Accumulation of 3,4,3′,4′-Tetrachlorobiphenyl and 2,4,5,2′,4′,5′- and 2,4,5,3′,4′,6′-Hexachlorobiphenyl in Juvenille Coho Salmon

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Juvenile coho salmon (*Oncorhynchus kisutch*) parr were fed 10 μg of equal proportions of 3,4,3′,4′-tetrachlorobiphenyl, 2,4,5,2′,4′,5′-hexachlorobiphenyl, and 2,4,6,2′,4′,6′-hexachlorobiphenyl per gram of food pellets for an initial 165 days. During the 165 days, the coho parr gained weight at a rate of 0.7% of body weight per day when fed the diet containing the chlorobiphenyls, compared to 1.0% of weight per day of control fish. Analyses at various time intervals indicated that significant differences in accumulated body concentrations of the chlorobiphenyls occurred only after 108 days of feeding. At 108 days, three fish averaged 1.4 ± 0.35 μg of each hexachlorobiphenyl and 0.65 ± 0.32 μg of the tetrachlorobiphenyl per gram wet whole body tissues. Analyses before and after a final 48 days of starvation showed different concentrations for the tetrachlorobiphenyl compared to the hexachlorobiphenyls in blood and tissues from white muscle, brain, stomach, lateral line dark muscle, spleen, spinal column, liver, pyloric caeca, heart, intestine, and adipose. Before starvation, concentrations of the tetrachlorobiphenyl ranged from a low of 0.09 μg/g wet white muscle to a high of 10.8 μg/g wet adipose tissues; concentrations of hexachlorobiphenyls were about twice those for the tetrachlorobiphenyl. After starvation, the concentrations of tetrachlorobiphenyl ranged from a low in white muscle of 0.24 μg/g tissue to a high in adipose of 33 μg/g tissue while concentrations of hexachlorobiphenyls were three to four times greater than those for tetrachlorobiphenyl. Other differences were found for the concentrations of chlorobiphenyls in the various tissues and blood when the fish were fed diets without added chlorobiphenyls during the final 48 days.

In recent years polychlorinated biphenyls (PCB) have been remarkably ubiquitous in the environment, and there is a growing concern about their effects on nature and man (*1–6*). They were first reported in fish by Jensen (*7*), and since then additional data have been collected about PCB in the aquatic life from many regions of the Northern Hemisphere (*8–12*).

Relatively little attention has been given to the effects of PCB entering fish via the food web as contrasted to PCB dissolved in water. At the low end of the food web, zooplankton from the open Atlantic Ocean were reported to contain 0.3–0.45 part per million (ppm, 10^{-6} g/g) of PCB (*11*). It is not clear whether PCB concentrations increase with increasing trophic levels of the marine food web; however, the top predators may have 10^7 times higher PCB levels than those in ambient waters (*13*).

Zitko and Hutzinger (*14*) reported that concentrations of PCB in hatchery-reared Atlantic salmon (*Salmo salar*) ranged from 0.2–3.7 μg/g wet whole fish. There was no buildup of PCB residues in fish fed dry food containing PCB at a concentration of 1.3 μg/g, but a concentration of 10 μg/g in the diet resulted in an equilibrium residue of 3.5–4 μg/g in hatchery salmon.

The work of Mayer et al. (*15*) showed that dietary doses of 1.45–14,500 μg PCB (as Aroclor 1254) per kg body wt per day given to coho salmon had no effect on growth, nor produced observable toxicosis until death occurred in the highest dosage group after 260 days. With channel catfish, they showed that PCB concentrations of 48 and 480 μg/g had no effect on growth, nor caused mortalities.

Johansson et al. (*16*) examined PCB fed in capsule form at the 10-ppm level based on the body weight of brown trout. Hutzinger et al. (*17*) examined single dose effects in brook trout of pure PCB isomers at levels of 460–1,200 ppm.

The present investigation involved feeding juvenile coho salmon parr a diet of 10 ppm (w/w) of isomerically pure chlorobiphenyls impregnated in food pellets for the purpose of determining in the fish growth effect and accumulation of the chlorobiphenyls, the effects of starvation stress on such accumulations, and the concentrations of chlorobiphenyls in various tissues after we replaced the contaminated by the control diet. These experiments are important to the further understanding of how young salmon deal with ingested chlorobiphenyls of specific known structures.

Experimental

Fish Rearing and Diets. Salmon parr (*Oncorhynchus kisutch*) were hatchery reared from the eggs of a single spawning fish. Approximately 220 cohorts, average weights of 0.4 gram, were placed into a rearing tank (10 × 14 × 49 in., fiberglass resin) with constant flowing water and were grown to an average weight of 2.9 grams (about 6 cm in length). A perforated divider, with 464 holes (3/16 in.)/ft², was placed in the tank and 50 cohorts were kept in each compartment. The remaining fish were removed and placed in another tank for later use. The 50 control fish having a total body weight of 145 grams were in the "upstream" compartment and the 50 test fish, fed chlorobiphenyls and weighing a total of 146 grams were placed in the "downstream" compartment nearest a drain.

The temperature of water in the tanks was controlled at 11–14°C by mixing dechlorinated municipal water and cold well water. The temperature was usually monitored several times weekly.

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Oregon Moist Fish Pellets (Formula II, Moore-Clark Co., LaConner, Wash.) served as the basic diet. The food fed to the test fish was prepared by mixing equal parts of 3,4,3′,4′-tetrachlorobiphenyl, 2,4,5,2′,4′,5′-hexachlorobiphenyl, and 2,4,6,2′,4′,6′-hexachlorobiphenyl (Analabs, Inc., North Haven, Conn.) in molecularly distilled (*18*) herring oil, mixing this with acetone, and adding the Oregon Moist Pellets (OMP) to make up a final mixture containing 10 μg total chlorobiphenyls per gram of final food pellets. The herring oil added 0.5% to the original fat content of the OMP. The volume of acetone used was 1.8 times the weight of pellets. The acetone was removed at 50–60°C with a rotary evaporator and reduced pressure with a water aspirator. The food fed to the control fish was prepared in the same manner but without chlorobiphenyls added to the herring oil.

The fish were fed three times weekly. Constant amounts of food equal to 3.5% of their initial total body weights were fed for the first 53 days, then increasing amounts were fed equal to 3.5% of their weights dependent on weekly body weight measurements throughout the remainder of the experiment. Weights of fish were measured on Mondays prior to feeding after a weekend without feeding.

Three fish from each group were sacrificed and analyzed on the 24th, 53rd, and 108th day. On days numbered 117, 137, 165, and 213, five or six fish from each group or subgroup were examined. (The 137-day fish were used for examinations of liver acid lipase and fatty acid analyses, the results of which are to be published elsewhere.) On the 165th day, the remaining chlorobiphenyl-fed fish were divided into two subgroups of nearly equal weights. During the next 48 days, one subgroup of treated fish was starved and the other was fed the so-called chlorobiphenyl free diet that was fed to the control fish. Similarly, the remaining control fish were subdivided on the 165th day, and one subgroup was fed as before and the other was starved. The experiment was terminated on the 213th day.

Analytical Procedures. All chemicals were either reagent grade or highest grade commercially available. Solvents were commercial glass distilled.

Fish taken for analyses were removed from the rearing tank on Monday mornings, prior to feeding the remaining fish. The assay fish were cut behind the head severing the spinal cord and then weighed. The fish were killed by cutting off the tail and draining (about 2–3 min) blood from the caudal vein into distilled water. The blood from three to five fish at the end of the experiment was combined in about 20 ml of water and stored until analyzed. All samples that were not analyzed immediately were stored at −70°C.

Whole body samples at room temperature were cut into small pieces (5–10 mm) and ground in a stainless steel Lourdes tissue homogenizer. Organs, other parts of fish, and aliquots of ground whole body samples were homogenized at room temperature in a Potter-Elvehjem tube with a Teflon pestle (Kontes Glass Co. No. K-886000). Two to five volumes of distilled water were accurately measured and added to facilitate homogenization. The stomach and intestinal tissues were flushed with distilled water under force using a hypodermic syringe, prior to homogenization and extraction.

Measured amounts of homogenates were transferred to 50-ml centrifuge tubes, and the lipids and lipophilic compounds were extracted from the homogenates with mixtures of chloroform and methanol by the method described by Hanson and Olley (*19*). Sonication was used for mixing the solids, aqueous methanolic phase, and chloroform. The tubes were centrifuged for 30 min at 2000 rpm in a Model PR-2 centrifuge (International Equipment Co., Boston), the chloroform was removed, another chloroform portion was added, and the procedure repeated. Combined chloroform extracts were dried over sodium sulfate, a measured aliquot was transferred to a tared graduated conical tube, and the chloroform evaporated at 35–40°C in a stream of dry nitrogen. The extracted lipids with chlorobiphenyls were dissolved in a known volume of *n*-hexane, and the lipids were gravimetrically quantitated for all samples up to and including those for 117 days. The 165-day samples and others were assumed to have lipid contents near enough to those of 117 days as not to affect adversely the subsequent clean up on a Florisil column.

An amount of the hexane solution (usually 0.5 ml) containing 20–40 mg of lipid extract was freed of lipids and cleaned up by chromatographing (*20*) through a column of Florisil (60–100 mesh, PR grade, Floridin Co.). In the case of very small samples of heart and spleen tissues, the entire extracts were concentrated and used. Florisil columns were made of ungraduated blanks of 5-ml size disposable glass serological pipets (special Kimble No. 72120-S2), packed at the tip with fine spun glass followed by 1.4 grams (9-cm height) of dry Florisil (used as received). The Florisil was then eluted once with 10 ml of hexane, the hexane solution of lipid extract was added followed by additional hexane, and a 10.5-ml hexane eluate was collected. The 10.5-ml eluate with the chlorobiphenyls was then either directly analyzed or concentrated to a suitable known volume and analyzed by gas–liquid chromatography (glc).

Glc was performed on a Nuclear-Chicago Pesticide Analyzer chromatograph, Model 4764, equipped with a 2-mm i.d. by 120-cm glass column packed with 1.5% SP-2250 plus 1.95% SP-2401 on acid-washed dimethylchlorosilane-treated Supelcon (100–120 mesh, Supelco, Inc.). A ^{63}Ni-electron capture detector was operated at 300°C. Temperatures of the injector port and the column oven were 245°C and 185°C, respectively. Nitrogen carrier gas flowed at 70 ml/min. Generally, 3.0 μl of solutions were injected into the glc column. The concentration of a chlorobiphenyl was determined by comparison of glc peak height of a sample to that of standard chlorobiphenyl at concentrations to give approximately the same peak heights. Peak heights were maintained at 20–50% of full scale on a 5-mv recorder. A standard was analyzed immediately prior to every experimental sample.

The extraction method was examined to determine the recovery efficiency toward chlorobiphenyls. This was done by injecting each of two salmon parr (27.2-gram and 28.2-gram fish) with a fluid (0.20 ml) containing ^{14}C-labeled chlorobiphenyls and 1% (w/v) bovine serum albumin in 0.9% saline. The fluid contained 0.10 μCi (3.2 μg) of equal weight proportions of 2,4,5,2′,5′-pentachlorobiphenyl-(2,4,5-trichlorobiphenyl-UL-^{14}C) and 2,5,2′,5′-tetrachlorobiphenyl-UL-^{14}C (Mallinckrodt Chemical Works, St. Louis, Mo.). Following the injections, the fish remained in the aquarium for 19 hr before they were sacrificed and examined. Livers and lateral line muscle beneath the skin were taken as representative of widely differing tissue structures. These tissues were subjected to the extraction method, and radioactivity was quantitated in the lipid extracts and in the extracted residual tissues. Measurements (3,400 and 27,500 dpm) revealed 98 and 99% recoveries of chlorobiphenyls from liver and lateral line muscle, respectively.

The extraction of replicate samples of the prepared diets for the test fish, and subsequent glc analysis, revealed 96–101% of the theoretical amounts of the chlorobiphenyls added to the pellets. Spiking the treat-

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pellets with an additional 8.3 ppm of the three chloro- biphenyls, followed by extraction and analysis, revealed a recovery of each of the two hexachlorobiphenyls and a recovery of the tetrachlorobiphenyl.

Standard solutions of about 20 μg/ml of chlorobiphenyls in hexane were periodically checked for stability by comparing analyses with freshly prepared solutions. At such low concentrations, losses due to adsorption on walls of glass containers can be considerable. New standards were employed when losses were found to exceed 5% (w/v).

Results

Chlorobiphenyls in Diets. Analysis of the diet prepared for the test group of fish showed that 1 gram of pellets contained 3.4 μg of 2,4,6,2′,4′,6′-hexachlorobiphenyl, 3.5 μg of 3,4,3′,4′-tetrachlorobiphenyl, and 3.2 μg of 2,4,5,2′,4′,5′-hexachlorobiphenyl, or a total of 10.1 μg/g of pellets. The control diet was similarly analyzed and found to contain 0.036 μg/g, 0.063 μg/g, and 0.028 μg/g, respectively, of the compounds which gave relative retentions on gas chromatograms that corresponded to those of the three chlorobiphenyls. Assuming that the glc peaks for the extracted compounds from the sample of control diet were due to chlorobiphenyls, then the three peak components represented 0.13 μg/g of pelleted food. Replicate analyses showed concentrations of 0.06–0.08 μg of three chlorobiphenyls per gram of control food at a time halfway through the investigation. At the same time, the test diet was shown to have 10.2 μg of chlorobiphenyls per gram which indicated no losses during storage.

Growth of Fish. An examination of growth data was made to judge the effect of the chlorobiphenyls on the fish. Because frequent individual handling of fish can possibly produce an additional undesirable stress variable in the experiments, the fish were weighed individually only on the 108th day and also when they were sacrificed for the periodic analyses. A statistical analysis of the distribution of body weights of 40 test fish and 44 control fish at 108 days showed that the variation between fish in both groups was too large to assign a satisfactory significant difference to the group mean weights. Statistics showed only 80% probability of weight differences between the 108-day fish and the 53-day fish which were sacrificed.

A log-linear regression analysis of the mean weights of each group for the 165-day period indicated that the chlorobiphenyl-fed fish grew at a rate of 0.7% of body weight/day (growth = 3.10 exp 0.007 t) while the control fish grew at 1.0% of body weight/day (growth = 2.90 exp 0.010 t). Analysis of covariance of the growth data was performed to test whether the regression lines for each group were the same and whether the slopes of those regression lines were the same. Results showed that these are the same less than 1% of the time (p = 0.99); therefore, the growth rates were different over the period of 165 days.

At the end of 165 days of regular feeding, six chlorobiphenyl-fed fish and six control fish in two subgroups, which were eventually starved, had mean weights (±std dev) of 11.1 ± 3.4 and 11.8 ± 3.3 grams, respectively. After 48 days of starvation, these fish weighed 7.9 ± 2.8 grams and 9.0 ± 2.7 grams, respectively. Similarly, after 165 days, two subgroups of five chlorobiphenyl-fed fish and five controls (continually fed control-type diets) weighed 11.6 ± 3.0 and 11.4 ± 3.1 grams, respectively. In contrast to the starved subgroups, the fed subgroups of fish after 48 days weighed 12.2 ± 3.9 and 12.5 ± 2.3 grams, respectively.

Accumulation of Chlorobiphenyls. Determinations of the chlorobiphenyls in whole body homogenates were made for each of three fish per group on days numbered 24, 53, 108. The results shown in Table I indicate that the three chlorobiphenyls accumulated in the fish with the passing of time, but that the tetrachlorobiphenyl accumulated slower than either of the hexachlorobiphenyls. The concentrations of the three chlorobiphenyls retained in the fish tissues were somewhat but not statistically different on the 24th and 53rd days; however, after 108 days, the concentrations of the two hexachlorobiphenyls were significantly different at the 90% confidence level (Student's t-test) from that of the tetrachlorobiphenyl in test fish. The combined concentrations of the chlorobiphenyls found in the control fish (0.16 ppm) were about 4.6% of those found in the test fish (3.5 ppm).

A consideration of the amounts of chlorobiphenyls found in the test samples relative to the amounts fed to the fish is important to understanding how the fish deal with ingested chlorobiphenyls rather than just considering the time dependent uptake levels of the compounds. In this case, the actual food uptake was not measured but, by judging from the amounts remaining on a few occasions between feeding periods, the administered food was estimated to be greater than 98% ingested. The figures in parentheses in Table I illustrate a percentage decrease in the amounts of chlorobiphenyls relative to ingested amounts with passing of time. For example, individual chlorobiphenyls and total chlorobiphenyls found in the test fish, relative to the amounts fed, decreased between 24 days and 108 days from 47–16% for tetrachlorobiphenyl and from 60–29% for the total chlorobiphenyl loads.

On the 117th day, five fish from each group were sacrificed and the concentrations of chlorobiphenyls determined in composite specimens of brain, liver, heart, spleen, spinal column, stomach and pyloric caeca, intes-

Table I. Analyses of Whole Juvenile Coho Salmon When Fed for 24, 53, and 108 Days[a]

Group[b]	Days	Body wt,[c] g	Lipid content, wt %	3,4,3′,4′-Cl_4-BP	2,4,5,2′,4′,5′-Cl_6-BP	2,4,6,2′,4′,6′-Cl_6-BP	Total
				Wet tissue,[c,d] μg/g			
Test	24	4.24 ± 0.85	7.60 ± 0.78	0.49 ± 0.16 (47%)	0.73 ± 0.18 (70%)	0.66 ± 0.24 (63%)	1.88 ± 0.58 (60%)
	53	5.26 ± 1.77	6.48 ± 0.62	0.59 ± 0.23 (30%)	0.93 ± 0.21 (47%)	0.90 ± 0.29 (45%)	2.42 ± 0.73 (41%)
	108	3.06 ± 1.15	3.8 ± 1.6	0.65 ± 0.32 (16%)	1.41 ± 0.35 (35%)	1.42 ± 0.35 (35%)	3.48 ± 1.02 (29%)
Control	24	3.79 ± 0.60	5.83 ± 0.39	0.059 ± 0.023	0.090 ± 0.033	0.050 ± 0.023	0.199 ± 0.079
	53	5.42 ± 2.08	7.38 ± 0.76	0.044 ± 0.006	0.047 ± 0.029	0.053 ± 0.012	0.144 ± 0.047
	108	7.87 ± 3.24	6.8 ± 1.7	0.107 ± 0.022	0.023 ± 0.008	0.030 ± 0.001	0.160 ± 0.031

[a] Abbreviations for chlorobiphenyls: 3,4,3′,4′-Cl_4-BP for the tetrachlorobiphenyl, and 2,4,5,2′,4′,5′-Cl_6-BP and 2,4,6,2′,4′,6′-Cl_6-BP for the hexachlorobiphenyl isomers. [b] Three fish per group. [c] Mean values ± std dev. [d] Percent of chlorobiphenyl amount found in fish relative to amount fed is given in parentheses.

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Table II. Concentrations of Chlorobiphenyls and Lipid Content in Tissues of Juvenile Coho Salmon from Test and Control Groups at 117 Days[a]

Tissue specimens	Lipid content, wt %	3,4,3',4'-Cl_4-BP	2,4,5,2',4',5'-Cl_6-BP	2,4,6,2',4',6'-Cl_6-BP	Total
		Wet tissue, μg/g			
Test group[b]					
Brain	7.1	0.15	0.31	0.38	0.84
Liver	2.9	0.25	0.35	0.50	1.1
White muscle	2.6	0.29	0.63	0.64	1.6
Intestines	4.3	0.30	0.79	0.82	1.9
Stomach and pyloric caeca	3.3	0.43	0.76	0.95	2.1
Spinal column	6.6	0.92	1.4	1.4	3.7
Heart	...	0.98	1.2	1.5	3.7
Lateral line muscle	6.1	0.77	1.8	1.9	4.5
Spleen	...	0.85	2.0	2.1	4.9
Adipose	73.0	10.6	19.3	18.8	48.7
Control group[c]					
Brain	6.6	0.020	0.009	0.017	0.046
Liver	3.9	0.034	0.014	0.037	0.085
White muscle	5.5	0.028	0.015	0.025	0.068
Intestines	6.6	0.098	0.032	0.069	0.20
Stomach and pyloric caeca	4.2	0.032	0.014	0.022	0.069
Spinal column	23.7	0.096	0.026	0.032	0.15
Heart	...	0.85	0.068	0.18	1.1
Lateral line muscle	11.4	0.10	0.058	0.077	0.24
Spleen	...	(2.0)	0.13	0.12	(2.3)
Adipose	73.5	0.30	0.19	0.36	0.85

[a] Data are for composite tissues from five fish per group. Chlorobiphenyls abbreviated the same as for Table I. [b] Individual fish weights: 4.48, 4.78, 4.92, 9.70, 11.7 grams. [c] Individual fish weights: 5.91, 8.01, 8.50, 11.8, 15.1 grams.

Table III. Concentrations of Chlorobiphenyls in Tissues of Juvenile Coho Salmon from Test Group at 165 Days[a]

Tissue	3,4,3',4'-Cl_4-BP	2,4,5,2',4',5'-Cl_6-BP	2,4,6,2',4',6'-Cl_6-BP	Total
	Wet tissue, μg/g			
White muscle	0.086	0.20	0.22	0.51
Brain	0.26	0.37	0.56	1.2
Stomach	0.34	0.70	0.76	1.8
Liver	0.47	0.75	0.88	2.1
Heart	0.43	0.86	0.93	2.2
Spinal column	0.66	1.5	1.5	3.7
Pyloric caeca	0.93	1.7	1.8	4.4
Lateral line	1.3	3.1	3.2	7.6
Intestine	1.9	3.8	3.9	9.6
Spleen	4.4	7.7	7.6	19.7
Adipose	10.8	20.1	20.2	51.1

[a] Data are for composite tissues from five fish weighing 6.9, 7.5, 12.4 15.2, 16.2 grams. Abbreviations for chlorobiphenyl same as for Table I.

tine, dark lateral line muscle, white muscle, and in adipose tissue (*21*) lining the digestive organs. The concentrations of chlorobiphenyls, as μg/g wet tissues, for the test or treated fish are listed in Table II, by ranking in increasing concentration of chlorobiphenyls, along with the data for control fish.

Data for the concentrations of chlorobiphenyls found in tissues from treated fish after 165 days are shown in Table III. The tetrachlorobiphenyl in all individual tissues was at lower concentrations than either isomer of the hexachlorobiphenyl. The same concentration ratios of the tetrachloro to hexachloro isomers were found in all other previous samples from chlorobiphenyl-fed fish, as shown in Tables I and II.

Starvation Stress. Tissue samples from composite specimens of parts of fish, starved during the final 48 days of the investigation, were analyzed simultaneously with the corresponding controls. Data for the starved test and control subgroups are presented in Table IV. Comparisons can be made with the data in Table III, which serve as a "zero" time reference for the chlorobiphenyl concentrations in the chlorobiphenyl-fed test fish at the beginning of the 48 days of starvation. The concentrations of chlorobiphenyls found tentatively in control samples obviously derive from the OMP pellets that served as the basic food. The data indicate that mobilization or transformations of chlorobiphenyls take place in the fish during starvation. For example, concentrations of chlorobiphenyls in the spleens were lowered by about one half and those in the adipose tissues increased by about five-fold during starvation. Also the finding of 9.2 μg of chlorobiphenyls per gram dry weight in blood after the starvation period is indicative of mobilization of these compounds. With the exception of spleen and perhaps lateral line dark muscle tissues, the chlorobiphenyl concentrations increased in tissues analyzed after starvation. During starvation, the greatest change in the chlorobiphenyl concentrations during the 48 days occurred in the heart.

Chlorobiphenyl Depletion. The effects of removing the chlorobiphenyls from the diet of test fish during the final 48 days, by feeding them the same basic diet given to the control fish, are indicated by the data presented in Table V. Mobilization of the three chlorobiphenyls is indicated by the change in concentrations found, for example, in the lateral line dark muscle tissues when comparing data in Table V to that in Table III. Levels of chlorobiphenyls in adipose tissue decreased markedly during the last 48 days of control diet feeding in fish that had been fed the chlorobiphenyls for 165 days. All tissues in the test fish had concentrations of the chlorobiphenyls that remained markedly higher than those in control fish after the feeding of control diets to both subgroups for the final 48 days. In contrast to the starved fish, the test fish fed diets without added chlorobiphenyls during the final period had concentrations of chlorobiphenyls in blood of 3.1 μg/g dry weight or roughly 34% of that in the starved test fish.

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Table IV. Concentrations of Chlorobiphenyls in Tissues of Juvenile Coho Salmon Starved 48 Days Following 165-Day Feeding Regimen[a]

	Test group				Control group			
	3,4,3',4'-Cl_4-BP	2,4,5,2',4',5'-Cl_6-BP	2,4,6,2',4',6'-Cl_6-BP	Total	3,4,3',4'-Cl_4-BP	2,4,5,2',4',5'-Cl_6-BP	2,4,6,2',4',6'-Cl_6-BP	Total
Tissue	Wet tissue, μg/g				Wet tissue, μg/g			
White muscle	0.24	0.75	0.74	1.7	0.013	0.020	0.020	0.053
Brain	0.32	1.24	1.37	2.9	0.011	0.011	0.014	0.036
Stomach	0.55	2.0	1.83	4.4	0.016	0.026	0.025	0.067
Lateral line	1.3	3.2	3.1	7.6	0.033	0.059	0.067	0.16
Spleen	1.4	3.4	3.0	7.8	0.11	0.12	0.11	0.34
Spinal column	1.4	4.0	3.7	9.1	0.014	0.023	0.023	0.060
Blood[b]	1.5	4.0	3.7	9.2	0.083	0.067	0.12	0.27
Liver	1.2	4.2	4.1	9.5	0.028	0.029	0.039	0.096
Pyloric caeca	2.0	5.8	5.5	13.3	0.066	0.10	0.14	0.31
Heart	5.1	13.4	13.0	31.5	0.25	0.50	0.46	1.2
Intestine	5.1	15.7	15.0	35.8	0.074	0.064	0.070	0.21
Adipose	33	112	102	247	0.32	0.40	0.47	1.2

[a] Data are given for composite tissues from five fish for each group. Abbreviations for chlorobiphenyls are the same as for Table I. [b] Chlorobiphenyl concentrations are based on dry weight of blood.

Table V. Concentrations of Chlorobiphenyls in Tissues of Juvenile Coho Salmon Fed Control Diets 48 Days Following 165-Day Feeding Regimen[a]

	Test group				Control group			
	3,4,3',4'-Cl_4-BP	2,4,5,2',4',5'-Cl_6-BP	2,4,6,2',4',6'-Cl_6-BP	Total	3,4,3',4'-Cl_4-BP	2,4,5,2',4',5'-Cl_6-BP	2,4,6,2',4',6'-Cl_6-BP	Total
Tissue	Wet tissue, μg/g				Wet tissue, μg/g			
Brain	0.13	0.45	0.50	1.1	0.004	0.006	0.007	0.017
White muscle	0.16	0.49	0.46	1.1	0.005	0.004	0.006	0.015
Liver	0.33	0.77	0.76	1.9	0.028	0.014	0.030	0.072
Blood[b]	0.42	1.38	1.29	3.1	0.068	0.040	0.078	0.19
Stomach	0.40	1.62	1.52	3.5	0.012	0.007	0.016	0.035
Heart	0.48	1.64	1.54	3.7	0.077	0.036	0.076	0.19
Spinal column	0.96	2.9	2.6	6.5	0.039	0.039	0.055	0.13
Intestine	1.1	3.1	2.9	7.1	0.043	0.022	0.045	0.11
Pyloric caeca	1.2	3.3	3.1	7.6	0.059	0.055	0.068	0.18
Spleen	1.5	5.6	5.0	12.1	0.15	0.095	0.19	0.44
Lateral line	2.0	6.0	5.8	13.8	0.043	0.040	0.052	0.14
Adipose	2.5	6.5	6.0	15.0	0.11	0.19	0.12	0.42

[a] Same as for Table IV.

Discussion

From periodic measurements of body weights, we found that the three chlorobiphenyls served to ($p = 0.99$) retard significantly growth of juvenile salmon during a period of 165 days. However, when both treated and control fish were starved for 48 days, there was no significant difference in weight loss between the two groups as determined by statistical analysis (Student's t-test) of the data. Also, by replacing the chlorobiphenyl-treated food with control food, continued feeding of both treated and control fish for 48 days resulted in no difference in growth between the two subgroups. It is interesting to note that Mayer et al. (*15*) reported no effect of PCB on growth of their coho salmon.

By selecting cohorts of nearly equal average weights for the subgroups to examine the effects of starvation and depletion of the chlorobiphenyls, it was possible to minimize the factors related to natural food selection differences of a fish community for the short period of time. It is recognized that a community of cohorts of fish establish a food "pecking order," which, by itself, can contribute to wide ranges in individual growth rates.

There were no mortalities of fish attributed to diet or to the chlorobiphenyl constituents throughout the investigation. This finding is in accord with those of Mayer et al. (*15*) and Zitko and Hutzinger (*14*) when PCB was given in diets to salmonids. The results support the view that chlorinated biphenyls may not have fatal effects on fish via the food web even though the concentrations in the food are considerably above those concentrations in water that were found by others to be fatal (*22*). Perhaps the reason for fatal effects from PCB dissolved in water is that the level of exposure to sensitive physiologic sites in fish may be much higher when fish are placed in water containing PCB as contrasted to the level of exposures resulting by way of actions through the food paths.

Analytical results of whole body concentrations of the chlorobiphenyls found in the fish (Table I) compared to the theoretical concentrations, assuming complete uptake (estimated 98%) from the diet for the 24-, 53-, and 108-day measurements, indicate that only 30–60% of the chlorobiphenyls in the diet were retained in the fish; the percentage decreased with time. This finding is not unrealistic because the food digestion efficiency for fish is not 100%. In the present work, it appears that saturation concentrations of chlorobiphenyls in tissues were being reached, particularly with respect to the tetrachlorobiphenyl in the fish. Zitko and Hutzinger (*14*) reported that 10 μg PCB/g resulted in an equilibrium residue in their experimental salmon. A complete material balance study

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of the chlorobiphenyls would help to understand the relationship of accumulated compounds to compounds that simply passed through the fish and were not taken up in the digestive tract. Such work was not practical in the present experiments.

There are differences in the accumulated concentrations of the tetrachlorobiphenyl compared to the hexachlorobiphenyls in the juvenile salmon. The differences are only significant for whole body concentrations when the fish were fed for 108 days. However, more frequent examinations and greater sample size may place the time shorter for significant differences. The fact that the hexachlorobiphenyls accumulated to a greater extent than the tetrachlorobiphenyl in the treated fish coincides with the suggestion of Jensen et al. (*23*) that the percentage of chlorinated biphenyls with high degrees of chlorination increases as mixtures of chlorinated biphenyls pass through the food web from lower to higher animals. These are indications that perhaps the biphenyls of lesser chlorination are metabolized more readily than biphenyls of greater chlorination. On the other hand, such differences can be explained by the phenomenon of partition coefficients of different chlorobiphenyls in aqueous media, as opposed to differences in their metabolism—decidedly an important factor when considering what occurs in the digestive tract of fish. Hutzinger et al. (*24*) point out the possible unusually greater solubility of 3,4,3′,4′-tetrachlorobiphenyl in water compared to the solubility of other chlorobiphenyls.

The importance of knowing the lipid content of tissues is stressed from the standpoint of the analytical procedure. When using Florisil chromatography for cleanup of the extracted compounds, one wants to avoid overloading the Florisil with lipids. As shown in Table II, the lipid content is quite variable among tissues; however, as pointed out, once the content is recognized the actual procedure can be adjusted to suit the sample in question. The adipose tissues, which function largely as lipid storage sites in the animals (*21*), will also store lipophilic compounds like the chlorobiphenyls. For this reason, the adipose tissue is a logical choice for locating the greatest amount of chlorobiphenyls in fish.

As seen in Table II, the lipids in the heart and spleen are not reported, as we were unable to obtain acceptable measurements of the extractable lipids from these organs. There appeared to be anomalous concentrations of the chlorobiphenyls in the spleen samples, especially those for the tetrachlorobiphenyl in control fish. We have no explanation for this except to point out the very small size of the spleens that, coupled with an expected high lipolytic activity of this organ, may produce special problems in analysis.

It is beyond the scope of this paper to examine the absolute amounts of each chlorobiphenyl in all of the parts of the fish and relate these to total body loads of the compounds, although the value of such an examination is understandable. For instance, what is the relationship of the apparently high concentration of chlorobiphenyls (19.7 μg/g) in the spleens (Table III) to total body loads? In a small organ like the spleen, a relatively insignificant proportion of chlorobiphenyls in the whole fish can become a rather large concentration value.

If we compare data for treated fish at 117 days and 165 days, as seen in Tables II and III, there appear to be fluctuations in the concentrations of the chlorobiphenyls for the same tissue. White muscle and heart tissues, for example, showed decreases in the concentrations with time. This change is related either to an actual wide range of concentrations due to individual differences in metabolic activities in fish at the two times, or to factors related to the differences in the weights of the organs, or a combination of these. The fact that at 117 days the sampled fish averaged 7.1 grams while at 165 days the sample contained fish which averaged 11.6 grams of body weight, the analyses must logically involve factors associated with organs of possible different sizes and different total biochemical activity per organ. Biological variations are considerably greater than the precision of chemical analyses such that many more specimens are required for analyses than are employed in this work to try to explain fully such observed differences. It is inappropriate to offer additional rationalizations at this time.

There are numerous examples of differences in the activities of metabolic systems relative to foreign compounds in a variety of test animals. These are, in turn, related to differences between species, age, states of maturity, and sex (*25-27*). Perhaps species and maturity differences contributed to our results on 165 days of growth when contrasted to the results of others. Also, our results are based solely on fish responses toward three specific compounds rather than the numerous compounds in commercial PCB mixtures. Such mixtures may produce different biological effects than those observed in the present work. It is known that the metabolic rate for chlorinated biphenyls is very slow in fish (*24*). The accumulation of chlorobiphenyls in tissues indicate that the fish are unable completely to metabolize or excrete these foreign compounds when the food contains 10 ppm (w/w) of the compounds. Future experiments are planned to determine whether actual metabolism of chlorobiphenyls occur in coho salmon parr or that the fish are simply failing to assimilate them equally. An important future experiment is to perform similar food path work on an advanced life stage of these fish in seawater, and to determine the effects modified by the salinity of the evironment.

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Development of a Portable Polarograph for Determination of Aldehydes in Automotive Exhaust and Production Plant Samples

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While commercial polarographic instruments are useful and sensitive laboratory analytical tools, they have found extremely limited applications in production facilities and in stream analysis due to the complex and fragile nature of the accompanying equipment. A truly portable polarograph has been developed which overcomes these difficulties with no sacrifice in sensitivity and accuracy. The instrument, with solid state electronics, consists of a small package of about 10 lb. The strip chart recorder of the commercial version has been replaced by direct digital readout. Scan time has been reduced to 1 min. The dropping mercury electrode, with its cumbersome reservoir and stand tube, has been replaced with a small hanging mercury drop electrode with a self-contained mercury supply. The instrument is especially useful for monitoring aldehydes in both aqueous and nonaqueous chemical plant process streams, automotive exhaust, and in air samples where aldehyde pollution is suspected.

The method of Lupton and Lynch (*1*) has been successfully employed for the polarographic determination of various aldehydes in a wide variety of samples. In 1970, the Automotive Laboratory of The Dow Chemical Company received several federal government contracts which required the determination of aldehydes as part of an automotive emissions survey involving a variety of engine load, speed, and fuel conditions. Several analytical techniques were tested for determination of aldehyde species in both particulate and condensate samples. Colorimetric and mass spectrometric techniques often demonstrated large interferences due to the large number of organic species present in the samples. When the polarographic method was applied, no interferences were observed and sensitivity down to 1 ppm was achieved. Several hundred samples were determined employing this technique, and it was established by differential pulse polarography that the predominant aldehyde species in most samples was formaldehyde. When catalytic mufflers were tested, the aldehyde content of the exhaust was significantly lower. Thus, it would be possible to monitor exhaust condensate for formaldehyde as a measure of the condition of proposed antipollution devices (i.e., is it time to replace the catalytic muffler system?).

Since an instrumental technique was desired which would be reasonably portable for use in various testing stations and garages, the standard polarographic system was not acceptable. Thus, work began on a truly portable polarographic system.

Procedure

Equipment. A Sargent Model XXI polarograph monitored the hanging mercury drop and mercury pool electrodes when recorded data was necessary for method development.

A Princeton Applied Research Model 170 electrochemistry system equipped with a Princeton Applied Research Model 172 Droptimer recorded all differential and derivative pulse polarograms.

A Metrohm Model E-410 hanging mercury drop electrode was employed for much of the work with the portable polarograph.

The equipment used in the construction of the portable polarograph is described in the Discussion section.

A Heath-Schlumberger Model SR-255B Recorder recorded polarograms with the portable polarograph.

Reagents. Acetate buffer, approximately pH4, prepared as an equimolar mixture of acetic acid and sodium acetate, 0.1*M* in water.

Hydrazine reagent, prepared as a 2% by weight aqueous solution of hydrazine sulfate.

Formaldehyde stock solution, prepared as an aqueous dilution of reagent grade material to approximately 100 ppm. This solution is stable for several weeks.

Analytical Procedures for Automotive Exhaust Sampling

Samples were obtained according to government contract specifications by pulling a portion of the exhaust stream through a gas scrubbing tower containing a known volume of water. The tower is immersed in an ice bath during sampling (at a known flow rate and for the desired time period). Once obtained, these samples have demonstrated shelf-lives (from a aldehyde stability point of view) of several months under ordinary laboratory conditions.

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