

## **Accumulation of PCBs in Hatchery Trout**

Prepared by:

Robert F. Carline  
U.S. Geological Survey, Biological Resources Division  
Pennsylvania Cooperative Fish & Wildlife Research Unit  
Merkle Laboratory  
University Park, PA 16802

Patrick M. Barry  
The Pennsylvania State University  
School of Forest Resources  
University Park, PA 16802

H. George Ketola  
U.S. Geological Survey, Great Lakes Science Center  
Tunison Laboratory of Aquatic Science  
3075 Gracie Road  
Cortland, NY 13045

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## EXECUTIVE SUMMARY

Routine testing in 1998 revealed polychlorinated biphenyls (PCBs) in edible tissues of trout from Pennsylvania Fish and Boat Commission (PFBC) hatcheries. PCB levels in some tissue samples exceeded thresholds for the issuance of consumption advisories. Preliminary testing also revealed low levels of PCBs in commercial trout diets. Although all PFBC hatcheries use the same commercial feeds, PCB levels in trout vary among hatcheries, which suggests that hatchery water supplies and trout feeds are both contributing to PCBs in trout flesh. This study focused on the role of fish feeds in the accumulation of PCBs in hatchery-reared trout. The objectives were to identify potential sources of PCBs in hatchery trout, to determine the relation between PCB concentrations in diets and body burdens of PCBs in hatchery trout, to determine if PCB concentrations in hatchery trout vary seasonally, and to determine the assimilation rate and net accumulation of PCBs in hatchery trout. A 6-month feeding trial using four experimental diets was employed to determine the relation between PCB concentrations in feed and fish fillets. A 12-month feeding trial using one diet was conducted to examine seasonal variation in PCB concentrations in trout fillets. And, a 3-week experiment was run to measure net absorption of PCBs by trout.

The study was conducted at the Benner Spring Fish Research Facility. We fed groups of rainbow trout four specially formulated diets with PCB concentrations ranging from 69 to 280 ng/g. Trout were sampled monthly to assess growth and PCB concentrations in fillets and in the remainder of the carcass. Semi-permeable membrane devices (SPMD) were employed in the hatchery supply water to detect the presence of PCBs. At the end of the feeding trial, chromic oxide was added to each of the diets, which were fed for 2 weeks. Analyses of feces for chromic oxide and PCBs were used to compute net absorption of PCBs. One group of trout was fed one of the diets for 12 months and these fish were sampled monthly to assess growth and PCB levels in fillets.

Trout grew from 22 g to 190 g after 6 months and there was no relation between growth and PCB concentrations in feed. After trout fed for one month, PCB concentrations in fillets reached levels proportional to PCB concentrations in their feed and remained at that level for the remainder of the trial. We found a positive relationship between concentrations of PCBs in feed and in trout fillets, which ranged from 54 to 94 ng/g. Net absorption of PCBs averaged 87% and was not affected by PCB concentration. The SPMDs revealed low levels of PCBs in hatchery supply water. We computed a mass balance budget for PCBs by accounting for the amount consumed, assimilated, and stored in tissues. This analysis suggested that the entire body burden of PCBs could be accounted for by PCBs in the feed and that uptake of PCBs from supply water was not an important source of contamination. There was no evident seasonal variation in PCB concentrations of trout fed for 12 months. After 6 months, PCB concentrations in fillets gradually increased, which was due in part to increases in the lipid content of trout flesh.

We conclude that the FDA standard of <200 ng/g (0.2 ppm) PCBs in finished fish feeds is adequate to ensure that trout raised to catchable size will have fillet concentrations <100 ng/g PCBs, the level at which Pennsylvania advises the public to

limit fish consumption to one meal per week or less.

## INTRODUCTION

Routine testing by the Pennsylvania Fish and Boat Commission (PFBC) and Department of Environmental Protection in 1998 revealed polychlorinated biphenyls (PCBs) in edible tissues of trout from PFBC hatcheries. Pennsylvania, along with other states bordering the Great Lakes, has adopted consumption advisory limits for PCBs and other contaminants in sport fish caught in the recreational fishery. The Pennsylvania agencies (PFBC, Department of Environmental Protection, and Department of Health) agreed that the advisory thresholds for Great Lakes sport fish should also be applied to sport fish from other inland water bodies and for hatchery-reared trout. If PCB concentrations in edible fish tissue equal or exceed 0.1 ppm, the Commonwealth's advisory suggests that the public should not eat more than one meal of fish per week. At 0.2 ppm, the public is advised to not eat more than one meal per month, at 1.0 ppm consumption should be limited to one meal every two months, and at 2.0 ppm no consumption is advised. Trout from one Pennsylvania hatchery in 1998 exceeded some of these advisory limits, and since then, trout from other hatcheries have occasionally exceeded advisory limits. No trout samples have exceeded the FDA's Action Limit, which is 2.0 ppm. These discoveries of PCBs in hatchery trout have prompted the PFBC to determine the source or sources of these contaminants.

The presence of PCBs in trout from hatcheries around the state suggests that there may be a common source of PCBs, e.g., the commercial fish diets, which contain fish meal and fish oils. PCBs are lipid soluble; hence, they may be found in elevated concentrations in fatty tissues but may not be detectable in water from which the fish are collected. Recent analyses of components of fish food and the finished product have confirmed the presence of PCBs, but concentrations of PCBs in finished feed never exceeded the FDA standard of 0.2 ppm. PCBs, like other contaminants, are biomagnified. Therefore, it would seem logical that hatchery trout could have higher concentrations of PCBs than those in their food. PCBs in hatchery supply water may also contribute to body burdens in trout.

This study focused on rates of PCB accumulation by hatchery trout in relation to PCB concentrations in the fish food. Our objectives were to identify potential sources of PCBs in hatchery trout, determine the relation between PCB concentrations in diets and body burdens of PCBs in hatchery trout, determine if PCB concentrations in hatchery trout vary seasonally, and determine the assimilation rate and net accumulation of PCBs in hatchery trout.

To determine the relation between PCB concentrations in fish diets and fish tissue, we conducted a 6-month feeding trial using four diets with different concentrations of PCBs. At the end of the 6-month trial, a 3-week experiment was conducted to determine the assimilation rate of PCBs. One diet group of trout was fed for an additional 6 months to determine if PCB concentrations varied by season.

## METHODS

The study was conducted at the Benner Springs Fish Research Station in Benner Township, Pennsylvania. Fish were cultured indoors in 15 circular tanks each holding 950 L of water. Initially, well water was supplied to each tank at a rate of 45 L/min; during month 6 of the study, inflow was reduced to 36 L/min. A screen-covered standpipe in the middle of each tank provided for outflow. Holes along the entire length of the standpipe allowed water from the entire water column to exit the tank. Overhead lighting consisted of fluorescent light fixtures covered with plastic plates. Photoperiod was adjusted weekly to simulate outdoor conditions. Light intensity was not measured; we estimated it was 300 lux over the culture tanks.

We used four different diets that had similar proximate compositions (Table 1), but they varied in source of the fish meal and fish oil and in concentration of PCBs. All diets were produced by Zeigler Bros., Inc., Gardiner, Pennsylvania. Diet 1 was designed to have the lowest PCB concentration; it differed from the other diets in that herring (species are not identified) meal and deodorized menhaden oil (crude fish oil that is cold filtered to remove stearine portion of the lipids and steam distilled to remove flavor and odor) were used. Diet 2 was made with menhaden meal and winterized menhaden oil (cold filtered only); these ingredients are normally used in trout diets supplied to the PBFC, with one exception. Feeds used for trout production are made with crude (unfiltered) menhaden oil, which is nutritionally equivalent to cold filtered oil (personal communication, T. Markey, Zeigler Bros., Inc.); hence, Diet 2 can be described as the standard production diet. Diets 3 and 4 were made of the same ingredients as Diet 2, except that Aroclor 1260 (Chem Service, Inc., West Chester, PA) was added so as to approximate 50% and 100% more PCBs than in Diet 2. Diets were made into 3/32-inch diameter and 5/32-inch diameter pellets. After 3 months of feeding, fish were switched from the smaller to the larger diameter pellets.

On July 3 and 5, 2000, rainbow trout were collected from hatchery raceways, brought indoors, anesthetized with MS-222 (tricaine methanesulfonate), and sorted by total length (115 - 135 mm). Sorted trout were placed 25 at a time into buckets and added serially to one of 15 tanks until each tank contained 300-350 fish. Although we intended to allocate 350 trout per tank, we discovered at the end of month 2 that not all tanks received the intended allocation. Three tanks of fish were assigned to each of the four diets used in the 6-month feeding trial, and three other tanks were used with Diet 3 in the 12-month feeding trial. In addition, one control tank with a continuous inflow had no fish. Tanks were randomly assigned to diet treatments.

At the onset of the feeding trials, fish were fed at a rate of about 1.5% of total body weight on the basis of the estimated fish biomass in each tank (Table 2). As fish grew, ration size was reduced to 1.1% of total body weight by month 6 and 0.75% by month 12. The amount of daily feed for each tank was adjusted for fish mortalities. Rations were weighed on a Mettler PL-3000 top-loading balance, and fish were fed twice daily.

Dissolved oxygen and temperature were measured in all tanks three times per week with a YSI Model 95 meter. Personnel from the Benner Spring Fish Research group collected water samples on an irregular basis from four randomly chosen tanks and analyzed them for ammonia nitrogen ( $\text{NH}_3$ -N) with an Accumet Model AR25 dual channel pH/Ion meter.

We deployed semipermeable membrane devices (SPMDs; supplied by Environmental Sampling Technologies, St. Joseph, MO) to determine the presence and approximate concentrations of PCBs in the hatchery supply water. These devices consist of flattened polyethylene tubing (ca. 91 cm long, 2.5 cm wide) filled with 0.91 g triolein, a purified lipid. Lipid-soluble compounds such as PCBs diffuse from the surrounding water across the polyethylene membrane and become concentrated in the triolein. Three SPMDs were placed in each of two stainless steel carriers; one carrier was suspended in the springhouse outside of the hatchery building and a second carrier was suspended in the control tank, which contained no fish. This arrangement allowed for sampling supply water before it entered the building and after it flowed through supply lines and the culture tank. SPMDs were deployed for 1-month periods for 12 months. During months 2 and 3, we also deployed a pair of SPMDs in one of the fish tanks receiving Diet 4 to determine if PCB uptake by SPMDs might be influenced by fish wastes. Each time an SPMD was exposed to air when it was transferred from its shipping container to a carrier or from a carrier to a shipping container, another SPMD in a shipping container (referred to as a trip blank) was also exposed to air to assess possible uptake of PCBs from the atmosphere. After SPMDs were removed from carriers, they were placed back into airtight containers and stored at  $-28^{\circ}\text{C}$  until they were shipped back to the supplier for processing.

Each month we weighed and measured a subsample of 35 trout from each tank to determine growth. From this subsample, 6 trout were retained for tissue analysis. Because mortality was lower than anticipated, we culled fish on three occasions to ensure that we had sufficient food for the duration of the trials. At the end of month 2, we measured all fish in each tank and reduced the total number to 274 by removing the smallest and largest individuals such that the mean length of the remaining fish was within 1 mm of the mean prior to culling. At the end of month 3, we used a similar procedure to reduce fish numbers to 65 in tanks used for the 6-month trial and to 100 fish in tanks used in the 12-month trial. At the end of month 10, we reduced fish numbers to 20 in the tanks used in the 12-month trial.

Trout retained for monthly tissue analyses were filleted and ribs were removed. Fillets that had been rinsed in distilled water and the remaining carcasses were separately wrapped in aluminum foil, dull side up, and labeled. Knives used for filleting and aluminum foil were rinsed with hexane and distilled water before processing each composite sample of fish. Tissues were stored at  $-28^{\circ}\text{C}$  until they were shipped to the analytical laboratory.

#### Net Absorption of PCB

At the end of the 6-month trial, a 3-week experiment was conducted to determine the net absorption rate of PCBs. We used the chromic oxide ( $\text{Cr}_2\text{O}_3$ ) indicator method for determining net absorption of PCBs (Austreng 1978). This method entailed addition of  $\text{Cr}_2\text{O}_3$  to the feed and analysis of feces for  $\text{Cr}_2\text{O}_3$  and the constituent of interest, which in this study was PCB. Because  $\text{Cr}_2\text{O}_3$  is not absorbed by fish, the amount of  $\text{Cr}_2\text{O}_3$  in a fecal sample can be used to compute the amount of feed the fish had eaten to produce that amount of feces. By knowing the amount of PCB in the feed and the amount in feces, one can compute the amount absorbed and retained by fish.

Each of the four experimental diets (with 1% added chromic oxide) was fed to three lots of trout for 3 weeks. Each lot of fish was comprised of 41 trout (mean weight = 190 g). During the second and third weeks of feeding, we collected feces from all fish in each lot by stripping the lower portion of the intestinal tract (Austreng 1978). Fecal samples were pooled to produce duplicate samples of about 50 g each from each lot. Feces and feed were stored at -28°C until analysis.

#### Determination of Chromic Oxide

Feed and fecal samples were dried at 95°C for 72 hours and ground in a stainless steel grinder. Dried fecal samples ranged from 2.5 to 4.4 g. Samples were oxidized to convert chromium from the chromic (+3) to the chromate (+6) state. To digest samples, up to 5 g were combined with 30 ml of nitric acid in a 500-ml flask and boiled until orange fumes evolved. Samples were cooled, 20 ml of perchloric acid was added, and the mixture was boiled until its color changed from green to yellow or orange. Digests were then diluted to 500 ml with de-ionized water. If necessary, the samples were then diluted to the 5-20 : g/ml range with a mixture of nitric and perchloric acid in the same ratio as that used for the digestion (30 ml of nitric acid, 20 ml of perchloric acid, and 450 ml of de-ionized water). Standards were also made with this same solution. The amount of chromium was determined at a wavelength of 357.9 nanometers by comparison to standards (0-20 : g/ml) using a Perkin Elmer (Analyst 300) atomic absorption spectrophotometer at CN Laboratories (Courtland, MN).

#### Computation of Net Absorption

Percentage net absorption of PCB was calculated using the indicator ( $\text{Cr}_2\text{O}_3$ ) formula of Austreng (1978):

$$A = 100 - \left| \frac{100 \times \text{Cr}_{\text{feed}} \times \text{PCB}_{\text{feces}}}{\text{Cr}_{\text{feces}} \times \text{PCB}_{\text{feed}}} \right|$$

where: A is the percentage net absorption,  
 $\text{Cr}_{\text{feed}}$  is the concentration of chromium in feed,  
 $\text{Cr}_{\text{feces}}$  is the concentration of chromium in feces,  
 $\text{PCB}_{\text{feed}}$  is the concentration of PCBs in feed, and  
 $\text{PCB}_{\text{feces}}$  is the concentration of PCBs in feces.

#### Extraction of PCBs from Semipermeable Membrane Devices

After SPMDs were exposed to the hatchery supply water for 30 days, they were placed in airtight containers, frozen, and shipped to Environmental Sampling Technologies (EST) for processing. The SPMDs were first brushed and rinsed with water to remove attached microorganisms. They were sequentially rinsed with 1 N HCL, water, hexane, water, acetone, and isopropyl alcohol, and then air dried. After the surfaces of the SPMDs were air-dried, they were immersed in hexane for 24 hours at

18<sup>0</sup> C. The dialysate was saved, and the SPMDs were re-immersed in fresh hexane for an additional 8 hours at 18<sup>0</sup> C. The combined dialysate was reduced to 1-2 ml by evaporation under ultra high purity nitrogen gas and transferred to ampules for shipping to the analytical laboratory.

### PCB and Lipid Analyses

Battelle Laboratories, Duxbury, Massachusetts, analyzed experimental feeds, fish tissues, fish feces, and SPMD extracts for PCBs and lipids. The PCB concentrations were determined using NOAA National Status and Trends (NS&T) analytical procedures, modified for low-level total PCB (as Aroclor) determination. Individual PCB congeners are also determined in the NS&T Program. Battelle scientists co-authored these documents and have collaborated with NOAA and the National Institute of Standards and Technology to develop and refine the methods over the years (Peven and Uhler 1993, 1998).

In summary, approximately 20 g of fish tissue homogenate or fish feed were spiked with surrogate internal standards (to monitor extraction efficiency), combined with sodium sulfate (a drying agent), and extracted three times with hexane using Tissumizer maceration techniques. An aliquot of each sample was also dried at 105 °C to calculate moisture content. All of the three extracts associated with a single sample were combined in an Erlenmeyer flask and again dried with sodium sulfate to ensure that all the associated water had been removed from the extract. Where applicable, the extract was concentrated to 10 mL using Kuderna-Danish apparatus and/or nitrogen concentration techniques, and an aliquot was removed for lipid content determination. SPMD samples were received as hexane extracts. These extracts were spiked with surrogate internal standards, concentrated to <1 mL and entered the sample processing scheme at this point. The solvent extract was concentrated to <1 mL, and solvent exchanged into methylene chloride. This raw extract was applied to an alumina cleanup column to remove gross impurities, including a fraction of the lipid content. The eluate from the column was again concentrated to approximately 1 mL, the exact volume was measured, and the extract was further purified using HPLC/GPC techniques. In this procedure, the extract is fractionated such that the target analytes (Aroclors) are isolated from the other impurities in the extract. In many cases, due to the high lipid content of the samples, the individual extracts were split into multiple 1 mL aliquots, and each individual aliquot was fractionated by HPLC. After fractionation, the aliquots associated with a single sample were combined, and the entire sample was concentrated to <1 mL under nitrogen. These cleaned extracts were solvent exchanged into hexane, spiked with recovery internal standards (to quantify surrogate recoveries), and transferred to the GC/ECD analyst for Aroclor analysis.

Sample extracts were analyzed using a Hewlett Packard gas chromatograph, model 6890, equipped with an electron capture detector (ECD), using a 60-m, 0.25-: m ID column, hydrogen carrier gas, EPC injector, and a 100-to 120-minute temperature program to enhance Aroclor identification. Before any field samples were analyzed, the GC/ECD was calibrated with a 5-level calibration of combined Aroclor 1016 and 1260 solution (to represent the PCB response in general) as well as a 1-level calibration

solution of a combined Aroclor 1254:1260 mixture. Additionally, 1-level calibration solutions of Aroclors 1221, 1232, 1242, 1248, 1254, 1262 and 1268 were analyzed to identify and confirm which Aroclor(s) were detected. All PCB values reported herein are based on the Aroclor 1254:1260 standard and were corrected on the basis of recovery of the surrogate PCB 34.

All laboratory batches of samples contained one procedural blank, one laboratory control spike sample, a matrix spike/matrix spike duplicate pair, and a duplicate sample. The procedural blank is solvent spiked with surrogate internal standard carried through the procedure with the authentic samples, and is intended to detect any laboratory-introduced contamination. The laboratory control spike sample is solvent spiked with surrogates and target analytes (in this case Aroclor 1016 and Aroclor 1260). The matrix spike and matrix spike duplicate are authentic field samples spiked with surrogates and target analytes (also Aroclor 1016 and Aroclor 1260). The laboratory control and matrix spike samples are intended to measure the laboratories efficiency and accuracy in the PCB analysis, and by comparing the results of these samples also determine the effect of the sample matrix (i.e., matrix spike sample). The results of the replicate analyses (matrix spike and spike duplicate) were used to obtain a measure of analytical precision. Duplicate samples were also analyzed for the determination of percent moisture and percent lipids to assess precision and representativeness of the data. For the SPMD batches only a procedural blank and a laboratory control sample were added to the authentic samples, because the samples were not extracted at the Battelle laboratory.

The extracts from a subset of the samples were submitted for detailed PCB congener characterization. The PCB congener analysis was performed by GC/low-resolution mass spectrometry (LRMS), with the MS detector operating in the selected ion-monitoring mode (SIM). The latest generation GC/LRMS instrumentation was used (Hewlett-Packard 6890A GC equipped with a Hewlett-Packard 5973 LRMS). The chromatographic separation was performed using a 60-m, 0.25-mm inner diameter, 0.25- $\mu$ m film thickness, DB-5MS fused silica capillary column. A 1- $\mu$ L sample extract is injected onto the instrument. The GC is equipped with an electronic pressure controlled (EPC) inlet for optimum sensitivity and reproducibility. Helium is used as the carrier gas, and the temperature program has been optimized to separate the 107 target PCB congeners. The following GC temperature program was used:

Initial temperature: 60/C

Initial time: 1 minute

Ramp Rate: 10/C/minute to 140/C; 1/C/minute to 220/C; 5/C/minute 290/C

Final temperature: 290/C; 10 minutes

The mass spectrometer was operated in the selected ion-monitoring mode (SIM) to provide the necessary sensitivity and selectivity. Each target congener was monitored using two ions — a primary ion for quantitation and a secondary ion, for structural identification and confirmation. Identification was based on chromatographic retention time and primary/secondary ion ratio criteria (i.e., identification of the peak as a PCB congener, the level of chlorination of that PCB congener, and the known

retention time characteristics of each congener from prior detailed GC/ECD retention time characterization/mapping).

The GC/MS analytical system was tuned with perfluorotributylamine (PFTBA), and calibrated with a 5-point calibration, with the analyte concentrations in a range of approximately 0.005 to 2 ng/: L. The validity of the initial calibration was monitored with a continuing calibration check analysis (a mid-level calibration standard) at least every 10 samples. The calibration solutions contain all 107 target base congeners and the internal standards. Quantification was performed by the method of internal standards, using the recovery and surrogate internal standard compounds, similarly to the PCB analysis by GC/ECD.

Dichloromethane was used to extract lipids from 30-g samples of homogenized tissue or feed. The final extract was brought to a volume of 15 mL. With a syringe, a subsample (500 : L) of the extract was removed and placed into a preweighed aluminum pan. The extract was then dried overnight in an oven at 105 °C, weighed, and later re-weighed to ensure constant weight. Lipid weight was computed from the following formula:

$$\text{Total lipid weight (mg)} = \frac{\text{Volume of sample extract (mL)}}{\text{aliquot vol(mL)}} \times \text{aliquot dry wt. (mg)}$$

Lipid weight was divided by dry weight of the sample and expressed as percentage dry weight.

### Statistical Analyses

All statistical analyses were performed with Minitab (2000) software. We used primarily one-way or two-way analysis of variance (ANOVA) and where appropriate, made pairwise comparisons with the Tukey method.

## RESULTS AND DISCUSSION

### Water Quality

Springs and wells from a limestone aquifer provide the water supply for the Benner Spring facility. The water has an average pH of 7.6, an alkalinity of 180 mg/L as CaCO<sub>3</sub>, and conductivity of 365 µS/cm (E. Myers, personal communication, PFBC). During the study, water temperature in culture tanks ranged from 9.9 to 11.0° C and averaged 10.5° C (SD = 0.18, N = 1,532). Dissolved oxygen ranged from 8.6 to 10.9 mg/L and averaged 10.0 mg/L (SD = 0.39, N = 1,398).

Ammonia nitrogen ranged from 0.02 to 0.12 mg/L and averaged 0.07 mg/L (SD =

0.03; N =32). The un-ionized form of ammonia is the greatest concern, because it is much more toxic to fish than the ionized form. Growth of trout may be affected when un-ionized ammonia reaches 0.0125 mg/L (Piper et al. 1982). We computed the concentration of un-ionized ammonia assuming a pH of 7.6 and a temperature of 10.5°C. When total ammonia nitrogen was 0.12 mg/L, the highest observed concentration, un-ionized ammonia was estimated at 0.001 mg/L, which is well below the toxic levels.

### Fish Health and Mortality

Rainbow trout remained healthy throughout the experiments. No therapeutics were administered. A few individuals were diseased or behaved abnormally; these fish were removed and treated as mortalities. Monthly mortality was consistently low; it ranged from 0 to 0.47% and averaged 0.17%.

### PCBs in Water Supply

We deployed SPMDs in triplicate in the springhouse, which is just outside of the Benner Spring Culture and Research facility, and in a rearing tank that had a constant flow of water, but no fish. Concentrations of PCBs in the SPMDs deployed in the springhouse ranged from 149 to 560 ng/SPMD and averaged 293 ng/SPMD for the 12 months (Table 3). PCB concentrations in the spring roughly paralleled the amount of spring flow, which was highest at the beginning of the study, steadily declined to February 2001, increased for two months, and then began declining.

Concentrations of PCBs in the rearing tank without fish ranged from 87 to 334 ng/SPMD and averaged 200 ng/SPMD, about 30% less than PCBs in the springhouse.

We deployed SPMDs in the source spring and inside the building in part to determine if the hatchery infrastructure, such as pipes, tanks, etc., might contribute PCBs to the water supply. These data indicate that no additional PCBs were added to the water supply. Rather, PCBs inside the facility were lower than in the source spring. It is possible that these differences are related to large differences in the amount of water flowing through the springhouse and the rearing tank. Spring flow averaged more than 12,000 L/min, whereas the exchange rate in the rearing tank was 45 L/min during the first 5 months of the study and 36 L/min thereafter.

During September and October 2000, we deployed two SPMDs in one of the tanks with fish receiving Diet 4 (highest PCB concentration) to determine if the presence of fish waste and possibly uneaten feed might influence PCB uptake by SPMDs. Concentrations of PCBs in these SPMDs ranged from 155 to 217 ng/SPMD and averaged 179 ng/SPMD. Thus, fish waste did not contribute to increased PCB uptake by SPMD. It is possible that the development of a biofilm (community of microorganisms) on the surface of the SPMD inhibited movement of PCBs across the membrane. We noted that the biofilms on the SPMDs from the tank with fish were thicker than those from the tank without fish.

We exposed SPMDs to air (trip blanks) each time a set of SPMDs was replaced. These trip blanks were intended to detect possible contamination of SPMDs from the atmosphere or from handling. Among 11 trip blanks, no PCBs were detected in eight of

them and three had PCB concentrations ranging from 54 to 67 ng/SPMD. Among all 11 trip blanks, the average PCB concentration was 17 ng/SPMD. We concluded that contamination of SPMDs did not substantially influence results.

### Six-Month Feeding Trial

*Diet Analyses.* Mean concentrations of PCBs in the experimental diets ranged from 69 to 280 ng/g (0.069 - 0.28 ppm)<sup>1</sup>. Our goal was to use diets containing a wide range of PCB concentrations, with one diet containing PCB concentrations well below those normally found in commercial feed, and at the high end, a diet that exceeded the FDA limit (0.2 ppm) for PCBs in finished feed. Both of these goals were met with the experimental diets.

We collected feed samples for analyses throughout the trial to determine if PCB concentration changed with storage time, because all feeds were manufactured at the same time and were kept frozen at the Benner Spring facility. We noted two trends in PCB concentration through time. The highest concentration of PCBs in Diet 1 (102 ng/g) was found in the initial samples taken from the four different containers in July 2000 (Table 4). Three of these four containers were re-sampled in subsequent months and mean concentrations ranged from 50 to 55 ng/g. Results from the other diets did not indicate high values from initial samples. It is possible that samples of Diet 1 were contaminated when they were taken from the storage containers. Alternatively, these high values may have been the result of analytical errors, although personnel from Battelle Laboratories did not note any unusual circumstances during analyses of these samples.

The second trend we noted was a tendency for PCB concentrations in Diets 2, 3, and 4 to decline in November and December 2000 (Table 4). Diet 3 continued to decline in February and March 2001 and increased thereafter. Here again, we have no explanation for these variations. Mean concentrations of PCBs differed significantly among diets (Two-way ANOVA;  $P < 0.001$ ) and among months ( $P < 0.001$ ). We used either means or medians to characterize PCB concentrations in feed in subsequent analyses.

*Growth.* Among trout in the four diet groups, final mean lengths ranged from 242 to 248 mm, and final mean weights ranged from 183.3 to 196.9 g (Table 5). There was no significant difference in mean length among diet groups (One-way ANOVA;  $P > 0.05$ ). There was a significant difference in final mean weights (One-way ANOVA;  $P < 0.05$ ), but none of the pairwise comparisons were significant (Tukey test;  $P > 0.05$ ). Trout fed on Diet 1 were the largest after 6 months, followed by trout on Diet 4, Diet 3, and Diet 2. Differences in final mean weight were not related to differences in ration size. Though we attempted to keep ration size the same for all tanks, there were some variations owing to differences in numbers of fish and their mean weights. Trout fed

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<sup>1</sup>Throughout the text we report PCB concentrations in ng/g to avoid excessive numbers of decimal points and zeros. To convert ng/g to parts per million (ppm) move the decimal point three places to the left, so that 100 ng/g equals 0.100 ppm.

Diet 1 had the lowest mean monthly ration size, yet they had the largest mean length and weight after 6 months. It is likely that this growth difference was due to differences in quality of the diets. Diet 1 was made from herring meal, while the other diets were made with menhaden meal. Herring meal consists of 68-72% protein while menhaden meal consists of 62-63% protein (personal communication, J. Johnson, Omega Protein, Reedville, VA), which could account for differences in growth. There was no indication that PCB concentration affected growth, because trout on Diet 4, which had the highest PCB concentration, were similar in size to trout fed Diet 2 and Diet 3.

*Lipid Content of Fillets.* Lipid content is routinely measured when fish tissue is analyzed for PCBs, because PCBs are lipid soluble and PCB concentrations in tissues can be affected by lipid content. Lipid content of fillets (expressed as percentage dry weight) increased gradually throughout the trial period (Table 6). We tested for differences in lipid content from August to January and found a significant difference among months (Two-way ANOVA;  $P < 0.001$ ). There was a marginally significant difference ( $P = 0.057$ ) among diets, because lipid content in Diet 3 tended to be lower than in the other diets.

*PCBs in Fillets.* At the onset of the feeding trial, rainbow trout fillets had an average PCB concentration of 48 ng/g or 0.048 ppm (Table 7). After one month, PCB concentrations in fillets increased among all diet groups and the amount of increase was roughly proportional to PCB concentrations in each of the diets. Thereafter, PCB concentrations in fillets varied monthly, but the rank order among diet groups generally remained the same (Figure 1). Because there was no significant change in fillet concentrations from month 1 to 6 (Two-way ANOVA;  $P = 0.12$ ), but there was a significant difference among diets ( $P < 0.001$ ), we computed median values of fillet concentrations from all samples collected from month 1 to 6 and used these medians to characterize PCB concentrations.

Median PCB concentrations were positively related to median concentrations in each diet (Figure 2), though the relation was clearly not linear. Pairwise comparisons indicated that concentrations of PCBs in fillets were not significantly different (Two-way ANOVA, Tukey comparison;  $P = 0.32$ ) for trout fed diets 3 and 4, but all other pairwise comparisons were significantly different ( $P < 0.01$ ). There was no relation between lipid content of fillets and PCB concentration ( $r^2 = 0.1$ ), which suggests that PCB concentrations in the diets rather than lipid composition were the primary factor influencing PCB concentrations in fillets.

*PCBs in Carcasses.* We selected a subsample of trout carcasses (whole body less fillets) and had it analyzed for PCBs and lipids so that we could characterize whole body burdens of PCBs. Monthly concentrations of PCBs in carcasses varied significantly among the four diets and among months (Two-way ANOVA;  $P < 0.001$ ; Table 8). At the onset of the study, trout carcasses averaged 143 ng/g (0.143 ppm). Among trout fed Diet 1, PCB concentrations declined through time and averaged 61 ng/g at the end of the 6-month feeding trial. Among trout fed Diet 2, PCB concentrations increased after two months and then declined; their mean PCB concentration was 143 ng/g, the same as at the onset of the trial. Fish fed Diets 3 and 4

had increases in PCB concentrations for the first two months and then concentrations declined after six months. Presumably this pattern was the result of fish accumulating new tissue faster than they were accumulating PCBs. Overall, mean concentrations of PCBs in carcasses were positively related to PCB concentrations in feeds.

#### Assimilation of PCBs

At the end of the 6-month feeding trial we conducted an experiment to estimate the assimilation rate or net absorption of PCBs from each of the four diets. On the basis of concentrations of chromic oxide and PCBs in feces, we calculated net absorption of PCBs from three replicate fecal samples from fish fed each of the four diets. Concentrations of PCBs in feces ranged from about 48 ng/g for fish fed Diet 1 to 99 ng/g for those fed Diet 4 (Table 9). There was a significant difference in mean PCB concentration among diets (One-way ANOVA;  $P < 0.01$ ) and the mean concentration from fish fed Diet 4 was significantly different than those from all other diets, but no other pairwise comparisons was significant (Tukey comparison;  $P > 0.05$ ). Within and among diet groups, net absorption varied little (Table 9). It ranged from 80.8% to 91.6% and averaged 87.0% for all diets. There was no significant difference in net absorption among the four diets (One-way ANOVA;  $P > 0.05$ ). Thus, 13% of the dietary PCBs were eliminated with feces and the remainder was assimilated and stored in body tissues.

#### PCB Intake and Storage Budget

We computed a mass balance budget to estimate the expected increase in body burdens of PCBs attributable to feed intake and compared the expected body burden with actual body burden as a means of inferring if PCBs in the water supply may have contributed to the total body burden. Thus, if actual body burden exceeded the expected body burden, one could infer that the difference was due to uptake from water. Conversely, if the expected body burden was less than the actual, one would infer that fish had metabolized some of the PCBs or otherwise eliminated them through some route other than the feces.

We performed these calculations for all diets in each of six months (Table 10). We weighed carcasses from 97 composite samples that were collected during the 6-month feeding trial and computed percentage of whole body weight as carcass and fillet. These percentages did not change as fish grew; therefore, we used a median from all samples. Fillets constituted 34.2% of whole body weight. Fillet and carcass weights were multiplied by their respective PCB concentrations to estimate starting PCB body burden. PCB absorption was computed from the amount of food eaten times PCB concentration in feed times the mean net absorption (87%). The absorbed PCBs were added to the initial body burden to estimate the expected body burden. We then used the mean weight of fish at the end of the month and calculated actual body burden. In those months where actual exceeded expected body burden, we inferred uptake from water. Among the 24 monthly computations, actual exceeded expected body burden eight times, while the reverse was true 16 times. On average, actual body burdens were 8.8% less than expected, which suggests that PCBs in the water supply did not measurably influence PCB concentrations in the fish or that PCB uptake from water was

more than offset by metabolic breakdown and elimination.

### Seasonal Variations in PCBs

Rainbow trout used in the 12-month feeding trial were fed diet 3 at the same rate as fish in the 6-month trial and from months 7 to 12, ration size was gradually decreased from about 1.0 to 0.7% per day (Table 11). Trout in the 12-month trial grew at rates nearly identical to those in the 6-month trial and after 12 months they averaged about 353 mm in total length and weighed about 620 g.

Fillets, which were analyzed for lipids and PCBs, were taken from these fish twice during the first 6 months and monthly thereafter. In October 2000 and January 2001 PCB concentrations in fillets of trout from the 12-month trial were similar to those of trout in the 6-month trial (Figure 3). This result was not unexpected, because these fish were treated in exactly the same manner, except that trout in the 12-month trial were held at somewhat higher densities (100 vs. 75 trout/tank starting in October 2000) than trout in the 6-month trial. We are treating all trout from the 6- and 12-month trials fed on diet 3 as true replicates for the purposes of describing changes in PCB concentration through time. The average PCB concentration in fillets increased at the start of the trial and then fluctuated at about 85 ng/g for the first 6 months after which concentrations gradually increased to 125 ng/g at the end of the 12 months (Figure 4). Lipids tended to increase through time, but the pattern of change in lipids did not closely follow that of PCBs (Figure 5). Therefore, the continued increase in PCBs from month 7 through 12 was not simply a function of lipid content of the fillets.

### Congener Analyses.

Collectively, PCBs consist of 209 individual chemicals (congeners) that vary with respect to the number of chlorine atoms and their locations on the biphenyl rings. Each congener is assigned a number starting at PCB 1 that has one chlorine atom to PCB 209 that has 10 chlorine atoms. These congeners vary in their biomagnification by animals and in their toxicity to animals. We selected six samples that were analyzed for 107 common congeners with the intent of documenting the congener makeup of the samples and of identifying gross differences in congener composition among samples.

Fillets from fish fed Diet 2 had more than 40 congeners with peak concentrations clustered around PCB 101 and PCB 153 (Figure 6). Congener patterns in fillets from fish collected after 6 and 12 months of feeding on Diet 3 (spiked with Aroclor 1260) were similar. The highest congener concentrations from fish fed for 12 months (Figure 7) were in the range of PCB 138 - PCB 153 and PCB 91 - PCB 118 in addition to several heptachloride congeners (starting at PCB 174).

Congener patterns in fillets resembled patterns in feeds with one exception. Congeners in fish fed on Diet 2 and congeners in Diet 2 showed similar peaks except that Diet 2 contained appreciable concentrations on PCB 3 - PCB 17 (Figure 8), while fillets had low concentrations of congeners in this range (Figure 6). Congener patterns in fillets and in Diet 3 were similar, except that PCB 7/9 was substantially higher in Diet 3 (Figure 9) than in fillets (Figure 7). The relatively low concentrations of congeners with few chlorine atoms in fish fillets may be related the fishes' ability to metabolize

these congeners. PCBs are transformed to hydroxylated metabolites in the liver and congeners with few chlorine atoms are more readily metabolized than those with large numbers of chlorine atoms (see Eisler 2000).

## SUMMARY AND IMPLICATIONS

We found a well-defined relationship between concentration of PCBs in feed and concentration in rainbow trout fillets among trout that had been fed four different diets for 6 months. PCB concentration in fillets generally changed to a new level after one month of feeding and remained at that level for the remaining 5 months. This suggests that if trout in a production setting were switched to a diet containing higher concentrations of PCBs, this change in diet could be detected within one month. Perhaps of more interest, is the question “would the opposite occur if trout were switched to a lower PCB diet”? We would expect PCB concentrations in fillets to decline after one month, providing the trout were continuing to grow. But, we have no way to predict if PCBs would stabilize in one month or if it would take several months for PCB concentrations in fillets to reach some new equilibrium with their food supply.

A major motivation for this study was to determine whether or not the FDA standard of 0.2 ppm PCBs in finished fish feed was adequate to ensure that trout raised to stocking size had PCB concentrations in their fillets less than the most stringent consumption advisory adopted by the Great Lakes states -- 0.06 ppm. In 1998 personnel from several Pennsylvania agencies agreed that adoption of the 0.06 ppm consumption advisory standard was unrealistic, because this standard was so close to the minimum detectable levels of PCBs (0.05 ppm). Instead, they decided to adopt 0.1 ppm as the most stringent consumption advisory with which the public was advised not to eat more than one meal per week of hatchery trout. More recently, the advisory to not eat more than one meal per week has been extended to all sport fish caught from Commonwealth waters owing to the widespread distribution wild fish with elevated levels of mercury and other contaminants. For the purposes of this discussion, we shall continue to use 0.1 ppm of PCBs as the critical level for issuance of consumption advisories.

Rainbow trout reared on Diet 2, the diet presumed to be typical of that supplied by commercial producers, had a mean PCB concentration of 0.07 ppm (70 ng/g) in fillets. None of the composite samples of fillets that were collected from this group of trout ever equaled or exceeded 0.1 ppm of PCBs. Thus, for rainbow trout fed a diet that averaged 0.126 ppm of PCBs and raised to a size typically stocked in spring (ca. 9.75 inches), concentrations of PCBs in fillets should remain below the 0.1 ppm consumption advisory. On the basis of the relation between PCB concentration in feed and fillets, we predict that at 0.2 ppm PCBs in feed, the average concentration in fillets would be about 0.08 ppm, although some samples could certainly exceed the 0.1 ppm standard for fillets. Hence, we conclude that the FDA standard of 0.2 ppm PCBs in finished feed is adequate to ensure that stocked trout will not exceed the 0.1-ppm consumption advisory standard, provided that the trout are not exposed to any other sources of PCBs.

A related question is whether or not trout raised to sizes larger than 9.75 inches would continue to remain below the 0.1 ppm standard. We did not conduct this experiment with the standard diet (Diet 2); hence, we can only speculate on this question. Data from the 12-month feeding trial in which Diet 3 was used, provides some insights. After 6 months of rearing with this PCB-spiked diet, PCB concentrations in fillets began to gradually increase for the next 6 months. If Diet 2 had been fed to fish for an additional 6 months we would expect PCB concentrations to increase and possibly some samples would exceed the 0.1 ppm standard, but we have no way to predict if the mean concentration would reach that standard. Analyzing trout of the size in question from Benner Spring Fish Culture Station would be the most direct way to address this question.

One of our concerns when designing this study was the possible contribution of PCBs by hatchery water supply. The presence of PCBs in the supply water was confirmed with the aid of SPMDs. We used a mass balance budget to compute possible contributions of PCBs from the supply water and on the basis of the results from this method, we inferred that the supply water did not substantially contribute to the PCB accumulation in trout. The most direct way to address the issue of contributions from supply water would be to use PCB-free diets. Because such diets were not available, we had to rely on an indirect method to assess the importance of contamination of the water supply. Although we concluded that contaminants in Benner Spring did not influence body burdens of trout at this facility, one cannot assume that other culture facilities are contaminant free.

We conducted a 12-month feeding trial to determine if PCB concentrations in fillets fluctuated seasonally. There was no indication that PCB concentrations varied with season, at least until trout were about 16 months old at the end of the 12-month feeding trial. If the experiment had been extended, some seasonal effects might have become evident. As female trout mature and produce eggs, lipids are moved from somatic tissues to gonadal tissue, and PCBs would move in a similar fashion. Eisler (2000) notes that spawning is a major route of PCB loss in female fish, because the eggs are rich in lipids. Thus, one would expect PCB concentrations in mature fish to vary seasonally.

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Table 1. Specifications for proximate composition of commercially produced fish food typically used by Pennsylvania Fish and Boat Commission culture stations.

Crude Protein - minimum	42%
Protein from fish meal, minimum	14%
Protein from non-vegetable sources, minimum	29%
Protein from vegetable sources, maximum	13%
Crude Fat	18-20%
Fish oil, minimum	4-5%
Fish oil - coated on outside of pellets	3%
Crude Fiber, maximum	4%
Ash, maximum	11%
Moisture, maximum	10%

Vitamin Pack	8lb./ton
Vitamin C	1.5 lb./ton
Choline Chlorine 50%	3.5 lb./ton
Trace Mineral Pack	2 lb./ton

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Table 2. Number of rainbow trout (N), mean length (L), and mean weight (W) at the beginning of the month, and monthly ration (R in g of feed/g of fish/day) for the 12 tanks of trout used in the 6-month feeding trial.

Date	Diet Types and Tank Replicates											
	1			2			3			4		
	A	B	C	A	B	C	A	B	C	A	B	C
5 JUL 2000												
N	302	331	304	350	344	319	325	345	342	326	352	347
L (mm)	124	124	124	124	124	124	124	124	124	124	124	124
W (g)	22.6	22.6	22.6	22.6	22.6	22.6	22.6	22.6	22.6	22.6	22.6	22.6
R (%)	1.54	1.46	1.62	1.41	1.47	1.55	1.44	1.44	1.40	1.50	1.35	1.42
7 AUG 2000												
N	294	324	298	341	338	313	316	339	336	316	346	341
L (mm)	146	142	142	142	140	139	144	140	141	139	141	139
W (g)	38.0	35.6	34.6	34.3	33.2	34.3	37.7	33.9	36.0	35.4	36.7	34.3
R (%)	1.30	1.34	1.40	1.40	1.42	1.46	1.38	1.47	1.45	1.46	1.34	1.48
7 SEP 2000												
N	274	274	274	274	275	274	274	274	274	274	274	273
L (mm)	164	164	162	160	161	159	159	161	161	160	162	159
W (g)	53.3	52.6	49.8	49.9	49.7	46.5	48.3	49.5	48.5	49.2	55	48.5
R (%)	1.30	1.36	1.38	1.42	1.43	1.57	1.48	1.39	1.42	1.42	1.33	1.46

## 5 OCT 2000

N	65	65	65	65	65	65	65	65	65	65	65	65
L (mm)	185	181	181	181	179	179	177	181	179	177	180	176
W (g)	79.2	74.2	74.7	71.5	70.6	63.5	68.3	70.4	69.4	68.3	70.8	66.5
R (%)	1.28	1.34	1.29	1.43	1.36	1.44	1.45	1.38	1.39	1.42	1.34	1.44

## 7 NOV

N	59	59	59	59	59	59	59	59	59	59	59	59
L (mm)	208	204	209	200	203	204	199	205	204	202	206	200
W (g)	113.4	109.9	116.4	100.5	110.1	106.8	101.2	108.1	108.1	104.8	112.1	104.1
R (%)	1.11	1.15	1.11	1.24	1.67	1.20	1.24	1.16	1.20	1.18	1.13	1.19

## 7 DEC

N	53	53	53	53	53	53	53	53	53	52	53	53
L (mm)	229	225	226	220	223	223	219	225	220	224	227	224
W (g)	154.5	148.2	150.8	139.3	144.6	141.1	137.7	149.3	138	146.2	150.3	146.5
R (%)	1.06	1.12	1.07	1.16	1.15	1.19	1.19	1.12	1.17	1.12	1.08	1.12

## 8 JAN 2001

L (mm)	252	244	248	243	242	241	239	244	244	243	250	244
W (g)	201.6	188.7	200.4	185.7	184.9	179.3	181.3	188.9	185.1	190.5	200.7	190.1

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Table 3. Concentrations of PCBs in semipermeable membrane devices (SPMD) in the springhouse at the Benner Spring Fish Culture Station and in a reference tank with flowing water and no fish. Dates shown are when SPMDs were initially deployed. SPMDs were replaced at approximately 30-day intervals.

Date	PCBs in Spring (ng/SPMD)	PCBs in Reference tank (ng/SPMD)
7-Jul-2000	560	357
	424	277
	440	237
9-Aug-2000	448	204
	352	198
	389	205
11-Sep-2000	396	186
	289	163
	312	166
11-Oct-2000	329	205
	405	222
	328	219
10-Nov-2000	405	271
	401	292
	359	299
10-Dec-2000	339	228
	328	236
	277	211
10-Jan-2001	180	107
	180	316
	168	213
8-Feb-2001	360	111
	149	112
	171	153

9-Mar-2001	211	170
	202	203
	188	200
11-Apr-2001	242	129
	250	131
	219	87
7-May-2001	181	134
	179	125
	259	103
8-Jun-2001	243	139
	211	334
	165	256
Mean	293	200

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Table 4. PCB concentrations (ng/g) of the four experimental diets fed to rainbow trout.

Month and year	Diet 1		Diet 2		Diet 3		Diet 4	
	Single samples	Monthly mean	Single samples	Monthly mean	Single samples	Monthly mean	Single samples	Monthly mean
5 JUL 2000	98		117		236		306	
	98		139		214		303	
	110		136		235		307	
	110		143		242		291	
	99		114		235		301	
	99	102	121	128	247	235	317	304
7 SEP 2000	50		117		231		259	
	56		144		208		340	
	58	55	133	131	275	238	303	301
5 OCT 2000	68		138		247		334	
	52		193		228		323	
	47	56	180	170	258	245	265	307
7 NOV 2000	49		118		175		243	
	50		90		181		213	
	53	51	97	102	190	182	224	227

7 DEC 2000	50		107		193		248	
	53		97		184		232	
	50	51	93	99	183	187	228	236
All samples								
Mean		69		126		220		280
SD		25		28		30		41
16 FEB 2001					181			
					135	158		
8 MAR 2001					157			
					150	154		
10 APR 2001					205			
					229	217		
7 MAY 2001					226			
					209	218		
7 JUN 2001					195			
					225	210		
Months 1-12								
Mean						210		
SD						34		

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Table 5. Mean lengths and weights of rainbow trout at the end of the 6-month feeding trial and mean monthly ration. There was no significant difference (One-way ANOVA;  $P > 0.05$ ) in mean lengths among diets; there was a significant difference ( $P < 0.05$ ) in mean weight among diets, but none of the pairwise comparisons were significant (Tukey comparison;  $P > 0.05$ .) Mean monthly ration varied significantly among month and diet type (Two-way ANOVA;  $P < 0.05$ ); values in columns followed by the same letter are not significantly different (Tukey comparison;  $P < 0.05$ ).

Diet	Final mean length (mm)	Final mean weight (g)	Mean monthly ration (g of feed/g of fish/day-%)
1	248	196.9	1.291a
2	242	183.3	1.359b
3	243	185.1	1.343b
4	246	193.8	1.321ab

Table 6. Percentage of lipids by dry weight in fillets of rainbow trout fed one of four diets. Each value represents the analysis of a composite sample of fillets from at least six fish. The values for 5 JUL 2000 were from a common lot of fish that was used to stock tanks assigned to each diet.

Date	Diet							
	1		2		3		4	
	Composite	Mean	Composite	Mean	Composite	Mean	Composite	Mean
5 JUL 2000	16.0							
	16.8							
	15.8							
	14.7							
	19.1	16.5						
7 AUG 2000	24.1		15.5		19.6		19.7	
	18.3		19.5		18.6		19.2	
	19.0	20.5	19.0	18.0	18.4	18.9	20.5	19.8
7 SEP 2000	26.0		18.0		22.0		16.9	
	19.0		21.0		21.6		14.4	
	18.8	21.3	20.0	19.7	17.1	20.2	20.8	17.4
5 OCT 2000	27.0		24.0		21.4		24.7	
	25.0		28.0		21.0		22.0	
	21.2	24.4	26.4	26.1	13.0	18.5	28.0	24.9

7 Nov 2000	23.9		23.2		20.2		19.1	
	23.1		23.8		19.6		22.3	
	23.7	23.6	18.4	21.8	23.9	21.2	21.0	20.8
7 DEC 2000	23.4		26.6		25.0		27.2	
	21.8		28.7		23.6		28.4	
	23.5	22.9	29.2	28.2	21.3	23.3	25.1	26.9
8 JAN 2001	31.1		29.4		31.0		29.4	
	28.9		35.9		27.3		27.2	
	30.3	30.1	29.0	31.4	28.5	28.9	26.9	27.8

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Table 7. Concentrations of PCBs (ng/g) in fillets of rainbow trout fed one of four diets. Each value represents the analysis of a composite sample of fillets from at least six fish. Fish sampled on 5 JUL 2000 were from a common lot of fish that were used to stock tanks at the start of the feeding trial.

Date	Diet			
	1	2	3	4
5 JUL 2000	49			
	51			
	45			
	44			
	49			
7 AUG 2000	56	52	108	98
	57	73	98	81
	57	69	82	101
7 SEP 2000	42	72	79	88
	45	50	85	69
	57	46	70	100
5 OCT 2000	62	80	77	88
	37	62	94	90
	123	82	91	118
7 NOV 2000	45	64	70	101
	47	64	67	122
	50	51	91	106
7 DEC 2000	54	86	100	88
	41	91	103	94
	52	94	61	86
8 JAN 2001	47	71	97	98
	49	85	84	70
	50	69	81	93

Table 8. Concentrations of PCBs and percentage lipid by dry weight of carcasses (whole body minus fillet) from rainbow trout fed four diets. Each value represents the analysis of a composite sample consisting of at least six fish.

Date	Diet 1		Diet 2		Diet 3		Diet 4	
	PCB (ng/g)	Lipid (%)	PCB (ng/g)	Lipid (%)	PCB (ng/g)	Lipid (%)	PCB (ng/g)	Lipid (%)
5 JUL 2000	129	33.5						
	159	30.0						
	142	35.7						
8 SEP 2000	121	42.0	175	44.7	259	50.0	276	34.5
			196	47.9	240	45.1	289	42.2
			202	42.6	267	44.6	247	43.5
7 NOV 2000	74	35.5	121	32.9	175	32.9	208	37.7
	85	36.3	123	38.4	156	34.9	179	36.5
	80	36.2	132	39.6	164	37.0	83	34.8
8 JAN 2001	65	41.4	119	42.8	154	38.4	194	40.5
	63	36.8	105	33.0	180	39.2	216	41.4
	56	38.5	117	42.4	134	41.1	227	42.3
10 APR 2001					173	40.5		
					187	42.7		
					193	48.9		
				196	47.2			

5 JUL 2001

212 45.9

232 45.2

Mean

SEP-JUL

78

38

143

40

195

42

213

39

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Table 9. Concentrations of PCBs in feces collected from rainbow trout fed one of four experimental diets and computer net absorption of PCBs

Diet	PCBs(ng/g)		Net absorption (%)	
	Composite samples	Mean	Composite samples	Mean
1	53		84.8	
	45		87.2	
	45	47.6	87.8	86.6
2	75		80.8	
	52		86.8	
	53	59.7	85.8	84.5
3	82		88.3	
	76		88.4	
	54	70.6	91.6	89.4
4	96		88.3	
	96		87.9	
	105	99.0	86.3	87.5
All samples				87.0

Table 10. Monthly estimates of initial body burdens of PCBs, PCB absorption, and actual body burdens at the end of each month. The feeding trial began in July 2000 and ended in January 2001.

Diet and month	Initial Conditions							Expected Final Conditions									Actual PCB Body burden (ng)	Actual vs. expected % difference	
	Whole body weight (g)	Fillet weight (g)	Carcass weight (g)	Fillet PCB conc. (ng/g)	Carcass PCB conc. (ng/g)	PCB Body burden (ng)	Monthly food intake (g/fish)	Food PCB conc. (ng/g)	PCB intake (ng)	PCB absorption (ng)	PCB Body burden (ng)	Whole body wt. (g)	Fillet weight (g)	Carcass weight (g)	Fillet PCB conc. (ng/g)	Carcass PCB conc. (ng/g)			
1	JUL	22.6	7.7	14.9	48	143.2	2501	15.3	53.2	814	708	3209	36.1	12.3	23.8	56.7	127.4	3726	13.9%
	AUG	36.1	12.3	23.8	56.7	127.4	3726	18.3	53.2	974	847	4573	51.9	17.8	34.2	48	111.6	4663	1.9%
	SEP	51.9	17.8	34.2	48	111.6	4663	24.1	53.2	1282	1115	5779	76	26.0	50.0	50	95.7	6085	5.0%
	OCT	76	26.0	50.0	50	95.7	6085	40.6	53.2	2160	1879	7964	113.2	38.7	74.5	47	79.9	7771	-2.5%
	NOV	113.2	38.7	74.5	47	79.9	7771	46.1	53.2	2453	2134	9905	151.2	51.7	99.5	49	70.6	9558	-3.6%
	DEC	151.2	51.7	99.5	49	70.6	9558	58.6	53.2	3118	2712	12270	196.9	67.3	129.6	49	61.3	11242	-9.1%
2	JUL	22.6	7.7	14.9	48	143.2	2501	14.2	126	1789	1557	4057	33.9	11.6	22.3	65	167.1	4481	9.5%
	AUG	33.9	11.6	22.3	65	167.1	4481	18.3	126	2306	2006	6487	48.7	16.7	32.0	56	190.9	7050	8.0%
	SEP	48.7	16.7	32.0	56	190.9	7050	24.1	126	3037	2642	9692	68.5	23.4	45.1	74	158.3	8869	-9.3%
	OCT	68.5	23.4	45.1	74	158.3	8869	40.6	126	5116	4451	13319	105.8	36.2	69.6	60	125.6	10915	-22.0%
	NOV	105.8	36.2	69.6	60	125.6	10915	46.1	126	5809	5053	15968	141.7	48.5	93.2	90	119.8	15532	-2.8%
	DEC	141.7	48.5	93.2	90	119.8	15532	58.8	126	7409	6446	21977	183.3	62.7	120.6	75	113.9	18439	-19.2%
3	JUL	22.6	7.7	14.9	48	143.2	2501	14.2	210	2982	2594	5095	35.9	12.3	23.6	96	199.4	5889	13.5%
	AUG	35.9	12.3	23.6	96	199.4	5889	18.8	210	3948	3435	9324	48.8	16.7	32.1	78	255.6	9509	2.0%
	SEP	48.8	16.7	32.1	78	255.6	9509	23.7	210	4977	4330	13839	69.1	23.6	45.5	87	210.3	11618	-19.1%
	OCT	69.1	23.6	45.5	87	210.3	11618	40.6	210	8526	7418	19035	105.8	36.2	69.6	76	164.9	14230	-33.8%
	NOV	105.8	36.2	69.6	76	164.9	14230	46.1	210	9681	8422	22652	141.7	48.5	93.2	88	165.3	19677	-15.1%
	DEC	141.7	48.5	93.2	88	165.3	19677	58.7	210	12327	10724	30401	185.1	63.3	121.8	87	165.6	25677	-18.4%
4	JUL	22.6	7.7	14.9	48	143.2	2501	14	280	3920	3410	5911	35.5	12.1	23.4	93	207	5964	0.9%
	AUG	35.5	12.1	23.4	93	207	5964	19	280	5320	4628	10593	50.9	17.4	33.5	86	270.7	10563	-0.3%
	SEP	50.9	17.4	33.5	86	270.7	10563	23.5	280	6580	5725	16288	68.5	23.4	45.1	99	213.6	11947	-36.3%
	OCT	68.5	23.4	45.1	99	213.6	11947	40.6	280	11368	9890	21837	107	36.6	70.4	110	156.5	15044	-45.2%
	NOV	107	36.6	70.4	110	156.5	15044	46.1	280	12908	11230	26274	147.7	50.5	97.2	89	184.5	22427	-17.2%
	DEC	147.7	50.5	97.2	89	184.5	22427	58.7	280	16436	14299	36726	193.8	66.3	127.5	87	212.4	32852	-11.8%

Table 11. Number of rainbow trout (N), mean length (L), and mean weight at the beginning of the month, and monthly ration (R in g of feed/g of fish/day) for three tanks of trout fed diet 3 for 12 months. Trout were not measured and weighed in October and November; lengths and weights shown are from trout in the 6-month feeding trial.

Date	Variable	Tank Replicate		
		A	B	C
4 JUL 2000	N	308	337	342
	L (mm)	124	124	124
	W (g)	22.6	22.6	22.6
	R (%)	1.55	1.43	1.34
7 AUG 2000	N	306	336	342
	L (mm)	134	143	147
	W (g)	36.3	35.8	38.6
	R (%)	1.42	1.42	1.29
7 SEP 2000	N	274	274	274
	L (mm)	158	159	161
	W (g)	47.9	49.0	54.5
	R (%)	1.42	1.43	1.32
5 OCT 2000	N	100	100	100
	L (mm)	178	177	181
	W (g)	69.5	67.5	72
	R (%)	1.40	1.42	1.38
7 NOV 2000	N	100	100	100
	L (mm)	203	203	203
	W (g)	105.8	105.8	105.8
	R (%)	1.2	1.2	1.2

7 DEC 2000	N	100	100	100
	L (mm)	221	221	221
	W (g)	141.7	141.7	141.7
	R (%)	1.2	1.2	1.2
8 JAN 2001	N	100	100	100
	L (mm)	244	242	248
	W (g)	188.8	184.7	190
	R (%)	1.18	1.19	1.17
16 FEB 2001	N	93	94	94
	L (mm)	272	270	272
	W (g)	275.4	272.9	273.2
	R (%)	1.00	1.01	1.00
8 MAR 2001	N	87	88	88
	L (mm)	286	285	290
	W (g)	310.8	310.6	312.9
	R (%)	0.98	0.99	0.97
10 APR 2001	N	80	81	81
	L (mm)	310	306	305
	W (g)	405.1	390.3	359.3
	R (%)	0.93	0.95	0.99
7 MAY 2001	N	74	75	75
	L (mm)	327	323	322
	W (g)	460.6	454.9	445.2
	R (%)	0.89	0.93	0.97
7 JUN 2001	N	14	14	14
	L (mm)	343	337	339
	W (g)	554.1	526.4	535.6
	R (%)	0.69	0.72	0.72
5 JUL 2001	N	8	8	8
	L (mm)	351	353	356
	W (g)	600.8	616.6	635.2
	R (%)	0.75	0.75	0.74

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Table 12 . Percentage lipid by dry weight and concentration of PCBs in fillets of rainbow trout used in the 12-month feeding trial with Diet 3. Composite values represent the analysis of a sample consisting of at least six fish.

Date	Lipids (% dry weight)		PCB (ng/g)	
	Composite	Mean	Composite	Mean
5 OCT 2000	23.0		110	
	22.0		92	
	23.0	22.7	79	94.0
8 JAN 2001	28.7		91	
	27.7		79	
	25.2	27.2	63	77.8
16 FEB 2001	32.5		112	
	30.1		113	
	21.7	28.1	72	99.0
8 MAR 2001	23.6		112	
	26.3		94	
	24.9	24.9	93	99.9
10 APR 2001	21.3		106	
	29.4		126	
	26.4	25.7	95	108.8
7 MAY 2001	29.6		114	
	29.4		127	
	28.2	29.1	86	108.8
7 JUN 2001	37.9		108	
	27.1		123	
	22.6	29.2	102	111.1
5 JUL 2001	29.3		132	
	23.1		110	
	21.6	24.7	132	124.7

Figure 1. Median concentrations of PCBs in fillets of rainbow trout fed four different diets for 6 months. Each data point for months 1 to 6 represents the median of three composite samples of fillets taken from at least six different fish.

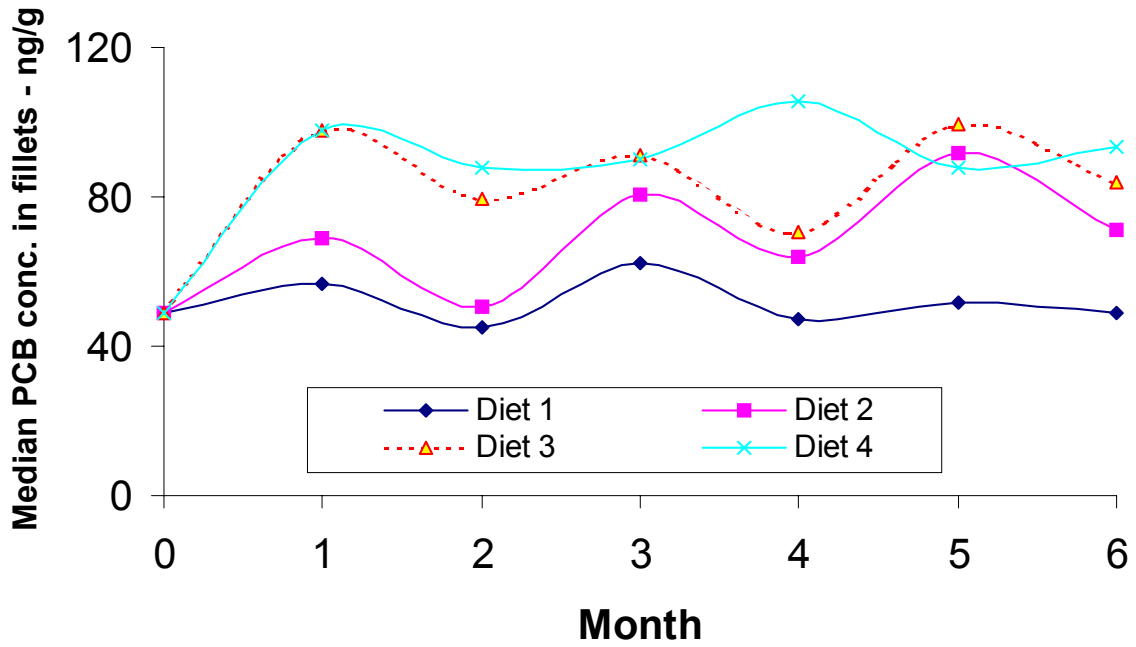


Figure 2. Median concentrations of PCBs in fillets of rainbow trout in relation to median concentrations of PCBs in four different diets. Each median data point represents 18 composite samples of fillets collected after 1 through 6 months (3 composite samples per month) of feeding.

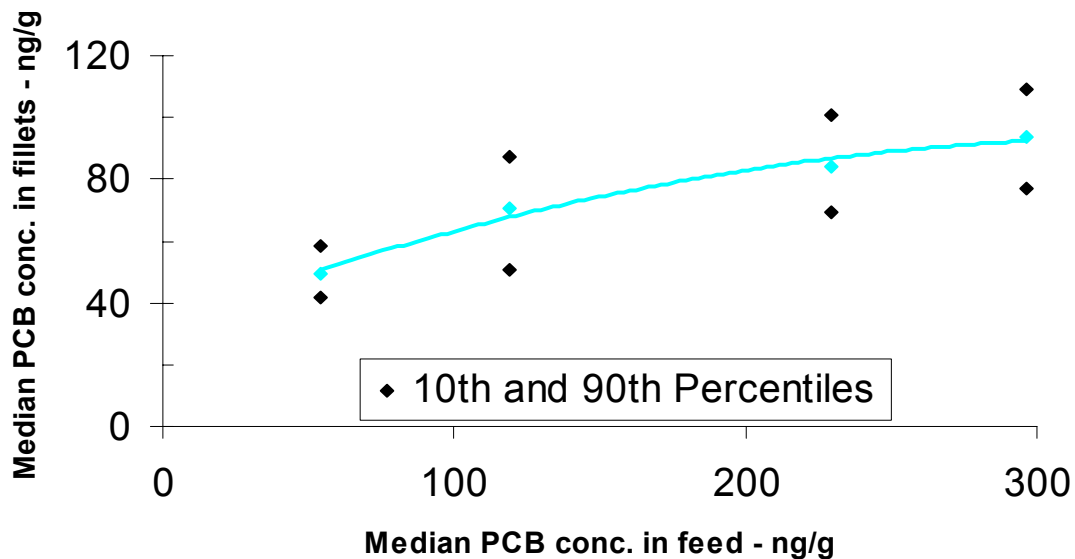


Figure 3. Concentrations of PCBs in fillets of rainbow trout fed Diet 3 for for 6 or 12 months. Each data point represents analysis of one composite sample of fillets.

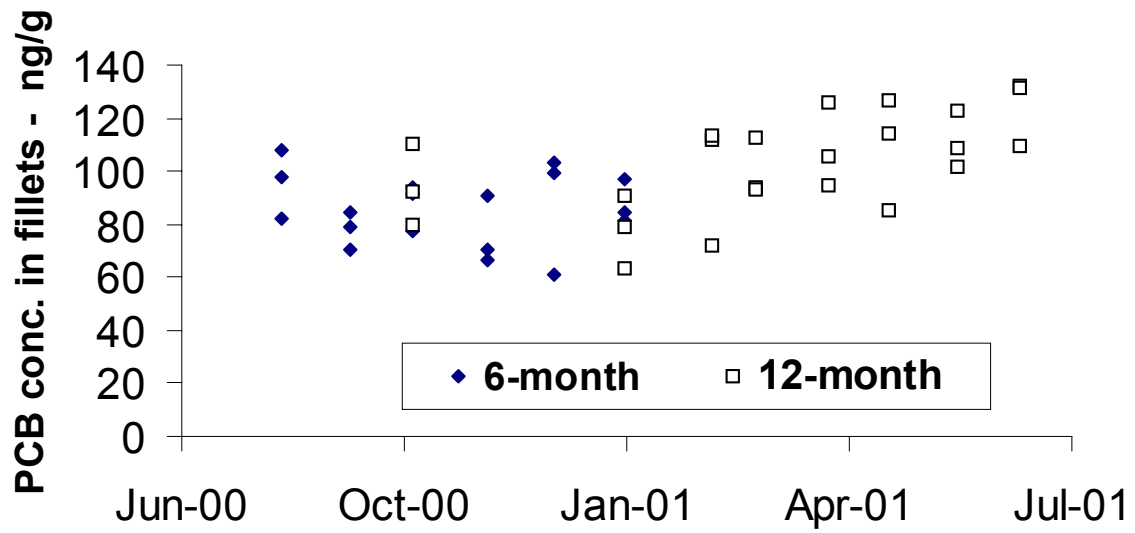


Figure 4. Median concentrations of PCBs in fillets of rainbow trout fed Diet 3 for 6 and 12 months.

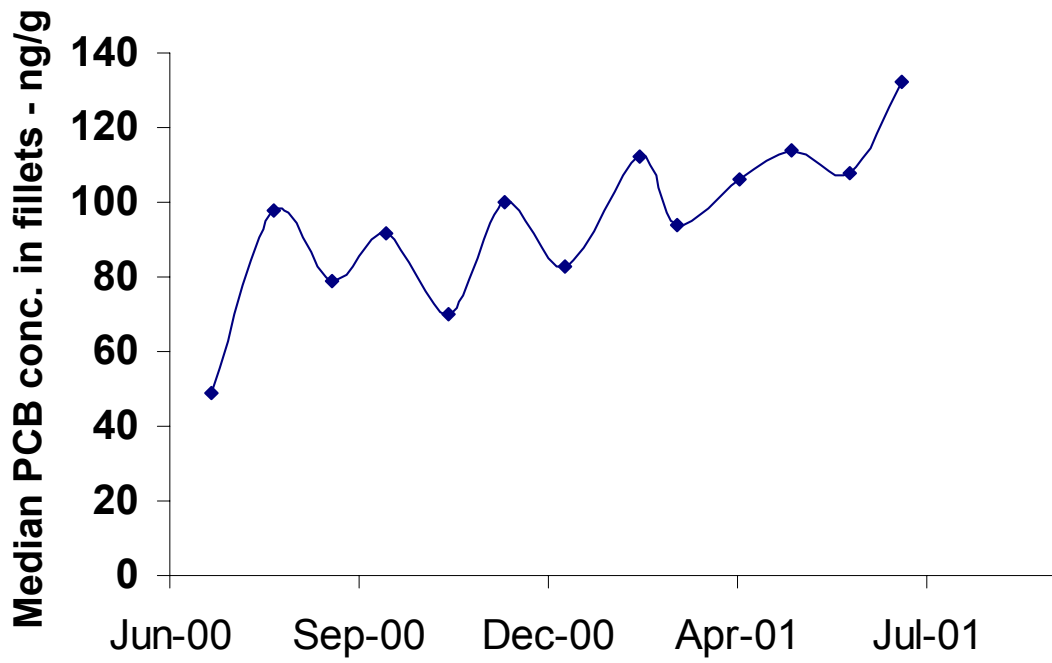


Figure 5. Mean lipid content of fillets of rainbow trout fed Diet 3 for 6 and 12 months.

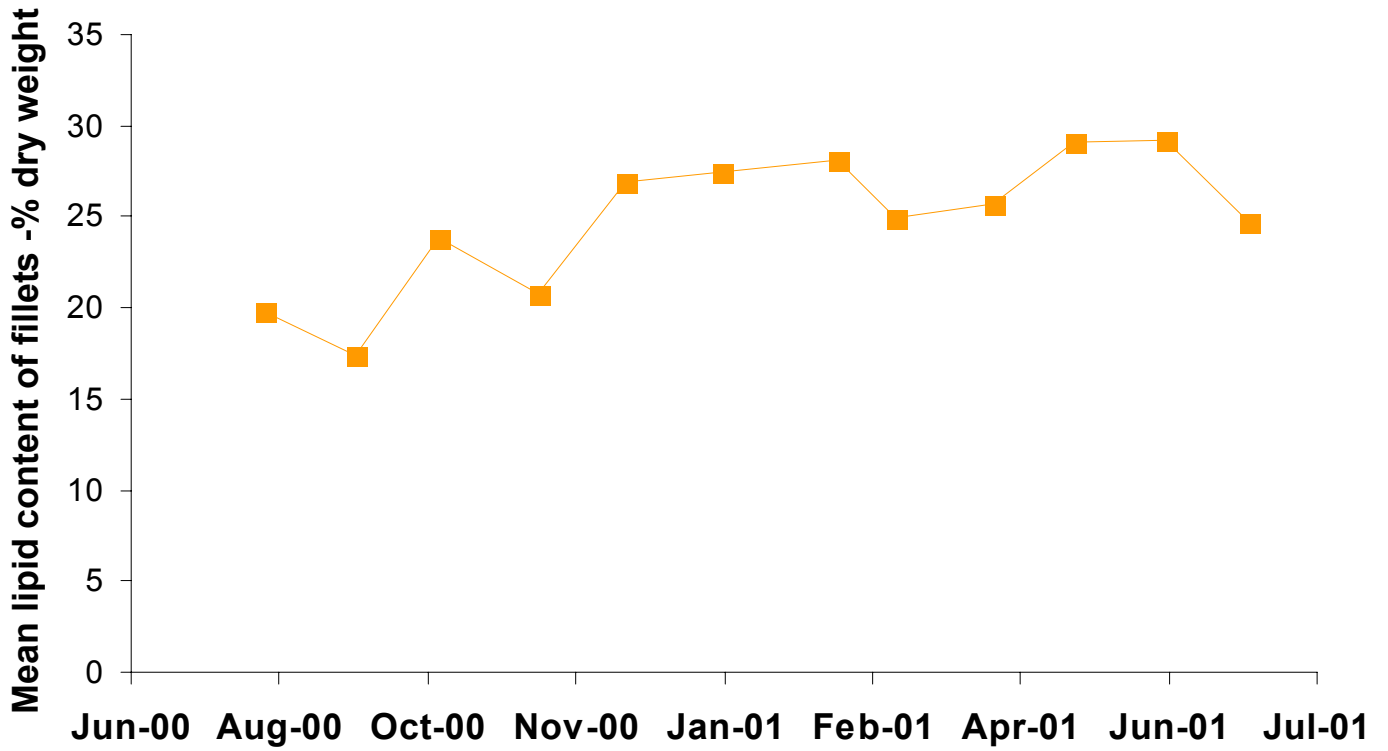


Figure 6. Relative proportions of selected PCB congeners in fillets of rainbow trout that had been fed Diet 2 for 6 months.

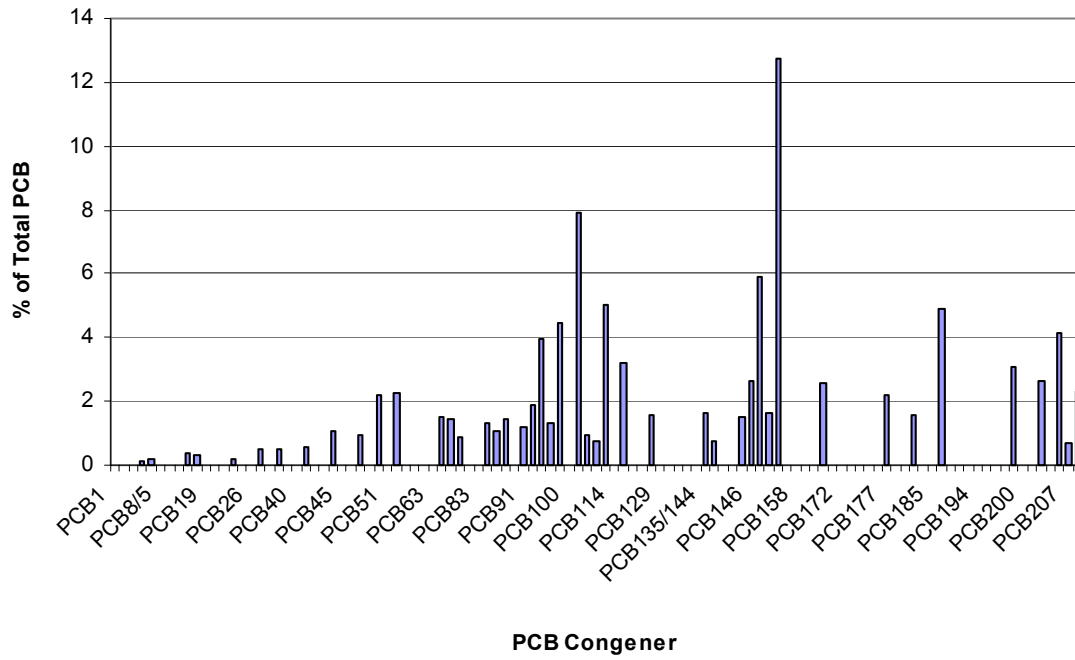


Figure 7. Relative proportions of selected PCB congeners in fillets of rainbow trout that had been fed Diet 3 for 12 months.

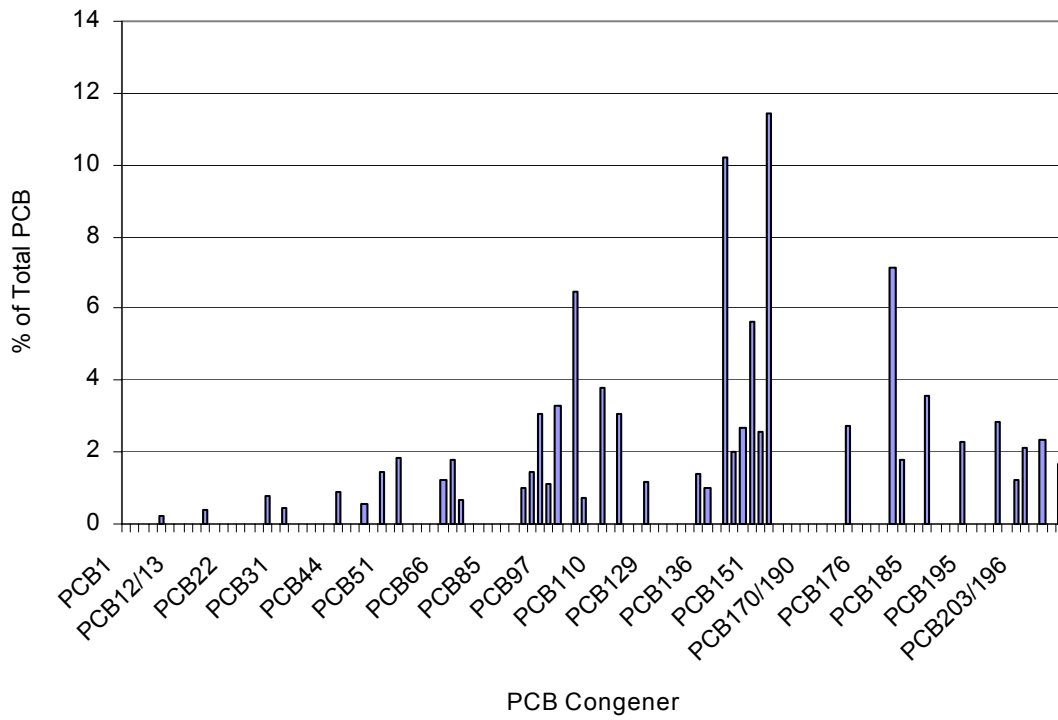


Figure 8. Relative proportions of selected PCB congeners in Diet 2.

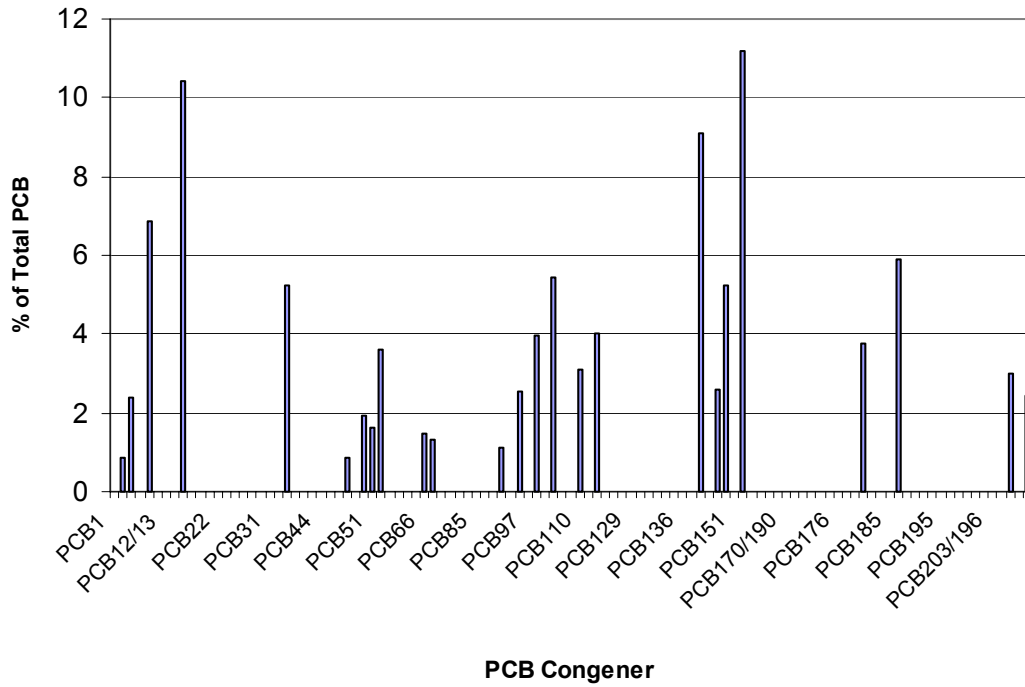


Figure 9. Relative proportions of selected PCB congeners in Diet 3.

