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Aquatic Toxicology 78 (2006) 176–185

TOXICOLOGY

www.elsevier.com/locate/aquatox

Biotransformation of polychlorinated biphenyls (PCBs) and bioformation of hydroxylated PCBs in fish

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Received 12 January 2006; received in revised form 23 February 2006; accepted 24 February 2006

Abstract

Hydroxylated PCBs (OH-PCBs) are a class of organic contaminants that have been found recently in the plasma of Great Lakes fish, the source of which is either bioformation from PCBs or accumulation from the environment. To address the potential for fish to biotransform PCBs and bioform OH-PCBs juvenile rainbow trout (*Oncorhynchus mykiss*; ~80 g) were exposed to dietary concentrations of an environmentally relevant mixture of PCBs. Eight OH-PCBs were found in the plasma of rainbow trout after 30 days of exposure to the PCBs, the relative pattern of which was similar to those observed in wild lake trout (*Salvelinus namaycush*) from Lake Ontario. Hydroxylated-PCBs were not found (detection limit 0.02 pg/g) in the food or control (not PCB-exposed) fish. A curvilinear log $t_{1/2}$–log K_{ow} relationship for recalcitrant PCBs was found, similar to previously reported relationships, although $t_{1/2}$ values were longer and shorter than studies using smaller fish or cooler temperatures, respectively. A number of PCB congeners fell below the log $t_{1/2}$–log K_{ow} relationship providing the first estimates of non-chiral PCB biotransformation rates in fish. Enantioselective degradation of the chiral congeners PCBs 91 and 136, also indicated biotransformation. Biotransformation of PCBs was structure-dependent with greater biotransformation of PCBs with vicinal hydrogen atoms in the *meta*/*para* positions, suggesting CYP 2B-like biotransformation. Other chiral congeners with a *meta*/*para* substitution pattern showed no enantioselective degradation but were biotransformed based on the log $t_{1/2}$–log K_{ow} relationship. The results of this study demonstrate that laboratory held rainbow trout can biotransform a number of PCB congeners and that bioformation is likely an important source of OH-PCBs in wild salmonids of the Great Lakes.

Keywords: Chiral; Metabolites; Rainbow trout; CYP2B-like

1. Introduction

Polychlorinated biphenyls (PCBs) are readily accumulated by aquatic organisms (Borga et al., 2005), and although banned decades ago, remain a concern in many aquatic systems (e.g., Great Lakes) (de Vault et al., 1996). Recently, hydroxylated PCBs (OH-PCBs) were found in a number of fish species from the Great Lakes (Campbell et al., 2003; Li et al., 2003), which present new concerns because their toxicity may be greater than their parent compounds (i.e., PCBs) (Purkey et al., 2004), particularly with respect to endocrine disruption (Gerpe et al., 2000; Carlson and Williams, 2001).

At issue is the source of OH-PCBs in biota; either formation by biotransformation of PCB congener residues in the organism (*bioformation*) or by uptake from the environment and accumulation to measurable levels. Hydroxylated PCBs found in fish may be derived as metabolites from CYP enzyme-mediated Phase I biotransformation of PCBs via an insertion of an OH-group (Yoshimura et al., 1987; Letcher et al., 2000). Fish are generally considered to have poor capability to biotransform PCBs (Matthews and Dedrick, 1984; Boon et al., 1989), which raises doubts about the relevance of this pathway in fish. However, recent work would suggest that fish do have some capacity to biotransform PCBs (Wong et al., 2002a, 2004), and the formation of OH-PCBs in marine fish scup (*Stenotomus chrysops*) exposed to PCB 77 has been reported (White et al., 1997). Hydroxylated PCBs have also been shown to be present in air and water of the Great Lakes region (Muir,

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doi:10.1016/j.aquatox.2006.02.033

personal communication), and could be accumulated by fish either directly from water or food. Although the OH group addition increases the water solubility of PCBs, they are still hydrophobic enough (some OH-PCB congeners would have a log octanol–water partition coefficient (K_{ow}) > 5) to accumulate in aquatic organisms (McKim et al., 1985), assuming that Phase II biotransformation was not rapid enough to eliminate these compounds as quickly as they are accumulated.

To address the potential of fish to biotransform PCBs and bioform OH-PCBs, juvenile rainbow trout (*Oncorhynchus mykiss*) were exposed to a combination of PCB congeners (Aroclors® 1242, 1254 and 1260, plus PCB 202 and 209) in their diet for 30 days followed by a depuration period of 160 days. The Aroclor® mixture serves as a realistic surrogate for the PCB congener profile to which salmonids are exposed in the Great Lakes (Niimi and Oliver, 1983) and included a large number of congeners (92 PCBs) with a large range of log K_{ow}s (approximately 4–8), chlorine number and substitution patterns (Hawker and Connell, 1988). PCB 202 and 209 were also added in excess to the food to insure that higher K_{ow} PCB congeners would be measurable in the fish to fully develop the log $t_{1/2}$–log K_{ow} relationship. PCB biotransformation was examined by three methods: comparison of PCB half lives ($t_{1/2}$s) to a log $t_{1/2}$–log K_{ow} relationship established for recalcitrant contaminants to estimate biotransformation rates (Fisk et al., 1998, 2000); assessing changes in enantiomeric fractions of chiral PCB congeners in the fish (Wong et al., 2002a); measurement of OH-PCBs, PCB metabolites, in the plasma of PCB-exposed and non-exposed fish. By utilizing these three methods, we expect to quantitatively address the long-standing issue of the ability of fish to biotransform PCBs and bioform OH-PCBs.

2. Methodology

2.1. Chemicals and food preparation

Aroclors® 1242, 1254 and 1260, PCBs 202 and 209 were purchased from AccuStandard (New Haven, CT, USA). OH-PCBs (listed in Fig. 1) and ^{13}C-OH-PCB recovery standards were purchased from Wellington Labs (Guelph, ON, Canada). All solvents (pesticide grade) were obtained from Caledon Laboratories (Georgetown, ON, Canada). Granular sodium sulfate (ACS grade) was obtained from EM Science (Gibbstown, NJ, USA) 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).

Food was spiked by suspending a known quantity of each PCB Aroclor® standard (1:1:1 ratio), and PCB 202 and 209 (10 μg/g of each Aroclor® mixture and 0.5 μg/g of each of PCB 202 and 209; PCBs 202 and 209 are low in Arcolor® standards and were added to assure data for higher chlorinated congeners) in 500 ml of hexane with 250 g of commercial fish food (3 Vigor Sinking Fish Feed, Corey Feed Mills, Fredericton, NB, Canada) and evaporated to dryness under pressure. Food was air dried for 24 h and stored at 10 °C in stopper sealed jars. Control food and food used for the depuration phase were treated in an identical manner but without addition of the PCBs. Concentrations of PCBs were determined in food (Table 1) using the same analytical techniques used for tissue.

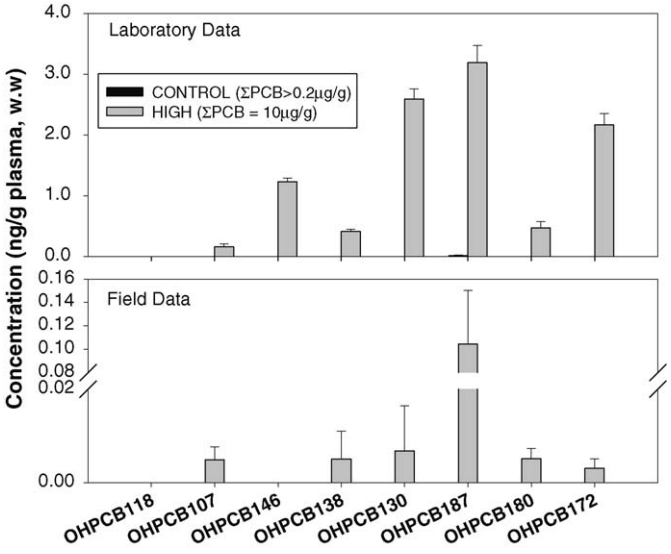

Fig. 1. Concentrations (mean ± 1S.E.; $n = 6$ for each treatment) of eight OH-PCBs in plasma of rainbow trout exposed to dietary PCBs (top, this study) and lake trout from the Great Lakes (bottom, from Campbell et al., 2003). Detection limits of all OH-PCBs for both studies were 0.2 pg/g.

2.2. 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 12 °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 14 days prior to the experiment. The Canadian Animal Care Guidelines were followed throughout the duration of fish husbandry.

2.3. Exposures

Two treatments were established, PCB-exposed and control (not PCB-exposed). Exposed juvenile rainbow trout (initial weights, ~80 g; $n = 85$) were fed PCB-spiked food for 30 d (uptake phase) followed by 160 d of non-spiked food (depuration phase). Control fish (initial weights ~80 g; $n = 85$) were fed non-spiked food throughout the experiment. Each treatment group was held in separate aquaria (85 fish per 300 L tank), which contained activated charcoal in nylon bags to absorb any dissolved PCBs or metabolites in the water. The daily rate of feeding was 1.5% of body weight, corrected to the new mean body weight of all treatments after each sampling period.

Six fish were weighed and sampled from each treatment for determination of PCB concentrations on days 0, 5, 10, 20, and 30 of the uptake period and on days 0, 5, 10, 20, 40, 80, and 160 of the depuration period. Because we only used one replicate for each treatment, pseudoreplication was addressed by using average concentration values to determine all rate constants. Liver and gastrointestinal (GI) tract (including the stomach, pyloric

Table 1
Chlorine substitution patterns, concentrations in food, K_{ow}, and depuration rates and biotransformation rates of polychlorinated biphenyls (PCBs) in juvenile rainbow trout

PCB	Chlorine substitution pattern[a]	Concentration. high food (ng/g w.w.)	K_{ow}[b]	Group[c]	Minimal depuration rate[d] (10^{-2} d^{-1})	Dep rate[e], k_d (10^{-2})	Depuration half life (days)	Biotransformation rate[f] (10^{-3} d^{-1})
4/10	2-2/26-	25.9 ± 2.5	4.74	4/4	1.5	0.99 ± 0.09	70 ± 6.2	
6	2-3	14.4 ± 0.7	5.06	4	1.1	1.3 ± 0.11	55 ± 4.9	
7/9	24/25	36.8 ± 1.9	5.06	4/4	1.1	0.96 ± 0.10	72 ± 7.4	
8/5	2-4/23	47.1 ± 2.1	5.02	4/4	1.1	0.91 ± 0.08	76 ± 6.7	
12/13	34/3-4	2.9 ± 0.1	5.26	4/4	0.94	0.83 ± 0.08	84 ± 8.6	
15/17	4-4/24-2	87.5 ± 2.4	5.28	3/4	0.93	0.73 ± 0.05	94 ± 6.5	
16	23-2	23.3 ± 0.6	5.16	4	1.0	1.0 ± 0.10	69 ± 6.7	
18	25-2	82.1 ± 2.7	5.24	4	0.96	1.1 ± 0.11	65 ± 6.8	
19	26-2	9.6 ± 0.4	5.02	5	1.1	1.0 ± 0.07	68 ± 4.9	
22	23-4	22.1 ± 0.4	5.58	4	0.76	0.83 ± 0.06	83 ± 5.7	
24/27	236/26-3	33.5 ± 0.9	5.4	4/4	0.85	0.83 ± 0.06	84 ± 5.6	
25	24-3	7.7 ± 0.2	5.67	4	0.72	0.82 ± 0.06	85 ± 6.0	
26	25-3	14.7 ± 0.3	5.66	4	0.72	0.71 ± 0.06	98 ± 7.6	
31/28	25-4/24-4	320.3 ± 6.7	5.67	4/3	0.72	0.68 ± 0.05	102 ± 8.1	
32	26-4	19.5 ± 0.5	5.44	4	0.83	0.69 ± 0.05	101 ± 7.6	
33/20/53	34-2/23-3/25-26	58.7 ± 1.2	5.6	4/4/5	0.75	0.75 ± 0.06	92 ± 6.7	
40	23-23	9.5 ± 0.2	5.66	4	0.72	0.85 ± 0.06	81 ± 5.7	**1.3**
41/71	234-2/26-34	99.9 ± 2.4	5.84	4/4	0.66	0.66 ± 0.05	104 ± 8.4	
42	23-24	33.3 ± 0.7	5.76	4	0.68	0.71 ± 0.06	97 ± 8.1	
43	235-3	47.4 ± 0.9	5.75	4	0.69	0.57 ± 0.06	122 ± 12.4	
44	23-25	57.9 ± 1.3	5.75	4	0.69	0.66 ± 0.05	106 ± 8.7	
45	236-2	11.6 ± 0.1	5.53	5	0.78	0.73 ± 0.06	95 ± 7.3	
46	23-26	5.4 ± 0.2	5.53	5	0.78	0.75 ± 0.05	92 ± 6.3	
47/48	24-24/245-2	78.5 ± 1.6	5.82	2/4	0.66	0.65 ± 0.05	107 ± 8.9	
51	24-26	9.1 ± 0.1	5.63	5	0.73	0.73 ± 0.06	95 ± 7.8	
52/49	25-25/24-25	160.9 ± 3.4	5.84	4/4	0.66	0.62 ± 0.05	111 ± 9.6	
56/60	23-34/234-4	134.0 ± 3.8	5.4	4/3	0.59	0.79 ± 0.06	88 ± 6.4	
59	236-3	8.2 ± 0.2	5.95	4	0.62	0.68 ± 0.06	102 ± 9.3	
63	235-4	8.2 ± 0.2	6.17	3	0.57	0.54 ± 0.06	127 ± 12.9	
64	236-4	28.6 ± 0.7	5.95	4	0.62	0.63 ± 0.06	110 ± 9.6	
70	25-34	86.2 ± 3.0	6.2	4	0.57	0.55 ± 0.06	126 ± 12.8	
74	245-4	46.3 ± 1.2	6.2	3	0.57	0.69 ± 0.06	101 ± 9.0	
76/98	345-2/246-23	11.8 ± 0.6	6.13	4/5	0.58	0.63 ± 0.06	109 ± 9.5	
82/151	234-23/2356-25	123.1 ± 4.0	6.42	4/5	0.53	0.63 ± 0.05	110 ± 9.6	**0.9**
83	235-23	12.4 ± 0.4	6.26	4	0.56	0.61 ± 0.05	113 ± 10.1	
87	234-25	83.0 ± 2.6	6.29	4	0.55	0.61 ± 0.06	114 ± 10.7	
91	236-24	24.3 ± 0.6	6.13	5	0.58	0.79 ± 0.07	88 ± 8.1	
92/84	235-25/236-23	83.8 ± 2.2	6.2	4/5	0.57	0.66 ± 0.06	106 ± 10.0	
95/66	236-25/24-34	314.6 ± 8.2	6.16	5/3	0.57	0.54 ± 0.05	128 ± 12.7	
97	245-23	29.7 ± 0.9	6.29	4	0.55	0.62 ± 0.06	113 ± 10.3	
99	245-24	31.4 ± 0.9	6.39	2	0.54	0.49 ± 0.03	141 ± 8.1	
101	245-25	62.8 ± 1.8	6.38	4	0.54	0.58 ± 0.06	120 ± 11.5	
105	234-34	56.8 ± 2.1	6.65	3	0.51	0.58 ± 0.06	120 ± 11.5	
107/147	235-34/2356-24	16.6 ± 0.6	6.68	3/2	0.51	0.60 ± 0.06	124 ± 12.1	
110	236-34	78.7 ± 2.6	6.48	4	0.53	0.60 ± 0.06	116 ± 10.7	
114	2345-4	16.3 ± 0.6	6.65	3	0.51	0.55 ± 0.01	126 ± 2.6	
118	245-34	90.4 ± 3.2	6.74	3	0.51	0.54 ± 0.06	129 ± 13.1	
128	234-234	9.8 ± 0.4	6.74	2	0.51	0.51 ± 0.05	135 ± 14.2	
129/178	2345-23/2356-235	52.7 ± 2.3	6.94	4/1	0.51	0.52 ± 0.05	132 ± 13.3	
132	234-236	40.2 ± 1.2	6.58	5	0.52	0.92 ± 0.06	75 ± 4.9	**4.0**
135	235-236	27.0 ± 0.8	6.64	5	0.51	0.60 ± 0.06	115 ± 10.9	**0.9**
136	236-236	17.9 ± 0.5	6.22	5	0.56	0.76 ± 0.06	92 ± 6.9	**1.9**
137	2345-24	10.1 ± 0.5	6.83	2	0.51	0.51 ± 0.06	137 ± 15.5	
138	234-245	143.1 ± 5.7	6.83	2	0.51	0.51 ± 0.06	136 ± 14.7	
141/179	2345-25/2356-236	129.0 ± 5.1	6.78	4/5	0.51	0.54 ± 0.05	128 ± 12.7	
144	2346-25	7.3 ± 0.3	6.67	5	0.51	0.55 ± 0.06	125 ± 13.0	
146	235-245	18.3 ± 0.7	6.89	1	0.51	0.50 ± 0.05	138 ± 14.7	
149/133	236-245/235-235	142.4 ± 5.0	6.76	5/1	0.51	0.61 ± 0.05	114 ± 10.2	**1.0**
153	245-245	86.7 ± 2.9	6.92	1	0.51	0.51 ± 0.05	136 ± 14.4	
156	2345-34	13.4 ± 0.7	7.18	3	0.52	0.48 ± 0.03	145 ± 9.0	
158	2346-34	20.9 ± 1.9	7.02	2	0.51	0.57 ± 0.03	131 ± 7.1	

Table 1 (*Continued*)

PCB	Chlorine substitution pattern[a]	Concentration. high food (ng/g w.w.)	K_{ow}[b]	Group[c]	Minimal depuration rate[d] (10^{-2} d^{-1})	Dep rate[e], k_d (10^{-2})	Depuration half life (days)	Biotransformation rate[f] (10^{-3} d^{-1})
163	2356-34	61.4 ± 2.5	6.99	2	0.51	0.54 ± 0.05	129 ± 12.7	
167	245-345	13.9 ± 0.7	7.27	1	0.52	0.48 ± 0.06	143 ± 16.3	
170/190	2345-234/23456-34	96.8 ± 4.3	7.36	2/2	0.53	0.53 ± 0.06	131 ± 13.9	
171	2346-234	11.4 ± 0.5	7.11	2	0.51	0.47 ± 0.05	147 ± 16.9	
172	2345-235	15.8 ± 0.7	7.33	1	0.53	0.53 ± 0.06	130 ± 13.8	
174	2345-236	19.9 ± 0.8	7.11	5	0.51	0.50 ± 0.05	140 ± 15.0	
175	2346-235	6.5 ± 0.4	7.15	1	0.51	0.53 ± 0.03	130 ± 8.3	
176/130	2346-236/234-235	37.3 ± 1.5	6.78	5/2	0.51	0.62 ± 0.05	122 ± 9.5	
177	2356-234	4.2 ± 0.2	7.08	2	0.51	0.49 ± 0.06	142 ± 16.6	
180/193	2345-245/2356-345	137.7 ± 6.3	7.44	1/1	0.54	0.55 ± 0.06	126 ± 12.6	
182	2345-246	40.1 ± 1.7	7.2	1	0.52	0.49 ± 0.05	140 ± 15.0	
183	2346-245	23.3 ± 1.0	7.2	1	0.52	0.58 ± 0.06	120 ± 11.4	
185	23456-25	66.3 ± 2.8	7.11	5	0.51	0.57 ± 0.05	121 ± 11.0	
187	2356-245	36.6 ± 1.5	7.17	1	0.52	0.49 ± 0.05	142 ± 15.1	
189	2345-345	3.1 ± 0.1	7.71	1	0.59	0.51 ± 0.05	135 ± 14.3	
194	2345-2345	23.2 ± 1.0	7.8	1	0.61	0.54 ± 0.06	128 ± 12.9	
195	23456-234	9.9 ± 0.5	7.56	2	0.56	0.54 ± 0.05	128 ± 12.7	
196/203	2346-2345/23456-245	23.8 ± 1.0	7.65	1/1	0.58	0.51 ± 0.05	137 ± 14.4	
198	23456-235	3.3 ± 0.1	7.62	1	0.57	0.58 ± 0.03	120 ± 6.4	
201	2356-2345	31.8 ± 1.3	7.62	1	0.57	0.48 ± 0.05	143 ± 14.7	
202/173	2356-2356/23456-23	9.5 ± 0.4	7.13	1/5	0.51	0.67 ± 0.04	104 ± 6.7	**1.6**
205	23456-345	2.2 ± 0.1	8	1	0.67	0.64 ± 0.08	108 ± 14.1	
206	23456-2345	2.3 ± 0.1	8.09	1	0.70	0.73 ± 0.10	95 ± 12.4	
207	23456-2346	1.3 ± 0.1	7.74	1	0.60	0.53 ± 0.05	130 ± 12.7	
208	23456-2356	10.3 ± 0.5	7.71	1	0.59	0.49 ± 0.05	141 ± 14.9	
209	23456-23456	1.9 ± 0.1	8.18	1	0.74	0.71 ± 0.12	97 ± 15.8	

Biotransformation rates are based on the difference between estimated minimum depuration rates (no biotransformation) based on the $\log t_{1/2}$–$\log K_{ow}$ relationship of recalcitrant PCBs and measured depuration rates.

[a] Chlorine substitution pattern is assigned using substitution ring 1-substitution ring 2 format, e.g., PCB 91 (2,2′,3,4′,6-pentachlorobiphenyl) represented as 236-24.

[b] K_{ow} values of all PCBs are from Hawker and Connell (1988).

[c] Congeners were grouped according to substitution pattern using rules from Boon et al. (1994, 1997).

[d] Minimum depuration rates = 0.693/half-life (d), where half-lives were determined from the equation $\log \text{half-life} = -3.7 + (1.5 \times \log K_{ow})(0.1 - \log K_{ow}{}^2)$, which assumes no biotransformation (Fisk et al., 2000).

[e] Measured depuration rates (±1S.E., coefficient of determination for the model shown in parentheses).

[f] Biotransformation rate = measured depuration rate − minimum depuration rate. A biotransformation rate was only calculated when the SE of the half life did not overlap the 95% CI of the regression.

caeca, spleen, intestines, and adipose fat associated with these organs as well as the gut contents), were separated from the carcass. Plasma was taken from each of the six fish sampled at day 30 of uptake and OH-PCB concentrations determined (described below). Tissues were weighed and then frozen at −20 °C until analyzed.

2.4. PCB analysis

Frozen whole fish samples minus the liver and gastrointestinal tract (to avoid PCBs from undigested food), were allowed to thaw at room temperature prior to extraction. Samples were homogenized using an AE-G225 food processor (American Eagle, Chicago, Illinois) and extracted on a Dionex ASE 200 (Sunnyvale, California). Extractions using ASE followed previously established methods (Dionex, 2004). PCBs 30 and 204 were added prior to extraction as recovery standards. Lipids were determined gravimetrically using 10% of the extract and were removed by acidified silica gel chromatography [8 g of 100% activated and then acidified silica gel (22 g of H_2SO_4 added to 78 g of silica gel and thoroughly mixed)] with 2 g of sodium sulfate on top. PCBs were eluted with 15% DCM in hexane (150 mL), fortified with 2,2,4-trimethylpentane, and evaporated to 1 mL final volume.

All samples were analyzed on a Hewlett-Packard (Wilmington, DE, USA) 5890 gas chromatograph (GC) with a ^{63}Ni-electron capture detector equipped with a 60 m DB-5 column (J&W Scientific, Folsom, CA, USA) following established methodology (Niimi and Oliver, 1983). Sample quantification was performed using multiple external standards obtained from the National Laboratory for Environmental Testing (1997). PCB recoveries were 80.5 ± 1.6% (mean ± 1S.E.) and all PCB concentrations were recovery corrected.

2.5. Chiral analysis

Chiral analysis was performed on all sample extracts by GC/mass spectrometry (MS) (Wong et al., 2002a, 2004) using three chiral columns to separate enantiomers; a Chirasil-Dex column (Varian, Walnut Creek, CA) for PCBs 91, 95, 136, 149,

and 174; a Cyclosil-B column (J&W Scientific) for PCBs 91, 95, 136, and 149; a BGB column (BGB Analytik, Adiswil, Switzerland) for PCBs 132 and 183.

Chiral PCB composition was expressed as enantiomer fractions (EFs) (Harner et al., 2000), defined as

$$\mathrm{EF} = \frac{A}{A+B} \quad (1)$$

where A and B are the (+) and (−) enantiomer concentrations, respectively, for PCBs 136, 149, 174, and 176, or the first (E1)- and second (E2)-eluting enantiomers for PCBs 91 and 95 (Wong et al., 2001) and PCB 183 (Wong et al., 2002b). The EFs of racemic standards were between 0.492 ± 0.002 and 0.507 ± 0.003. Enantiomer separation and precision of EFs was verified by periodic analysis of chiral PCBs in standardized reference materials (Wong et al., 2002b) and was found to be ±0.032 (95% confidence).

2.6. OH-PCB analysis

Blood was collected from anesthetized fish sampled from both the control and high exposures on day 30. Whole blood was transferred to epindorf vials and centrifuged for 5 min at 10,000 rpm on an IEC Micromax centrifuge (Needham Hts, MA) to separate plasma, which was stored in cryovial tubes at −20 °C prior to analysis. Plasma samples were thawed on the day of analysis and plasma was pooled ($n = 2$ and 3 pools per treatment) to ensure sufficient plasma for OH-PCBs detection. The method used for OH-PCB extraction, clean-up and analysis of PCBs and OH-PCBs in plasma was identical to those detailed previously (Buckman et al., 2004).

Samples were analyzed and followed previously established methods (Sandau et al., 2000), using Agilent 6890/5973 GC–MS (Palo Alto, CA, USA) with electron capture negative ionization (EC-NI) and a fused silica HP5-MS column (30 m × inner diameter × film thickness), Agilent Technologies). The detection limits for OH-PCBs were 0.2 pg/g and the average percent recovery of the organic phase (PCBs) and aqueous phase (OH-PCBs) recovery standards were $79.8 \pm 1.6\%$ and $68.8 \pm 2.4\%$, respectively, and concentrations were recovery corrected. Concentrations of OH-PCBs in food were determined in the same manner as plasma samples.

2.7. Data analysis

Growth rates were determined by fitting all fish weight data to an exponential model (ln fish weight = $a + b \times$ time (d); where a is the intercept and b is the growth rate). Differences between whole body growth rate constants were tested for homogeneity of slope and parallelism in an analysis of covariance using general linear model in SYSTAT (version 10, SPSS Ltd.). Student's t-test and Turkey's honestly significant difference (HSD) test was used to compare percent lipid differences between treatments and different sampling times within treatments at the $p < 0.05$ level of significance.

All concentrations were lipid-based, as lipid content increased over the experiment, and corrected for growth by multiplying the concentration by a factor of $(1 + b \times \text{time})$, where b is the growth rate (Fisk et al., 1998), prior to calculation of bioaccumulation parameters. 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 a constant). Half-life ($t_{1/2}$) values were calculated using $\ln 2/k_d$.

We analyzed biotransformation of PCBs using three methods. The first method, which was achiral, and qualitative, assessed biotransformation of PCBs by measuring OH-PCBs (Fig. 1) in the plasma of PCB-exposed and non-exposed fish at day 30 of uptake. The second method, also achiral but quantitative in that it produced biotransformation rates for biotransformed congeners, compared $t_{1/2}$s 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 (Niimi and Oliver, 1983; Muir et al., 1992). Congeners that have a depuration rate that is greater (and a $t_{1/2}$ that is lower) than that established for its K_{ow} based the on the depuration rates of the recalcitrant PCB are considered to be biotransformed (i.e., fall below the $t_{1/2}$–$\log K_{ow}$ relationship). Subtracting this minimal depuration rate based on the congeners K_{ow} from the experimental-determined depuration rate provides an estimate of biotransformation. 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. This method has previously been successful in demonstrating biotransformation of polychlorinated alkanes by rainbow trout (Fisk et al., 2000). The last method was qualitative as well as quantitative, and chiral, and assessed biotranformation by examining changes in EFs of chiral congeners (Wong et al., 2002a, 2004). Minimum biotransformation rates can be calculated for chiral congeners using changes in EF over time (Wong et al., 2002a) using nonlinear least squares to fit:

$$\mathrm{EF}(t) = \frac{1}{(1 + (-)_0/(+)_0 \exp(-kt))} \quad (2)$$

where k is the minimum biotransformation rate constant and $(-)_0/(+)_0$ the initial enantiomer composition, assumed to be unity (i.e., racemic at start).

3. Results and discussion

3.1. Fish health

Exposure to the PCB Aroclor® mixtures did not appear to influence the health of the rainbow trout. Mortalities were minimal (five fish out of a total of 170), and coloration and behavior of the treatment fish was consistent with control fish. No significant differences ($p < 0.05$) in whole fish growth rates or lipid content were found between treatment and control populations.

3.2. PCB biotransformation

Based on the $t_{1/2}$–log K_{ow} relationship and chiral methods for determining biotransformation, juvenile rainbow trout were found to biotransform a number of PCB congeners (Tables 1 and 2). However, the methods used to identify biotransformation identified different sets of congeners being biotransformed (Table 2). OH-PCBs were found in the plasma of PCB-exposed fish (Fig. 1), further indicating biotransformation of PCBs, although identifying the parent compound is difficult and discussed below. Other studies have also provided evidence that fish can selectively biotransform certain PCBs, but the list of congeners studied is short (PCBs 52, 77, 91 and 136) and biotransformation rates were only provided for PCBs 91 and 136 using chiral methods (White et al., 1997; Nichols et al., 2001; Wong et al., 2002a, 2004).

3.3. OH-PCB metabolites

OH-PCBs were present in the plasma of rainbow trout exposed to the environmentally-relevant group of PCB congeners for 30 days (Fig. 1). Of the OH-PCB congeners analyzed (Table 2), 3-OH-PCB 118 was the only metabolite that was not present in the plasma of high dosed rainbow trout. Since OH-PCBs were below detection limits (0.2 pg/g for all OH-PCB congeners) in food and control fish, besides a minor amount of 4-OH-PCB 187 (<3 pg/g) in control fish, biotransformation of the PCBs is the likely source of the OH-PCBs in the treatment fish.

The PCB parent compounds of the OH-PCBs observed in this study are not easy to determine, as there are several metabolic mechanisms by which OH-PCBs may be formed. These mechanisms include direct insertion of a hydroxyl group, formation of a 2,3-arene oxide intermediate with the possibility of an intramolecular hydrogen migration (NIH shift), rearrangement of a keto-enol intermediate, or the de-chlorination of an arene-oxide intermediate to form different OH-PCB metabolites (Sandau et al., 2000). The parent congeners of the eight OH-PCBs found in this study, by assuming direct insertion of a hydroxyl group, were not biotransformed based on chiral analysis and analysis of PCB biotransformation using the log $t_{1/2}$–log K_{ow} relationship (results below). It is, however, possible that these metabolites were formed by mechanisms other than direct insertion of a hydroxyl group, but it was impossible to determine the mechanism using the methods in this study. This study was also limited by the number of OH-PCB standards available at the time of analysis, and many unidentified peaks, which could be other OH-PCBs, were present in addition to the nine OH-PCBs analyzed. Thus, biotransformation may produce measurable amount of OH-PCB but have an inconsequential influence on bioaccumulation and observed concentrations of PCBs in fish.

The OH-PCB pattern found in the rainbow trout from this experiment was very similar to those reported in Great Lakes fish plasma (Campbell et al., 2003) but very different than the pattern in abiotic matrices of Great Lakes air, water and snow (D.C.G. Muir, personal communication). This would suggest that these OH-PCBs found in wild fish are most likely due to formation and not bioaccumulation. However, bioaccumulation may be an important exposure route of other OH-PCBs, those not quantified in this study, but found in abiotic matrices.

3.4. Log $t_{1/2}$–log K_{ow} relationships

A curvilinear relationship between $t_{1/2}$ and log K_{ow} was found in this experiment (Figs. 2 and 3) when all PCB congeners were analyzed. The slopes for the quadratic regression of this relationship ($b_1 = 1.38$, $b_2 = -0.1$, $r^2 = 0.89$) were nearly identical to slopes reported previously for a regression based on the same subset of congeners using smaller fish (~5 g starting weight) ($b_1 = 1.45$, $b_2 = -0.1$, $r^2 = 0.86$) (Fisk et al., 1998) and different water temperatures (~8 °C) ($b_1 = 1.39$, $b_2 = -0.1$, $r^2 = 0.78$) (Buckman et al., 2004) (Fig. 2). However, the intercept differed between experiments, with the greatest intercept, and longer $t_{1/2}$s

Table 2
Biotransformation rates of PCB congeners in juvenile rainbow trout exposed to PCBs using $t_{1/2}$–log K_{ow} relationship and chiral methods of calculating biotranformation rates

PCB	Chlorine substitution pattern[a]	K_{ow}[b]	Group[c]	Biotransformation rate (10^{-3} d^{-1}) based on log $t_{1/2}$–log K_{ow} relationship	Chiral biotransformation rate[d] (10^{-3} d^{-1})
40	23-23	5.66	4	1.3 ± 0.6	N/A
82/151	234-23/2356-25	6.42	4/5	0.9 ± 0.5	N/A
91	236-24	6.13	5	2.0 ± 0.7	2.0 ± 0.2
131	2346-23	6.58	5	2.2 ± 0.6	N/A
132	234-236	6.58	5	4.0 ± 0.6	0.0
135	235-236	6.64	5	0.9 ± 0.6	N/A
136	236-236	6.22	5	1.9 ± 0.6	6.3 ± 0.5
158	2346-34	7.02	2	0.7 ± 0.3	N/A
149/133	236-245/235-235	6.76	5/1	1.0 ± 0.5	N/A
199	23456-236	7.20	5	1.4 ± 1.0	N/A
202/173	2356-2356/23456-23	7.13	1/5	1.6 ± 0.4	N/A

[a] Chlorine substitution pattern is assigned using substitution ring 1-substitution ring 2 format, e.g., PCB 91 (2,2′,3,4′,6-pentachlorobiphenyl) represented as 236-24.
[b] K_{ow} values of all PCBs are from Hawker and Connell (1988).
[c] Congeners were grouped according to substitution pattern using rules for grouping established by Boon et al. (1994,1997).
[d] Chiral biotransformation rates were calculated using changes in EF over time (Wong et al., 2002a). Congeners with a N/A (not applicable) are not chiral and cannot be assessed with this method.

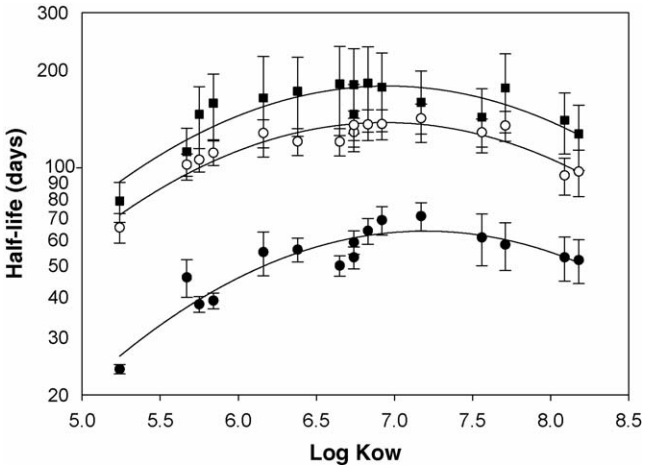

Fig. 2. Log $t_{1/2}$ of 16 recalcitrant polychlorinated biphenyl (PCB) congeners in juvenile rainbow trout versus log K_{ow}. PCB $t_{1/2}$s derived 80 g fish held at 12 °C (this study) are open circles, for 5 g fish held at 12 °C are closed circles (Fisk et al., 1998), and for 5 g held at 8 °C are closed squares (Buckman et al., 2004). The second order regression (solid lines) for the open circles was $\log t_{1/2} = -2.62 + (1.38 \log K_{ow}) - (0.1 \log K_{ow}^2)$, $r_2 = 0.89$; for closed circles was $\log t_{1/2} = -3.40 + (1.45 \log K_{ow}) - (0.1 \log K_{ow}^2)$, $r^2 = 0.87$); for closed squares was $\log t_{1/2} = -2.59 + (1.39 \log K_{ow}) - (0.1 \log K_{ow}^2)$, $r^2 = 0.78$. The K_{ow} value of PCBs congeners are from Hawker and Connell (1988).

in the 8 °C experiment (Buckman et al., 2004), followed by the larger fish at 12 °C (this study) and the small fish at 12 °C (Fisk et al., 1998). Thus, $t_{1/2}$s of PCBs are longer at colder temperatures and for larger fish, consistent with previously studies on water temperature (Buckman et al., 2004) and fish size (Fisk et al., 1998). It is also apparent from this study and others (Fisk et al., 1998; Mackay and Fraser, 2000; Buckman et al., 2004) that the curvilinear relationship with more rapid depuration of PCBs with log $K_{ow} > 7$, as compared to PCBs with lower log K_{ow} values is robust. It is believed that the $t_{1/2}$s of recalcitrant compounds should increase linearly with K_{ow} (Hawker and Connell, 1988). However, it is likely that K_{ow} is not a perfect surrogate for lipids and may explain the curvilinear nature of these relationships; K_{ow}s for super hydrophobic chemicals are inaccurate, or rather the K_{ow} does not reflect the behavior of the chemical very well (Chiou, 1985; Gobas et al., 1989; Fisk et al., 1998; Schüürmann and Klein, 2003).

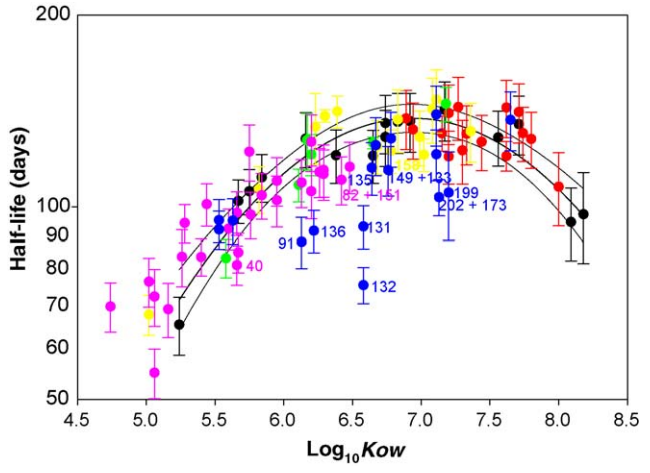

Fig. 3. Log $t_{1/2}$ of 92 PCBs in juvenile rainbow trout versus log K_{ow}. Black circles represent the 16 recalcitrant PCB congeners used to derive the log $t_{1/2}$–log K_{ow} relationship. Red circles: Group I congeners (no vicinal hydrogen atoms); yellow: Group II congeners (vicinal hydrogen atoms only in *ortho* and *meta* positions of the biphenyl ring and greater than or equal to 2 chlorines in *ortho* positions); green circles: Group III congeners (vicinal hydrogen atoms only in *ortho* and *meta* positions of the biphenyl ring and less than 2 *ortho* chlorines); magenta circles: Group IV congeners (vicinal hydrogen atoms in the *meta* and *para* positions of the biphenyl ring and has two or less *ortho* chlorines); and blue circles: Group V congeners (vicinal hydrogen atoms in the *meta* and *para* positions of the biphenyl ring and has greater than 2 *ortho* chlorines). Labeled congeners had $t_{1/2}$ significantly lower than the regression for the 16 recalcitrant PCBs. The K_{ow} value of PCBs congeners are from Hawker and Connell (1988).

The log $t_{1/2}$–log K_{ow} relationship demonstrated and quantified biotransformation of a number of PCB congeners with biotransformation rates ranging from 0.6×10^{-3} to 4.0×10^{-3} d^{-1} (Table 2) (Fisk et al., 2000). As far as we are aware, these are the first reported non-chiral biotransformation rates for PCBs in fish.

The PCBs that were biotransformed in rainbow trout were largely based on chlorine substitution patterns. PCB congeners can be assigned to one of five structural groups previously described (Boon et al., 1994, 1997): Group I, congeners without vicinal H atoms (side-by-side on the same ring); Group II, congeners with vicinal H only in the *ortho* and *meta* positions and two or more *ortho* Cl atoms; Group III, same as Group II, but with one or less *ortho* Cl; Group IV, congeners with vicinal H in the *meta* and *para* positions with two or less *ortho* Cl; Group V, same as Group IV, but with three or more *ortho* Cl atoms (Table 1). These groupings have demonstrated that PCB biotransformation in mammals is dependent on chlorine substitution patterns (Kannan et al., 1995; Norstrom et al., 1992; Hoekstra et al., 2002; Li et al., 2003). In this study, there was no biotransformation of the Group I, II, or III congeners, except PCB 158 (Group II) (Tables 1 and 2). Group I congeners are typically not easily biotransformed (McFarland and Clark, 1989; Boon et al., 1992), while Groups II and III are likely biotransformed via CYP 1A enzyme mediated processes. Induction of CYP 1A by certain organic contaminants, most planar, is well established for fish (Bucheli and Fent, 1995) and has been used as a biomarker of exposure to non-*ortho* congeners (Masuda et al., 2001) but has rarely, if ever, been tied directly to PCB biotransformation. CYP 1A activity may be important if it is induced, which would seem unlikely in this study given low exposure to non-*ortho* PCB congeners associated with induction (Safe, 1990). Most of the congeners that were biotransformed belonged to Groups V and IV. The observed biotransformation is consistent with degradation via CYP 2B-like isoforms (Boon et al., 1994), based on the parent compound chlorine substitution patterns. Thus, our results suggest that CYP 2B-like mediated processes likely dominated biotransformation of PCBs in the rainbow trout, in contrast with current literature suggestions that CYP 2B-like isoforms are not inducible in fish (Elskus et al., 1994) nor important for biotransformation of PCBs in fish (Boon et al., 1997). However, we must point out that we cannot conclusively demonstrate CYP 2B catalytic activity in this study and inducibility may not be necessary for catalytic activity of CYP 2B-like isoforms. In addition, is has been suggested that

the critical factor to PCB biotransformation, mainly observed in other vertebrate species, is the availability of adjacent unsubstituted carbon atoms on at least one of the rings, preferably at the 3,4-positions (Matthews and Dedrick, 1984) which is consistent with CYP 2B-like mediated biotransformation.

3.5. Chiral data

The modification of chiral PCB EFs in the fish over the course of this experiment provided further evidence of PCB biotransformation in fish, however only two of nine chiral PCBs (PCBs 91 and 136) had changes in EFs (Fig. 4). Both are Group V congeners, which agree with other results from this study, that is CYP 2B-mediated biotransformation. These results were also consistent with previous experiments using rainbow trout (Wong et al., 2002a) and field measurements for a number of fish species (Wong et al., 2001). Biotransformation rates of $2.0 \pm 0.2 \times 10^{-3}\ d^{-1}$ and $6.3 \pm 0.5 \times 10^{-3}\ d^{-1}$ for PCBs 91 and 136, respectively, were found using changes in EFs in the fish over time (Table 2). This rate for PCB 91 agrees with the value calculated via the $\log t_{1/2}$–$\log K_{ow}$ relationship, whereas the value of PCB 136 is considerably higher. Wong et al. (2002a) found a similar biotransformation rate of $2.7 \pm 2.0 \times 10^{-3}\ d^{-1}$ for PCB 136 in rainbow trout. The high biotransformation rate

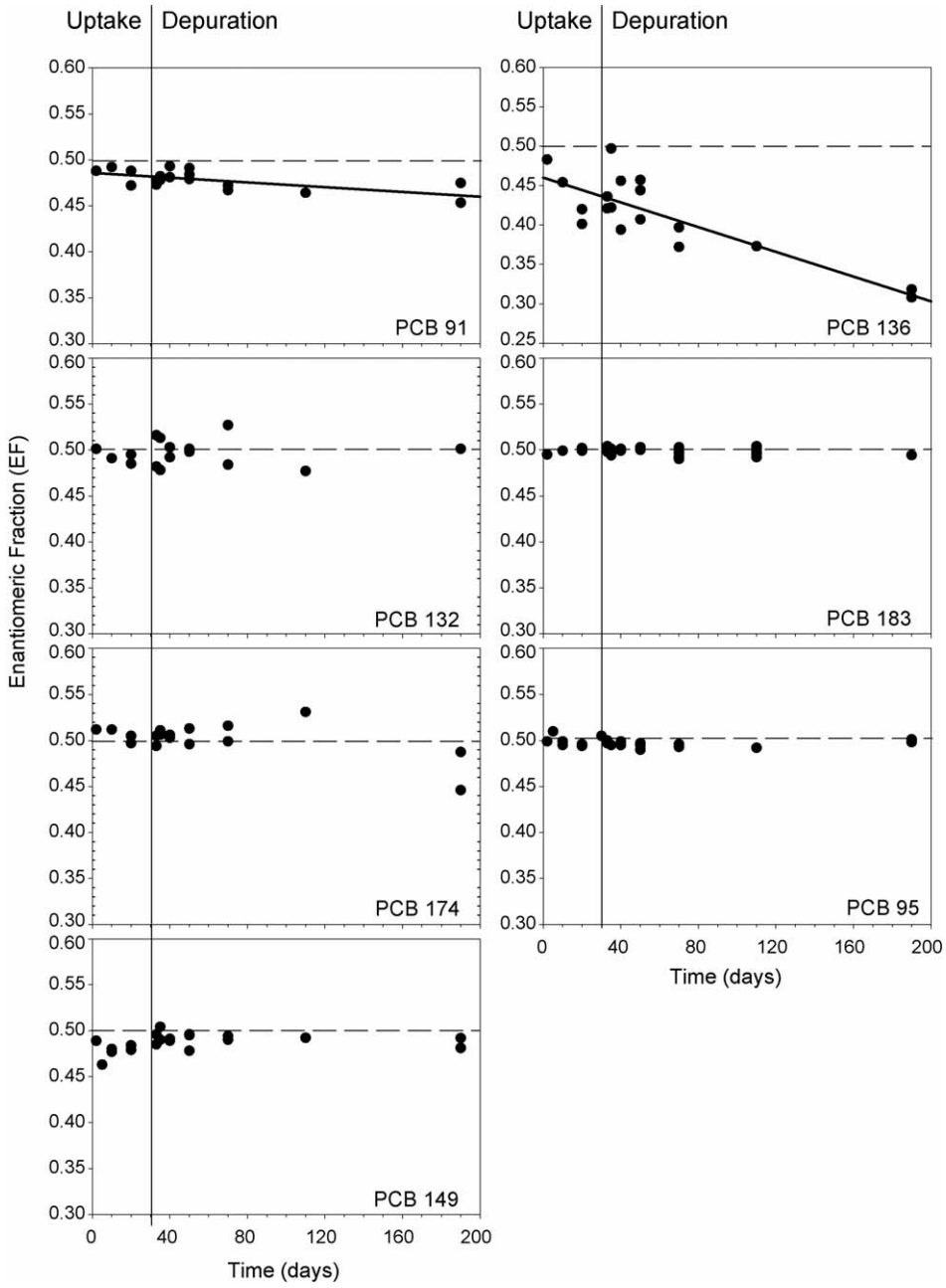

Fig. 4. Mean (±1S.E.; $n = 6$) EFs of PCB atropisomers in rainbow trout over 30 days of PCB dietary exposure to PCBs followed by 160 days of depuration. Dashed lines represent a racemic signature (EF = 0.50).

for PCB 136 determined via EF methods in this study may have been confounded analytically due to the large number of congeners in this study, most of which were not present in the Wong et al. (2002a) work.

Other Group V chiral congeners (PCBs 95, 132, 149, and 174) were found to be racemic in the fish throughout the course of the experiment. PCB 95 is known to be recalcitrant in rainbow trout (Wong et al., 2002a). However, PCBs 132, 149 and 174 were found to be biotransformed based on the $t_{1/2}$–log K_{ow} relationship. This would suggest that both enantiomers of these congeners are biotransformed at the same rate, that is, biotransformation was achiral. It has been suggested that the use of chiral analysis in the determination of biotransformation in fish may be a more sensitive tool than indirect measurements of biotransformation, especially in cases where biotransformation is slow (Wong et al., 2002a). However, that suggestion implicitly assumes enantioselective biotransformation and would miss achiral biotransformation.

There has been a large range of PCB congener $t_{1/2}$s in fish published in literature (Niimi and Oliver, 1983; Coristine et al., 1996; Fisk et al., 1998; Buckman et al., 2004). The $t_{1/2}$s of PCBs observed in this study are much shorter than those reported in Niimi and Oliver (1983). While the $t_{1/2}$s for the di- and trichlorinated PCB congeners seem to be relative range with those reported in Niimi and Oliver (1983), the $t_{1/2}$s of congeners with greater than three chlorines are greater as compared with this work. Recent laboratory studies (Coristine et al., 1996; Fisk et al., 1998; Buckman et al., 2004) reported $t_{1/2}$s similar to those reported in this study for all homologue groups, although $t_{1/2}$s seem to vary slightly with ambient water temperature as well as fish size (Fig. 2).

This is not the fist study to attempt to demonstrate that fish are able to biotransform PCBs (Melancon and Lech, 1976; Herbst et al., 1978; McKim and Heath, 1983; Elskus et al., 1994; White et al., 1997; Nichols et al., 2001). However, these past studies did not measure biotransformation rates and examined biotransformation of single or a few PCB congeners. Melancon and Lech (1976) were the first to identify a polar metabolite of tetrachlorobiphenyl in bile from rainbow trout. White et al. (1997) found low concentrations ($\leq$124 ng/g w.w.), relatively to the 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 the bile of marine scup (*Stenotomus chrysops*) exposed to 0.1 mg/kg of PCB 77. Nichols et al. (2001) found that rainbow trout fed fathead minnows dosed with 2,2,5,5-tetrachlorobiphenyl were able to absorb and biotransform this compound. The results of this study, addresses the long-standing issue of the ability of fish to biotransform a large number of PCB congeners and form hydroxylated PCBs.

In conclusion, the results of our study demonstrate that PCB biotransformation and hydroxylated PCB formation is fish is possible. While it may be true that the ability of fish to biotransform PCBs is restricted compared to birds and mammals, the mechanisms involved appear to be similar in all species and dependent upon chlorine substitution pattern. This has been noted previously in a review on pharmacokinetics of PCBs by Matthews and Dedrick (1984), but is now supported with this work.

Acknowledgements

This work was supported by grants from the Canadian Chlorine Coordinating Committee (C4) to Aaron T. Fisk, Scott B. Brown and Keith R. Solomon and to Charles Wong. We thank Angela Baitz, Colin Darling (fish maintenance and sampling) and Sean Backus (OH-PCB analysis) from Environment Canada, Burlington, ON. We also thank Derek Muir (Environment Canada, Burlington, ON) for insightful input.

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