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Role of Temperature and Enzyme Induction in the Biotransformation of Polychlorinated Biphenyls and Bioformation of Hydroxylated Polychlorinated Biphenyls by Rainbow Trout (*Oncorhynchus mykiss*)

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Hydroxylated PCBs (OH−PCBs) are metabolites of polychlorinated biphenyls (PCBs) that have recently been found in the plasma of Great Lakes fish. Studies have shown that the ability of laboratory-held rainbow trout (*Oncorhynchus mykiss*) to bioform OH−PCBs from dietary mixtures of PCB congeners is complex and may be attributed to factors such as temperature and/or enzyme induction. Past studies have also suggested that CYP1A- and 2B-like enzymes are the likely mechanism for forming OH−PCBs, but this has not been directly studied in a controlled setting. To address these issues, we exposed rainbow trout (∼80 g) to dietary concentrations of a mixture of three Aroclors (1248, 1254, and 1260), at three water temperatures (8, 12, and 16 °C), as well as additional PCBs known to induce CYP1A- and CYP2B-like isoforms in mammals. PCB half-lives in trout were inversely related to water temperature, but biotransformation of PCBs was positively related to water temperature. Thirty-one OH−PCBs were observed in trout plasma after 30 days of dietary exposure to the Aroclor mixtures, although approximately 40% of the ∑OH−PCBs concentrations were OH−PCB for which no standards were available. Concentration of OH−PCBs in the trout plasma increased with increasing temperature and with the addition of CYP2B-like inducing congeners but not with the addition of CYP1A-inducing congeners to food. The results of this study provide the first *in vivo* evidence that rainbow trout are responsive to CYP2B-like induction by PCBs and that this enzyme system can influence PCB concentrations and OH−PCB formation in fish.

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Introduction

There has been an increase in studies focusing on metabolites of PCBs, hydroxylated PCBs (OH−PCBs), in the environment. Concentrations of OH−PCBs have been reported in a number of fish species (*1*−*2*). Because fish are believed to have limited ability to biotransform PCBs compared with mammals and birds (*3*), the presence of OH−PCBs in fish has led to questions about the source of these compounds and their potential effects in fish.

Several studies have demonstrated biotransformation of PCBs by fish (*4*−*13*). For example, recent studies have demonstrated that fish slowly metabolize PCB 77 (3,3′,4,4′-tetrachlorobiphenyl (*14*) and PCB 52 (2,2,5,5-tetrachlorobiphenyl) (*15*) and form OH−PCBs after exposure to PCB 77 (*14*). Buckman et al. (*13*) also showed that rainbow trout exposed to a mixture of PCBs (Aroclors 1248, 1254, and 1260) bioformed seven OH−PCB congeners. The pattern of OH−PCBs detected in trout plasma (*13*) were similar to the pattern of OH−PCBs found in wild lake trout (*Salvelinus namaycush*) from the Great Lakes (*1*) suggesting that biotransformation of PCBs is the likely source for OH−PCBs in wild fish. However, in a study using smaller fish and a cooler water temperature (8 °C) (*16*), no OH−PCB metabolites were found in the plasma of rainbow trout exposed to the same Aroclor mixtures.

Due to the variability in results reported in literature, it seems likely that bioformation of OH−PCBs may be dependent upon factors, such as temperature, that may limit the ability of fish to biotransform PCBs. Temperature has been demonstrated to be a significant factor in the elimination of other xenobiotics (*17*−*19*) and can influence biotransformation enzyme activity (*20*). However, it has been suggested that the effect of temperature on biotransformation may be limited for the more persistent substances such as PCBs (*21*).

Induction of cytochrome P450 (CYP) enzymes may also affect the rate of biotransformation of PCBs in fish. Because the metabolism of PCBs is likely achieved by the involvement of CYP enzymes, CYP induction may enhance the biotransformation of specific PCBs. This has been noted for other compounds (*22*−*24*). Non-*ortho* substituted PCBs (77, 126, and 169) are a sub-group of PCB congeners that are known to be the most potent inducers of the CYP1A enzymes and are considered the most toxic congeners (*25*). In addition, there are a number of PCBs that are known to induce CYP2B enzymes in mammals, although there is some debate as to whether these compounds can induce CYP2B-like isoforms in fish (*26*). Concentrations of both CYP1A-inducing and CYP2B-inducing congeners have been observed in Great Lakes fish (*27*) and could potentially affect the ability of fish to biotransform PCBs to OH−PCB metabolites.

To clarify mixed findings reported in the literature, we examined the extent to which temperature may influence the biotransformation of a large number of PCB congeners in rainbow trout (*Oncorhynchus mykiss*). The influence of CYP induction (CYP1A- and CYP2B-like isoforms) on the rate of biotransformation was also assessed. Depuration rates and biotransformation rates of PCBs as well as concentrations of 68 OH−PCBs and OH−PCB homologue group concentrations that include concentrations of unknown OH−PCBs (OH−PCBs without quantification standards) are provided.

Methodology

Chemicals and Food Preparation. Aroclors 1242, 1254, and 1260 and PCBs 202 and 209 were purchased from AccuStan-

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TABLE 1. PCB Concentrations (arithmetic mean ± 1 SE) in (A) Food and Trout Carcass and (B) Trout Plasma, Both at Day 30 (final day of uptake)

(A) Food and Carcass

homologue group	control food	PCB-exposed food	CYP1A-exposed food	CYP2B-exposed food	control 8 °C carcass	control 12 °C carcass	control 16 °C carcass	PCB-exposed 8 °C carcass	PCB-exposed 12 °C carcass	PCB-exposed 16 °C carcass	CYP1A-exposed 12 °C carcass	CYP2B-exposed 12 °C carcass
di-	8.0 ± 0.71	180 ± 13	210 ± 5.1	200 ± 2.1	54 ± 5.9	44 ± 4.9	41 ± 5.1	1200 ± 220	970 ± 98	910 ± 120	1200 ± 75	910 ± 88
tri-	20 ± 0.32	780 ± 57	840 ± 6.7	810 ± 35	100 ± 8.4	76 ± 6.4	74 ± 4.4	3700 ± 850	2900 ± 290	2800 ± 4000	3300 ± 290	2500 ± 230
tetra-	64 ± 0.45	1300 ± 82	1600 ± 74	1500 ± 98	250 ± 13	220 ± 14	200 ± 13	5200 ± 1100	4000 ± 450	4000 ± 470	4800 ± 360	4000 ± 540
penta-	27 ± 1.1	900 ± 94	1500 ± 38	1500 ± 130	190 ± 9.3	160 ± 8.7	140 ± 7.9	6200 ± 1600	4700 ± 650	4800 ± 740	6100 ± 580	5000 ± 830
hexa-	12 ± 0.32	1000 ± 120	1800 ± 43	1900 ± 160	86 ± 8.8	64 ± 5.9	67 ± 6.2	6900 ± 2100	5200 ± 780	5500 ± 1000	6900 ± 870	5300 ± 910
hepta-	2.9 ± 0.42	580 ± 82	1100 ± 33	1100 ± 96	22 ± 7.6	16 ± 3.9	17 ± 3.8	4400 ± 1500	3200 ± 500	3400 ± 780	4100 ± 650	2700 ± 560
octa-	2.2 ± 0.05	180 ± 24	340 ± 14	350 ± 31	23 ± 6.8	16 ± 4.1	18 ± 3.8	1900 ± 650	1300 ± 200	1500 ± 380	1700 ± 330	1200 ± 250
nona-	0.31 ± 0.04	24 ± 3.5	45 ± 0.61	48 ± 4.0	5.4 ± 0.92	2.2 ± 0.42	2.8 ± 0.54	280 ± 100	180 ± 31	230 ± 70	260 ± 59	160 ± 30
deca-	0.23 ± 0.14	42 ± 25	150 ± 8.6	160 ± 15	6.2 ± 1.1	2.4 ± 0.53	3.5 ± 0.61	890 ± 320	500 ± 84	740 ± 200	700 ± 160	420 ± 77
∑PCBs	180 ± 2.1	6600 ± 550	1000 0 ± 260	1000 0 ± 760	1000 ± 330	930 ± 210	902 ± 132	38447 ± 10579	28736 ±3780	30000 ± 5100	3600 0 ± 4000	2800 0 ± 4400
CYP1A-Inducing PCB Congeners												
PCB 77	ND[a]	5.3 ± 1.2	8.9 ± 1.4	4.8 ± 1.3	ND	ND	ND	320 ± 120	330 ± 44	260 ± 120	890 ± 140	290 ± 53
PCB 126	ND	ND	11 ± 2.1	ND	ND	ND	ND	ND	ND	ND	240 ± 88	ND
PCB 169	ND	ND	12 ± 1.8	ND	ND	ND	ND	ND	ND	ND	210 ± 68	ND
CYP2B-Inducing PCB Congeners												
PCB 87	2.8 ± 0.13	130 ± 13	110 ± 5.9	220 ± 20	24 ± 2.4	32 ± 2.4	35 ± 3.5	400 ± 38	430 ± 62	450 ± 74	460 ± 81	790 ± 160
PCB 99	1.6 ± 0.12	45 ± 5.3	40 ± 4.2	78 ± 7	23 ± 3.1	31 ± 1.8	33 ± 2.6	220 ± 32	290 ± 60	330 ± 43	330 ± 58	520 ± 100
PCB 101	3.2 ± 0.21	100 ± 10	100 ± 5.8	160 ± 13	58 ± 5.2	83 ± 8.9	87 ± 9.6	800 ± 100	840 ± 100	880 ± 110	910 ± 100	1300 ± 290
PCB 153	1.9 ± 0.11	130 ± 22	150 ± 14	270 ± 23	54 ± 4.6	71 ± 6.5	73 ± 9.1	780 ± 130	990 ± 150	1100 ± 190	1000 ± 160	1600 ± 410
PCB 180	1.1 ± 0.14	240 ± 35	240 ± 16	500 ± 45	33 ± 2.2	41 ± 4.7	47 ± 5.8	1000 ± 200	1200 ± 200	1200 ± 430	1100 ± 210	1800 ± 620
PCB 183	0.21 ± 0.01	36 ± 5.1	35 ± 3.8	70 ± 6.6	8.4 ± 0.83	10 ± 1.4	11 ± 2.1	200 ± 43	240 ± 39	240 ± 57	240 ± 47	520 ± 100
PCB 194	0.12 ± 0.01	40 ± 5.5	38 ± 7.0	83 ± 8.1	8.2 ± 0.71	7.7 ± 4.1	8.1 ± 4.4	300 ± 60	320 ± 54	390 ± 100	300 ± 62	600 ± 180

(B) Plasma

homologue group	control 8 °C	control 12 °C	control 16 °C	PCB-exposed 8 °C	PCB-exposed ~12 °C	PCB-exposed 16 °C	CYP1A-exposed 12 °C	CYP2B-exposed 12 °C
di-	22 ± 0.61	21 ± 0.61	35 ± 4.4	37 ± 1.1	42 ± 4.9	48 ± 3.9	56 ± 3.3	190 ± 93
tri-	77 ± 17	47 ± 4.3	46 ± 1.6	200 ± 4.2	230 ± 26	250 ± 10	200 ± 6.2	220 ± 14
tetra-	100 ± 29	59 ± 10	59 ± 6.8	290 ± 6.8	310 ± 31	360 ± 15	300 ± 11	330 ± 81
penta-	17 ± 4.4	13 ± 1.7	13 ± 1.6	84 ± 2.1	90 ± 8.4	110 ± 3.5	91 ± 3.0	88 ± 26
hexa-	43 ± 12	42 ± 7.1	38 ± 6.4	310 ± 12	320 ± 33	390 ± 19	370 ± 17	350 ± 120
hepta-	20 ± 6.2	24 ± 7.5	19 ± 4.6	300 ± 9.0	290 ± 28	400 ± 15	340 ± 6.3	380 ± 22
octa-	7.0 ± 1.7	15 ± 5.4	6.6 ± 0.93	59 ± 2.6	55 ± 6.2	100 ± 23	340 ± 260	390 ± 340
nona-	3.3 ± 1.2	5.0 ± 1.6	2.4 ± 0.94	15 ± 0.21	16 ± 0.21	24 ± 1.0	19 ± 0.91	15 ± 7.5
deca-	5.0 ± 1.5	5.5 ± 0.22	1.5 ± 0.92	55 ± 6.5	51 ± 6.1	75 ± 7.7	94 ± 45	58 ± 29
PCB	400 ± 100	300 ± 50	280 ± 32	1700 ± 48	1800 ± 180	2200 ± 120	2200 ± 290	2400 ± 560
CYP1A-Inducing PCB Congeners								
PCB 77	ND	ND	ND	16 ± 3.2	23 ± 4.4	23 ± 5.8	30 ± 5.2	17 ± 3.3
PCB 126	ND	ND	ND	ND	ND	ND	12 ± 2.6	ND
PCB 169	ND	ND	ND	ND	ND	ND	11 ± 1.9	ND
CYP2B-Inducing PCB Congeners								
PCB 87	6.6 ± 2.0	3.3 ± 0.51	3.9 ± 0.50	25 ± 0.72	27 ± 3.0	29 ± 5.5	28 ± 0.81	40 ± 1.5
PCB 99	4.1 ± 1.0	2.2 ± 0.43	2.2 ± 0.24	12 ± 0.31	13 ± 1.3	14 ± 3.1	13 ± 0.52	25 ± 1.0
PCB 101	8.8 ± 2.5	4.0 ± 0.11	4.9 ± 0.61	28 ± 0.71	29 ± 2.5	31 ± 8.2	30 ± 1.0	54 ± 1.6
PCB 153	5.9 ± 1.3	5.0 ± 0.64	5.3 ± 0.91	44 ± 1.4	44 ± 3.7	44 ± 16	49 ± 1.6	76 ± 3.8
PCB 180	5.1 ± 1.2	5.9 ± 1.4	6.2 ± 1.3	95 ± 2.6	89 ± 8.5	95 ± 32	100 ± 0.42	230 ± 7.2
PCB 183	1.0 ± 0.34	1.4 ± 0.43	0.51 ± 0.11	9.0 ± 0.21	9.7 ± 1.0	7.5 ± 3.8	8.1 ± 0.92	31 ± 1.3
PCB 194	1.2 ± 0.32	1.4 ± 0.22	1.4 ± 0.31	18 ± 0.91	16 ± 1.9	15 ± 7.8	23 ± 0.71	56 ± 1.6

[a] ND = Non-detectable concentrations (below limits of detection 0.1 ng/g (w/w)). PCB-exposed = 10 μg/g of each of Aroclor (1242, 1254, and 1260) and 0.5 μg/g of PCBs 202 and 209. CYP1A-exposed = same as PCB-exposed with additional CYP1A-inducing PCB congeners (PCBs 77, 126, and169 (10 ng/g each)). CYP2B-exposed = same as PCB-exposed with additional CYP2B-inducing PCB congeners (PCBs 87, 99, 101, 153, 180, 183, and 194 (10 ng/g each)).

dard (New Haven, CT); OH−PCB standards and ^{13}C-OH−PCBs were from Wellington Labs (Guelph, ON, Canada) and AccuStandard; and all solvents (pesticide grade) were from Caledon Laboratories (Georgetown, ON, Canada). Granular sodium sulfate (ACS grade) was obtained from EM Science (Gibbstown, NJ) and was heated at 550 °C for 16 h prior to use. Pesticide-grade dry silica (60−200 mesh) was obtained from ACP (Montreal, PQ, Canada).

Fish food was prepared following established methods for bioaccumulation experiments (*13*) and is summarized in the Supporting Information. Four types of food were produced: control (no PCBs added), PCB-exposed (10 μg/g of each of Aroclors 1242, 1254, and 1260 plus 0.5 μg/g of PCB 202 and 209), CYP1A-exposed (same PCB mixture as PCB-exposed food plus 10 ng/g of CYP1A-inducing PCB congeners 77, 126, and 169), and CYP2B-exposed (same PCB mixture as PCB-exposed food plus 10 ng/g of PCB congeners that induce CYP2B enzyme activity in mammals, PCBs 87, 99, 101, 153, 180, 183, and 194). Concentrations of PCBs in food are reported in Table 1.

Fish Husbandry. Juvenile rainbow trout (initial weights, ~80 g; Rainbow Springs Trout Hatchery, Thamesford, ON; Stevenson strain) were held in de-chlorinated, flow-through water at 8, 12, or 16 °C (±0.5 °C) and a 12 h light and dark cycle. Trout were maintained on non-spiked food at a feeding rate of 1.5% of the average body weight of the fish for 1 month prior to the experiment. The Canadian Animal Care Guidelines were followed throughout the duration of fish husbandry.

Exposures. Juvenile rainbow trout (initial weight, ~80 g; n = 85 per tank) were tagged with individual pit tags and weighed 20 days prior to commencement of exposure. Eight treatments were established: three control (fed food with no PCBs added, held at 8, 12, or 16 °C), three PCB-exposed (fed PCB-spiked food and held at 8, 12, or 16 °C), a CYP1A-exposed (fed CYP1A-dosed food and held at 12 °C), and a CYP2B-exposed (fed CYP2B-dosed food and held at 12 °C). Rainbow trout in three treatments (PCB-, CYP1A-, and CYP2B-exposed) were fed PCB-spiked food (treatment food described above) for 30 days (uptake phase) followed by 300 days of

non-spiked food (depuration phase) at a feeding rate of 1.5% of the average body weight of the fish, readjusted for each sampling day. Each treatment group was held in separate aquaria (300 L), the outlet of which contained activated charcoal in nylon bags to absorb any dissolved PCBs or metabolites in the water. On days 0, 5, 10, 20, and 30 of the uptake phase and days 5, 10, 20, 40, 80, 160, and 300 of the depuration phase, six fish from each treatment (control and PCB-exposed (8, 12, and 16 °C) and CYP1A-exposed and CYP2B-exposed (12 °C)) were sacrificed by cervical section and sampled for determination of PCB concentrations. Liver and gastrointestinal (GI) tract (including the stomach, pyloric caeca, spleen, intestines, and adipose fat associated with these organs as well as the gut contents), were separated from the carcass to avoid contamination from PCBs in undigested food. Blood was taken from each of the six fish sampled at day 30 of uptake prior to sacrifice, to determine OH−PCB concentrations. Whole blood was transferred to Eppendorf vials and centrifuged for 5 min at 10000 rpm on an IEC Micromax (model #3590, Rotor #851) (Needham Hts, MA) centrifuge to separate plasma. Tissues and plasma were weighed and then frozen at −80 °C until analyzed.

PCB, OH−PCB, and EROD Analyses. PCB extraction and analysis in fish and food followed previously established methods (*13*), which are summarized in the Supporting Information. Note the recovery of PCB 30, added to fish prior to extraction, was 82.36% ± 2.11% (mean ± standard error (SE)), and concentrations were corrected for recovery. The method used for OH−PCB extraction, cleanup, and analysis of PCBs and OH−PCBs in plasma is detailed previously (*28*). The average percent recovery of the organic phase (PCBs in plasma) was 79.8 ± 1.6% (mean ± 1 SE) based on PCBs 30 and 204. Five ^{13}C internal standards (^{13}C 4-OH-12, ^{13}C 4-OH-29, ^{13}C 4-OH-61, ^{13}C 4-OH-120, and ^{13}C 4-OH-187) from various homologue groups were used to determine the recovery of OH−PCBs in the plasma and are discussed below. OH−PCB concentration data were recovery corrected to compare homologue group levels. No OH−PCBs were detected in the food. Analysis of liver samples for EROD enzyme activity was carried out with post-mitochondrial supernatants as described previously (*29*) with modifications (*30*).

Data Analysis. Individual growth rates were determined by subtracting the natural log of the initial weight from the natural log of the final weight and dividing by the day of experiment plus twenty (because fish were tagged and weighed 20 days before experimentation began). As growth dilution can cause differences in concentration between individual fish, all concentrations were corrected for growth by multiplying the fish concentrations by a factor of ($1 + b_i \times$ time), where b_i is the individual growth rate (*31*).

All bioaccumulation parameters used lipid-based concentrations as lipid content increased over the duration of the experiment. Depuration rate (k_d) constants for PCBs were determined by fitting the data to a first-order decay curve (ln concentration $= a + k_d \times$ time (d), where a is the intercept). Half-life ($t_{1/2}$) values were calculated using $\ln 2/k_d$.

Differences between whole body growth rate constants and depuration rates were tested for homogeneity of slope and parallelism in an analysis of covariance using the general linear model in SYSTAT (version 10, SPSS Ltd.). Tukey's Honestly Significant Difference (HSD) test was used to compare percent lipid differences between treatments at the $p < 0.05$ level of significance.

We analyzed biotransformation of over 90 PCBs using two methods. The first method assessed biotransformation of PCBs by measuring 68 OH−PCBs and unknown OH−PCBs in the plasma of fish at day 30 of uptake. The second method produced biotransformation rates for biotransformed PCB congeners (*31*). This method compared $t_{1/2}$ values of the PCBs of interest with those of 16 known recalcitrant PCBs (PCBs 18, 28, 44, 52, 66, 101, 105, 118, 128, 138, 153, 187, 189, 195, 206, and 209), which had maximum chlorine substitution in the *meta* and *para* positions of the biphenyl rings and thus should have no significant biotransformation and the slowest elimination of all the PCB congeners, varying with the log K_{ow} of the congener (*31*, *32*). Biotransformation was deemed to be significant for a congener when the standard error of its $t_{1/2}$ fell below the 95% confidence intervals of the regression between log $t_{1/2}$ and log K_{ow} of the 16 known recalcitrant PCBs. Subtracting this minimal depuration rate based on the congeners K_{ow} from the experimentally determined depuration rate provides an estimate of the biotransformation rate of that congener.

Results and Discussion

Growth, Liver Size, and Survival. Not surprisingly, whole fish and liver growth rates were significantly different between temperature treatments, with faster growth rates at higher temperatures. Differences in growth rates were found between 8 °C treatments (whole fish growth rate ≈ 9.1, liver growth rate ≈ 5.1) and both the 12 °C treatments (whole fish growth rate ≈ 10.0, liver growth rate ≈ 5.5) and 16 °C treatments (whole fish growth rate ≈ 12.5, liver growth rate ≈ 6.6) ($p < 0.05$), but there were no differences in growth rates between 12 °C treatments and 16 °C treatments. No differences in whole fish or liver growth rates were found between Aroclor exposed and control juvenile rainbow trout populations ($p > 0.05$). The liver somatic index (LSI) was significantly different ($p < 0.05$) between temperatures with a larger LSI at colder temperatures (2.2−2.7 at 8 °C) and LSI decreasing with increasing temperature (between 1.7 and 1.8 at 12 °C and at 1.5 at 16 °C). However, the LSI of fish exposed to PCBs after 30 days were not significantly different from controls ($p > 0.05$). Liver somatic indices also decreased over the course of the experiment in all groups ($p < 0.05$) as fish grew bigger. Mortality was minimal in all treatments and ranged from 1.3 and 6.0%.

Half-lives of Recalcitrant PCBs. Half-lives of recalcitrant PCB congeners were dependent on temperature and longer half-lives were found for fish held in cooler water. Half-lives ranged between 130 and 263 days (mean r^2 for depuration rates was 0.85) for the 8 °C treatment, between 102 and 210 days (mean $r^2 = 0.83$) for the 12 °C treatments, and between 94 and 190 (mean $r^2 = 0.88$) for the 16 °C PCB-exposed treatment (Supporting Information, Table 1). Half-lives of recalcitrant PCB congeners from the CYP1A- and CYP2B-exposed treatments did not differ from the 12 °C PCB-exposed treatment.

Curvalinear relationships between $t_{1/2}$ and log K_{ow} of recalcitrant PCB congeners (listed above) were found at all temperature ranges and all PCB-exposed treatments (Figure 1). The coefficients of the quadratic regressions of this relationship were nearly identical between all treatments [($b_1 = 1.11$, $b_2 = -0.1$, $r^2 = 0.83$; 8 °C) ($b_1 = 1.13$, $b_2 = -0.1$, $r^2 = 0.85$; 12 °C) ($b_1 = 1.13$, $b_2 = -0.1$, $r^2 = 0.86$; 16 °C) ($b_1 = 1.10$, $b_2 = -0.1$, $r^2 = 0.84$; CYP1A-exposed, 12 °C) ($b_1 = 1.12$, $b_2 = -0.1$, $r^2 = 0.82$; CYP2B-exposed, 12 °C), as well as the coefficients reported previously for the same subset of congeners in similar sized (*13*) and smaller fish (~5 g starting weight) (*16*, *31*). However, the intercept of the regression was greater in the 8 °C treatment compared with the 12 and 16 °C treatments, and thus $t_{1/2}$ values were greater at lower temperatures. These $t_{1/2}$ values, and relationships with water temperatures, are consistent with previous studies (*13*, *16*, *31*), but it has been suggested that the effect of temperature on elimination and metabolism may be limited for the more persistent substances such as PCBs (*21*, *33*). The results of this study suggest that the effects of temperature on the $t_{1/2}$ values of recalcitrant PCBs do not vary with log K_{ow}.

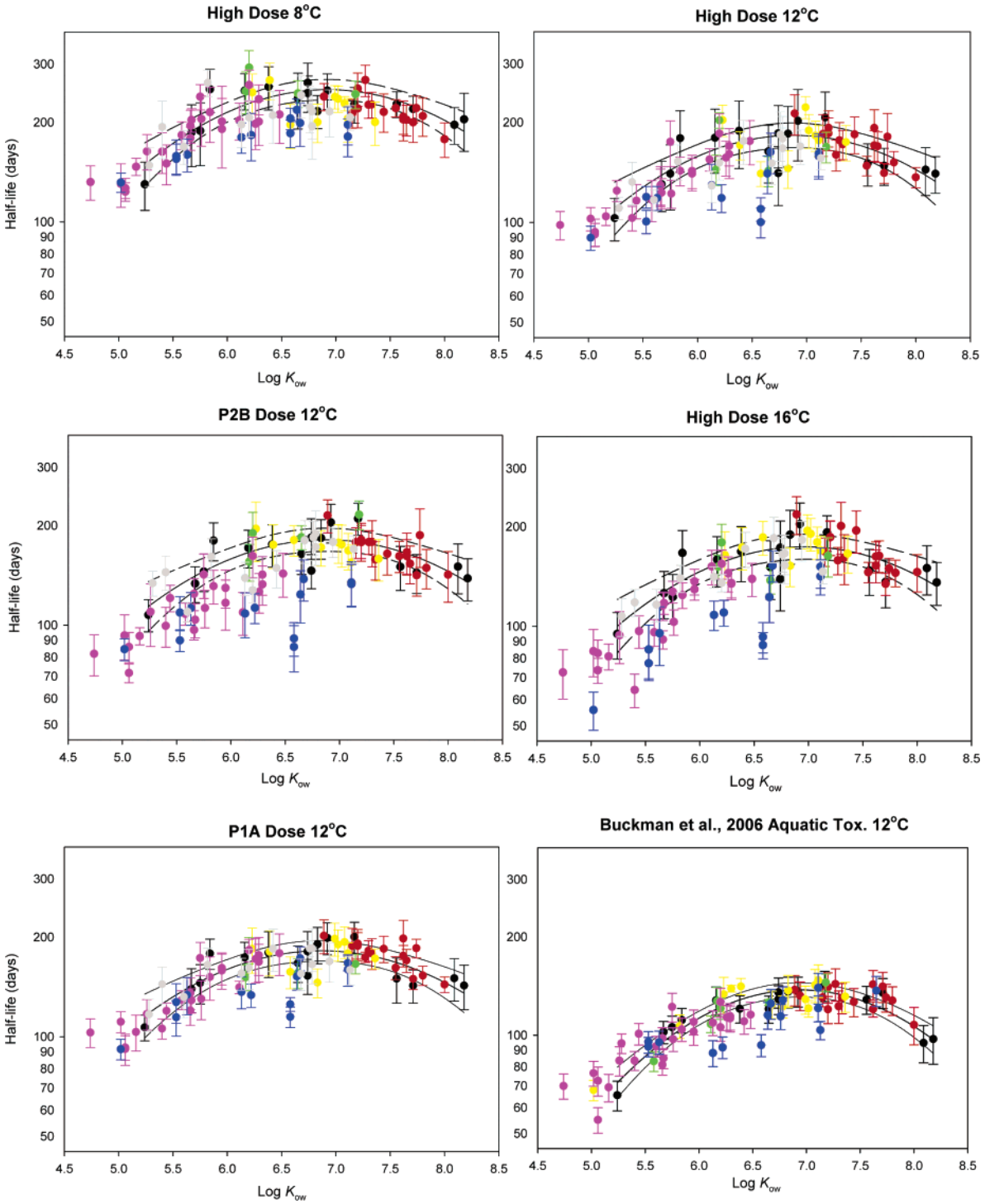

FIGURE 1. Log half-life ($t_{1/2}$) of 92 polychlorinated biphenyl (PCB) congeners in rainbow trout vs log octanol/water partition coefficient (K_{ow}). PCB-exposed dose = 10 μg/g of each Aroclor mixture (1:1:1 ratio of Aroclors 1242:1254:1260 and 0.5 μg/g of PCBs 202 and 209. CYP1A-exposed dose equals the same as the PCB-exposed dose with additional CYP 1A-inducing congeners (PCBs 77, 126, and 169, 10 ng/g each). CYP2B-exposed dose equals the same as the PCB-exposed dose with additional CYP2B-inducing congeners (PCBs 87, 99, 101, 153, 180, 183, and 194). Black circles represent 16 recalcitrant PCB congeners (PCBs 18, 28, 44, 52, 66, 101, 105, 118, 128, 138, 153, 187, 189, 195, 206, and 209) which had maximum chlorine substitution in the *meta* and *para* positions of the biphenyl rings. Red circles represent Group I congeners (no vicinal H atoms); yellow circles represent Group II congeners (vicinal H atoms only in *ortho* and *meta* positions of the biphenyl ring and greater than or equal to 2 chlorines in *ortho* positions); Green circles represent Group III congeners (same as Group II but with less than 2 *ortho* chlorines); Magenta circles represent Group IV congeners (vicinal H atoms in the *meta* and *para* positions of the biphenyl ring with 2 or less *ortho* chlorines); Blue circles represent Group B congeners (same as Group IV but with greater than 2 *ortho* chlorines). Lines represent quadratic regressions for filled black circles only.

PCB Biotransformation and Metabolite Formation. In several treatments (12 °C PCB-exposed, 16 °C PCB-exposed, CYP1A-exposed, and CYP2B-exposed), a number of PCB congeners fell below the log $t_{1/2}$ − log K_{ow} relationship derived from the 16 recalcitrant PCBs, indicating that they were biotransformed (Figure 1) (*31*, *32*). The PCBs that were biotransformed by rainbow trout had similar chlorine substitution patterns, indicating that this is an important

factor influencing PCB biotransformation. PCB congeners can be assigned to one of five structural groups previously described (*34*, *35*): Group I, congeners without vicinal hydrogen (H) atoms (side-by-side on the same ring); II, congeners with vicinal H atoms only in the *ortho* and *meta* positions and 2 or more *ortho* Cl atoms; III, same as II, but with 1 or less *ortho* Cl; IV, congeners with vicinal H atoms in the *meta* and *para* positions with 2 or less *ortho* Cl; and V, same as IV, but with 3 or more *ortho* Cl atoms. Biotransformation appears to be limited to congeners belonging to Group IV and Group V (congeners with vicinal H atoms in the *meta* and *para* positions). The effect of substitution pattern on biotransformation of PCBs has been observed previously in fish (*2*, *16*) as well as in other mammalian species (*36*−*38*).

Biotransformation of PCBs by rainbow trout increases with increasing temperature and with exposure to CYP2B-inducing congeners (Figure 1). Little biotransformation was observed at 8 °C as most congeners fell within the 95% CI of the regression line. Biotransformation of some congeners of Group V was found at 12 °C; these congeners fell below the 95% CI of the regression line, and significant biotransformation of Group IV and V was found at 16 °C. Biotransformation rates for PCB congeners that fell below the log $t_{1/2}$ − log K_{ow} relationship derived from the 16 recalcitrant PCBs are reported in Supporting Information Table 1 and ranged from 0.3 −to 3.2 × 10^{-2}/*d*, increasing with increasing temperature. In the 12 °C treatments, the rate of biotransformation of PCBs was greater in the CYP2B-exposed treatment as compared with the CYP1A- and PCB-exposed treatments.

A range of OH−PCBs were present at day 30 in the plasma of rainbow trout in every treatment group (Supporting Information Table 2), providing further evidence of PCB biotransformation by the trout. ∑OH−PCB concentrations in the plasma also were related to temperature with the highest concentrations in the 16 °C PCB-exposed treatment (5.5 ± 0.5 ng/g), followed by the 12 °C PCB-exposed treatment (2.8 ± 0.2 ng/g) and finally the 8 °C PCB-exposed treatment, which had sum OH−PCB concentrations (∼700 pg/g) slightly greater than those of the controls (∼500 pg/g). Several OH−PCB congeners in a number of treatments had concentrations above 100 pg/g (Figure 2, Supporting Information Table 2). All control treatments had ∑OH−PCB concentrations equal to ∼500 pg/g, despite differences in water temperature, and are in the range reported for lake trout from the Great Lakes (*1*). The control treatment foods had concentrations of PCBs (Table 1) due to the fish oils used in the manufacture of the food itself. In fact, control fish had whole body ∑PCB concentrations of nearly 1 μg/g at day 30 of accumulation (Table 1), which was approximately 30 times less than that for PCB-exposed fish but similar to values observed in Great Lakes salmonids (*39*). PCB concentrations in the plasma of rainbow trout from control fish were approximately five to eight times less than PCB-exposed fish (Table 1), so, in effect, the control treatments can be considered a "low-dosed" treatment.

Concentrations of OH−PCB were higher in the PCB-exposed fish than in controls and is either due to more PCBs available for biotransformation and/or that the high PCB concentrations in the PCB-exposed treatments may have induced enzyme kinetics to increase PCB metabolite formation. No differences in OH−PCB concentration between the control treatments at the various temperatures were found, suggesting that temperature and enzyme induction via higher exposure were needed to increase OH−PCB formation. These need to be considered when assessing OH−PCB and PCBs in wild fish, where PCB concentrations are likely to be much lower.

Homologue group OH−PCB concentrations in all treatments were in the order of tetra- > tri- > di- > penta- > hexa- > hepta- > mono- > octaachlorinated. No OH−PCBs from the nonchlorinated homologue group were found. Unknown OH−PCB congeners, substitution patterns unknown due to a lack of standards, were also found in the di-, tri-, tetra-, penta-, hexa-, and heptachlorinated OH−PCB homologue groups with the largest number, 6, in the dichlorinated OH−PCB homologue group, which reflects the lack of standards in this group and the highest concentration of unknown congeners in the tetrachlorinated OH−PCB homologue group (see Supporting Information Table 2 for concentrations). In some treatments, the concentrations of unknown OH−PCBs were 50% of the total ∑OH−PCB concentration. The large number of tetrachlorinated OH−PCB is likely related to the fact that these lesser chlorinated PCBs are more easily biotransformed but have enough Cl to be hydrophobic enough to be bioaccumulated.

Recovery standards of di- and trihydroxy PCBs were low (31.6−38.6%), and the errors in reported concentration due to poor recovery of these PCBs may be large. These recoveries were likely low because the methods used to extract OH−PCBs were developed for more chlorinated OH−PCBs (penta and hexa) and may be unsuitable for extracting the more polar mono-, di-, and trihydroxy PCBs. It is possible, if not likely, that additional less-chlorinated unknown OH−PCBs congeners are bioformed by rainbow trout but are at concentrations too low, due in part to extraction procedures, to be detected and quantified. Recoveries of all other OH−PCB ^{13}C recovery standards were above 75%.

The ratio of ΣOH−PCBs/ΣPCBs in fish from the PCB-exposed treatments in this study were similar to those found in wild fish, although concentrations in this study were greater (*1*−*2*). This would suggest that bioformation is the dominant pathway of OH−PCB accumulation in wild fish since OH−PCBs are nondetectable in the fish food. This is not the first study to demonstrate that fish are able to bioform OH−PCBs. Although most studies have provided indirect evidence of PCB biotransformation in fish (*5*, *6*, *8*, *9*, *15*), White et al. (*14*) found low concentrations (≤124 ng/g w/w) relative to PCB exposure concentration of two major hydroxylated metabolites (5-OH-tetrachlorobiphenyl and 4-OH-tetrachlorobiphenyl) in the gallbladder, as well as two minor metabolites (6-OH-tetrachlorobiphenyl and 2-OH-tetrachlorobiphenyl) in bile of marine scup (*Stenotomus chrysops*) exposed to 0.1 mg/kg of PCB 77. Another study (*16*) provided direct evidence of PCB metabolism in rainbow trout via OH−PCB formation in a PCB-dosing experiment and calculated biotransformation rates of PCBs being biotransformed. However, this is the first study to directly demonstrate that temperature can influence the rate of biotransformation in fish.

Effect of Cytochrome P450 Induction on Biotransformation of PCBs. There was no difference in EROD activity at day 0 between the control, PCB-exposed, and CYP-exposed treatments compared to controls regardless of temperature (mean EROD activity was 2.2 ± 0.8 pmol/min/mg microsomal protein). At day 30, EROD activity was elevated in both the CYP1A- and CYP2B-exposed treatments (49 and 31 pmol/min/mg microsomal protein, respectively) and no difference in EROD activity was observed relative to controls (∼ 3.3 pmol/min/mg microsomal protein) in any of the PCB-exposed treatments. EROD activity remained elevated in the CYP1A-exposed treatment until day 70 (40 days of depuration) of the experiment while EROD activity in the CYP2B-exposed treatment returned to levels observed in the controls by day 50 (20 days of depuration) of the experiment. Exposure to cytochrome P450 inducing PCBs was found to be a significant factor in the biotransformation of PCBs in the rainbow trout (Figure 1, Figure 2, and Supporting Information Table 2) but only by CYP2B-like-inducing congeners. While the addition

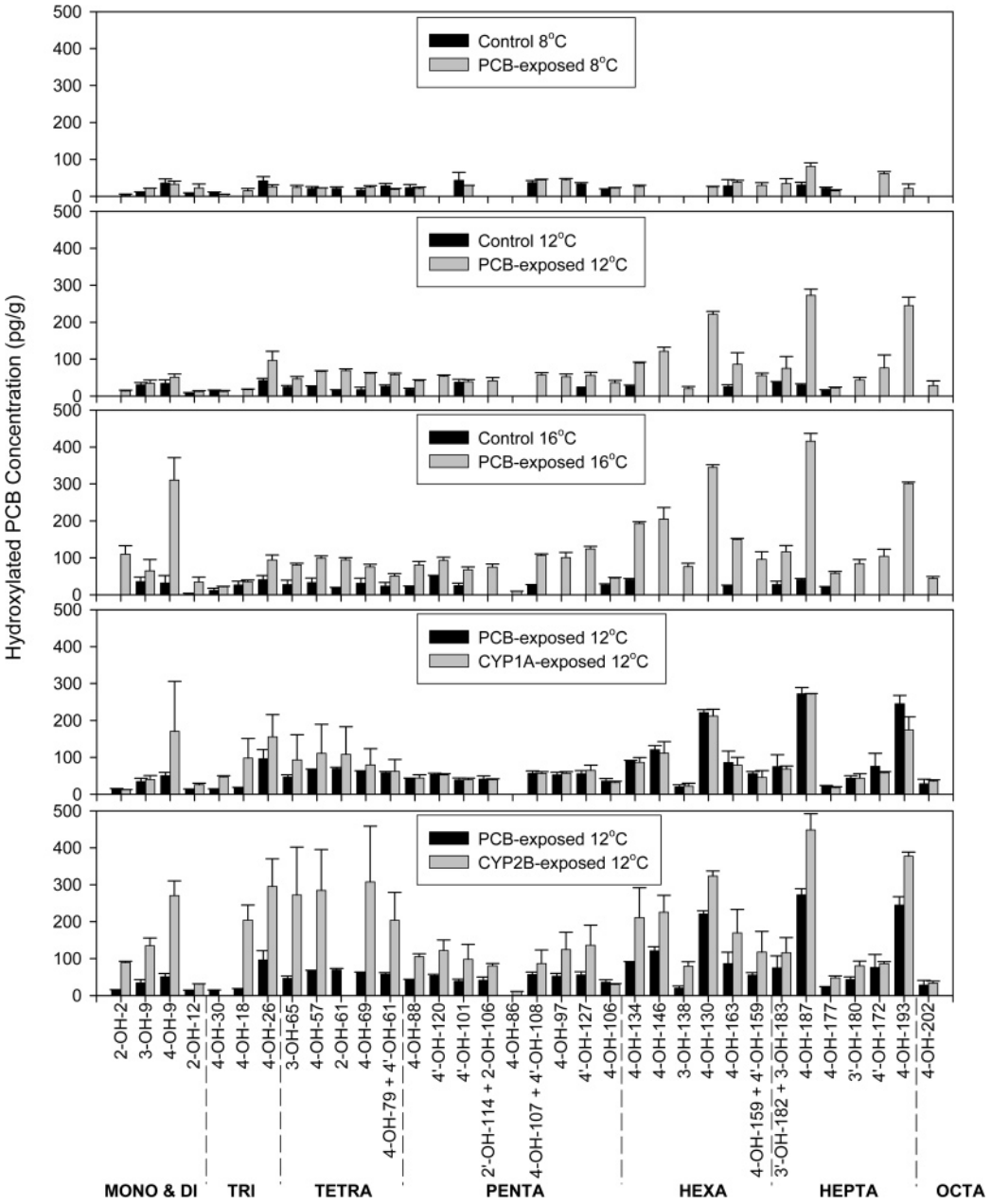

FIGURE 2. Pattern of OH−PCBs found in rainbow trout plasma at day 30 of dosing with an Aroclor mixture of PCBs. PCB-exposed dose was 10 mg/g of each Aroclor mixture (1:1:1 ratio of Aroclors 1242:1254:1260 and 0.5 mg/g of PCBs 202 and 209). CYP1A-exposed dose equals the same as the PCB-exposed dose with additional CYP1A-inducing congeners (PCBs 77, 126, and 169, 10 ng/g each). CYP2B-exposed dose equals the same as the PCB-exposed dose with additional CYP2B-inducing congeners (PCBs 87, 99, 101, 153, 180, 183, and 194).

of CYP1A-inducing congeners to the Aroclor dosed food increased EROD activity, it did not affect biotransformation of any PCB congeners by trout relative to the 12 °C PCB-exposed treatment. However, the addition of CYP2B-inducing congeners to Aroclor dosed food resulted in elevated biotransformation rates and EROD activity and an increase in the number of PCB congeners being biotransformed. Induction of EROD is an indication of CYP1A activity, and this activity usually stimulates increased metabolism of PCBs with vicinal hydrogens in the *ortho−meta* positions of the biphenyl (*35*); however, in this study, there was no indication of increased biotransformation of these compounds. In this study, the biotransformation of PCBs observed in all treatment groups, including PCB-exposed, CYP1A-exposed, and CYP2B-exposed treatments were limited to congeners with vicinal hydrogen atoms in the *meta−para* positions on the biphenyl rings (Group IV and Group V) indicating biotransformation similar to CYP2B-like biotransformation in mammals. No biotransformation of any of the Group I, Group II, and Group III congeners was observed in any treatment despite the addition of CYP1A-inducing congeners and EROD induction. Further, ∑OH−PCB concentrations in the CYP2B-like exposed treatment were 5.9 ± 0.6 ng/g, whereas concentrations in the CYP1A-exposed treatment were similar to those observed in the 12 °C PCB-exposed treatment and were 2.6 ± 0.3 ng/g. Finally, the pattern of OH−PCBs observed in the CYP1A- and CYP2B-like exposed treatments was slightly different from the pattern of OH−PCBs observed in the PCB-exposed treatments (Figure 2) and, in general, augmented the formation of tri- and tetrachlorinated OH−PCB congeners.

Prior to this study, the influence of CYP induction on the biotransformation rates of PCBs in fish had never been tested in vivo. Cytochrome P450s are thought to be the primary

enzymes responsible for the biotransformation of PCBs to their hydroxylated form, which is strongly directed by the number and position of chlorination on the biphenyl rings (*3*). In mammals, biotransformation of PCBs occurs by either (1) direct insertion of an HO-group in the *meta*-position or (2) via formation from a *meta*−*para*-epoxide (or arene oxide) intermediate (*40*, *41*). The position of the halogen substituents may determine which CYP enzyme catalyzes the transformation. Halogen substituents at one or both *para* positions seem to favor metabolism by CYP1A, whereas an *ortho* halogen often impedes metabolism by CYP1A. Phenobarbital-inducible CYP2B and CYP2B-like forms preferentially metabolize PCB congeners with adjacent, unsubstituted *meta*−*para* carbons and at least one *ortho*-halogen (*14*). The role of CYP in PCB metabolism for fish has not been established directly, although several studies indicate the involvement of CYP1A in the metabolism of non-*ortho* substituted PCBs (*10*, *14*). In addition, direct evidence for CYP2B enzymes in fish is limited (*6*, *42*). Indirect evidence suggests that induction of cytochrome isoforms could affect biotransformation of PCBs due to the fact that most observed biotransformation of PCBs by fish is limited to PCB congeners with vicinal H atoms in the *meta*/*para* positions on the biphenyl rings (*13*), congeners that would be associated with CYP1A biotransformation. The results of this experiment suggest that isoforms other than CYP1As are responsible for the majority of biotransformation observed in rainbow trout.

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Supporting Information Available

Analysis methods and depuration and biotransformation rates and statistics (Table S1) and concentrations of OH−PCBs in blood plasma (Table S2) are given. This material is available free of charge via the Internet at http://pubs.acs.org.

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