Sublethal Effects of Structurally Related Tetrachloro-, Pentachloro-, and Hexachlorobiphenyl on Juvenile Coho Salmon

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■ Four groups of cohorts of juvenile coho salmon (*Oncorhynchus kisutch*) were fed 2,5,2',5'-tetrachlorobiphenyl, 2,4,5,2',5'-pentachlorobiphenyl, and 2,4,5,2',4',5'-hexachlorobiphenyl together at combined total concentrations of 1, 2, and 12 parts per million in food pellets. Salmon fed 12 ppm chlorobiphenyls gained weight significantly ($P < 0.05$) faster during a one-month treatment than salmon in a control group. Whole body concentrations of the three chlorobiphenyls in the fish during a 72-day period indicated a possible steady-state condition for the chlorobiphenyls when administered at the concentration of 1 ppm, but not at 2 ppm or greater in the food. Biphenyls chlorinated in the 2,5- and 2,4,5-positions on the aromatic rings were accumulated to a similar extent in the fish. Rearrangement of chlorine atoms from the 2,5-positions to the 3,4-positions in isomeric tetrachlorobiphenyls resulted in an apparent decrease in the accumulation of the compound in young salmon. For the first time a pentachlorobiphenyl was found to cause enhanced activity of the hepatic aryl hydrocarbon hydroxylase system in treated fish.

Polychlorinated biphenyls are well recognized as persistent contaminants in the environment. Chlorobiphenyls are quite resistant to chemical and enzymatic degradations (*1*). Chlorobiphenyls possess a wide range of molecular structures, which can be classified according to the degrees of chlorination. The structural arrangements of chlorine atoms on the biphenyl molecule are expected to have considerable influence on the resistance of the molecules to chemical and enzymatic alterations, especially in biotransformations.

Few experiments have been reported on the metabolism of chlorobiphenyls in fish. A review by Hutzinger et al. (*1*) furnishes information on the chemistry of these compounds in fish and other animals, in addition to providing references to studies on the uptake, accumulations, and metabolism of chlorinated biphenyls. Biochemical and physiological effects, such as enlargement of liver, altered cholesterol metabolism, blood anaemia, and hyperglycemia, in brown trout treated with polychlorinated biphenyls after 43 days were observed by Johansson et al. (*2*). Analyses of chlorobiphenyls in Atlantic salmon after 243 days of treatment with a polychlorinated biphenyl (Aroclor 1254) in food showed a reduction or disappearance of the chlorobiphenyls having low degrees of chlorination (*1*). However, Hutzinger et al. (*3*) found no evidence for the excretion of hydroxylated chlorobiphenyls from brown trout which had consumed 4-chloro-, 4,4'-dichloro-, 2,5,2',5'-tetrachloro-, and 2,4,5,2',4',5'-hexachlorobiphenyl in food.

Chlorobiphenyls, like other foreign aromatic compounds, are believed to be metabolized via an enzymatic system that involves aryl hydrocarbon hydroxylation. Evidence supporting this view is the observation that hydroxy chlorobiphenyls are excreted from animals fed chlorobiphenyls (*3*). Although certain chlorinated biphenyls are depurated from treated fish in spite of the lack of detectable hydroxylated metabolites, further work is needed to understand the role of molecular structure of chlorobiphenyls in the accumulations in fish and the effects that these compounds have on biochemical systems such as the aryl hydroxylases.

Chlorobiphenyls in organisms from various trophic levels in the marine food web are ready sources of these contaminants in higher levels of marine fish (*4*). An understanding of the fate of chlorobiphenyls after they are transported through food to fish will enable predictions to be made about food web interactions and persistence of different chlorobiphenyls in fish. The present work is a continuation of a related study (*5*) of specific structurally pure chlorobiphenyls fed to young coho (*Oncorhynchus kisutch*) salmon.

In examining growth rates of young salmon fed chlorobiphenyls in diets, we found that small populations (50–100 fish) of cohorts establish divergent ranges of body weights during the course of experimentation. For example, 105 salmon fry weighed 0.3–0.4 g each at the start of an experiment, while in one year in captivity they grew to have a weight range of 3.6–19.6 g, according to a certain feeding schedule (*5*). The resulting wide range may be related to a natural aggressive behavior of some fish to establish territorial dominance, which would allow dominant fish to get more food than subordinate fish (*6–8*).

In the present research, we designed our experiments to take into account the problem of a wide weight range due to the presence of dominant and subordinate fish that results in individual differences among fish to feed and consume chlorobiphenyls in food. The purpose of the research was to examine the relationship of dietary concentrations of the chlorobiphenyls to growth of individual young salmon of uniform weight distribution, to examine fish of uniform weight distribution for accumulations of three structurally related chlorobiphenyls of different degrees of chlorination, and to make a preliminary determination of the effect of a pentachlorobiphenyl on enzymatic activities of an aryl hydrocarbon hydroxylase.

Experimental

Juvenile coho salmon were reared in a manner previously described (*5*). The fish were cohorts held in reserve from the initial study, and they were nearly one year older when used. The fish were fed diets at a rate of 2% of body weights each day, five days per week. At several intervals, the fish were individually identified and weighed, and the amount of food fed was adjusted to the weight of fish remaining after the removal of fish for analyses.

Experiments were designed to determine the influence on fish growth of and accumulations in fish of 1, 2, and 12 ppm of structurally related chlorobiphenyls which were added to the source of food, Oregon moist pellets (*9*). The additives were 2,5,2',5'-tetrachlorobiphenyl, 2,4,5,2',5'-pentachlorobiphenyl, and 2,4,5,2',4',5'-hexachlorobiphenyl, which are among the principal components of Aroclor 1242, 1248, or 1254 (*10*). We included 12 ppm chlorobiphenyls in the diets to allow us to relate the present results to the previous work (*5*).

Treatment of Fish. To examine fish fed the three concentrations of chlorobiphenyls vs. a control group, we selected four groups of fish weighing as nearly alike as possible, with similar standard deviations of mean weights. This was done by selecting 64 fish having the narrowest weight range from among 105 cohorts, dividing them into two groups of 32, and measuring the individual fish weights according to each group. Next, the groups were divided in half, and groups of 16 fish

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were sorted to give a distribution of about the same number of low, medium, and high weight fish in each group. Each fish was branded with an identifying code mark, which was made using rapid cold-brands with branding tools at temperatures near that of liquid nitrogen (*11*). At the start of feeding food-impregnated chlorobiphenyls, the groups ranged in weight from 11 to 20 g; their mean weights ± standard deviation were 15.6 ± 2.0 g. There were 400 ml of water/g of fish in separate rearing tanks for each group at the start of the feeding. Such water space was believed to be sufficient to preclude problems from overcrowding (*8*).

When the fish were taken from the rearing tanks for analyses, they were identified by the cold-brand markings and selected in such a way that the lowest weight, the highest weight, and weights closest to the mean weight of the group were used. This method of selection was designed to negate the effects of the individual differences of fish to consume food superimposed on the growth effects associated with the dietary chlorobiphenyls.

At different times during the feeding period, the fish were identified, their weights recorded, and selections made for analyses. The fish were killed by placing them in water containing 200 ppm (w/v) of MS-222 (ethyl *m*-aminobenzoate methanesulfonate) anesthetic until their movements ceased, then wrapping them in aluminum foil, quick freezing them at −60 °C in a freezer, and holding them at −60 °C until analyzed. Generally, four fish were taken for analysis each time, and individual measurements were made for the concentrations of the three chlorobiphenyls in homogenized samples of each whole fish.

Chlorobiphenyl Analyses. The methods of analyses were essentially the same as reported previously (*5*). A Virtis Model 45 homogenizer (the Vertis Co., Gardiner, N.Y.) was employed to thoroughly grind and mix the tissues. Weighed aliquots of homogenized tissue samples were extracted with chloroform–methanol–water mixtures, and subsequent concentrated extracts were analyzed by electron capture gas-liquid chromatography. Standards of the three chlorobiphenyls (Analabs, Inc., North Haven, Conn.) were analyzed prior to each experimental sample.

Statistical analyses of covariance *F* test (*12*) and multiple comparisons of all pairs of regression coefficients (*13, 14*) were performed on growth data. Student's *t* test was performed on the data for the accumulations of chlorobiphenyl in fish tissues.

Aryl Hydroxylation. The effect of a pentachlorobiphenyl on aryl hydrocarbon hydroxylation was determined by comparing hepatic enzymatic activities for two treated fish with those for two control fish. All four fish were coho salmon which had been maintained in laboratory freshwater tanks for 3 years. During this experiment, the water temperature for the fish was 11–12 °C. The treated fish were injected intraperitoneally with 0.2 ml of corn oil containing 10 mg of 2,4,-5,2',5'-pentachlorobiphenyl, while the control fish received only corn oil. The last feeding of the fish was 3.5 h after the injections. Pairs of treated and control fish were sacrificed at 45 and 69 h after the injections. Immediately after sacrificing, the livers were excised and quickly chilled in ice-cold 0.25 M-sucrose. Assays for aryl hydrocarbon hydroxylase, as benzo[a]pyrene hydroxylase, were performed on 15 000 × g (20 min) supernatant fractions from homogenates of 1 part liver in 14 volumes of cold 0.25 M-sucrose. The enzyme assay method of Nebert and Gelboin (*15*), but using radioactive labeling techniques, was applied to incubation mixtures at 25 °C, which is approximately the optimum temperature of related enzymes in fish (*16*). Assay blanks were performed using heat-denatured portions of the 15 000 × g supernatant. The quantitation of the products of the enzymic reaction was made by performing parallel assay reactions using ^{14}C-labeled-benzo[a]pyrene (Amersham/Searle Corp.) as the substrate and determining from scintillation counts and specific radioactivity (21 mCi/mmol) of the substrate the picomoles of phenolic and other hydroxylated products produced by the reaction. Protein analyses were performed on the 15 000 × g supernatant fractions by the Lowry method as modified by Miller (*17*).

Results and Discussion

Studies of biological and biochemical effects of chlorobiphenyls often utilize complex mixtures of PCB's, and for this reason it is difficult to ascribe some biochemical effects of PCB's to specific or joint action of such undefined groups of compounds. The present study with individual chlorobiphenyls is an effort to alleviate some of the uncertainties associated with complex mixtures and learn how specific structural entities in PCB's relate to certain observed effects on fish.

Effects on Growth. Body weights of the fish were determined at each time interval when fish were selected for analyses. The data are summarized in Table I. The rate of growth of each fish which remained until 17 or 35 days was determined from the slope of a linear regression plot of wet body weight and time, using a 700 Series Wang calculator programmed for point plotting and regression analysis. The data according to each group are given in Table II. An analysis of covariance *F* test showed that the regression coefficients were

Table I. Body Weights of Salmon Fed 1, 2, and 12 ppm Chlorobiphenyls in Diets

Time, days	No. of fish	Concentration of chlorobiphenyls in diets, ppm			
		0	1	2	12
		Mean body weights, g[a]			
0	64	15.1 ± 1.8	15.7 ± 2.2	15.8 ± 1.8	15.6 ± 2.3
3	64	16.3 ± 3.5 (5.8%)	16.4 ± 3.2 (3.8%)	16.8 ± 3.0 (7.0%)	16.3 ± 3.3 (6.5%)
7	48	16.7 ± 1.4 (5.0%)	17.1 ± 3.1 (8.2%)	18.0 ± 2.6 (11%)	17.3 ± 2.7 (12%)
17	31	19.1 ± 1.5 (25%)	20.5 ± 3.2 (38%)	21.0 ± 3.7 (33%)	21.3 ± 3.2 (37%)
35	16	23.4 ± 1.1 (55%)	25.1 ± 3.9 (59%)	25.3 ± 1.6 (60%)	28.1 ± 2.3 (68%)
72	4	(20.7)[b]	(31.1)[b]	(23.5)[b]	(36.4)[b]

[a] Means ± standard deviation. Percent weight gains relative to zero time are given in parentheses. [b] Weight of single fish, which is not necessarily representative of a group.

Table II. Growth Rates of 31 Individual Experimental Coho Salmon Fed 1, 2, and 12 ppm Chlorobiphenyls

Control	ppm treated		
	1	2	12
	Gram per day[a]		
0.242	0.272	0.211	0.331
0.154	0.229	0.478	0.390
0.198	0.336	0.309	0.376
0.228	0.267	0.220	0.367
0.203	0.453	0.271	0.264
0.233	0.215	0.172	0.236
0.204	0.255	0.274	0.284
...	0.343	0.334	0.449
	Mean		
0.208	0.296	0.284	0.337
	SD		
0.030	0.078	0.095	0.072

[a] Slope of linear regression curve for body weights to 17 or 35 days.

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significantly different ($F = 3.164$, $d/$ 3 and 88, $P < 0.05$) (*12*). A multiple comparison of all pairs of regression coefficients showed that the control group was significantly different from the 12 ppm group ($P < 0.05$) (*13, 14*). All other comparisons did not show a significant difference. In this way, we found that the fish fed 12 ppm of chlorobiphenyls gained weight significantly faster than those in the control group. Thus, calculations for mean growth rates between groups of treated fish showed no significant difference—not even for a comparison of the 1 ppm treated group to the 12 ppm treated group. The treatment of fish, therefore, with these particular chlorobiphenyls resulted in a greater gain in body weights during one month of feeding than did fish receiving the basal control diet. The physiological events that result in the observed weight gain are not readily explained at this time but constitute an attractive area for future research.

When the results of the present work are compared to those of the previous work (*5*), both the ages of the fish and the solubilities of the chlorobiphenyls may have been factors in the observed differences. The molecular structures of the chlorobiphenyls were not the same between present and previous experiments. Tetrachlorobiphenyl isomers have different solubilities in water (*1*).

Chlorobiphenyl Accumulations in Salmon Bodies. Prior to feeding chlorobiphenyl-treated food pellets to the experimental fish, the foods were extracted as in the case of fish tissues, and analyses were performed by gas-liquid chromatography as previously reported (*5*). The results of the food analyses are shown in Table III. The GLC of the basal control diet extract gave numerous peaks at high sensitivity of detection, but only the three peaks corresponding to the retention times of the reference standards were quantitated for comparisons to the chlorobiphenyl-treated diets. The GLC pattern of the control fish is presumed to be a reflection of the ubiquity of lipophilic contaminants in the basal food pellets.

Our results indicate that a need exists in research for fish foods that are free of organic contaminants. In the present study, the concentrations of chlorobiphenyls were one to two orders of magnitude greater than ubiquitous foreign substances in the food, but these substances become important factors when one wishes to use concentrations much less than 1 ppm of chlorobiphenyls or other compounds in animal food. However, not enough is known to permit the preparation of a synthetic clean balanced diet for salmon that is free of the interfering contaminants. In fact, it is likely to take years to develop a clean diet that is without nutritional deficiencies.

By plotting the total combined concentration of the three chlorobiphenyls found in the experimental fish against time, it appeared that nearly constant values were reached for fish fed at the 1 ppm level (Figure 1), beginning as early as about 2 weeks; however, this was not true for fish fed 2 ppm chlorobiphenyls or more. Each point in Figure 1 is based on three or four fish except for the points at 72 days, which are for single fish. The plateau line for the lowest concentration of chlorobiphenyls between 17 and 35 days indicates a possible steady-state equilibrium condition, whereby the rates of uptake and storage of chlorobiphenyls in the fish are equal to the rates of metabolism and depuration, or residues are partitioned into water to meeting equilibrium requirements.

The total food ingested in 2 weeks amounts to 20% of the total weight of fish. Thus, if there were no excretions and ignoring weight gains, the total body burden of chlorobiphenyls theoretically would be 0.2 ppm (wt/wt) for the 1 ppm concentration in the food. This 0.2 ppm is in good agreement with the data in Figure 1. Residues do not increase further with additional feeding; thus, an equilibrium has been reached. At 2 weeks, the group receiving 2 ppm has equivalent body concentrations; one-half of the amount ingested must have been eliminated or otherwise excreted or lost. The 12 ppm group had less than six or 12 times the concentrations in the whole bodies of the 2 ppm or 1 ppm groups, respectively. If the chlorobiphenyls are taken up equally from the food, then the results suggest an ability of the fish to metabolize or excrete chlorobiphenyls at low concentrations. There is no reason to believe that less food was ingested by the 12 ppm group than by the other groups, because the fish generally consumed all food pellets that were given to them. The fish were healthy and they ate vigorously.

Data for the individual chlorobiphenyls are expected to show the effect of molecular structure on accumulations occurring in fish. Earlier work showed that 3,4,3',4'-tetrachlorobiphenyl accumulated in coho parr at about one-half of the rate for the 2,4,5,2',4',5'- and 2,4,6,2',4',6'-hexachlorobiphanyl isomers (*5*). The concentrations of 2,5,2',5'-tetrachloro-, 2,4,5,2',5'-pentachloro-, and 2,4,5,2',4',5'-hexachlorobiphenyl found in the present experimental fish are shown in Tables IV–VI, respectively.

In Table IV we show a progressive increase of 2,5,2',5'-tetrachlorobiphenyl with time at concentrations above 1 ppm

Table III. Analysis of Chlorobiphenyls in Prepared Food Pellets

Theoretical concn, ppm	Found concentration, μg/g food: 2,5,2',5'-Tetrachlorobiphenyl	2,4,5,2',5'-Pentachlorobiphenyl	2,4,5,2',4',5'-Hexachlorobiphenyl	Total
0[a]	0.027	0.063	0.018	0.11
1	0.38	0.30	0.31	0.99
2	0.69	0.73	0.65	2.07
12	4.07	3.82	3.82	11.7

[a] Control.

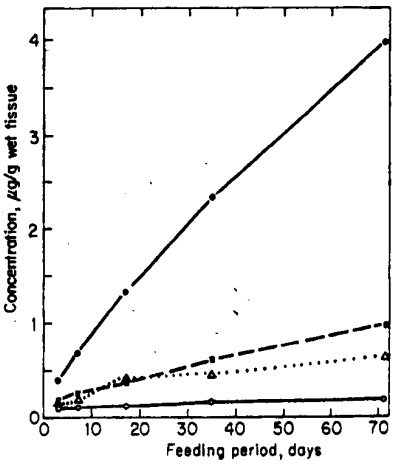


Figure 1. Total concentrations of accumulated 2,5,2',5'-tetrachlorobiphenyl, 2,4,5,2',5'-pentachlorobiphenyl, and 2,4,5,2',4',5'-hexachlorobiphenyl in salmon throughout feeding period
Fish fed chlorobiphenyls at (●) 12 ppm, (■) 2 ppm, (▲) 1 ppm, (○) control level

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chlorobiphenyls in the food. The tetrachlorobiphenyl increased from one and one-half to twofold in the fish as the feeding interval was doubled during 72 days. A significant accumulation of tetrachlorobiphenyl in the treated fish was found as early as 3 days. A concentration of 1.2 ppm (wt/wt) in fish is reached in 72 days when feeding the highest level in food. Analytical results for the pentachlorobiphenyl (Table V) and the hexachlorobiphenyl (Table VI) were nearly identical and also nearly the same as for the tetrachlorobiphenyl. Student's t test showed no significant differences among paired data for the chlorobiphenyl accumulations. It appears that a chlorine atom occupying the 4(4')-position of the chlorobiphenyls has no effect on bioaccumulations in these fish when the 2,5,2',5'-positions are also occupied by chlorine.

The comparative results for the tetrachlorobiphenyl conflict with what was found previously (*5*). Data in Table VII are given for comparison of two tetrachlorobiphenyl isomers. Because 2,4,5,2',4',5'-hexachlorobiphenyl is common to the previous and present experiments, it serves as a marker to allow the comparison to be made. The chlorine atoms in the 2,5-positions of the tetrachlorobiphenyl seem to allow the molecules to accumulate much more, perhaps twice as fast, than the molecules with chlorine atoms in the 3,4-positions. This is readily illustrated by comparing the ratios in Table VII of the concentrations of tetrachlorobiphenyls to that of the hexachlorobiphenyl.

It appears that the placement of chlorine atoms at the 2,5-positions on the aromatic nucleus hinders the reactions involved in biotransformations. One explanation of bioaccumulations is that the stereo configuration of the molecule hinders the fixation of the substrate to an aryl hydrocarbon hydroxylase, which may be involved in the first step of metabolism of chlorobiphenyls; thereby, the molecule with a hindered structure may accumulate in an organism. On the other hand, the greater water solubility of 3,4,3',4'-tetrachlorobiphenyl compared to other chlorobiphenyls, as noted by Hutzinger et al. (*1*), is a factor in reducing the quantity accumulated. But according to what is presently understood, the difference in accumulations of the 2,5,2',5'- and 3,4,3',4'-tetrachlorobiphenyls is more plausibly related to a preferential loss of the 3,4,3',4'-isomer as a result of higher water solubility and a lower partition coefficient between fish and water. A similar explanation can be made for the relative accumulations of other components of PCB's in fish.

Zinck and Addison (*18*) intravenously administered 2-, 3-, and 4-chlorobiphenyl to two species of skate (*Raja radiata* and *R. ocellata*) and found that the 3-isomer was excreted to a greater extent than the other two isomers. Here, too, the 3-position for chlorination of biphenyl is perhaps involved so as to enhance the hydrophilic character of the compound. A determination of a complete material balance for chlorobiphenyls entering and leaving fish could possibly lead to an understanding of the role of solubility, gastrointestinal absorption, and other physiological processes related to uptake and bioaccumulations of chlorobiphenyls.

It is interesting to find in Table VII that the concentrations of 2,4,5,2',4',5'-hexachlorobiphenyl in cohorts of salmon one year different in age were nearly the same. This is likely in part a reflection of the closeness of the feeding regimens. Over a period of time, the regimens allowed nearly identical absolute quantities per unit body weight of the compounds to be fed to the fish at the levels of 10 and 12 ppm.

Table IV. Concentrations of 2,5,2',5'-Tetrachlorobiphenyl in Juvenile Coho Salmon Fed 1, 2, and 12 ppm Combined Chlorobiphenyls in Diets

Days	Concentration in diets, ppm			
	0[a]	1	2	12
	Concentration in wet tissue, ng/g[b]			
3	29 ± 4	42 ± 7	58 ± 5	142 ± 24
7	28 ± 6	66 ± 8	93 ± 9	252 ± 16
17	24 ± 1	144 ± 24	122 ± 23	450 ± 87
35	45 ± 17	139 ± 35	198 ± 22	775 ± 82
72	45	180	300	1230

[a] Control diet, zero ppm theoretical, 0.1 ppm found. [b] Mean values, ± standard deviations, for three or four fish analyzed at 3—35 days; single fish analyzed at 72 days.

Table V. Concentrations of 2,4,5,2',5'-Pentachlorobiphenyl in Juvenile Coho Salmon Fed 1, 2, and 12 ppm Combined Chlorobiphenyls in Diets

Days	Concentration in diets, ppm			
	0[a]	1	2	12
	Concentration in wet tissues, ng/g[b]			
3	51 ± 10	53 ± 6	65 ± 5	133 ± 19
7	47 ± 9	78 ± 9	93 ± 9	218 ± 17
17	46 ± 2	156 ± 17	140 ± 20	444 ± 76
35	69 ± 16	162 ± 42	214 ± 21	782 ± 68
72	82	235	355	1380

[a] Same as in Table IV. [b] Same as in Table IV.

Table VI. Concentrations of 2,4,5,2',4',5'-Hexachlorobiphenyl in Juvenile Coho Salmon Fed 1, 2, and 12 ppm Combined Chlorobiphenyls in Diets

Days	Concentration in diets, ppm			
	0[a]	1	2	12
	Concentration in wet tissue, ng/g[b]			
3	32 ± 10	46 ± 10	59 ± 16	130 ± 18
7	25 ± 6	57 ± 12	72 ± 11	214 ± 13
17	38 ± 9	146 ± 28	121 ± 26	444 ± 106
35	58 ± 12	151 ± 29	197 ± 16	775 ± 87
72	62	190	322	1380

[a] Same as in Table IV. [b] Same as in Table IV.

Chlorobiphenyl Induction of Aryl Hydrocarbon Hydroxylation. Certain chlorobiphenyls are metabolized in birds and mammals (*3*), and there are indications from studies with industrial PCB's (Aroclor 1254) that some chlorobiphenyls are metabolized extremely slowly in fish (*1*). If the metabolism involves the biochemical transformations that are associated with other aromatic hydrocarbons, then a first step could be hydroxylation, perhaps via an arene oxide intermediate (*19*). Such oxide intermediates are more likely to be formed from chlorobiphenyls of low chlorination as opposed to highly chlorinated biphenyls, because two adjacent nonchlorinated positions on the biphenyl nucleus are a possible prerequisite to the reaction (notwithstanding dechlorination). Fish are generally recognized to possess enzymic systems capable of xenobiotic metabolism (*16, 20, 21*). Male and female trout (*Salmo fario*) were shown by Creaven et al. (*22*) to possess hepatic microsomal hydroxylase activity toward biphenyl. However, there is no evidence reported for an association between aryl hydrocarbon hydroxylase and chlorobiphenyls in fish.

Data in Table VIII show the enzyme inductive effects of single doses of the pentachlorobiphenyl in two male coho salmon. At 45 and 69 h after the treatment, hepatic enzymatic activities of test fish were 2.6 and 2.4 times greater, respectively, than those of control fish. Although these data are obtained from only four salmon livers, we routinely measure benzo[a]pyrene hydroxylase in our laboratory and find that replications among assays for a like group of fish have standard deviations which are ±37% of the mean assay values.

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Table VII. Concentrations of Chlorobiphenyls in Juvenile Coho Salmon Fed 10–12 ppm Chlorobiphenyls

		μg chlorobiphenyl/g wet tissue			
Expt	Days	3,4,3',4'-Tetrachlorobiphenyl	2,5,2',5'-Tetrachlorobiphenyl	2,4,5,2',4',5'-Hexachlorobiphenyl	Ratio[d]
I[b]	53	0.59 ± 0.23	...	0.93 ± 0.21	0.6
I	108	0.65 ± 0.32	...	1.41 ± 0.35	0.5
II[c]	35	...	0.78 ± 0.08	0.78 ± 0.09	1.0
II	72	...	1.23	1.38	0.9

[a] Concentration of tetrachlorobiphenyl divided by concentration of hexachlorobiphenyl. [b] Ten ppm of equal portions of 3,4,3',4'-tetrachloro-, 2,4,5,2',4',5'-, and 2,4,6,2',4',5'-hexachlorobiphenyl, feeding 3.5% body weight/day, 3 days/week (5). [c] Twelve ppm of equal portions of 2,5,2',5'-tetrachloro-, 2,4,5,2',5'-pentachloro-, and 2,4,5,2',4',5'-hexachlorobiphenyl, feeding 2.0% body weight/day, 5 days/week.

Table VIII. Aryl Hydrocarbon (Benzo[a] pyrene) Hydroxylase Activity in Hepatic Tissues of 3-Year Old Coho Salmon Treated with 10 mg of 2,4,5,2',5'-Pentachlorobiphenyl

	Fish			Time after treatment,[a]	Hydroxylase activity,
Group	Sex	Wt, g	Length, cm	h	pmol/mg protein/30 min
Test	♂	240	33	45	180 ± 18
Control	♀	225	30	45	68 ± 3
Test	♂	167	26	69	49 ± 2
Control	♂	164	26	69	20 ± 8

[a] See Experimental section.

Thus, in the present case, the effect is significant for in vivo induction of the enzymic system by a single dose of pentachlorobiphenyl in salmon.

The induction of an aryl hydrocarbon hydroxylase in fish by a chlorobiphenyl has been demonstrated for the first time, using 2,4,5,2',5'-pentachlorobiphenyl. It is tempting to speculate on the basis of our research with a single biphenyl that certain other aromatic substrates, including certain other chlorinated biphenyls, may alter the activities of the aryl hydrocarbon hydroxylases.

Sanborn et al. (23) exposed green sunfish (*Lepomis cyanellus*) to aqueous solutions of pure ^{14}C-labeled chlorobiphenyls to study metabolism. They found percentage distributions of 18.39, 98.84, and 99.41%, respectively, for 2,5,2'-trichlorobiphenyl, 2,5,2',5'-tetrachlorobiphenyl, and 2,4,5,2',5'-pentachlorobiphenyl in the fish as parent materials, and the remainder of radioactivity occurred as polar residues. Thus, sunfish are apparently able to metabolize the chlorobiphenyls, especially the trichlorobiphenyl. Zinck and Addison (*18*), on the other hand, showed that a large fraction of monochlorobiphenyls was excreted from skate when the compounds were administered by intravenous injection. Although they did not indicate the excretion of polar derivatives of the chlorobiphenyls, that possibility remains an open question. Aside from the above species differences, different routes of metabolism could be involved in fish depending on the mode of entry of the compounds into the organisms.

PCB's induce microsomal enzymes in higher animals, notably in rats (*24–26*). The practical implication of such induced enzymatic activities is that the animals may be expected to metabolize many foreign compounds at elevated rates. The rates of metabolism of PCB's are very slow in fish. That a chlorobiphenyl induces the microsomal enzyme system for aryl hydrocarbon hydroxylation suggests that aromatic hydrocarbons from the environment, such as those occurring in petroleum, are more readily metabolized in fish which have been preexposed to chlorobiphenyls. The present evidence suggesting that the chlorobiphenyls alter the activities of the aryl hydrocarbon hydroxylase in fish is an important area for future research.

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