

AN ABSTRACT OF THE THESIS OF

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Title: ACCUMULATION OF DIETARY POLYCHLORINATED
BIPHENYL BY RAINBOW TROUT (*Salmo gairdneri*)

Abstract approved: _____
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This study was conducted to investigate the accumulation of dietary polychlorinated biphenyls (PCB's) by a Mt. Shasta strain of rainbow trout (*Salmo gairdneri*). A commercial mixture of PCB's (Aroclor 1254) was added to the diet at a level of 15 ppm on a dry weight basis. Accumulation of PCB's was measured using a gas-liquid chromatograph (GLC) equipped with an electron capture (EC) detector.

The relative concentration of PCB's in the extractable lipid reached an equilibrium at 95 parts per million (ppm) after 8 weeks of dietary exposure. On a whole-fish basis, the concentration of PCB's increased with increases in the lipid content, which stabilized after 24 weeks to give the trout a concentration of 8.5 ppm PCB's. Absolute quantities ($\mu\text{g PCB/fish}$) increased as the fish grew, and after 32 weeks the trout contained an average of 636 $\mu\text{g PCB/fish}$.

Total retention of PCB's from the diet after 32 weeks of exposure was 68%.

PCB's were found to be distributed at a constant level in the lipid fraction of various tissues. However, due to the variation in lipid content of different tissues, large variation of PCB concentration on a whole-tissue basis existed.

Elimination of PCB's from the trout was very slow if it occurred at all. Rainbow trout that were fed the PCB diet for 16 weeks and then the control diet for 16 weeks did not appear to eliminate any PCB residues during the latter period, although the relative concentration (ppm) decreased due to a simple dilution effect as the trout grew. Fish maintained on the PCB diet for 32 weeks and then starved for 8 weeks contained essentially the same quantity of PCB's before and after starvation. While the lipid content decreased by approximately one-half, the concentration of PCB's in the remaining lipid doubled.

Peak patterns of PCB's isolated from the trout were identical to those of the Aroclor 1254 standard at all stages of the experiment, indicating that rainbow trout do not selectively absorb or metabolize PCB's.

No differences were observed in the lipid content, growth, or size of the liver of fish on the PCB diet and control diet. Microscopic

examination of liver tissues did not reveal any damage from ingestion of PCB's. No mortalities were attributed to PCB toxicity.

Accumulation of Dietary Polychlorinated Biphenyl
By Rainbow Trout (Salmo gairdneri)

by

Andrew James Lieb

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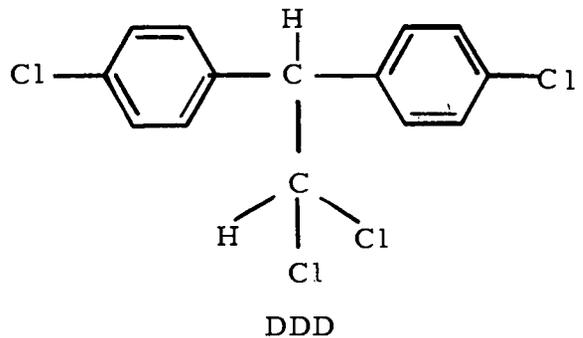
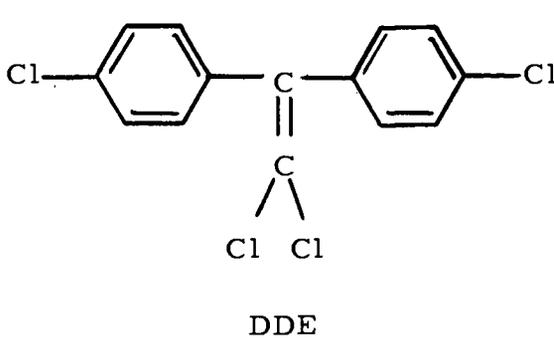
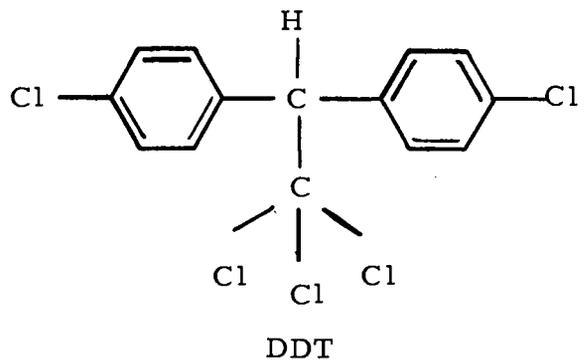
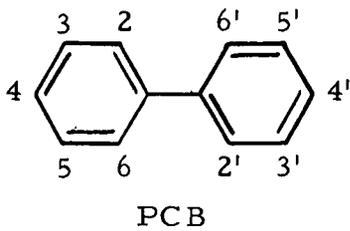
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ACCUMULATION OF DIETARY POLYCHLORINATED BIPHENYL
BY RAINBOW TROUT (Salmo gairdneri)

REVIEW OF LITERATURE

Polychlorinated biphenyls (PCB's) are a mixture of compounds derived from chlorination of the biphenyl molecule. The basic structure and numbering system of the substituted biphenyl is shown below with chlorination possible at any of the numbered positions. Structurally PCB's resemble the chlorinated hydrocarbon insecticide DDT and its analogs.



Two hundred and ten combinations of chlorine substitution of the biphenyl system are theoretically possible assuming that the starting product is only biphenyl and no reaction other than chlorination occurs. It is not certain how many of these do appear in significant amounts in technical products, but Aroclor 1254 was separated into 69 distinguishable peaks by Sissons and Welti (1971) and 71 peaks by Herring (1973).

PCB's are manufactured in the United States exclusively by Monsanto and they are sold along with chlorinated terphenyls under the trade name of Aroclors. PCB's are also manufactured in Italy, France (Phenoclor), Germany (Clophen), Japan (Kaneclor), Great Britain, and the U. S. S. R. (Cook, 1972).

Commercially available Aroclors are designated by four digits, the first two digits describe molecular types; 12 indicates that the mixture consists of chlorinated biphenyl, 54 chlorinated terphenyl, and 25 and 44 are mixtures of chlorinated biphenyl and terphenyl. The last two digits represent the percent chlorination. Thus, Aroclor 1254 is a chlorinated biphenyl containing 54 percent chlorine by weight. Commercially available biphenyls from Monsanto are Aroclors 1221, 1232, 1242, 1248, 1254, 1260, and 1268. As the average chlorine content of the Aroclor increases, the more highly chlorinated isomers become more abundant. Aroclor 1221 consists mostly of biphenyl, mono- and dichlorinated biphenyl (Willis and

Addison, 1972), while Aroclor 1254 contains predominately isomers of tetra-, and penta-, hexa-, and heptachlorobiphenyl (Bagley et al., 1970).

Chemical and Physical Properties

PCB's are chemically inert, are not hydrolyzed by water, and resist alkalies, acids, and corrosive materials. The physical properties of PCB's are low vapor pressure, low water solubility, and high dielectric constant. Boiling points range from 278° C for Aroclor 1221 to 415° C for Aroclor 1268 (Plenning, 1930). PCB's are highly soluble in lipids and non-polar organic solvent. Thus, PCB's have the necessary physical and chemical properties for persistence and accumulation in the food chain (Peakall and Lincer, 1970). The physical states of PCB's at 21° C vary from colorless oily liquids to more viscous and increasingly darker liquids to black resinous solids with increasing chlorination (Interdepartmental Task Force, 1972).

PCB's have many industrial applications due to their unique physical and chemical properties. Some of the main uses are as insulation in electrical capacitors and electrical transformers, hydraulic lubricants, fluids in heat transfer systems, plastizers, and many minor uses, such as, wax extenders, adhesives, dusting agents,

and carbonless reproducing paper (Nisbet and Sarofim, 1972).

Production

Annual production of PCB's in the U. S. A. reached a high of 43,000 metric tons in 1970 (anonymous, 1971) and Japan produced about 12,000 metric tons in 1970 (Isono, 1971). Data on the production of PCB's in other countries were not found.

It is estimated that only 20% of the 1970 sales in North America represent a net increase in the amount of PCB's in service. The remainder is assumed to be introduced into the environment in the form of $1.5-2 \times 10^3$ tons per year into the atmosphere, $4-5 \times 10^3$ tons per year into fresh and coastal waters, and 1.8×10^4 tons per year into dumps and land fills (Nisbet and Sarofim, 1972).

Due to the huge loss of PCB's into the environment, Monsanto in September, 1970 voluntarily restricted sales to operations where the disposal of the end products may be controlled (Gustafson, 1970).

Distribution in the Environment

Wildlife

Knowledge of PCB's in the environment has steadily increased since Jensen (1966) first identified them as the previously unknown substances found during the course of pesticide residue analysis.

Jensen (1966), found that PCB's occurred in the Swedish natural environment at the same levels as the chlorinated pesticides. In a later report, Jensen et al., (1969) showed the greatest contamination by PCB's occurred in industrial regions and in the food-chain species found in such areas. Investigation of the food-chain (fish to seal, fish to guillemot, fish to heron, and fish to white tail eagle) revealed increases from prey to predator of at least ten fold. For the eagle and heron, the increase was nearly 100 fold. They also reported higher residues of the more highly chlorinated compounds and suggested that PCB's of lower chlorine content are metabolized or excreted faster so that a proportional increase of the former takes place as PCB's pass through the food chain.

PCB distribution and occurrence in the Rhine River and coastal areas of the Netherlands are described by Koeman et al. (1969). They found that most of the PCB's present in fish and seabirds living in the Wadden Sea originated in the Rhine River. Again, compounds chlorinated to a lesser extent did not occur as frequently as residues as the more highly chlorinated compounds.

In California, Risebrough et al. (1968) found that residues of PCB's are considerably higher in animals of San Francisco Bay than in those from the more remote Farallon Islands, 27 miles west of the Golden Gate Bridge.

Peregrines and merlins trapped on the western slope of Lake

Michigan contained 52 and 196 ppm PCB respectively. Levels of DDD and DDT found in these specimens were lower than values obtained previously, presumably as a result of interference by PCB's in earlier determinations (Risebrough, 1970).

While investigating a fish kill, Duke et al. (1970) detected PCB's (Aroclor 1254) in the biota, sediment, and water of the Escambia River estuary near Pensacola, Florida. One source of PCB was traced to a leak from an industrial plant near which levels in sediments reached 486 ppm. Levels in the water decreased with distance from the plant, indicating that PCB's due to their low solubility in water are readily adsorbed onto sediments. Nimmo et al. (1971) found that pink shrimp (Penaeus duorarum) and fiddler crab (Uca minax) would accumulate PCB residues from the sandy silt of Escambia Bay, and that the amount of PCB's in the shrimp or crab was directly related to the amount in the sediments.

In Canada, Zitko et al. (1972) also reported that higher levels of PCB's are associated with industrial coastal areas. Muscle of Atlantic salmon (Salmo salar) caught off Greenland contained 0.20 ppm PCB; whereas, salmon landed in Canada had 0.45 to 0.62 ppm PCB. In all samples the gas-liquid chromatography (GLC) peak pattern resembled most closely that of Aroclor 1254.

Samples of young terns from Long Island Sound contained concentrations of PCB higher than DDE at 25 and 2.1 ppm,

respectively, while eight species of fish caught by the terns contained 1.0 and 0.19 ppm PCB and DDT, respectively (Hays and Risebrough, 1972).

Residues of PCB's in the eggs of 46 species of wild birds in Great Britain were reported by Prestt et al. (1970) to be generally below 5 ppm, but there were notable exceptions. For example, residues in heron (Andea cinerea) eggs averaged 5 ppm, but 10 of 101 eggs contained more than 10 ppm, including one with 40 and one with 80 ppm. Zitko and Choi (1972), reported levels of PCB in eggs of double-crested cormorants (Phalacrocorax auritus), herring gulls (Larus argentatus), and black ducks (Anus rubripes) from Fatpot Islands, New Brunswick to be 17.2, 5.45, and 9.1 ppm, respectively.

Lake Erie fish analyzed during 1970-1971 all contained detectable residues of PCB's with the average level for the species tested ranging from 0.08 to 4.4 ppm. Coho salmon averaged 2.1 ppm (Carr et al., 1972).

Water

Effluents from sewage treatment plants have been shown to contain significant amounts of PCB's. In Sweden, Ahling and Jensen (1970) detected PCB's in sewage sludge, while Holden (1970) estimated that approximately a ton of PCB's per year are introduced into the Clyde estuary in Scotland and the same amount into the

Thames estuary in England from sewage outfalls.

Veith and Lee (1971) found PCB's discharged from sewage treatment plants on the Milwaukee River with concentrations as great as 2.07 and 2.8 $\mu\text{g}/\text{l}$. In California, Schmidt et al. (1971) estimated that several sewage treatment plants discharge over a ton of PCB's per year into the Pacific Ocean.

Food

Since November 1969 the F.D.A. has carried out PCB analyses on all raw agricultural commodities sampled under the pesticide surveillance program. For 18 months, July 1, 1970 to September 30, 1971, a total of 3,505 samples were analyzed, and 684 samples of fish, cheese, milk, shell eggs and fish byproducts were found to contain PCB's. Fish samples contained PCB's most frequently with 363 out of 670 samples containing from a trace to 35.3 ppm (Kolbye, 1972).

Milk samples exceeding FDA guidelines for maximum PCB concentration have been prevented from shipment in several instances. The source of contamination in some cases was discovered to be Aroclor 1254 used in the formulation of a sealing agent applied to silo walls (Skrentny et al., 1971). In a follow-up study, Fries (1972) sampled milk from six farms with PCB contaminated silos and found residues to exceed the FDA guideline of 0.2 ppm in milk (equivalent

to 5 ppm in fat) when silage was fed. When silage was not fed, the levels of PCB's in milk were always lower than the guideline.

On the basis of the investigations of PCB's in animal feeds, packaging material, silos, and "bread basket surveys" the FDA has set tolerances for PCB's. Maximum acceptable levels are: milk and dairy products 2.5 ppm (fat basis), eggs 0.5 ppm, finished animal feeds 0.5 ppm, animal feed components (including fish meal) 5.0 ppm, fish (edible portion) 5.0 ppm, infant and junior foods 0.1 ppm, and food packaging material 5.0 ppm. Furthermore, PCB's are no longer permitted for use in machinery, equipment, etc., where any possible contamination of food products could occur (Fine, 1972).

The most serious contamination of food with PCB's occurred in Japan. In October 1968, an epidemic took place characterized by acniform skin eruptions, dark-brownish pigmented nails, increased eye discharge, and swelling of the upper eyelids. The cause of the symptoms, termed "Yusho" or rice oil disease, was rice oil contaminated with PCB's (Kuratsune et al., 1972). Concentrations of PCB's (Kanochlor 400) were between 2,000 and 3,000 ppm with average amounts of PCB's ingested by patients estimated to be about 2 grams (Yoshimura, 1971).

Human Levels

Analytical results of 637 samples of human adipose tissue from 38 cities in 18 states show 31.1% contain measurable amounts of PCB's and 19.6% contain trace amounts with the balance being negative (Yobs, 1972). Other investigators, report that 41-45% of the general U. S. population have 1.0 ppm or more PCB in their adipose tissues and that the PCB's appear to be the more highly chlorinated Aroclors (Price and Welch, 1972).

Toxicity and Accumulation of PCB'S

Birds

An indication of the avian toxicity of PCB's was obtained by feeding Aroclor 1254 to Bengalese finches. Estimated dose rate for 50% mortality at 56 days was 254 mg/kg/day. All birds dying from PCB toxicity had enlarged kidneys and some displayed leg paralysis or body and wing trembling before death. PCB's were 1/13 as toxic as DDT (Prestt et al., 1970). The cause of death among chicks in a recently painted battery was traced to an epoxy-resin paint formulation containing Aroclor 1242. Subsequent feeding studies revealed hydropericardium and enlarged livers present in chicks that died. PCB's fed at 0.01% caused only slight toxic effects, but at 0.02% distended abdomen and labored respiration were evident.

Growth depression in chicks in direct proportion to levels of PCB's fed have been demonstrated (Flick et al., 1965; Rehfeld et al., 1971), but are reversible when PCB's are removed from the diet (Rehfeld, 1972b). Accumulation of PCB's in adipose tissue increases significantly as the levels in the diet increase. GLC profiles have shown that birds are better able to metabolize PCB's containing fewer chlorines per molecule and that more highly chlorinated PCB's are more readily stored (Rehfeld, 1972a).

Tests of six PCB mixtures containing 32 to 62% chlorine show that toxicity increases with percent chlorination when tested on pheasant, duck and quail. In general PCB's were similar to DDE in toxicity (Heath et al., 1970).

Polychlorinated biphenyls supplied by three different manufacturers: Phenoclor (France), Clophen (Germany) and Aroclor (USA) gave strikingly different results for toxicity tests with chickens. All three formulations contained 60% chlorine. At a dietary dosage of 400 ppm for 60 days all 24 birds fed Phenoclor died, with Clophen 22 of 24 died, with Aroclor only 3 of 24 died. All birds fed Phenoclor and Clophen displayed liver necrosis, whereas none on Aroclor did (Vos and Koeman, 1970). Differences were explained by the identification of chlorinated dibenzofurans as contaminants in Phenoclor and Clophen (Vos et al., 1970).

Since the chlorinated pesticides are known to cause egg-shell

thinning (Peakall, 1970), PCB's have naturally caused concern regarding their possible effects on bird reproduction. Tests with Aroclor 1254 on pheasants (Phasianus colchicus) revealed that weekly weight changes of hens, fertility and hatchability of eggs, mortality of adults, and egg shell thickness were not affected by PCB's given orally. However, total reproductive success was depressed. Mortality of chicks from hatching to 6 weeks of age was significantly greater in offspring of hens receiving 50 mg PCB weekly and surviving chicks were significantly lighter (Dahlgren and Linder, 1971). Anti-androgenic-like effects of Aroclor 1254 when fed to cockerels at 250 ppm were noted by Platonow and Funnell (1971). A striking difference was noted in the comb size and testicular weight.

Calcium metabolism in birds is intimately related to reproductive metabolism and is regulated by steroids to a large extent. PCB's have been shown to induce hepatic hydroxylating enzymes that increase the metabolism of these reproductive steroids (Bitman et al., 1972; Lincer and Peakall, 1970). Risebrough et al. (1968) found PCB's had an oestradiol degrading potential on a weight basis of approximately 5 times that of DDE or DDT. However, different metabolites were produced by PCB induced enzymes than with DDE and DDT.

The evidence available to date suggests that PCB's are not a cause of the egg-shell thinning observed in many populations of birds

(Peakall, 1971). No correlation between thinning and PCB content has been found in eggs of the great blue heron (Ardea herodias) (Vermeer and Reynolds, 1970) or in eggs of the white pelican (Pelecanus erythrorhynchos) (Anderson et al., 1969). Whenever a correlation between PCB's and thin egg shells has been observed, PCB has been isolated concomittantly with DDE (Anderson et al., 1969).

Insects

The toxicity of PCB's to insects is also related to the chlorine content, but in the reverse order to the results obtained for birds. PCB's chlorinated to a lesser extent are more toxic to flies than PCB's with a higher chlorine content, and the toxicity of mixtures containing more than 48% chlorine were very low. PCB's are much less toxic than dieldrin or DDT, but PCB's increase the toxicity of DDT or dieldrin when administered jointly (Lichtenstein et al., 1969).

Aquatic Invertebrates

Marine organisms at the bottom of the food chain, have been shown to markedly concentrate PCB's from their aqueous environment. A diatom, Cylindrotheca closterium, concentrated PCB's up to 1,100 above levels added to media (Keil et al., 1971). The growth rates of two species of marine diatoms Thalassiosira pseudonana

and Skeletonema costatum were reduced by PCB's in media, whereas, a green algae Dunaliella tertiolecta and two fresh water algae Euglena gracilis and Chlamydomonas reinhardtii were not inhibited. The sensitivity of these species to PCB's paralleled their sensitivity to DDT (Mosser et al., 1972).

A daphnid (Daphnia magna) exposed to Aroclor 1254 for 4 days at 1.1 ppb in water accumulated concentrations of PCB 48,000 times greater than those in water while mosquito larvae exposed to 1.5 ppb Aroclor 1254 for 24 hours accumulated 19 ppm or a 12,600 fold magnification factor (Sanders and Chandler, 1972).

Uptake of PCB's from seawater by Gammarus oceanicus was found to be dependent on the total surface area of the skeleton and on the concentration of PCB in water. After 4 to 6 hours of exposure, the rate of uptake decreased (Wildish and Zitko, 1971). Lethal concentration of Aroclor 1254 was as low as 0.01 $\mu\text{g}/\text{l}$ and during molting the shrimp were particularly vulnerable (Wildish, 1970).

Mammals

Grant et al. (1971a) studied male rats orally dosed with Aroclor 1254 and found PCB's in all tissues analyzed with the greatest concentration in the adipose tissue. The GLC pattern of extracted residues was different than that of the standard mixture administered, indicating that the less chlorinated PCB's are metabolized to a greater

extent than the more highly chlorinated PCB's. Metabolism of PCB's was thought to take place in the liver because of decreased PCB metabolism in rats treated with carbon tetrachloride. In another study with rats fed 100 ppm Aroclor 1254, Curley et al. (1971) found a steady build up of PCB's in all tissues. After 240 days, over 1,000 ppm of PCB had accumulated in the rats (whole body basis) with the more highly chlorinated PCB's more common.

The ability of PCB's to cross the placenta of pregnant rabbits orally dosed with Aroclors 1221 and 1254 was reported by Grant et al., (1971b). Accumulation of Aroclor 1254 in fetal tissue was much greater than Aroclor 1221. Villeneuve et al. (1971) indicated that Aroclor 1254 when orally dosed is fetotoxic to rabbits at 12.5 mg/kg and above. Lower levels did not induce any adverse effects in rabbit fetuses, but did increase liver weight and induce microsomal enzymes. Aroclor 1221 at levels up to 25 mg/kg did not produce fetotoxic effects.

Dermal toxicity studies by Vos and Beems (1971) showed hyperplasia and hyperkeratosis of the follicular epithelium of rabbit ear skin after application of Phenoclor DP6 and Clophen A60, whereas, Aroclor 1260 caused minimal hyperplasia and hyperkeratosis. Subsequent studies revealed contamination of Phenoclor, and Clophen with tetra- and penta-chlorodibenzofurans (Vos et al., 1970).

Rhesus monkeys fed 300 ppm Aroclor 1248 for 3 months lost

hair from the head, neck, and back with isolated acneform lesions developing on areas devoid of hair. Swollen eyelids and lips were observed with a discharge exuding from the eyes. Liver hypertrophy (2.3% of body weight in controls, 4.5% in PCB group) was attributed mainly to a proliferation of the smooth endoplasmic reticulum. Hyperplasia and dysplasia of the gastric mucosa were suggestive of an eventual neoplastic transformation (Allen and Norback, 1973).

Fish

Two estuary fish, pinfish (Lagodon rhomboides) and spot (Leiostomus xanthurus), exposed to 5 ppb Aroclor 1254 in water died within 14 to 45 days. Spot appeared unaffected by exposure to 1 ppb for periods of up to 56 days. Symptoms exhibited by fish prior to death were not typical of organochlorine pesticide poisoning, pinfish developed fungus-like lesions on their body and spot ceased feeding, became emaciated, and developed ragged fins and lesions. Spot rapidly stored PCB's and maximum levels were reached in 12 to 28 days. Thereafter, the relative amount (ppm) was constant, but the absolute amount (μg) continued to increase as the fish grew. Maximum concentration was 37,000 times water levels. When transferred to water free of PCB's, Aroclor 1254, was slowly lost from tissues. After 84 days relative concentration (ppm) dropped 73%,

and absolute amounts dropped 61%. The relative amount of each isomer did not change during the experiments (Hansen et al., 1971).

Goldfish lost their appetite, turned from bright orange to pale yellow, lost coordination, and turned to a side position in water containing 0.5 ppm Clophen (Germany). Time for 50% mortality ranged from 5 days at 4 ppm to 21 days at 0.5 ppm in water. PCB's in tissues ranged from 250 ppm to 324 for 0.5 and 4.0 ppm, respectively (Hattula and Karlog, 1972).

Zitko (1970) determined Aroclor 1221 and 1254 to be lethal to Atlantic salmon (Salmo salar) at concentration above 2 mg/l and indicated that PCB's may be less toxic than chlorinated hydrocarbons.

Mayer et al. (1972) reported acute toxicity of PCB's to be low for cutthroat trout (Salmo clarki) with 96 hr. LC50 values ranging from 1,170 to 60,900 $\mu\text{g}/\text{l}$ for Aroclors 1221 to 1260. The acute toxicity of Aroclor 1254 and 1260 was much less than that of the less chlorinated PCB's. They felt that the toxicity data are misleading in that concentrations are considerably above the true solubilities of PCB's. Acute oral toxicity of Aroclors 1242, 1248, 1254 and 1260 to rainbow trout (Salmo gairdneri) was in excess of 1500 mg/kg.

Chronic toxicity tests on rainbow trout, channel catfish (Ictalurus punctatus) and bluegill (Lepomis macrochirus) revealed the less chlorinated PCB's to be more toxic than the more highly

chlorinated PCB's. The rainbow trout were found to be the most susceptible. Uptake in catfish appeared biased toward the more highly chlorinated components, and elimination was more rapid for the less chlorinated components after exposure was terminated.

Coho salmon (Onchorhynchus kisutch) fed dietary doses of 0.048, 0.48, 4.8, 48, and 480 μg Aroclor 1254/g of food did not display altered growth nor any other observable effects until death occurred in the highest levels after 260 days. Levels of PCB's in the fish plateaued after 112 days in the 0.048, 0.48, 4.8 $\mu\text{g}/\text{kg}$ groups at 0.47, 0.5, and 3.83 ppm, respectively. The two highest treated groups leveled off after 200 days at 57 and 659 ppm, respectively.

Hutzinger et al. (1972) investigated the metabolic behavior of pure mono-, di-, tetra-, and hexachlorobiphenyl isomers in pigeons, rats, and brook trout. Their results showed the conversion of 4 chloro-, 4,4' dichloro, and 2,2', 5,5' tetrachlorobiphenyl isomers into monohydroxylated derivatives by the rat and pigeon, whereas, no hydroxymetabolites were detected in the excreta of the brook trout. No hydroxylated products of 2,2', 4,4', 5,5' hexachlorobiphenyl were detected in the excreta of pigeons, rats, or brook trout.

EXPERIMENTAL

Equipment and Supplies

The gas chromatograph used throughout this study was a Varian Aerograph Series 1400 equipped with a tritium electron capture (EC) detector, Varian Aerograph Instruments, Inc., Walnut Creek, CA, connected to a chromocoder strip chart recorder, Barber-Coleman Co., Rockford, IL.

The Florisel, 60-100 mesh, used for sample cleanup is produced by Floridin Co. and was purchased from Fisher Scientific Co., Fairlawn, NJ.

The three syringes used were 5 μ l, 10 μ l and 100 μ l Hamilton syringes manufactured by Hamilton Co., Inc., Whittier, CA.

All solvents used were obtained from Mallinckrodt Chemical Works, St. Louis, MO. The petroleum ether and hexane were "Nanograde" quality, and the methylene chloride was redistilled reagent grade. Granular anhydrous sodium sulfate was also obtained from Mallinckrodt.

Source of PCB's and Oils

The commercial source of PCB's was Aroclor 1254 supplied by Monsanto Chemical Co., St. Louis, MO. Aroclor 1254 is a mixture of biphenyls with varying degrees of chlorination, having an average chlorine content of 54%.

Alaskan salmon oil was obtained from Moore-Clark Co., Anacortes, WA and the soybean oil was purchased from Central Soya Co., Chicago, IL.

Preparation of Diets

The control diet was prepared by the method of Castell et al. (1972), which briefly consists of mixing 35 g of the complete dry mix, as shown in Table 1, with 65 g of water and forming cubes of a size suitable for the trout to eat. Feed consumption was calculated on a dry-weight basis. The diet containing PCB's was prepared in the same manner as the control diet except PCB's were added to the salmon oil at a concentration of 300 mg/kg to bring the final level of PCB's in the diet to 15 ppm on a dry weight basis. The fish were fed twice daily.

Feeding Trials

Fish used in this feeding trial were a Mt. Shasta strain of rainbow trout (Salmo gairdneri). They were hatched and raised at the Food Toxicology and Nutrition Laboratory of the Department of Food Science and Technology. Water at this facility is supplied from a well at a year-around temperature of 11-12^oC. The trout were fed a fat-free diet for 3 months after hatching and were then transferred to a diet containing salmon oil as the source of lipid until experiments

Table 1. Composition of semi-synthetic trout diet.

Ingredient	Percent
Casein	49.5
Gelatin	8.7
Dextrin	15.6
Salmon oil	5.0
Soybean oil	5.0
Mineral mix ^{a/}	4.0
Carboxymethylcellulose	1.3
Cellulose (Alphacel) ^{b/}	7.7
Vitamin mix ^{c/}	2.0
Choline chloride (70%)	1.0
Vitamin E conc. (α -tocopherol 330 I. U. /g)	0.2

^{a/} Modified Barnhart-Tomarelli (3) salt mix (0.002% NaF and 0.02% CoCl₂ added).

^{b/} Nutritional Biochemicals Corporation, Cleveland, Ohio.

^{c/} Vitamins supplied at the following levels (mg/kg): thiamin (HCl), 64; riboflavin, 144; niacinamide, 512; biotin, 1.6; Ca D-pantothenate, 288; pyridoxine (HCl), 48; folic acid, 19.2; menadione, 16; cobalamine (B₁₂), 0.159; i-inositol (meso), 2500; ascorbic acid, 1200; para-amino-benzoic acid, 400; Vitamin A concentrate (250,000 I. U. /g), 200; Vitamin D₂ (500,000 I. U. /g), 8.

were begun. At the start of the feeding trial the trout were 14 weeks old and weighed an average of 0.77 grams.

Two hundred forty trout were started on the PCB diet and 160 on the control diet. At the end of 16 weeks, half of the trout on the PCB diet were tagged and transferred to the control diet. The two groups of trout were raised in separate 10 gallon tanks with flow rates of one gallon of water per minute. After 16 weeks both groups were transferred to identical 3 ft diameter 100 gallon tanks with flow rates of 5 gallons of water per minute. Samples of 20 fish were taken from each group every four weeks for the first 12 weeks and then the samples were reduced to ten fish every 4 weeks for a total of 32 weeks. At the end of 32 weeks, feeding of the trout was discontinued. After 8 weeks of starvation, two fish that had been on the PCB diet were analyzed for PCB content. At each sampling the fish in each tank were weighed by netting and placing them in a tared container of water. All samples were wrapped in aluminum foil and frozen until ready for analysis.

Extraction of Lipids

The number of fish used for each lipid extraction varied due to the growth of the fish. Ten fish from each group were homogenized and extracted at the 4 week sampling period and five fish for 8, 12, 16 and 20 week periods. At the 24, 28 and 32 week samplings, five fish

were homogenized and 50 g aliquots were taken for the lipid extractions.

The method used for extraction of fat was a modification of the procedure outlined in section 211.13f of the Pesticide Analytical Manual vol. 1 of the FDA (1968). Briefly, this method consisted of weighing 25-50 grams fish, grinding on a high speed blender, adding 100 grams anhydrous Na_2SO_4 and blending until the sample and Na_2SO_4 were mixed. One hundred fifty mls of petroleum ether was added and blended with the mixture for two minutes. The supernatant was decanted through a column of anhydrous Na_2SO_4 , 25 mm x 50 mm long and collected in a round-bottom flask. The procedure was repeated until 1000 ml of petroleum ether had been used in extraction of the fat. After evaporation of the petroleum ether the percent lipid in the fish was determined.

Sample Cleanup

The Florisil column chromatography technique of Bills and Sloan (1967) was employed with slight modifications to separate PCB's from the lipid material. A glass column 2.2 cm x 50 cm equipped with a Teflon stopcock was tightly packed with 3 cm glass wool, followed by 11.5 cm of Florisil and finally 2.5 cm of anhydrous Na_2SO_4 . The Florisil was heated for 13 hours at 130°C before use. Pretreatment of the packed column consisted of eluting with 50 ml

methylene chloride followed by 50 ml of petroleum ether. Fat samples of 0.5 g or less were placed on top of the column, washed into the column with three 5 ml portions of solvent, and eluted with a total of 250 ml of petroleum ether. The elute was collected from the column in a round bottom flask and evaporated on a rotary evaporator for transfer to a volumetric flask. Hexane was employed to bring the eluate up to a final volume of 1, 5, 10 or 25 ml, depending upon the concentration of PCB's in the sample.

GLC Separation of PCB's

The fish samples and standard solutions were analyzed on a 6 ft x 1/4 in O. D. glass column packed with 2% SE-30 and 2% QF-1 on 70/80 mesh Anakrom ABS, held at 180°C with a 25 ml/min flow of nitrogen. The injection port was at 240°C and the detector at 220°C.

Pathological Examination

At the end of the feeding trial, a fish pathologist examined liver tissue sections from 20 fish on the PCB diet and 20 fish on the control diet under a microscope. Bouin's fixative was used to preserve small pieces of liver which were later stained with hematoxylin and eosin from which sections were made by the method of Humason

(1963). Livers, gills, stomachs, muscle and visceral adipose were removed from these fish and analyzed for PCB residues.

RESULTS AND DISCUSSION

The GLC separation of Aroclor 1254 is shown in Figure 1. Similar chromatograms have been obtained by other investigators using similar columns and conditions (Armour and Burke, 1970; Tas and de Vos, 1971). Each peak does not necessarily represent a single compound but may contain several isomers and/or compounds of different chlorine content (Bagley et al., 1970; Koeman et al., 1969; Herring 1973). Quantitative data was obtained by comparing the height of peaks 4, 5, 6, 7, 9, 10, and 12 of an Aroclor 1254 standard with the peaks of a sample. Each of these peaks was assumed to be representative of the entire PCB mixture (Risebrough et al. 1970; Vermeer and Reynolds, 1970).

Accumulation of PCB's

The relative concentration (ppm) of PCB's in the lipid fraction of trout increased rapidly for the first 8 weeks of dietary exposure and then tended to equilibrate at approximately 95 ppm as shown in Figure 2. Rapid linear accumulation followed by an equilibrium between intake and elimination is consistent with the results obtained when PCB's were fed to coho salmon (Mayer et al., 1972). Goldfish and rainbow trout fed DDT showed this same trend, and the level at which equilibrium took place was dependent upon the dietary level of

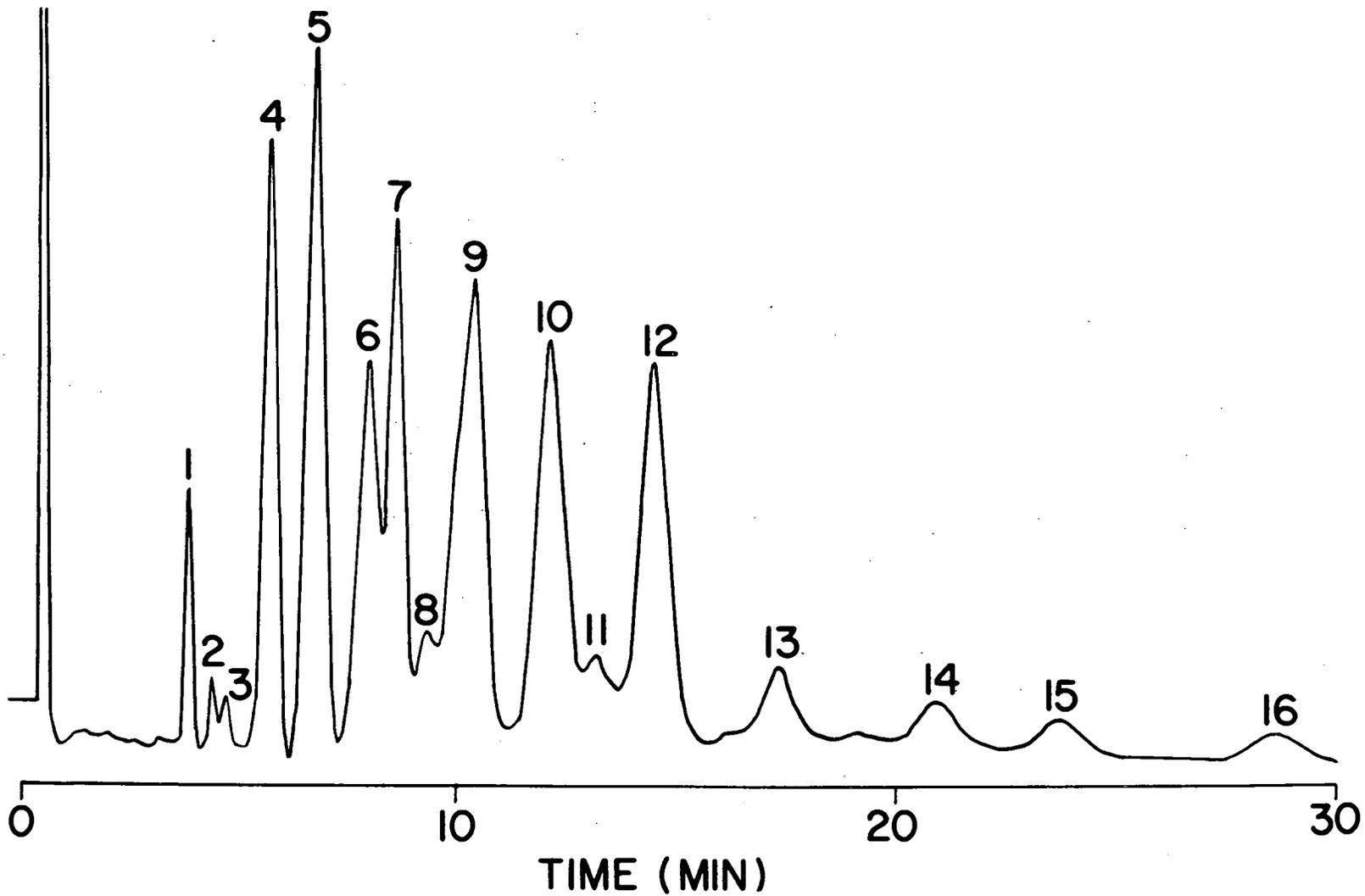


Figure 1. Gas chromatogram of Arochor 1254.

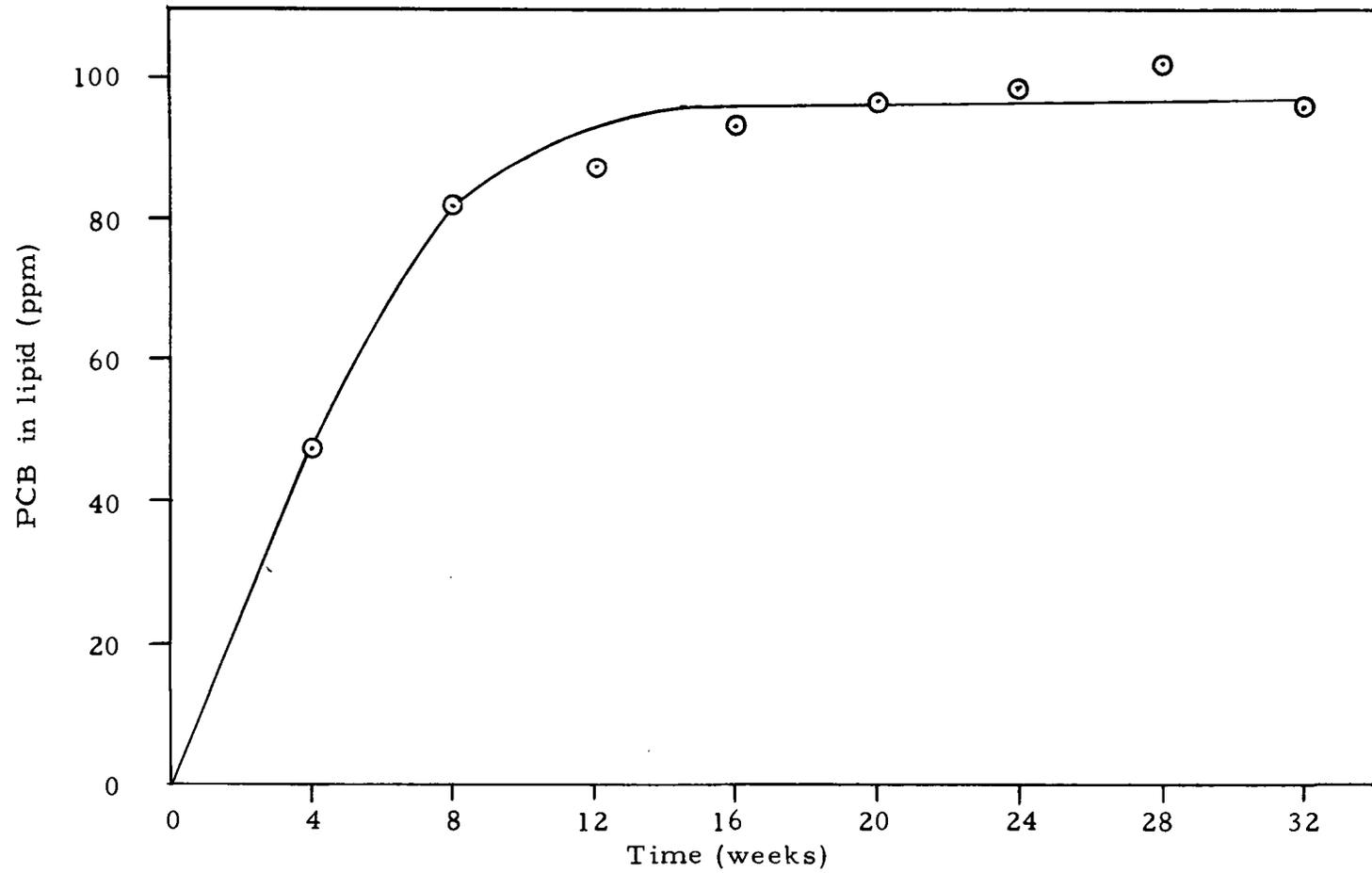


Figure 2. PCB in lipid fraction of rainbow trout.

DDT (Grzenda et al., 1970; Macek et al., 1970). The control fish contained less than 1 ppm background PCB in the lipid.

Figure 3 shows the level of PCB's on a whole-fish basis. Relative amounts (ppm) of PCB's accumulated in the trout continued to increase as the percent lipid increased (Table 2). The two flat areas on the curve correspond to the pattern of changes in the lipid content of the trout as they grew. The rapid increase of lipid content in the fish after 16 weeks was probably due to the transferring of the fish to larger tanks. As fish grow too large for a tank, vigorous feeding is inhibited, and transferring to a larger tank increases their ability to feed. As the percent lipid content of a trout becomes stable, the relative concentration of PCB's would be expected to equilibrate, as it appears to have after 24 weeks (Figure 3).

Table 2. Lipid content of rainbow trout (percent).

Time(weeks)	PCB diet	Control diet	PCB to control diet ^{a/}
4	4.45	4.42	--
8	5.58	5.57	--
12	5.71	5.71	--
16	5.64	6.18	--
20	7.47	6.59	6.84
24	8.51	7.86	8.15
28	8.45	8.84	9.32
32	8.52	8.25	8.40

^{a/} Transferred from PCB diet to control diet at end of 16 weeks.

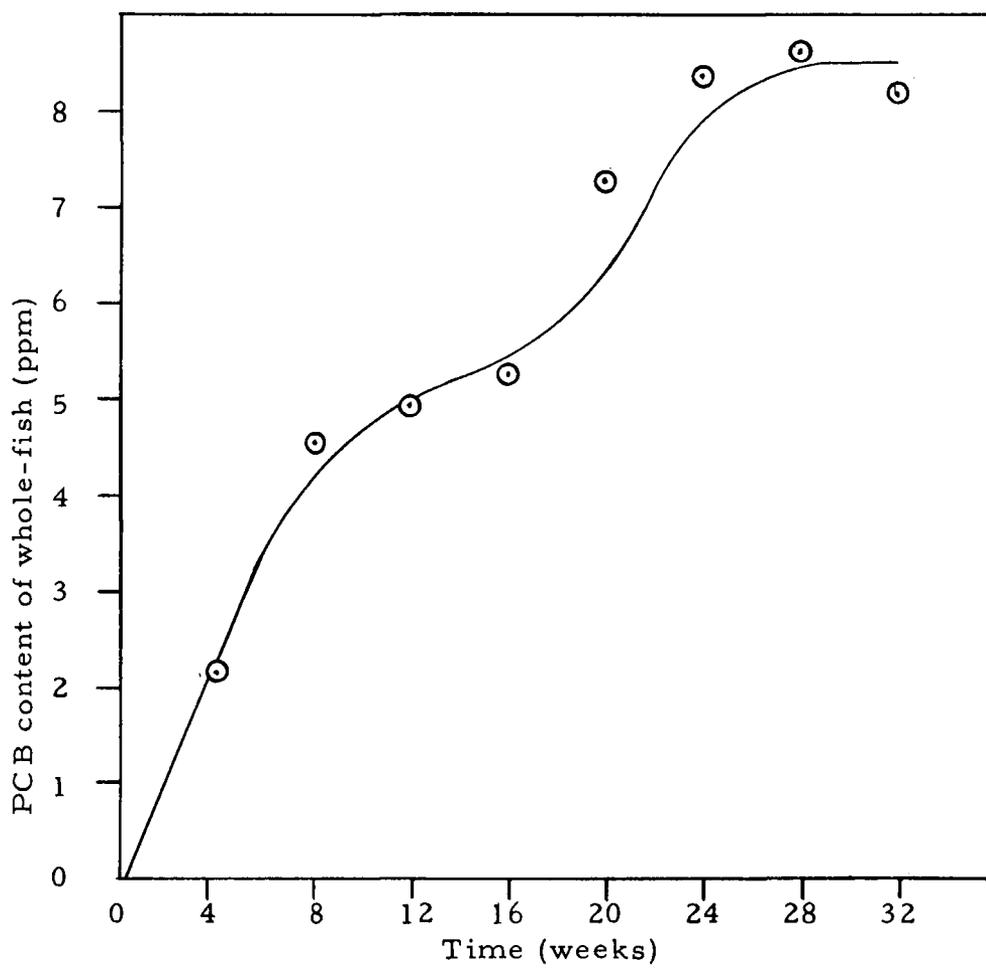


Figure 3. Concentration of PCB in rainbow trout (ppm, whole-fish basis).

While the relative concentration of PCB's reached equilibrium, Figure 4 shows that the absolute quantities (μg PCB/fish) rapidly increased as the trout grew. The increase in absolute quantities present in the trout closely followed the growth curve (Figure 5).

Relative amounts of PCB's in the lipid of the rainbow trout removed from the diet containing Aroclor 1254 at 16 weeks decreased rapidly. As shown in Figure 6, the level of PCB's in the extractable lipids dropped from 94 ppm to 13 ppm after 16 weeks on the control diet, but what appears to be a decrease in PCB's is merely a dilution of PCB's by the rapid growth of the rainbow trout. The actual μg of PCB's/fish remained constant, as shown in Figure 4. The inability of the rainbow trout to eliminate PCB's at a reasonable rate is surprising when compared to other animals. Catfish have been demonstrated to eliminate PCB's of lower chlorine content more effectively than the higher chlorinated isomers (Mayer et al., 1972). Spot after 84 days in water free of PCB's eliminated 61% of the PCB's from their body with no change in the relative ratios of each component (Hansen et al., 1971). Macek et al. (1970) predicted the half-life of DDT and dieldrin in rainbow trout to be 160 and 40 days respectively. During 168 days of exposure the fish exposed to 0.2 mg/kg of DDT per week assimilated and retained 20% and those exposed to 1.0 mg/kg per week retained 24%.

Table 3 shows the percent retention of PCB's available from

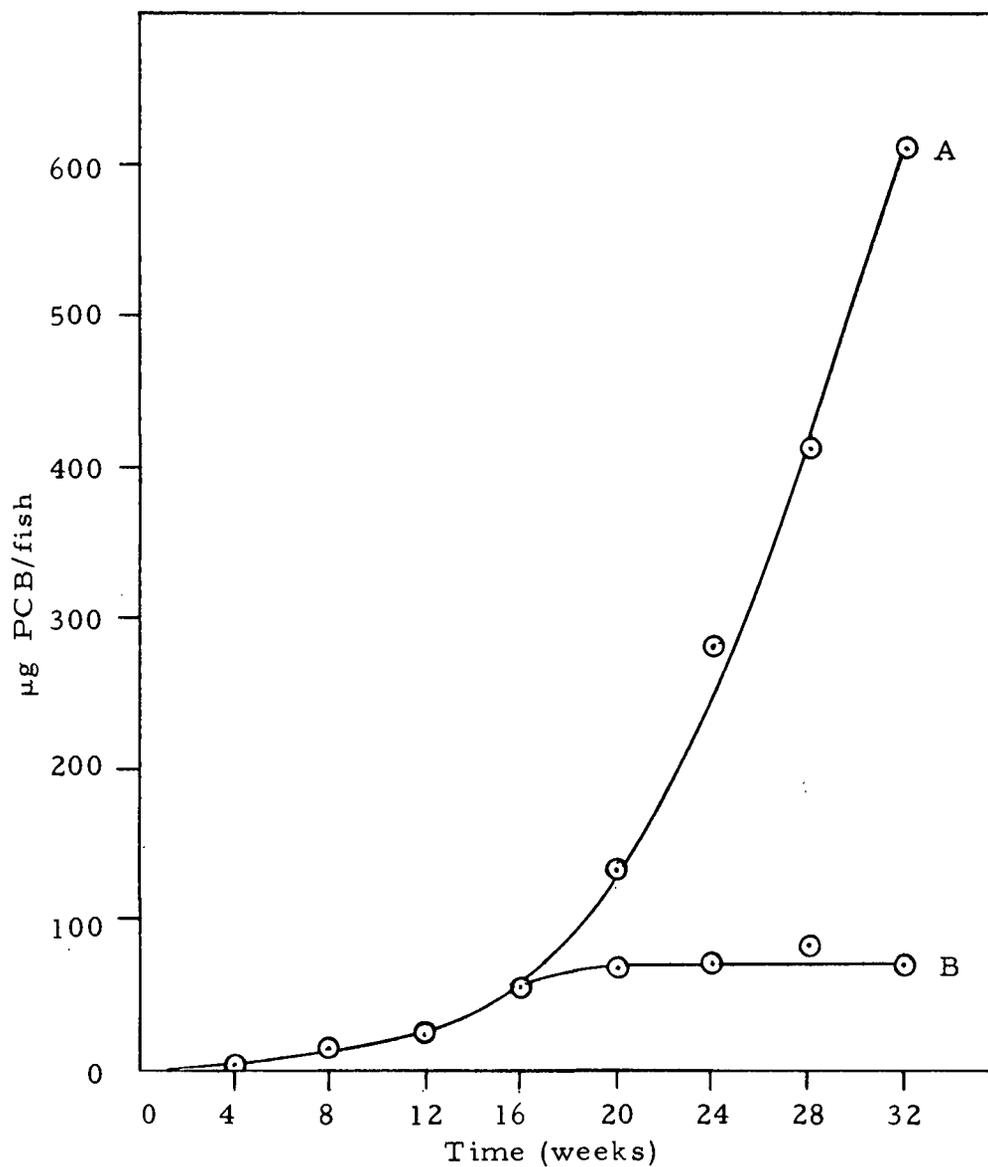


Figure 4. Total amount of PCB per fish (A) Fish on diet containing 15 ppm PCB. (B) Fish removed from diet containing 15 ppm PCB at end of 16 weeks.

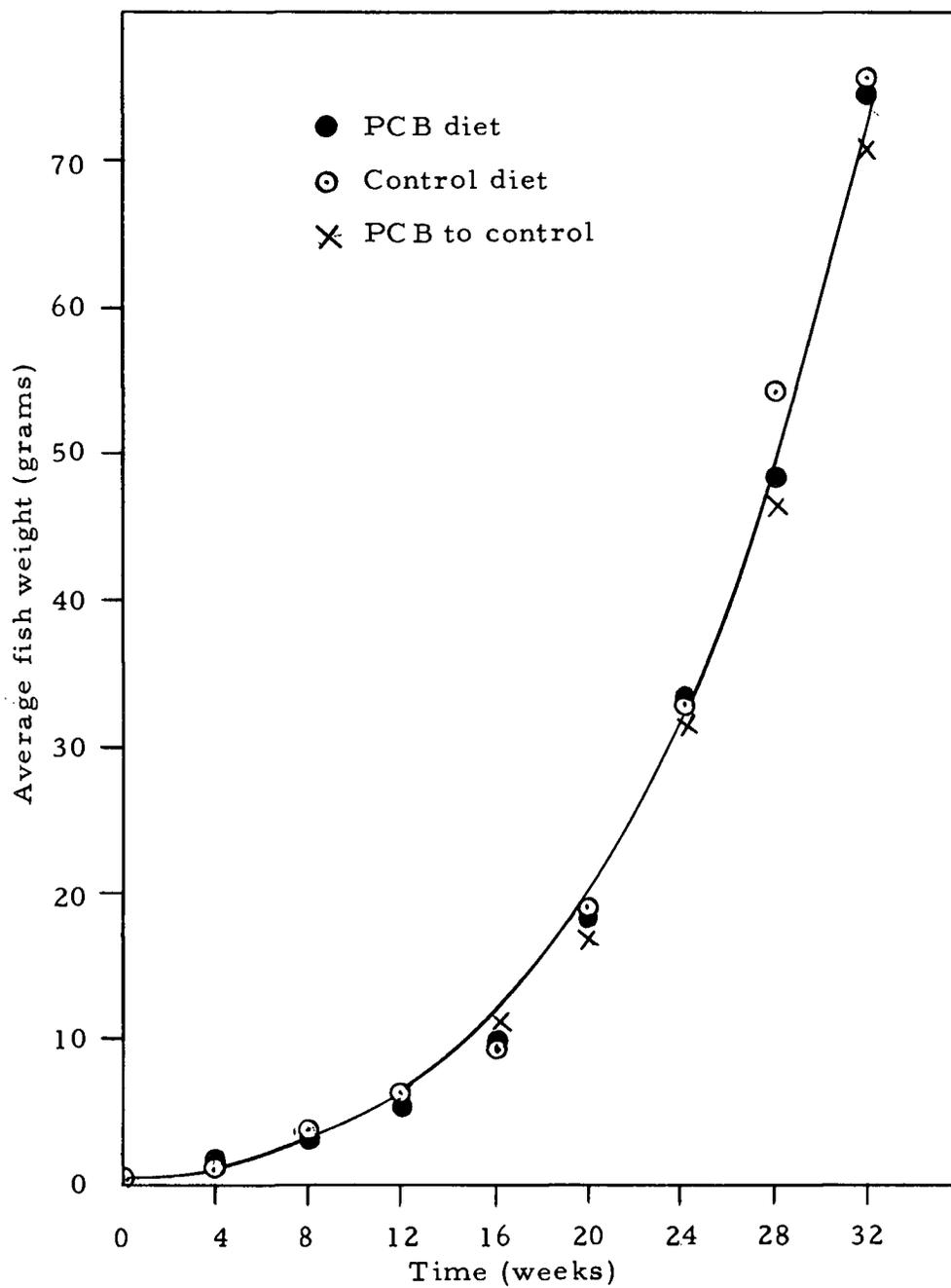


Figure 5. Growth of rainbow trout on three diet regimes.

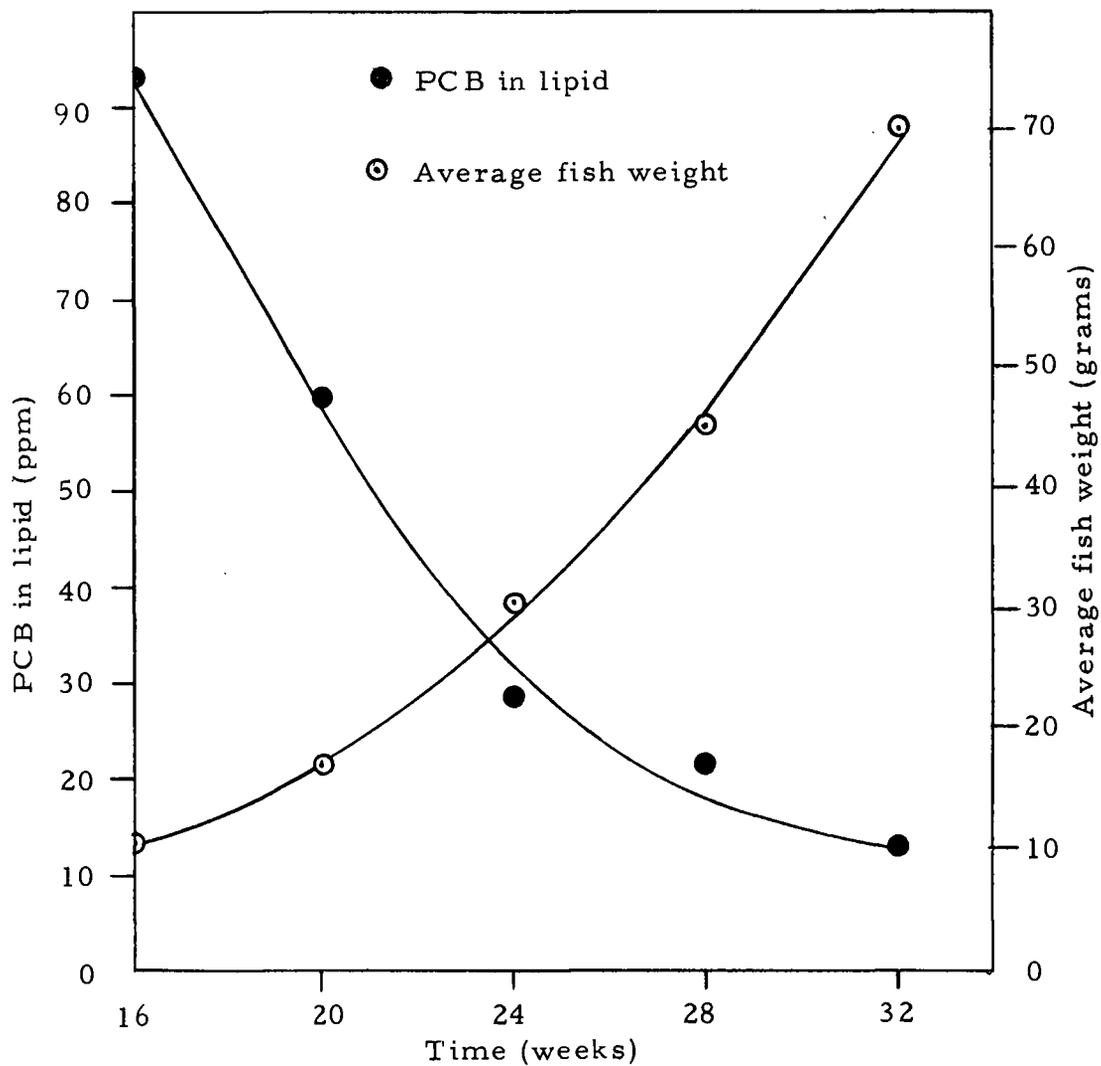


Figure 6. Concentration of PCB in lipid and growth of rainbow trout removed from diet containing PCB after 16 weeks.

the diet on a monthly basis, assuming that all PCB's absorbed were retained. While there is a large monthly variation, the total retention for 32 weeks (635.8 $\mu\text{g}/\text{fish}$ retained from 937.4 $\mu\text{g}/\text{fish}$ ingested) was 67.8%. Thus it appears that retention by rainbow trout is much greater for PCB's than for DDT.

Analysis of rainbow trout that had been on the PCB diet for 32 weeks and then starved for 8 weeks revealed that PCB's were not eliminated from the trout during the starvation period. While the lipid content decreased from 8.52% to 4.65% of the body weight, the relative concentration of PCB's in the lipid increased from 96 ppm to 190 ppm. The absolute quantities of PCB's in the fish after starvation was essentially the same as at the end of 32 weeks on the PCB diet at 635.8 $\mu\text{g}/\text{fish}$ and 617.5 $\mu\text{g}/\text{fish}$, respectively.

Metabolism

The observation that certain PCB peaks disappear or are reduced in size (usually the lower chlorinated PCB's) when PCB's are isolated from various species has been interpreted by some investigators to be indicative of metabolism of those PCB components (Grant et al., 1972; Bailey and Bunyan, 1972). Chromatograms of PCB's isolated from trout at all stages of the experiment were identical to those of the Aroclor 1254 standard, indicating that rainbow trout do not selectively absorb or metabolize PCB's. Hutzinger et al.

Table 3. Retention of ingested PCB's by rainbow trout.

Time (weeks)	Average μg PCB consumed/fish/4 week period	Total μg PCB accumulated/fish	$\Delta\mu\text{g}$ PCB/fish at end of 4 week period	% retention of PCB at end of 4 week period
4	11.0	3.2	3.2	29
8	19.9	15.0	11.8	59
12	31.6	26.2	11.2	35
16	45.4	55.1	28.9	64
20	98.7	133.2	78.1	79
24	191.1	276.4	143.2	74
28	196.9	404.0	127.6	65
32	<u>342.8</u>	635.8	231.8	68
Total ingested	937.4			

(1972) found that rats and pigeons formed hydroxymetabolites of pure 4-chloro-, 4,4'-dichloro-, and 2,2',5,5'-tetrachlorebipenyl, but they could not detect any hydroxymetabolites formed by brook trout. It is possible that trout do not have the mechanisms necessary to metabolize or eliminate PCB's absorbed into their bodies.

Distribution

The relative concentration of PCB's in the lipid fraction of various parts of the trout's body were very similar as shown in Table 4. Holden and Marsden (1967) published a similar finding for wild porpoises in which residues in tissues varied widely, but were in close agreement when expressed in terms of extractable lipid.

The lowest concentrations of PCB's were found in the liver, which does not agree with reports for other animals and fish. Distribution of residues in spot exposed to PCB's in water showed the liver to contain the highest concentrations after adipose tissue. This was also found with rats (Grant et al., 1971a; Curley et al., 1971). However, Macek et al. (1970) found that the levels of DDT were higher in the muscle than in the liver of rainbow trout fed DDT.

The lower levels of PCB's in the liver could be related to the inability of the trout to metabolize PCB's. Animals that have been found to contain higher levels of PCB's in their livers have also been demonstrated to metabolize or eliminate PCB's from their body,

Table 4. Distribution of PCB's in various tissues of the rainbow trout.

Tissue	% of lipid in tissue	Concentration of PCB in lipid (ppm)	Concentration PCB in tissue (ppm)
Viseral adipose	92.8	111	103.0
Gill	9.7	113	11.0
Muscle	2.7	104	2.8
Stomach	6.5	104	6.8
Liver	3.5	57	2.0
Whole fish	8.5	96	8.2

whereas we have demonstrated that rainbow trout do so, slowly, if at all.

Physiological Changes

The affinity of organochlorine compounds for lipids has been well established, and the relationship of exposure to these compounds and lipid content has been investigated more recently. Buhler et al. (1969) reported the lipid content of coho salmon increased as the DDT content of the diet increased. Macek et al. (1970) found that feeding rainbow trout either DDT or dieldrin at 1.0 mg/kg per week would significantly increase the lipid content in the fish. With rats fed PCB's, the lipid content as well as the size of the liver increased (Grant et al., 1970a). Table 1 shows that the lipid content of the rainbow trout fed PCB's was not very different from that of the controls. At the end of 32 weeks the fish on PCB's had 8.5% lipid and the controls had 8.3% lipid. Increases in the size of livers of fish on the PCB diet were not significantly larger than the controls at 1.48 and 1.46 percent of body weight, respectively.

Macroscopic examination of the fish both internally and externally did not reveal any noticeable differences, and microscopic examinations of liver tissues did not show any damage from the ingestion of PCB's. The growth curves of the trout from each group, as shown in Figure 5, reveal that growth was not inhibited by PCB's

in the diet. No mortalities were attributed to the ingestion of PCB's.

Conclusions

The following conclusions were drawn from the data that resulted from this investigation:

1. The relative concentrations (ppm) of PCB's became constant but absolute quantities increased as the trout grew.
2. PCB's were found at fairly constant level in the lipids of various tissues.
3. The elimination of PCB's from the body of rainbow trout occurred very slowly if at all.
4. Chromatograms of samples and standards were identical throughout the experiment suggesting that PCB's are metabolized slowly if at all.
5. The percent retention of PCB's from the diet was greater than that of DDT.
6. Pathological examination revealed no damage or abnormalities and no mortalities occurred from the ingestion of PCB's.

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