fecal tritium curve rather than to the urine curve. AFM_1 is apparently associated with the protein fraction in milk (Allcroft and Carnaghan, 1963). AFM_1 may also associate with protein fractions in the ruminal environment and be carried through the intestine in a form similar to that secreted in milk. According to AFM_1 analysis, the content in milk increased up to 3 or 4 days during chronic dosing at which time it plateaued. The radioactive secretion in milk reflects a similar pattern. Considering both radioactivity and chemical analysis, milk is obviously not a principal excretory route for aflatoxin. Evaluation of total distribution patterns is not possible with these data in that labeled materials by all excretory routes after 96 hr accounted for less than 15% of the AFB_1 dose in each animal. The tritium unaccounted for may be present in tissue. A variety of tissues are currently being analyzed.

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Accumulation of Dietary Polychlorinated Biphenyls (Aroclor 1254) by Rainbow Trout (*Salmo gairdneri*)

Andrew J. Lieb, Donald D. Bills,* and Russell O. Sinnhuber

The accumulation of PCB's (Aroclor 1254) by a Mt. Shasta strain of rainbow trout (*Salmo gairdneri*) from a dietary level of 15 ppm was determined using a gas chromatograph equipped with an electron capture detector. The relative concentration (parts per million) of PCB's in the fish stabilized while absolute quantities (micrograms of PCB/fish) increased as the fish grew. The total retention of PCB's from the diet was 68% for a 32-week feeding period. The distribution of PCB's was fairly constant in the lipid portion of various tissues. PCB's did not appear to be eliminated from the trout after PCB exposure ceased even when the fish were starved. The fish did not appear to be adversely affected by the PCB's and no mortalities were attributed to PCB toxicity.

Polychlorinated biphenyls (PCB's) are industrial chemicals which are widely distributed in the environment (Jensen *et al.*, 1969; Risebrough *et al.*, 1968). Due to their chemical and physical characteristics, PCB's are persistent, and they accumulate in the food chain in much the same manner as the organochlorine pesticides. Many species of fish and wildlife contain concentrations of PCB's that possibly could cause adverse effects. Of particular importance are those species of fish used for human consumption and animal feed. Atlantic salmon (*Salmo salar*) caught off Canada contained 0.45–0.62 ppm of PCB's (Zitko *et al.*, 1972). Lake Erie fish analyzed during 1970–1971 contained detectable residues of PCB's with average levels for different species ranging from 0.08 to 4.4 ppm. Coho salmon from Lake Erie averaged 2.1 ppm of PCB's (Carr *et al.*, 1972). This study was initiated to follow the accumulation in and to determine possible adverse effects of dietary PCB's on rainbow trout.

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EXPERIMENTAL PROCEDURES

Preparation of Diets. The control diet was prepared by the method of Castell *et al.* (1972), which consists of mix-

Table I. Composition of Semisynthetic Trout Diet

Ingredient	%
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 concd (α-tocopherol 330 I.U./g)	0.2

[a] Modified Barnhart–Tomarelli (1966) salt mix (0.002% NaF and 0.02% $CoCl_2$ added). [b] Nutritional Biochemicals Corporation, Cleveland, Ohio. [c] Vitamins supplied at the following levels (mg/kg): thiamin (HCl), 64; riboflavine, 144; niacinamide, 512; biotin, 1.6; calcium D-pantothenate, 288; pyridoxine (HCl), 48; folic acid, 19.2; menadione, 16; cobalamine (B_{12}), 0.159; isoinositol (meso), 2500; ascorbic acid, 1200; *p*-aminobenzoic acid, 400; vitamin A concentrate (250,000 I.U./g), 200; vitamin D_2 (50,000 I.U./g), 8.

ing 35 g of the complete dry mix, as shown in Table I, with 65 g of water and forming cubes of a size suitable for consumption by the trout. The diet containing Aroclor 1254 (supplied by Monsanto Chemical Co.) 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.

Feeding Trial. 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, Oregon State University, Corvallis, Ore. Water at this facility is supplied from a well at a year-round temperature of 11–12°. 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 were begun. At the start of the feeding trial the trout were 14 weeks old and weighed an average of 0.77 g.

The PCB diet was administered to 240 trout, and 160 trout were maintained on the control diet. The two groups of trout were raised in separate 10-gal tanks with flow rates of 1 gal of water/min. The fish were fed twice daily. At the end of 16 weeks, half of the trout on the PCB diet were tagged and transferred to the control diet. All fish were then transferred to identical 100-gal tanks, 3 ft in diameter with flow rates of 5 gal of water/min. Samples of 20 fish were taken from each group every 4 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 Aroclor 1254 diet were analyzed for PCB's. At each sampling the entire population of fish in each tank was weighed by netting and weighing in a tared container of water. Sacrificed fish were wrapped in aluminum foil and frozen until analyzed.

Extraction of Lipid and PCB's. The number of fish used per extraction varied as the fish grew. Ten fish from each group were homogenized and extracted at the 4-week sampling period and five fish for the 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 lipid extraction was a modification of the procedure outlined in the "Pesticide Analytical Manual" (1968). Briefly, this method consisted of weighing 25–50 g of fish, grinding on a high-speed explosion-proof blender, adding 100 g of anhydrous Na_2SO_4, and blending until the sample and Na_2SO_4 were mixed. Petroleum ether (150 ml) (Nanograde, Mallinckrodt) was added and blended with the mixture for 2 min. The supernatant was decanted through a column of anhydrous Na_2SO_4, 25 mm × 50 mm, and collected in a round-bottomed flask. The procedure was repeated until 1000 ml of petroleum ether had been used. After evaporation of the petroleum ether, the lipid was weighed, and the per cent lipid in the sample was calculated. Compared to Soxhlet extraction, lipid and PCB's were recovered at an efficiency of 97%.

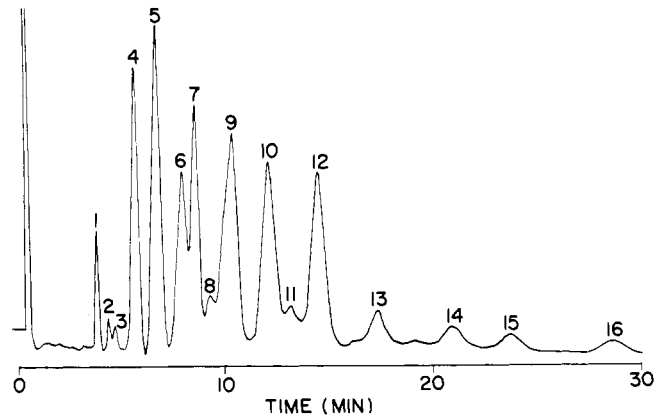

Figure 1. Gas chromatographic separation of Aroclor 1254.

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 × 50 cm equipped with a Teflon stopcock was tightly packed with 3 cm of 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 hr at 130° before use. The packed column was pretreated with 50 ml of methylene chloride (distilled over glass) followed by 50 ml of petroleum ether. Fat samples of 0.5 g or less were placed on the column and eluted with a total of 250 ml of petroleum ether. The eluate was collected in a round-bottomed flask and evaporated on a rotary evaporator for transfer to a volumetric flask. Hexane (Nanograde, Mallinckrodt) was employed to bring the eluate to a final volume of 1, 5, 10, or 25 ml, depending upon the concentration of PCB's in the sample. Recovery of Aroclor 1254 through this cleanup procedure approaches 100%. Florisil must not be activated in excess of the above heat treatment if quantitative recovery of all PCB components is to be attained.

Gas Chromatographic Analysis. A Varian Aerograph Series 1400 gas chromatograph equipped with a tritium electron capture detector was used in all analyses. The glass column was 6 ft × 1/8 in. i.d. packed with 2% SE-30 and 2% QF-1 on 70–80 mesh Anakrom ABS. The flow rate of nitrogen carrier gas was 25 ml/min. The column, injection port, and detector temperatures were 180, 240, and 220°, respectively.

Pathological Examination. At the end of the feeding trial, liver tissue sections from 20 fish on the PCB diet and 20 fish on the control diet were examined. Small pieces of liver were fixed in Bouin's solution and stained with hematoxylin and eosin. Sections were made by the method of Humason (1962). Livers, gills, stomachs, muscle, and visceral adipose were removed from these fish and analyzed for PCB residues.

All analyses throughout this experiment were conducted in duplicate or, when sample size permitted, in triplicate. Results of replicate analyses for PCB residues in tissues agreed within ±5% maximum in all cases.

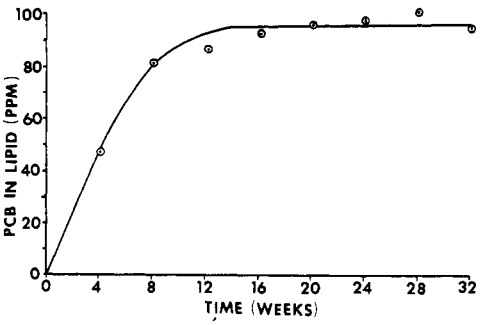

Figure 2. PCB in lipid fraction of rainbow trout.

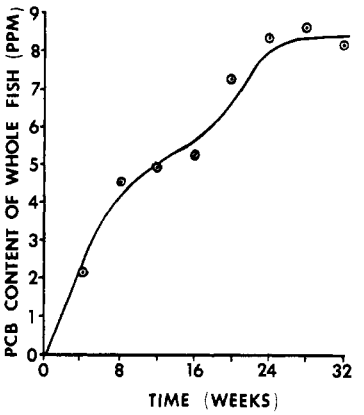

Figure 3. Concentration of PCB in rainbow trout (parts per million, whole-fish basis).

RESULTS AND DISCUSSION

The gas chromatographic 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). Quantitative data were 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 (parts per million) 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 DDT (Grzenda *et al.*, 1970; Macek *et al.*, 1970). The control fish contained less than 1 ppm of background PCB in their lipids.

Figure 3 shows the level of PCB's on a whole fish basis. Relative amounts (parts per million) of PCB's in the whole fish continued to increase as the per cent lipid increased (Table II). 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 transferring the fish to larger tanks. In a small tank, vigorous feeding is inhibited, and transferring to a larger tank increased the feed intake of trout. As the per cent lipid content of the trout became stable after 24 weeks, the relative concentration of PCB's appeared to equilibrate (Figure 3).

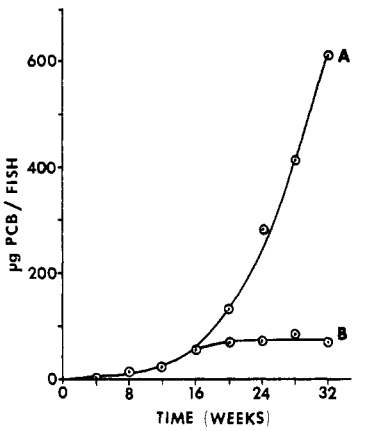

Figure 4. Total amount of PCB per fish: (A) fish on diet containing 15 ppm of PCB; (B) fish removed from diet containing 15 ppm of PCB at end of 16 weeks.

Table II. Lipid Content (%) of Rainbow Trout

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 to control diet at end of 16 weeks.

While the relative concentration of PCB's reached equilibrium, Figure 4 shows that the absolute quantities (micrograms of PCB/fish) continued to increase as the trout grew. The increase in absolute quantities of PCB's 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 to 13 ppm after 16 weeks on the control diet. However, the decrease in the relative concentration of PCB's was apparently only a dilution of PCB's by the rapid growth of the trout since the absolute amount 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 in contrast to studies with other species of fish. Catfish have been demonstrated to eliminate PCB's of lower chlorine content more effectively than the more highly chlorinated isomers (Mayer *et al.*, 1972). Spot, an estuarine fish, eliminated 61% of the absolute concentration of PCB's from their body with detectable changes in the relative ratios of PCB components after 84 days in water free of PCB's (Hansen *et al.*, 1971). The Fish-Pesticide Research Laboratory (Schoettger, 1973) reported that when juvenile lake trout were fed Aroclor 1248 at concentrations of 0.1, 1.8, and 6.0 ppm in the diet for 320 days and then fed uncontaminated food for 60 days, residues declined 31, 15, and 12%, respectively. This finding would suggest that there is a very limited mechanism for removal of PCB residues in this species of trout.

Table III shows the per cent retention of PCB's available from the diet on a monthly basis. While there is a large monthly variation, the total retention for 32 weeks was 67.8%. It appears that retention of PCB's by rainbow trout is much greater than DDT.

Analysis of rainbow trout that had been on the PCB diet for 32 weeks and then starved for 8 weeks revealed

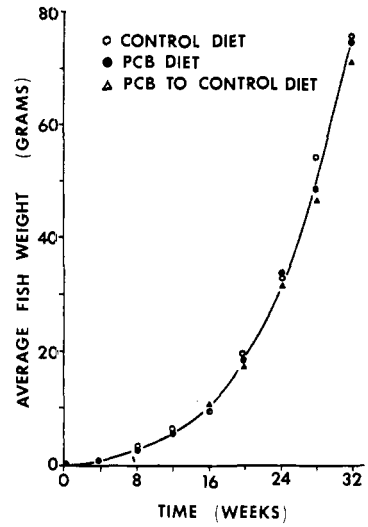

Figure 5. Growth of rainbow trout on three diet regimens.

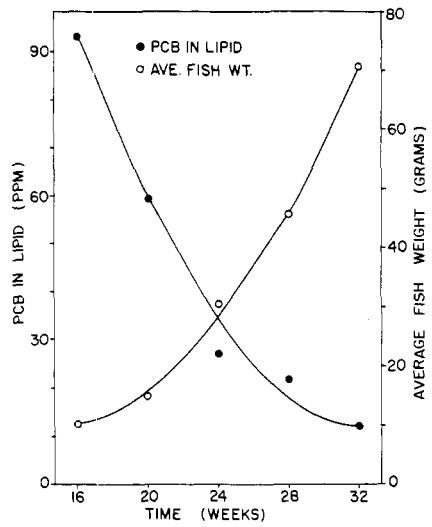

Figure 6. Concentration of PCB's in lipid and growth of rainbow trout removed from diet containing PCB's after 16 weeks.

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 to 190 ppm. The absolute quantities of PCB's in the fish after starvation (617.5 μg/fish) were essentially the same as at the end of 32 weeks on the PCB diet (635.8 μg/fish).

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.*, 1971; Bailey and Bunyan, 1972). Chromatograms of PCB's isolated from trout at all stages of the experiment were identical with those of the Aroclor 1254 standard, indicating that rainbow trout do not selectively absorb or metabolize PCB's. Hutzinger *et al.* (1972) found that rats and pigeons formed hydroxy metabolites of pure 4-chloro-, 4,4′-dichloro-, and 2,2′,5,5′-tetrachlorobiphenyl, but they could not detect any hydroxy metabolites formed by brook trout. It is possible that trout do not have a hydroxylating mechanism for metabolizing PCB's.

Distribution. The relative concentrations of PCB's in the lipid fraction of various trout tissues except the liver were very similar as shown in Table IV. Holden and Marsden (1967) reported that tissue residues of PCB's in

Table III. Retention of Ingested PCB's by Rainbow Trout

Time, weeks	Av μg of PCB consumed/ fish per 4-week period	Total μg of PCB accum./fish	% retention of total PCB ingested
	11	3	27
8	23	15	48
12	32	26	41
16	45	55	51
20	99	133	64
24	191	276	69
28	197	404	68
32	343	636	68
Total ingested	938		

Table IV. Distribution of PCB's in Tissues of Rainbow Trout

Tissue	% lipid in tissue	Concn of PCB in lipid, ppm	Concn of PCB in tissue, ppm
Visceral adipose	92.8	111	103
Gill	9.7	113	11.3
Muscle	2.7	104	2.8
Stomach	6.5	104	6.8
Liver	3.5	57	2.3
Whole fish	8.5	96	8.2

wild porpoises 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. This finding does not agree with reports for other animals and fish. In an estuarine fish, spot, which has been exposed to PCB's in water, the liver contained the highest concentration after adipose tissue (Hansen *et al.*, 1971). This observation has also been made for rats (Grant *et al.*, 1971; Curley *et al.*, 1971).

The lower levels of PCB's in the liver might 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 eliminate PCB's from their body, 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.*, 1971). Table II shows that the lipid content of rainbow trout fed PCB's was not very different than that of the controls. At the end of 32 weeks, the livers of fish on PCB's contained 8.5% lipid and the controls contained 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% 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 disclose any damage or changes 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.

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Chemical and Metabolic Characteristics of 1-Naphthyl β-D-Glucoside

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1-Naphthyl β-D-glucoside was stable under a variety of conditions encountered in metabolism studies. No degradation occurred when stored in methanol or in Tris-HCl buffer (pH 7.0) for 1 week at −20, 0, and 25°. The glucuronide of 1-naphthol was degraded by about 30% after 1 week in the buffer at 25°, but was equal in stability to the glucoside in all other experiments. When 1-naphthyl-^{14}C glucoside was given orally to rats, 67% of the radiocarbon was eliminated in urine after 24 hr. Of this, 28% was the administered compound, 35% 1-naphthyl glucuronide, 15% 1-naphthyl sulfate, and 14% was 1-naphthol. Cleavage of the glucoside linkage was possibly the sole initial step in the metabolism of the compound. However, 1% of a 1-naphthyl glucoside-*glucose*-^{14}C dose was tentatively identified as 1-naphthyl glucuronide, indicating that oxidation of the sugar moiety of intact 1-naphthyl glucoside may have taken place. 1-Naphthol-^{14}C given as a single oral dose was eliminated from rats more rapidly, 90% of the dose in the 0–24-hr urine, than was its glucoside conjugate. 1-Naphthyl glucuronide constituted 81% of the radiocarbon while 17% was 1-naphthyl sulfate and only 1.6% was free 1-naphthol.

Defining the metabolic pathways of pesticides in various biological systems is essential if proper evaluations of their safety are to be conducted. This has long been recognized and has been attempted at varying degrees since the introduction of synthetic organic pesticides. Major emphasis has been placed on metabolites formed by oxidation and/or hydrolysis, and which exist in the free form. Other metabolites, however, are biosynthesized from pesticides which also are potentially toxicologically significant. Conjugation is one of the more important mechanisms of pesticide metabolism, especially in the substituted phenyl carbamate insecticides (Dorough, 1970; Kuhr, 1970). With these chemicals, the majority of the terminal residues in animals and plants may exist as conjugates, many of which contain the intact carbamic acid ester.

Glucuronidation is the most important conjugation mechanism in animals while glucosylation is a major conjugation reaction in plants. Insects, unlike other animals, form glucosides rather than glucuronides. These reactions have been recently investigated in our laboratory using 1-naphthol-^{14}C as a substrate, and insect and rat liver homogenates as enzyme sources (Mehendale and Dorough, 1972a,b). The current study is an extension of these studies, but rather than being concerned with the biosynthesis of glycosides, this report deals with the fate of 1-naphthyl glucoside in rats and with the development

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