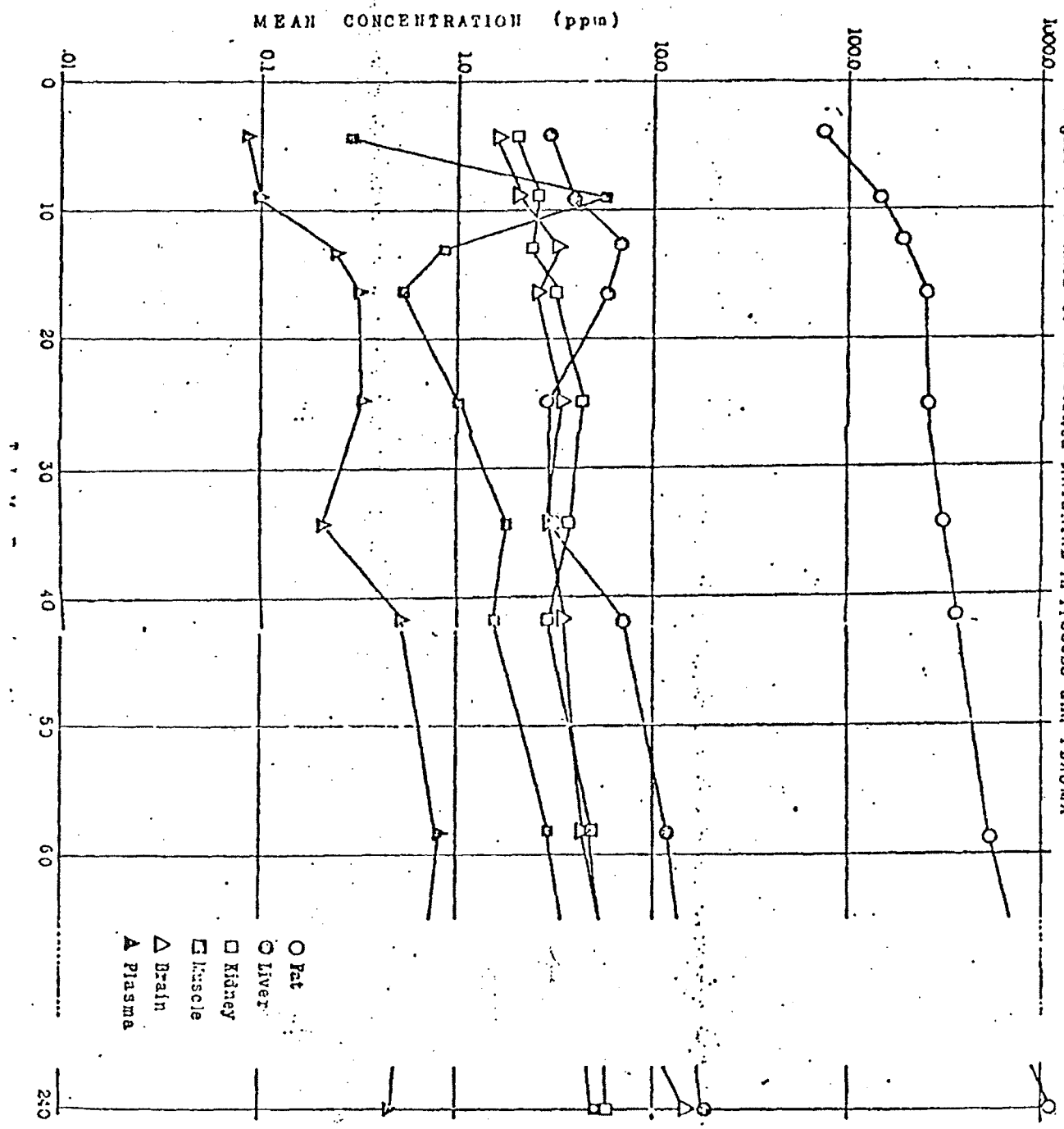
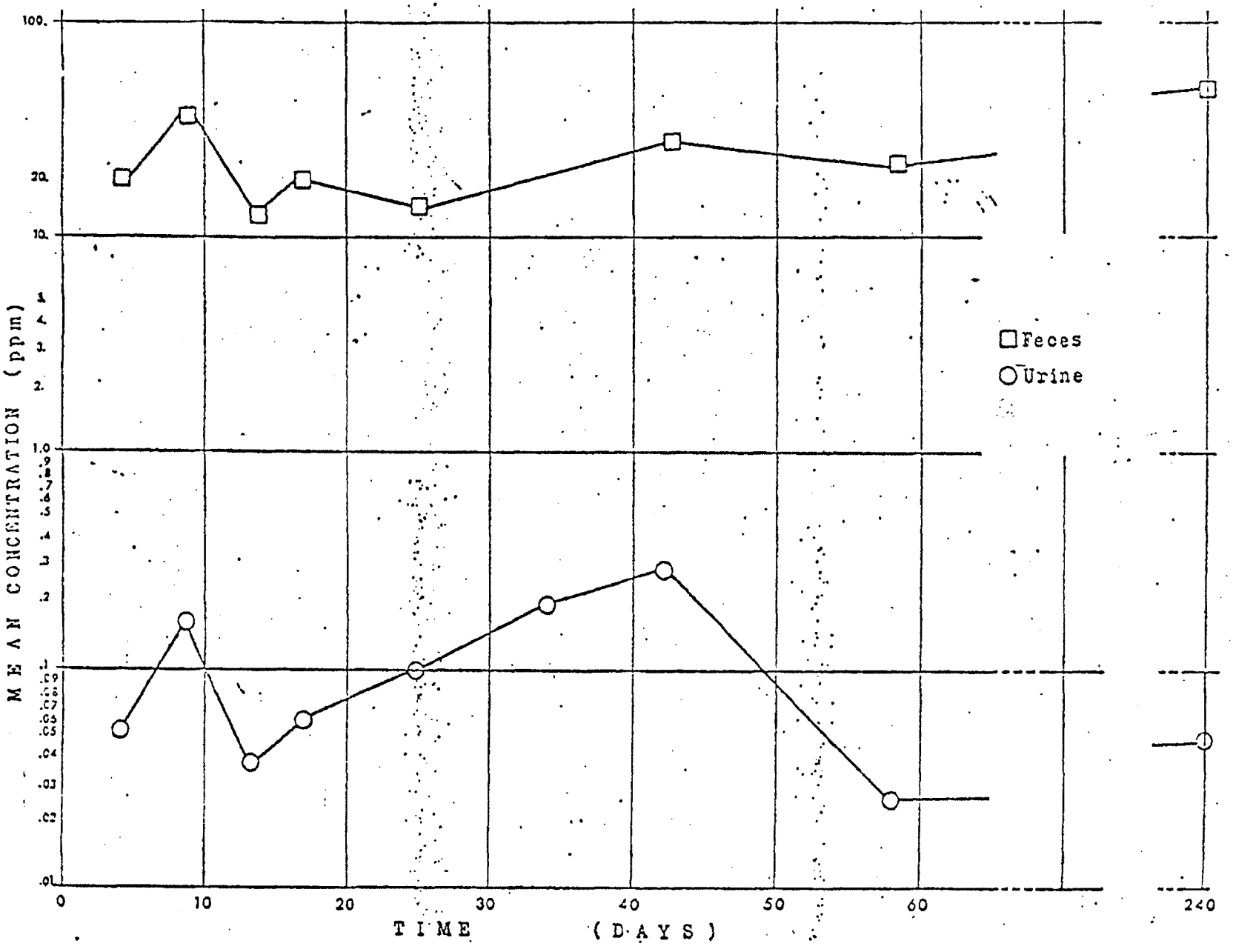


Figure 2. STORAGE of PCB-Derived MATERIAL in TISSUES and PLASMA

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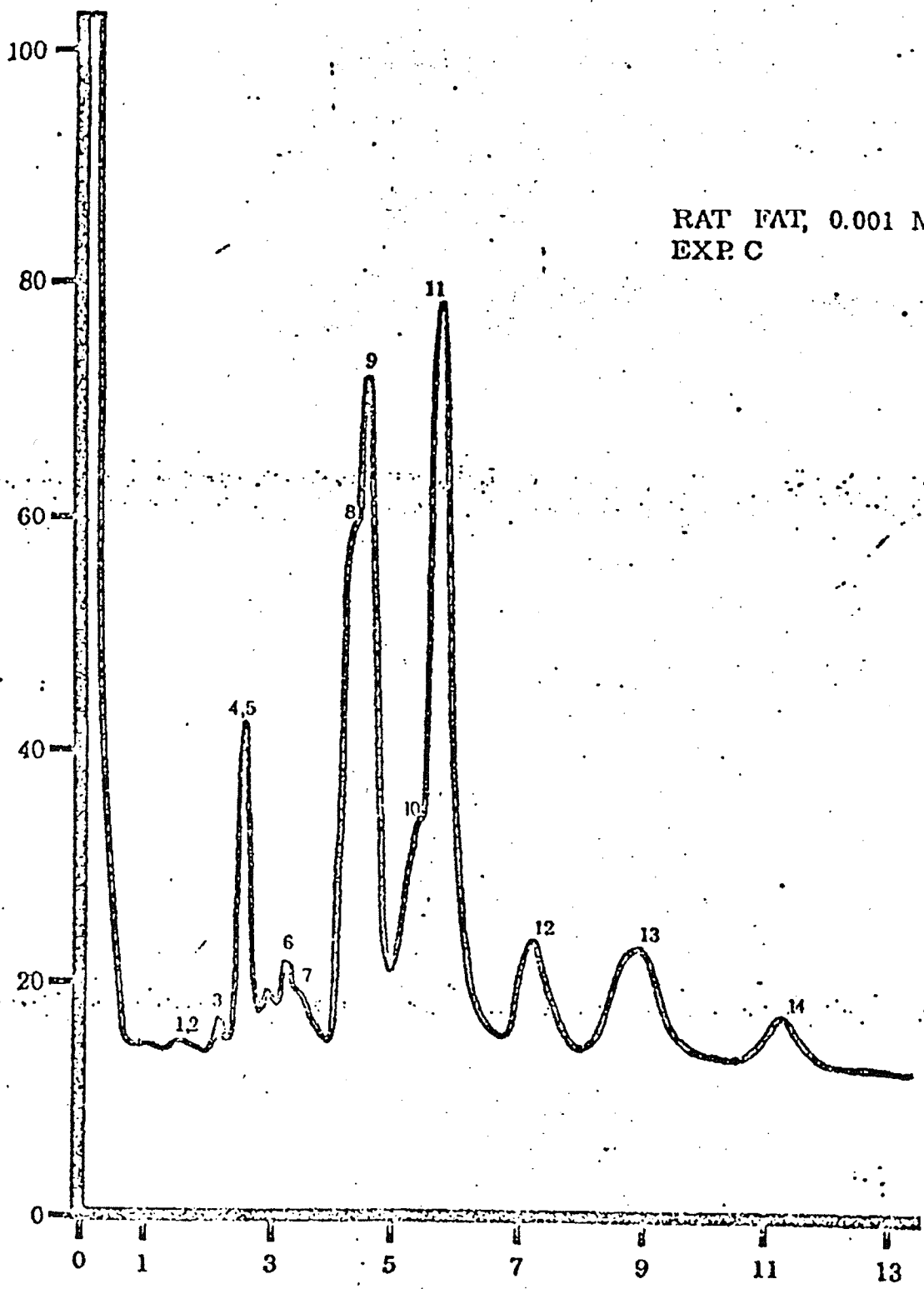
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Figure 5. EXCRETION of PCB and PCB-Derived MATERIAL in FECES and URINE

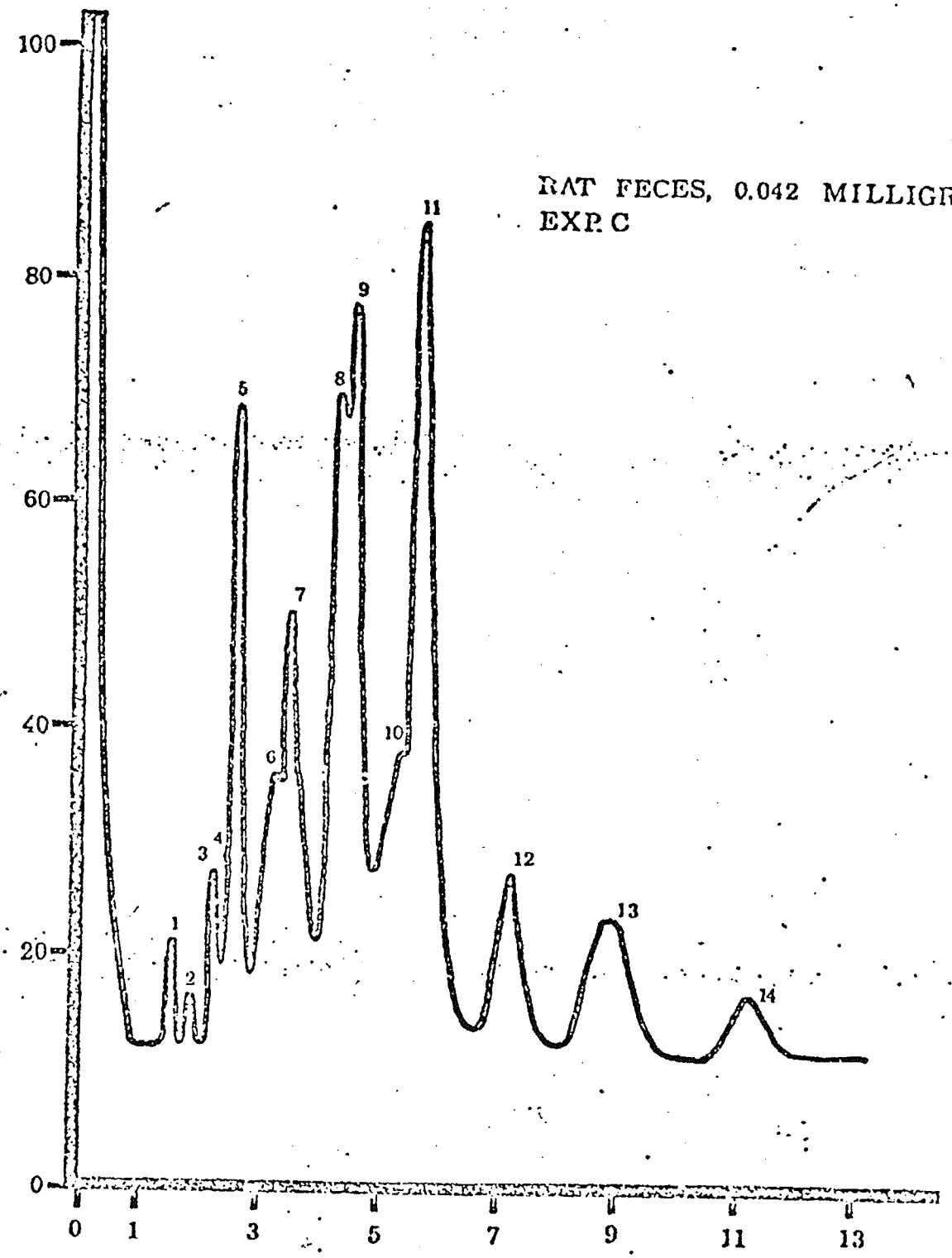


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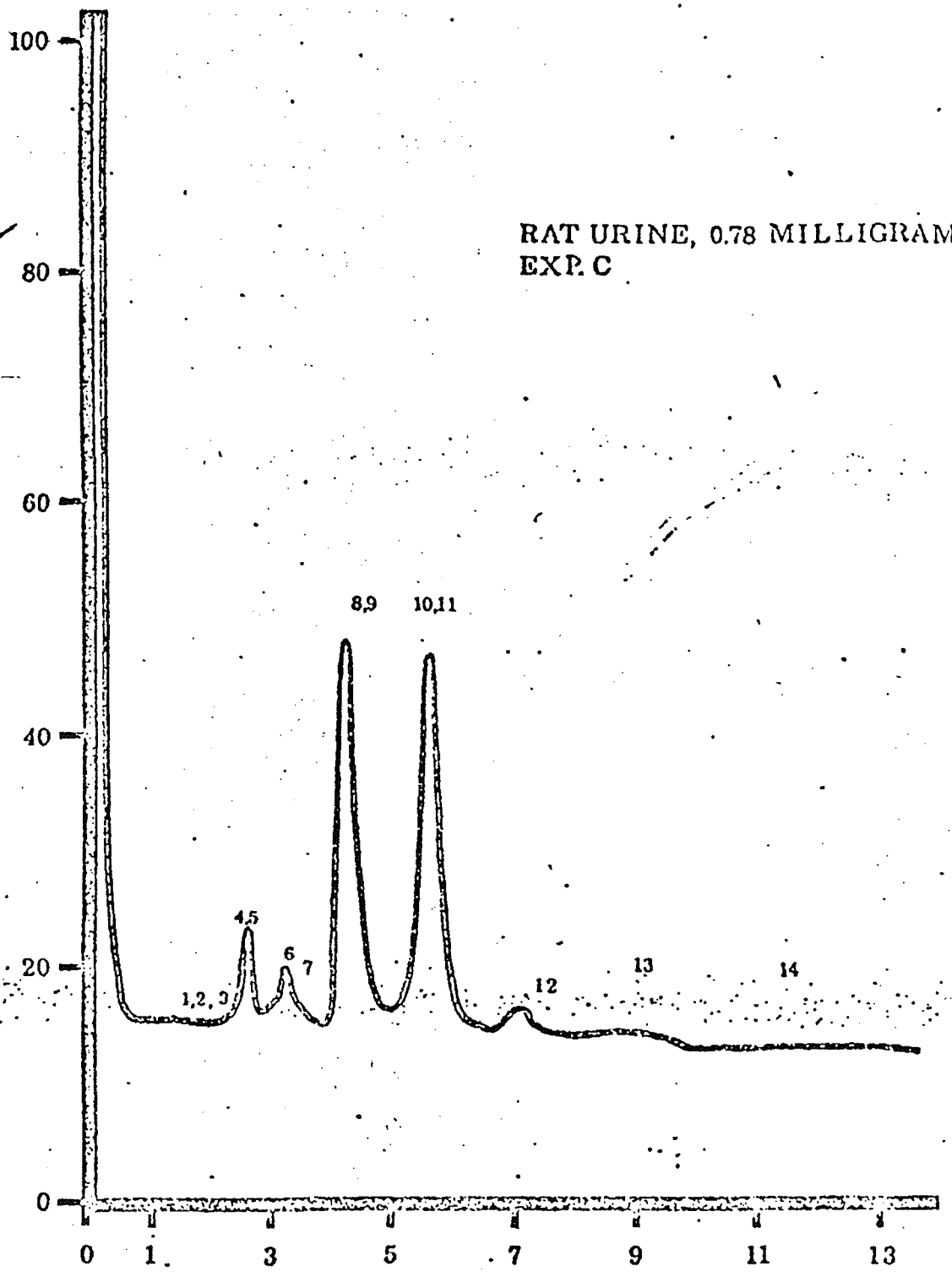
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Fig. 4



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APPENDIX II

Experiments to date not included in Manuscript

Experiment No.	No. of Rats	Sex	Age at Onset of experiment (days)	Wt. (Gms)	dosage/route
Ar 7-70-B12	4 (analyzed)	F	30	224*	100 ppm Diet for 247 days * 8.1 mg/kg/day
Ar-7-70-B12	3 (analyzed)	F	30	224*	100 ppm in Diet for 300 days * 8.1 mg/kg/day
Ar-7-70-B12	3 (analyzed)	F	30	224*	100 ppm in Diet for 366 days * 8.1 mg/kg/day
Ar-7-70-B10	4 (analyzed)	F	30	240*	20 ppm in Diet for 280 days * 1.6 mg/kg/day
Ar-8-71-B	A study to determine placental transfer, excretion in milk and consequent tissue storage in weanling rats of Aroclor 1254 when the compound is given to the mother rat during organogenesis.				
Ar 7-70-G4	The effect of Aroclor 1260 upon reproduction in female rats when the compound is given during organogenesis.				

* Average weight of group and average rate of food consumption for group.

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Additional residue studies, including experimental design and resulting data, are listed on the following pages:

Experiment No.	Tissue	Mean ppm (4 animals)	± SE
Ar-7-70-B12	Fat	778	72.4
	Liver	9.9	1.16
	Kidney	5.1	.95
	Muscle	1.22	.104
	Brain	6.96	.68
	Plasma	1.6	.311

When compared to males at the same dosage level for an equal amount of time, Student's t-test shows significant difference in brain and liver storage. When these data are compared to data from male rats on the same dosage for an equal amount of time (see manuscript) the Student's t-test shows significant differences for brain and liver storage.

	Tissue	Mean ppm (3 animals)	± SE
Ar-7-70-B12	Fat	1022	127
	Liver	14.6	0.69
	Kidney	2.8	0.21
	Muscle	1.2	0.11
	Brain	9.3	0.57
	Plasma	2.1	0.43

When these data are compared with 8 mo. study test for significant difference shows $p < 0.05$ for liver only.

	Tissue	Mean ppm (3 animals)	± SE
Ar-7-70-B12	Fat	820	216
	Liver	22.5	2.51
	Kidney	4.7	.24
	Muscle	2.9	.96
	Brain	11.2	1.71
	Plasma	3.0	0.78

When these data are compared with 10 mos. study t-test for significances show $p < 0.05$ for liver and kidney.

	Tissue	Mean ppm (4 animals)	± SE
Ar-7-70-B10	Fat	274	48
	Liver	2.2	.10
	Muscle	.32	.06
	Kidney	.60	.05
	Brain	2.6	.50
	Plasma	.42	.06

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The results of a study with Aroclor 1254(R) to determine placental transfer, rates of excretion in milk and consequent tissue distribution and storage levels in fetuses and weanling rats following oral dosage to the mother daily on the 7th through the 15th day of gestation at 10 mg/kg and 50 mg/kg, respectively by stomach tube is summarized as follows:

Fetus Analysis: Samples were taken by Caesarian section on the 20th day of pregnancy. The mean concentrations of Aroclor are given for the respective dosages as ppm.

	Controls	10 mg/kg	50 mg/kg
Mother 1	0	1.42	2.42
Mother 2	0	1.24	2.90
Mother 3	0	1.14	2.93

10 mg/kg

	Wt. at onset (gms)	Age (days)
Mother 1	254	90
Mother 2	265	90
Mother 3	258	90

50 mg/kg

Mother 1	242	90
Mother 2	244	90
Mother 3	261	90

Tissue Analysis: Samples were taken from 21 day-old male and female weanling rats. The concentrations in the tissues for three animals are given in ppm.

	Controls		10 mg/kg		50 mg/kg	
	Males	Females	Males	Females	Males	Females
Liver	<0.05	<0.05	4.06	2.85	17.36	21.63
			3.28	8.28	25.56	12.02
			2.70	4.11	11.80	15.99
		Mean $\bar{X}$	3.35	5.08	18.24	16.55
		S.E. $\pm$	0.39	1.64	4.00	2.79

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	Controls		10 mg/kg		50 mg/kg	
	Males	Females	Males	Females	Males	Females
Brain	<0.08	<0.08	1.96	1.94	10.16	10.48
			2.28	2.72	5.44	3.89
			2.24	1.24	7.47	6.75
		Mean $\bar{X}$	2.16	1.97	7.69	7.04
		S.E. ±	0.10	0.43	1.37	1.91
Kidney	<0.04	<0.04	1.52	1.54	7.53	2.41
			2.42	2.19	6.73	2.25
			1.14	2.32	6.70	4.10
		Mean $\bar{X}$	1.69	2.02	6.99	2.92
		S.E. ±	0.38	0.24	0.27	0.59

Milk Analysis: Milk samples were taken from suckling rats 11 days after the last dose to the mother. Concentrations are given in ppm.

	Controls	10 mg/kg	50 mg/kg
Range	0	16.48 -24.90	45.80-100.29
Mean	0	20.60	66.35
S.E.		± 1.59	± 8.36

Ar 7-70-G4

The results of a study with Ar 1260 show consequent tissue distribution and storage levels in weanling rats following oral dosage to the mother daily on the 7th through 15th day of gestation at 50 mg/kg by stomach tube is summarized as follows:

Note: One female and one male taken from each of 3 litters for analysis.

Tissue	Sex	Mean (3 animals)	± S.E.
Kidney	M	7.38	2.56
	F	5.44	1.41
Brain	M	7.53	1.04
	F	7.76	2.54
Liver	M	12.80	1.72
	F	16.13	6.75

Weights of Mothers 284, 264, and 288 gm

Mean weight of Mothers 278 grams

DSW 030589

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