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TOXICOKINETICS OF THREE POLYCHLORINATED BIPHENYL TECHNICAL MIXTURES IN RAINBOW TROUT (*ONCORHYNCHUS MYKISS*)

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Abstract—Accumulation and depuration parameters of polychlorinated biphenyls (PCBs) in fish have been reported only for a few congeners. As well, there is little information on the ability of fish to biotransform PCBs. To address these issues, juvenile rainbow trout (*Oncorhynchus mykiss*) were exposed to dietary concentrations of three Aroclor® mixtures (1248, 1254, 1260) in food for 30 d followed by an additional 160 d of nonspiked food at 8°C. Accumulation, depuration, and potential biotransformation of 92 PCB congeners were assessed. Half-lives ($t_{1/2}$) of PCB congeners ranged from 79 to 182 d, assimilation efficiencies ranged from 40 to 50% and biomagnification factors (BMF) ranged from 2.9 to 6.9. No evidence of significant biotransformation of any PCB congeners was found. All 92 congeners fell on the same $t_{1/2}$ to K_{ow} relationship as 16 preselected PCB congeners previously shown to persist in fish and no hydroxylated PCB metabolites (OH-PCBs) were detected in the plasma after 30 d of exposure. These findings suggest that OH-PCBs observed in feral fish may be accumulated from sources other than internal metabolism of the parent congeners, at least for juvenile fish at cool temperatures. Because $t_{1/2}$s in this experiment were slower than $t_{1/2}$s reported in other work, water temperature also may be an important factor in determining the $t_{1/2}$s of all PCB congeners in fish.

Keywords—Biotransformation Polychlorinated biphenyls Hydroxylated polychlorinated biphenyls Water temperature

INTRODUCTION

Despite being banned or restricted in most industrial countries since the 1970s, polychlorinated biphenyls (PCBs) continue to be the predominant persistent organochlorine contaminant in fish from the Great Lakes (North America) [1–3] and other regions. Concentrations of PCBs in Great Lakes biota declined significantly between 1970 and 1990 [1,2], but since have remained relatively constant [3]. Thus, chronic exposure to PCB contamination in the aquatic environment continues to be a concern.

A possible 209 PCB congeners differing only in the number and position of the chlorine substitutions on the biphenyl rings exist [4]. These congeners cover a wide range of octanol–water partition coefficient (K_{ow}) values, making PCB congeners a good surrogate for examining bioaccumulation and elimination of halogenated organics. Although there have been studies examining the toxicokinetics (i.e., uptake, elimination, biotransformation) of PCBs in fish, information is restricted to a limited number of congeners [5–8].

Elimination rates and bioaccumulative properties of PCBs largely are influenced by metabolic biotransformation in homeotherms [9]. Although fish are not believed able to efficiently biotransform organochlorine contaminants such as PCBs via cytochrome P450 (CYP) pathways [4], significant concentrations of OH-PCBs recently were measured in lake trout (*Salvelinus namaycush*) from the Great Lakes [10,11]. This raises questions about the possible source and effects of this class of PCB metabolite in fish [12]. As there is little information on the bioaccumulation potential of OH-PCBs or the capability of fish to form OH-PCBs from their parent PCB congener, it is impossible to assess the source of these hydroxylated compounds in the Great Lakes salmonids. In the few studies that have investigated PCB metabolism in fish, the level of metabolite production was minimal or below the levels of detection [8,13]. It is believed that for fish to metabolize, a PCB congener via the CYP1A enzyme, it must be planar (one or no *ortho* chlorines) [14] or have adjacent *ortho* and *meta*-positions that are not substituted with a chlorine atom [4,15,16]. Nonplanar congeners, with two or more chlorine atoms, substituted in the *ortho* positions, would be metabolized by the cytochrome P4502B-like (CYP2B) enzymes [17]. Because direct evidence for CYP2B-like isozymes in fish is limited [14], it is possible that fish are accumulating some or most of the OH-PCBs from water or food and not forming them from the parent PCB congeners.

Recently, a model has been developed to predict the potential for biotransformation of hydrophobic contaminants in juvenile rainbow trout from log half-life ($t_{1/2}$) to log K_{ow} relationships [18,19]. This relationship has been developed from recalcitrant organochlorines, mainly PCBs, for which elimination solely is partition based [18]. This model is based on the premise that chemicals that are biotransformed will have $t_{1/2}$s that fall below the log $t_{1/2}$ and log K_{ow} relationship for recalcitrant compounds [18]. Fisk et al. [19] used this model to show biotransformation of polychlorinated alkanes in juvenile rainbow trout (*Oncorhynchus mykiss*). Therefore, it is expected that if a PCB is biotransformed, it will fall below the log $t_{1/2}$ to log K_{ow} relationship for recalcitrant PCBs, similar to findings with polychlorinated alkanes.

We investigated the toxicokinetics (dietary accumulation and elimination) of 92 PCB congeners in juvenile rainbow

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Table 1. Hydroxylated polychlorinated biphenyl (OH-PCB) compound, number of chlorines, and chemical structure of polychlorinated biphenyl metabolites found in the blood plasma of lake trout from the Great Lakes (North America) [15,16] and scanned in the blood plasma of rainbow trout in this study

OH-PCB	No. Cl	Chemical structure
4-OH-PCB 107	5	4-OH-2,3,3′,4′,5-pentachlorobiphenyl
4-OH-PCB 108	5	4-OH-2′,3,3′,4′,5-pentachlorobiphenyl
3-OH-PCB 118	5	3-OH-2,3′,4,4′,5-pentachlorobiphenyl
4-OH-PCB 130	6	4-OH-2,2′,3,3′,4′,5-hexachlorobiphenyl
3-OH-PCB 138	6	3-OH-2,2′,3′,4,4′,5-hexachlorobiphenyl
4-OH-PCB 146	6	4-OH-2,2′,3,4′,5,5′-hexachlorobiphenyl
4-OH-PCB 172	7	4-OH-2,2′,3,3′,4′,5,5′-heptachlorobiphenyl
3-OH-PCB 180	7	3-OH-2,2′,3′,4,4′,5,5′-heptachlorobiphenyl
4′-OH-PCB 187	7	4-OH-2,2′,3,4′,5,5′,6-heptachlorobiphenyl

trout. A water temperature of 8°C was chosen as the holding temperature to compare the $t_{1/2}$ to K_{ow} relationship of this study to the relationship found previously where fish were held at a water temperature of 12°C [18]. Fish in the Great Lakes are exposed to temperatures of 8°C for a good portion of the year, necessitating toxicokinetic investigations of PCBs at this temperature. The ability of these fish to biotransform PCBs was evaluated by comparing the log $t_{1/2}$ to log K_{ow} relationships of these congeners to the $t_{1/2}$ to K_{ow} relationships of known recalcitrant PCBs [18]. The ability of fish to biotransform PCBs based on chlorine number and substitution pattern was assessed due to the large number of PCB congeners available. Plasma levels of OH-PCBs were measured to verify if OH-PCB metabolites observed in Lake Ontario lake trout [10] are present in the plasma due to biotransformation of the parent PCB congener. Results of this experiment provide an important step in better understanding the toxicokinetics of PCBs and the source of OH-PCBs in Great Lakes fish.

METHODS

Chemicals

Aroclor 1242, 1254, and 1260; PCBs 202 and 209; and CYP-inducing mixture (containing congeners 77, 126, and 169) were purchased from AccuStandard (New Haven, CT, USA). The OH-PCBs used as calibration standards (Table 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). American Chemical Society–grade granular sodium sulfate (Na_2SO_4) was obtained from EM Science (Gibbstown, NJ, USA). Pesticide-grade dry silica (60–200 mesh) was obtained from ACP (Montreal, PQ, Canada). The SX-3 biobeads (200–400 mesh) used in gel permeation chromatography columns were purchased from Bio-Rad Laboratories (Hercules, CA, USA).

Food preparation

Food was spiked by suspending a known quantity of each PCB Aroclor standard (1:1:1 ratio) and a known quantity of PCB 202 and 209, dissolved in 500 ml of hexane, with 250 g of commercial fish food (3 Vigor Sinking Fish Feed, Corey Feed Mills, Fredericton, NB, Canada) and slowly evaporating to dryness using a roto-evaporator. 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. The Aroclor mixture serves as a realistic surrogate for the PCB congener profile that salmonids are exposed to in the Great Lakes [5] and incorporates a wide range of congeners (92 PCBs) covering a large distribution of log K_{ow}s (4–7) and chlorine substitution patterns. Three exposure treatments were used: A control (no Aroclor added), a low dose (1 μg/g of each Aroclor mixture and 0.05 μg/g each of PCB 202 and 209), and a high dose (10 μg/g of each Aroclor mixture and 0.5 μg/g each of PCB 202 and 209). Both PCB 202 and 209 were added to provide more PCB congeners of higher K_{ow} values in order to better interpret the log $t_{1/2}$ to log K_{ow} relationship. Concentrations of individual PCB congeners were determined in control and spiked food to give exposure concentrations using the same analytical techniques used to determine concentrations in the rainbow trout tissue (Table 2). An additional high-dose exposure (hCYP), which was identical to the original high-dose treatment but included CYP1A type inducers (PCB 77, PCB 126, and PCB 169; 10 ng/g, wet wt for each congener), was added to determine whether induction of the CYP1A-related enzymes affects the rate of biotransformation of PCB congeners in juvenile rainbow trout. The concentration of the CYP1A-type inducer PCBs was chosen based on past studies, which demonstrated that dietary accumulation of PCB 126 (10 ng/g, [wet wt] in fish food) was sufficient to induce CYP1A transcription and subsequent enzyme activity in juvenile rainbow trout at only 5 d of exposure [13].

Fish husbandry

The current Canadian Animal Care Guidelines were followed throughout the duration of fish husbandry. Juvenile rainbow trout (Rainbow Springs Trout Hatchery, Thamesford, ON, Canada; Stevenson strain) were acclimated for 10 d at 8°C and maintained on nonspiked food at a feeding rate of 1.5% of the average body weight of the fish.

Exposures

Juvenile rainbow trout (initial weights, 5–10 g) were fed spiked food for 30 d (uptake phase), followed by 160 d of nonspiked food (depuration phase). Forty-five trout were used in the low-dose treatment and 135 fish in each of control, high, and hCYP treatments. The additional 90 fish in these treatments were necessary to provide sufficient plasma for OH-PCB analysis in blood plasma at day 30 of uptake. The low dose was excluded from the analysis of OH-PCBs in blood, because the concentrations of the parent PCBs were small (1 ng/g of each Aroclor) and it was hypothesized that the concentration of OH-PCB in these fish would be low and likely below the detection limits. Each treatment group was held in separate aquaria (300 L) with flow-through (1 L/g fish/d) dechlorinated city of Burlington (ON, Canada) tap water at an average temperature of 8°C. Activated charcoal, in nylon bags, was added to each tank to absorb any PCBs and metabolites directly from 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. Fish were fed slowly to ensure all food was eaten and that no food remained at the bottom of the aquaria. Aquaria were cleaned daily to remove any feces or leftover food that was not ingested. Three fish were sampled from each treatment for determination of PCB concentrations on days 0, 5, 10, and 30 of the uptake period and on days 0, 5, 10, 20, 40, 80, and 160 of the depuration period. Sampled fish were separated into liver, gastrointestinal tract (including the stomach, pyloric caeca, spleen, intestines,

and adipose fat associated with these organs as well as the gut contents), and carcass (whole fish minus liver and gastrointestinal tract). Each tissue was weighed and then frozen at −20°C until time of analyzed. The carcass and liver were combined and analyzed for PCB concentrations. To avoid PCBs from undigested food, the gastrointestinal tract was removed and not analyzed.

PCB analysis

Frozen whole fish samples minus the gastrointestinal tract were freeze-dried for 7 d prior to extraction. Methods of PCB extraction and analysis followed previously established procedures [20] with a few modifications. Briefly, samples were homogenized in approximately 50 ml of hexane and 2 g of sodium sulfate using a PT 10/35 polytron (Brinkmann, Switzerland). Both PCBs 30 and 204 were added as internal standards prior to homogenization. Homogenized samples were centrifuged and the supernatant, which contained PCBs and lipids, was decanted and condensed under a stream of ultra pure N_2. Lipids were determined gravimetrically using 10% of the extract and remaining lipids removed from the extract by gel-permeation chromatography columns. The eluent was concentrated and transferred to a silica gel column (8 g of 100% activated silica gel with 2 g of sodium sulfate on top). The PCBs were eluted with hexane, fortified with 2,2,4-trimethylpentane, and evaporated to 1,000 μl final volume. The PCB 166 was added to each vial prior to gas chromatography analysis as a performance standard.

All samples were analyzed on a Hewlett-Packard (Wilmington, DE, USA) 5890 gas chromatograph with a ^{63}Ni-electron capture detector. Compound separation was completed using a 60 m × 0.25 mm (internal diameter) DB-5 column (internal film thickness 0.25 μm; J&W Scientific, Folsom, CA, USA) with H_2 carrier gas (at a constant flow rate of 0.91 ml/min) following established methodology [5]. Nitrogen was used as the makeup gas for the electron capture detector (detector temperature: 325°C). Sample quantification was performed using multiple external standards obtained from the National Laboratory for Environmental Testing (Environment Canada, Burlington, ON). Recoveries were 86.92% ± 3.08% (mean ± standard error [SE]) based on PCB 30 and concentrations were not corrected for recovery.

OH-PCB analysis

Blood from 90 fish from each of the control, high, and hCYP exposure treatments on day 30 of uptake was collected after fish were anesthetized in a diluted clove oil bath (50 mg/L). After anesthetizing fish, tails were cut off and dipped in heparin solution to prevent clotting, and blood was drained into a large test tube. Blood from 30 fish was pooled to form three replicates for each treatment. Blood was spun down using a PR-7000 centrifuge (International Equipment, Needham Heights, MA, USA) at 4°C and 700 *g* for 5 min. Plasma was decanted into a cryovial and stored at −80°C until extracted.

The method used for OH-PCB extraction, clean-up, and analysis of PCBs and OH-PCBs in plasma was similar (differing only in recovery standards) to those detailed previously [21]. Briefly, plasma samples (mass range 2.0–7.0 g) were thawed and measured into 50-ml glass centrifuge tubes, spiked with $^{13}C_{12}$-labeled recovery standards (4′-OH-PCB 120, 4-OH-PCB 187 and nonlabeled PCB 30 and 204), mixed, and allowed to equilibrate. Proteins were denatured using 2-propanol/HCl and contaminants were extracted using a conventional liquid: liquid extraction technique using methyl *t*-butyl ether (MTBE) and hexane (1:1 ratio by volume). The combined organic phase was reduced in volume and partitioned with KOH (0.5 M). The neutral and basic compounds (PCBs) were removed in the organic phases while the acidic compounds (OH-PCBs) were deprotonated with 12 M HCl and isolated in the aqueous phase. The aqueous phase was washed with hexane and the hexane was transferred to the organic phase fraction. The washed aqueous phase (containing OH-PCBs) was then acidified and back-extracted with methyl *t*-butyl ether:hexane, derivatized with diazomethane, and cleaned up on an acidified silica gel column (22% H_2SO_4, 5 g).

The organic phase (containing neutral PCBs) went through two clean-up steps including the removal of lipids, using acidified silica gel columns as above, and a second column with florisil (8 g, 1.2% deactivated) to remove any other biological impurities. Organic and aqueous fractions were reduced to a final volume of approximately 1,000 μl and 50 μl, respectively, and a performance standard (PCB 166) was added.

Samples were analyzed and followed previously established methods [21], using gas chromatography–mass spectroscopy with electron capture negative ionization performed on an Agilent Technology (Palo Alto, CA, USA) 6890 gas chromatograph equipped with an Agilent 5973 mass spectrometer. Helium was used as the carrier gas and all injections were made in pulsed splitless mode. The gas chromatography was fitted with a fused silica HP5-mass spectrometry column ([5%-phenyl]methylpolysiloxane, 30 m × 0.25 mm internal diameter × 0.25 μm film thickness, Agilent Technologies). Methane was used as the reagent gas. The detection limits for OH-PCBs were 0.2 pg/g and the average percent recovery of the organic phase (PCBs) recovery standards was 79.8 ± 1.6% (mean ± 1 SE). The percent recovery of the aqueous phase (OH-PCBs) recovery standards was 68.8 ± 2.4% (mean ± 1 SE). No corrections were made to the concentration data.

Data analysis

Growth rates were determined by fitting all fish weight data to an exponential model (ln fish wt = $a + b \times$ time [d]; where a is a constant and b is the growth rate). 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 ($1 + b \times$ time), where b is the growth rate [18]. Many of the congeners used were found at significant concentrations (>1 ng/g [wet wt]) in the nonspiked food (Table 2) and control rainbow trout, a situation encountered in other similar studies [18]. For those PCBs that were of comparable concentration in both food and control fish throughout the entire experiment, a mean concentration was determined in the control fish and subtracted from all exposed fish concentrations. For PCBs that showed a significant increase in concentration in control fish during the experiment, concentrations in the exposed fish were corrected by subtracting the mean concentration of the control fish for the same collection day.

Depuration rate (k_d) constants 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 $\ln 2/k_d$. Absorption efficiency (α) was determined by fitting the data to the integrated form of the kinetic rate equation for constant dietary exposure using iterative nonlinear regression [22]

Table 2. Concentrations (ng/g, wet wt) of polychlorinated biphenyl (PCB) congeners (mean ± 1 standard error, $n = 3$) in the fish food of the control, low, high, and high + CYP1A-inducing compounds (hCYP) treatment

Congener[a]	Control	Low	High	hCYP[b]
PCB 4/10	<0.01	12.6 ± 1.3	52.8 ± 3.2	61.7 ± 1.7
PCB 6	0.7 ± 0.2	18.8 ± 1.1	61.0 ± 1.0	91.2 ± 2.8
PCB 7/9	0.4 ± 0.3	11.7 ± 0.5	48.8 ± 1.1	68.2 ± 0.7
PCB 8/5	6.1 ± 6.1	72.7 ± 4.4	182 ± 3.2	279 ± 8.3
PCB 12/13	0.2 ± 0.0	4.0 ± 0.2	16.0 ± 1.6	17.3 ± 0.7
PCB 15/17	1.3 ± 0.4	66.8 ± 3.6	218 ± 2.6	339 ± 14.7
PCB 16	0.6 ± 0.1	28.8 ± 0.9	94.4 ± 2.9	138 ± 5.3
PCB 18	1.5 ± 0.4	54.2 ± 2.5	162 ± 2.8	252 ± 8.5
PCB 19	0.3 ± 0.1	8.6 ± 0.5	36.6 ± 1.2	46.4 ± 1.0
PCB 22	0.4 ± 0.0	32.0 ± 2.0	109 ± 1.3	177 ± 10.7
PCB 24/27	0.2 ± 0.1	8.3 ± 0.4	33.8 ± 0.8	47.4 ± 1.3
PCB 25	0.0 ± 0.0	8.0 ± 0.5	31.4 ± 0.4	51.1 ± 2.6
PCB 26	0.3 ± 0.0	17.0 ± 1.4	55.6 ± 0.6	95.4 ± 5.3
PCB 31/28	3.4 ± 0.6	135 ± 0.3	415 ± 5.3	678 ± 37.4
PCB 32	0.4 ± 0.1	21.8 ± 1.5	74.2 ± 1.0	121 ± 4.0
PCB 33/20/53	1.4 ± 0.2	69.8 ± 4.8	225 ± 3.3	351 ± 17.5
PCB 40	0.4 ± 0.1	11.4 ± 0.3	40.8 ± 0.4	62.0 ± 2.1
PCB 41/71	1.0 ± 0.1	37.1 ± 2.6	132 ± 1.5	200 ± 10.2
PCB 42	0.5 ± 0.1	17.7 ± 1.3	58.3 ± 0.7	90.5 ± 5.2
PCB 43	1.3 ± 0.2	29.2 ± 2.1	92.5 ± 2.4	140 ± 6.9
PCB 44	2.8 ± 0.4	58.4 ± 3.7	184 ± 2.8	265 ± 15.9
PCB 45	0.2 ± 0.0	12.6 ± 0.4	47.1 ± 0.8	67.6 ± 2.0
PCB 46	0.1 ± 0.0	5.5 ± 0.0	23.2 ± 0.8	32.9 ± 1.8
PCB 47/48	1.1 ± 0.2	31.7 ± 2.6	104 ± 1.6	161 ± 5.8
PCB 51	0.1 ± 0.0	4.1 ± 0.1	16.3 ± 1.3	28.2 ± 1.4
PCB 52/49	6.1 ± 0.7	86.6 ± 5.9	268 ± 5.2	378 ± 22.8
PCB 54/29	0.0 ± 0.0	2.8 ± 0.2	15.1 ± 0.4	16.6 ± 1.2
PCB 55	0.1 ± 0.0	2.0 ± 0.1	7.7 ± 0.4	12.1 ± 0.7
PCB 56/60	1.9 ± 0.3	40.6 ± 2.8	135 ± 1.0	199 ± 12.5
PCB 59	0.0 ± 0.0	2.6 ± 0.3	9.1 ± 0.5	16.3 ± 0.8
PCB 63	0.2 ± 0.0	3.6 ± 0.2	15.5 ± 0.7	25.1 ± 1.3
PCB 64	0.4 ± 0.1	17.3 ± 1.2	56.1 ± 0.3	87.6 ± 5.1
PCB 70	2.7 ± 0.6	60.0 ± 4.5	188 ± 3.5	275 ± 17.9
PCB 74	1.4 ± 0.1	31.2 ± 2.1	97.2 ± 2.2	158 ± 8.7
PCB 76/98	0.1 ± 0.0	4.9 ± 0.3	21.8 ± 0.1	25.3 ± 1.2
PCB 82/151	1.2 ± 1.2	51.5 ± 3.4	181 ± 2.1	228 ± 11.6
PCB 83	0.8 ± 0.1	6.8 ± 0.6	26.9 ± 0.1	37.1 ± 1.9
PCB 87	1.7 ± 0.3	51.9 ± 3.7	187 ± 1.9	244 ±16.1
PCB 91	0.7 ± 0.1	14.7 ± 0.9	51.4 ± 1.0	71.8 ± 3.8
PCB 92/84	2.8 ± 0.5	56.7 ± 3.2	198 ± 2.2	248 ±13.3
PCB 95/66	11.4 ± 1.5	212 ± 4.1	697 ± 8.0	985 ± 56.6
PCB 97	1.2 ± 0.2	31.7 ± 2.1	111 ± 1.3	149 ± 8.1
PCB 99	2.8 ± 0.2	36.9 ± 2.7	123 ± 1.2	167 ± 7.3
PCB 101	5.0 ± 0.6	90.6 ± 6.3	305 ± 3.5	412 ± 21.5
PCB 105	0.9 ± 0.1	35.9 ± 2.4	135 ± 1.3	177 ± 13.5
PCB 107/147	<0.01	15.0 ± 1.1	59.2 ± 0.6	72.8 ± 6.5
PCB 110	2.8 ± 0.4	85.3 ± 5.9	313 ± 3.2	400 ± 25.1
PCB 114	0.2 ± 0.0	9.7 ± 0.7	38.7 ± 0.4	48.7 ± 2.5
PCB 118	3.1 ± 0.4	71.1 ± 4.8	251 ± 3.0	324 ± 19.9
PCB 119	2.0 ± 0.6	4.4 ± 0.7	12.8 ± 0.4	17.5 ± 0.3
PCB 128	0.2 ± 0.0	6.0 ± 0.4	26.9 ± 0.1	32.6 ± 1.9
PCB 129/178	0.4 ± 0.0	18.3 ± 1.8	72.5 ± 0.8	91.7 ± 4.4
PCB 132	0.8 ± 0.1	42.1 ± 3.1	153 ± 0.7	185 ± 12.8
PCB 135	0.7 ± 0.1	21.2 ± 1.2	74.8 ± 1.7	91.1 ± 4.5
PCB 136	0.5 ± 0.0	21.1 ± 1.2	78.8 ± 1.2	95.9 ± 6.1
PCB 137	0.2 ± 0.0	6.5 ± 0.9	27.4 ± 1.0	35.7 ± 2.1
PCB 138	3.0 ± 0.2	91.8 ± 8.3	346 ± 5.9	427 ± 21.5
PCB 141/179	1.5 ± 0.1	55.5 ± 4.3	208 ± 2.3	259 ± 14.8
PCB 144	0.2 ± 0.1	8.7 ± 0.6	37.6 ± 0.5	49.0 ± 2.3
PCB 146	1.0 ± 0.1	19.1 ± 2.1	71.3 ± 1.0	90.3 ± 4.3
PCB 149/133	2.1 ± 1.2	99.2 ± 6.1	352 ± 0.9	437 ± 21.7
PCB 153	4.7 ± 0.3	95.4 ± 6.9	332 ± 4.9	423 ± 20.3
PCB 156	0.3 ± 0.0	15.5 ± 1.7	63.0 ± 0.7	81.8 ± 6.3
PCB 158	0.5 ± 0.0	9.9 ± 1.0	42.2 ± 2.2	64.7 ± 8.1
PCB 163	0.7 ± 0.1	38.5 ± 3.5	142 ± 0.7	182 ± 10.0
PCB 167	0.2 ± 0.0	6.2 ± 0.6	28.5 ± 0.5	36.5 ± 1.7
PCB 170/190	0.6 ± 0.1	49.2 ± 4.0	192 ± 1.6	241 ± 15.7
PCB 171	0.1 ± 0.0	13.3 ± 1.5	54.4 ± 0.8	69.4 ± 3.6
PCB 172	0.1 ± 0.0	9.2 ± 1.0	36.9 ± 0.7	49.0 ± 2.7
PCB 174	0.5 ± 0.0	25.2 ± 2.1	92.6 ± 1.8	119 ± 6.3
PCB 175	0.5 ± 0.0	1.8 ± 0.3	9.6 ± 0.6	19.8 ± 1.1
PCB 176/130	<0.01	17.2 ± 1.3	66.1 ± 0.8	81.7 ± 4.3

Table 2. Continued

Congener[a]	Control	Low	High	hCYP[b]
PCB 177	0.2 ± 0.1	57.3 ± 4.2	250 ± 2.9	268 ± 9.4
PCB 180/193	2.3 ± 0.6	86.5 ± 6.5	330 ± 3.2	415 ± 24.0
PCB 182	1.1 ± 0.3	23.8 ± 1.9	94.1 ± 1.0	122 ± 4.6
PCB 183	0.6 ± 0.1	21.7 ± 1.6	89.3 ± 1.4	112 ± 8.0
PCB 185	0.6 ± 0.1	43.6 ± 3.5	167 ± 1.9	212 ± 11.3
PCB 187	0.7 ± 0.1	23.0 ± 2.0	84.4 ± 1.5	110 ± 4.4
PCB 189	0.3 ± 0.2	1.8 ± 0.2	8.9 ± 0.2	10.7 ± 0.6
PCB 194	0.2 ± 0.0	19.0 ± 1.6	77.7 ± 0.7	99.7 ± 6.9
PCB 195	0.1 ± 0.0	8.1 ± 0.7	35.9 ± 0.5	45.2 ± 2.8
PCB 196/203	0.7 ± 0.1	23.9 ± 1.8	93.4 ± 0.8	119 ± 4.8
PCB 197	0.0 ± 0.0	1.5 ± 0.2	5.3 ± 0.2	6.5 ± 0.2
PCB 198	0.0 ± 0.0	1.7 ± 0.3	6.8 ± 0.4	9.4 ± 0.2
PCB 199	88.0 ± 0.0	16.6 ± 11.2	92.6 ± 75.1	48.5 ± 17.3
PCB 201	0.3 ± 0.0	19.9 ± 1.5	75.9 ± 0.9	99.1 ± 4.0
PCB 202/173	0.1 ± 0.0	4.8 ± 0.7	22.4 ± 0.1	27.2 ± 1.0
PCB 205	0.3 ± 0.1	1.6 ± 0.2	6.9 ± 0.3	8.6 ± 0.7
PCB 206	0.1 ± 0.0	2.0 ± 0.2	8.8 ± 0.1	11.3 ± 0.4
PCB 207	0.0 ± 0.0	1.0 ± 0.1	4.3 ± 0.1	5.7 ± 0.4
PCB 208	0.3 ± 0.0	6.1 ± 0.5	27.3 ± 0.4	36.2 ± 2.4
PCB 209	0.1 ± 0.1	57.7 ± 4.0	64.1 ± 0.9	248 ± 9.5

[a] Congeners with two numbers coelute and may not be of equal concentration.
[b] hCYP-inducing treatment had approximately 10 ng/g each of PCB 77, PCB 126, and PCB 169 added to food although we were not able to quantify these congeners in the chromatograms due to coelution with other compounds.

$$C_{fish} = (\alpha F C_{food}/k_d) \times [1-\exp(-k_d t)] \quad (1)$$

where F is the feeding rate (F = 0.015 g food/g of fish/d, lipid normalized), C_{fish} is the concentration in the fish (lipid normalized), C_{food} is the concentration in the food (lipid normalized), and t is time (d). Equilibrium biomagnification factors (BMF_{eq}) were predicted from the equation

$$BMF = \alpha F/k_d \quad (2)$$

We used the average concentration for each sampling day to determine all rate constants. Steady state was assumed when a significant increase in concentration was not observed over three consecutive time intervals and did not increase thereafter.

Differences between whole body growth rate constants and depuration rates were tested for homogeneity of slope and parallelism in an analysis of covariance using general linear model in Systat (Ver 10, SPSS, Chicago, IL, USA). Tukey's honestly significant difference test was used to compare percent lipid differences between treatments at the $p < 0.05$ level of significance. Dunnetts test ($p < 0.05$) was used to compare liver somatic indices (LSI) of treatments to control fish.

We analyzed biotransformation of PCBs by using two methods. The first compared the $t_{1/2}$s of the PCBs of interest with those of 16 known recalcitrant PCBs in juvenile rainbow trout [18]. The 16 recalcitrant PCB congeners in the Fisk et al. [18] represent congeners with maximum chlorine substitution in the *meta* and *para* positions of the biphenyl rings. Therefore, these congeners should have the slowest elimination and greatest bioaccumulation potential of all the PCB congeners in fish [5,23]. Biotransformation of PCBs also was assessed by measuring the plasma of fish at day 30 of uptake for the OH-PCBs listed in Table 1.

RESULTS

Fish health

Exposure to the PCB Aroclor mixtures did not appear to influence the health of the rainbow trout. Mortalities were minimal (3 fish in total), and coloration and behavior of the treatment fish were consistent with control fish.

No differences in whole fish or liver growth rates were found between treatment and control juvenile rainbow trout populations (Table 3). Fish were larger at the end of the experiment compared to the start and contaminant concentrations therefore were growth normalized. Mean LSI were not different in any of the exposure treatments relative to the control treatment at any sampling period during the experiment. However, LSI decreased over the course of the experiment in all groups.

Lipid content of fish was not different between treatment groups on any sampling day. In all treatments, lipid percentage in the trout increased steadily during the uptake phase of the

Table 3. Growth parameters (mean ± 1 standard error [SE]) of juvenile rainbow trout exposed to three Aroclor® mixes of polychlorinated biphenyls. There were no significant differences between treatments, however, liver somatic index (LSI) decreased by day 190. The growth rates (± SE) were calculated using the equation ln weight = $a \pm b$·time (days), where b is the growth rate (r^2 for the model is shown in parentheses)

Treatment	Body (10^{-3}/d)	LSI (%)			% Mortality
		Day 5	Day 30	Day 190	
Control	7.8 ± 1.6 (0.47)	2.1 ± 0.2	2.2 ± 0.2	1.2 ± 0.1	0
Low	6.8 ± 0.9 (0.63)	2.7 ± 0.5	1.8 ± 0.1	1.2 ± 0.2	2.2
High	7.0 ± 1.1 (0.55)	2.3 ± 0.3	2.3 ± 0.3	1.5 ± 0.3	0.7
hCYP[a]	7.4 ± 1.2 (0.55)	2.6 ± 0.1	3.0 ± 0.6	1.2 ± 0.1	0.7

[a] hCYP = high + CYPIA-inducing compounds.

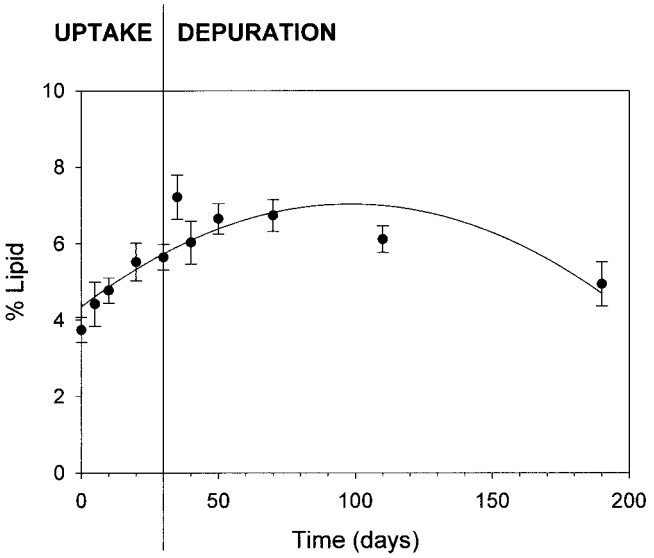

Fig. 1. Lipid percent in whole body rainbow trout minus gastrointestinal tract versus time in days. Treatments were pooled as no differences were found between treatments on any sampling day except day 20 where the high dose was significantly lower than the other treatments. All values are the mean ± 1 standard error of 12 fish.

experiment but decreased during the depuration phase (Fig. 1). To account for varying lipid levels throughout the experiment, lipid normalized concentration data were used to determine all bioaccumulation parameters.

Bioaccumulation parameters

All 92 PCB congeners were above the limits of detection of 0.1 ng/g in dosed fish after 5 d of exposure to spiked food. Uptake rates were similar between most PCB congeners and only the dichlorinated congeners (PCB 4/10, 7/9, 6, 8/5, and 12/13) had reached steady state concentrations by day 30 of exposure. Figure 2 shows the representative accumulation of several PCB congeners from different homologue groups by rainbow trout during a 30-d exposure and a 160-d depuration. Absorption efficiencies of PCBs ranged from 40.0 to 50.0% and BMFs of all PCBs included in this study were >1 (Table 4). Biomagnification factors calculated using the absorption efficiencies derived above (see *Methods* sections) were >1 for all PCB congeners analyzed.

Figure 3 shows that the $t_{1/2}$s derived from the low-dose group are longer than those obtained from the high-dose and hCYP-dose group. Bioaccumulation parameters were developed from high- and hCYP-treatment data only due to problems with the low-dose treatment (see *Discussion* section). Depuration of all PCB congeners was observed at day 35 of the experiment, 5 d after changing trout from PCB-dosed food to nonspiked food. Depuration rate constants of PCB congeners based on log linear relationships between concentration and time were highly significant and consistent between the high and hCYP treatments, but varied among congeners (Table 4). The $t_{1/2}$ values were dependent on the octanol–water partition coefficients (K_{ow}) (Fig. 3), having a curvilinear relationship with log K_{ow} values ($r^2 = 0.80$, $p < 0.001$) (Fig. 3).

Biotransformation

All PCB congeners analyzed fell on the same curvilinear log $t_{1/2}$ to log K_{ow} relationship as the 16 recalcitrant PCB congeners used to determine the log $t_{1/2}$ to log K_{ow} relationship

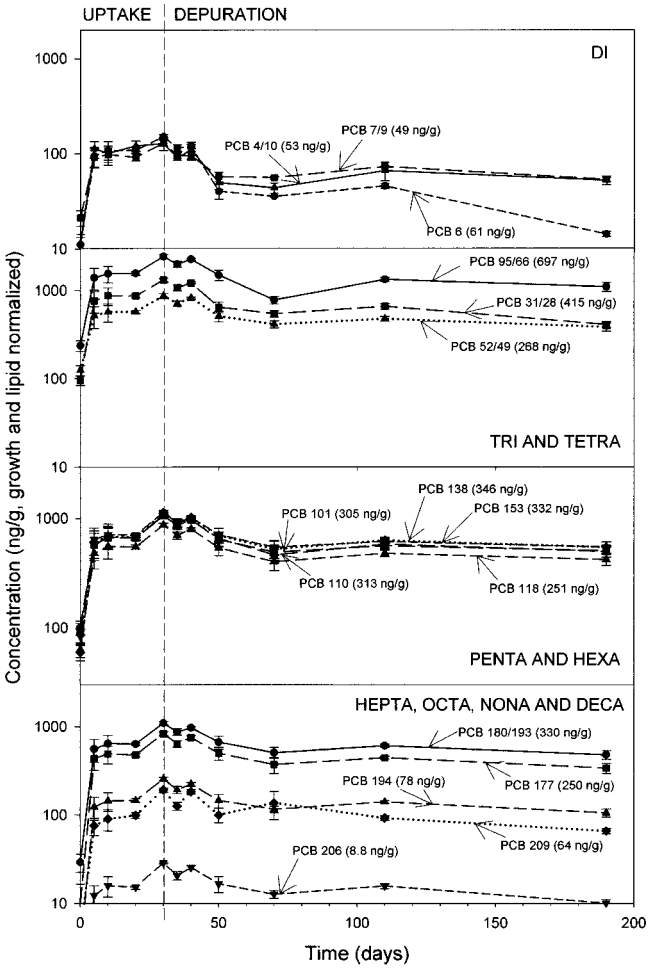

Fig. 2. Dietary accumulation and depuration curves of selected polychlorinated biphenyl (PCB) congeners from each homologue group in juvenile rainbow trout. Data represent uptake and depuration of selected congeners of the high-dose treatment. Congeners were selected based on their highest concentration in each homologue group. Each point is the mean concentration ± 1 standard error of three fish carcasses (minus gastrointestinal tract). Exposure concentrations (wet wt) are provided beside each PCB congener.

(Fig. 3). As well, concentrations of 3-OH-PCB 118 (3-OH-2,3′,4,4′,5-pentaCB) and 4-OH-PCB 172 (4-OH-2,2′,3,3′,4,5,5′-heptaCB) were near levels of detection of >0.2 pg/g in both the high and hCYP treatments, but we were unable to quantify these congeners and could not perform statistical tests on these results due to the large variability within the chromatogram itself and the small sample size. None of the other hydroxylated metabolites in Table 1 were present above the level of detection in any treatment despite a detection limit of 0.2 pg/g. However, significant concentrations of PCBs were found in the blood plasma (ΣPCB ~ = 872–1,020 ng/g wet wt in high-dose fish) at day 30 of exposure.

DISCUSSION

Fish health

Decreasing LSI over the course of the experiment has been noted in other bioaccumulation experiments using juvenile fish [18,23]. In other studies, often there is an increase in LSI accompanying exposure to Ah-receptor active compounds [23]. However, this was not the case in our study.

The variable increase and decrease in lipid content in our

experiment was unexpected as previous studies with similar sized rainbow trout have found that lipids tended to increase throughout the duration of the experiment [18]. Feeding and growth rates in our study remained constant and food was the same brand throughout the experiment. No obvious explanations exist for the lower lipid content after transition to the nonspiked food. The initial increase in lipid content may be related to the dietary adjustment from hatchery food and feeding rates to those used in the acclimation period prior to experimentation. Longer acclimation may have been necessary for fish to equilibrate lipid content before dosing.

Bioaccumulation parameters

The uptake and depuration curves (Fig. 2) in this experiment are typical of bioaccumulation studies of hydrophobic compounds [18,23]. In the uptake phase, it appears that all congeners had reached relative steady state by day 20 but that there was a sudden increase by day 30. This anomaly may be due to the larger fish size at days 10 and 20 of the experiment. The larger fish may have been feeding above the assumed feeding rate of 1.5% and, therefore, accumulating more contaminant than smaller fish as those after day 20. Typically, accumulation experiments examining uptake kinetics of recalcitrant hydrophobic contaminants in rainbow trout of similar size do not reach steady state after 30 d [18]. Therefore, it is not surprising that only the dichlorinated congeners with shorter $t_{1/2}$s had reached steady state by day 30 of uptake.

The PCB absorption efficiencies in this experiment are very similar to other studies [18], including those using different species [24]. Absorption of PCBs into fish tissue from food sources is an important mechanism in bioaccumulation of hydrophobic compounds with K_{ow}s > approximately 4. With decreasing water solubility and increasing K_{ow}, there is greater accumulation from food [25]. Previous studies also have demonstrated that the rate of residue accumulation and bioconcentration in fish increases with temperature [26]. The enhanced accumulation may be due to greater metabolic requirements and ventilation rates [8]. In our study, we fed trout PCB-contaminated food. The effect of temperature on dietary absorption is unknown. The absorption efficiencies of PCBs (40–61%) we found at a water temperature of 8°C were similar to those reported by Fisk et al. (31–49%) [18] at water temperatures of 12°C [18], using fish of the same size, suggesting that changes in water temperature regimes (at least between 8°C and 12°C) has a minimal effect on the efficiency of PCB accumulation in fish via dietary exposure.

The BMFs >1 indicate that these compounds can biomagnify within a food web [18,25]. The BMFs in this study (1.8–15.0) were twice that reported by Fisk et al. [18] and are due to the longer $t_{1/2}$s in this study (discussed below, this section). This would suggest that, if feeding rates were similar, PCB BMFs would be greater in colder waters.

Because there were significant concentrations of PCB congeners present in the nonspiked food (>1 ng/g), the rainbow trout continued accumulating PCB congeners during the depuration phase. This uptake resulted in slower observed depuration rates with apparent higher absorption efficiencies. Although we attempted to correct for this, the PCB depuration rates determined for the low treatment were nearly double those in the higher exposure groups. The PCBs in the nonspiked food had little effect on the depuration phase of the high-dose treatments because of the relatively insignificant amount of contamination from the nonspiked food. The influence of PCBs in the nonspiked food on the apparent depuration rates of the low-exposure treatment has been observed previously [18]. Due to this confound, the low-dose treatment was not used for toxicokinetic analysis.

The curvilinear relationship found between $t_{1/2}$ and K_{ow} has been observed previously in other studies [18]. The regression coefficients for the quadratic regressions drawn between log $t_{1/2}$ and log K_{ow} were nearly identical to relationships reported for a smaller subset of PCB congeners in juvenile rainbow trout by Fisk et al. ($b1_{Buckman} = 1.39$; $b2_{Buckman} = -0.1$, $r^2 = 0.78$; $b1_{Fisk} = 1.45$, $b2_{Fisk} = -0.1$, $r^2 = 0.86$) [18,19]. This relationship confirmed that the PCBs with a log K_{ow} near 7.0 had the greatest $t_{1/2}$s in fish as suggested previously [18].

The more rapid depuration of PCBs with log $K_{ow} > 7$, as compared to PCBs with lower log K_{ow} values is not easily explained but has been observed in several other studies using either water or food exposure [18,27]. In general, it is believed that the $t_{1/2}$s of recalcitrant compounds should increase linearly with K_{ow} [28,29]. The consistency of the relationship between the treatments and with past experiments provides support that this was not an anomaly. Possible explanations for this curvilinear relationship include: K_{ow} may not be a good surrogate for lipids; K_{ow}s for super hydrophobic chemicals are inaccurate, or rather the K_{ow} does not reflect the behavior of the chemical very well; and length of exposure was insufficient to allow equilibrium between fish compartments [18,30–32].

Temperature may have a profound influence on the depuration rate of PCBs when this study is compared to other similar studies at the same dose [18]. The same may be true for other organochlorines in fish. The PCB half-lives, of the high and hCYP doses of this study, were approximately double those reported for juvenile rainbow trout held in 12°C water [18]. The longer $t_{1/2}$s at the lower temperature of 8°C may be a result of slower eliminations rates through various pathways (i.e., gill, fecal, metabolism). Temperature also has a consistent effect across the entire range of K_{ow} (Fig. 4), suggesting that chemicals have a proportional impact on all of the major routes of elimination. Niimi and Palazzo [6] showed that temperature influenced the elimination rate of hexachlorobenzene (HCB). At 4°C there was no elimination of hexachlorobenzene, but at 12°C and 18°C, $t_{1/2}$s were 173 and 198 d, respectively.

Biotransformation

No OH-PCBs were found above the limit of detection (0.2 pg/g) in the plasma of rainbow trout at day 30 of uptake and the $t_{1/2}$ values of all 92 PCB congeners observed in this experiment fell on the log $t_{1/2}$ to log K_{ow} relationship previously derived from 16 recalcitrant PCB congeners [18]. These results indicate that biotransformation is minimal or below the level of detection using the methods of this study. It also may be possible that if metabolites are formed, they are formed elsewhere, such as in the liver or conjugated in the bile, and subsequently eliminated [8] before they can accumulate to measurable levels. The results of this study suggest that OH-PCBs measured in lake trout from the Great Lakes [10,11] may have been accumulated from sources other than the net result of biotransformation. Another possibility is extremely slow metabolism of the PCB parent compounds to the hydroxylated form by trout and concentrations observed in the wild may be accumulated over long periods of time (years).

In contrast, there is evidence that fish can selectively biotransform PCBs [7,8,13]. Recent studies suggest that fish may be able to slowly metabolize PCB 77 (3,3′4,4′-tetrachlorobi-

Table 4. Bioaccumulation parameters for all 92 polychlorinated biphenyl (PCB) congeners analyzed in juvenile rainbow trout; high + CYPIA-inducing compounds (hCYP)

PCB	Treatment	Food concn. (ng/g wet wt)	K_{ow}	Group[a]	No. Cl atoms	Dep rate (10^{-2})	Half-life (days)	Absorp. eff. (%)	BMF[b]
4/10	High	53	4.74	4	2	0.4 ± 0.2 (0.22)	183 ± 79.8	44 ± 5	5.8
	hCYP	62				0.7 ± 0.1 (0.61)	93 ± 17.7	61 ± 5	4.6
6	High	61	5.06	4	2	1.2 ± 0.2 (0.72)	56 ± 8.0	43 ± 3	1.9
	hCYP	91				1.3 ± 0.1 (0.82)	53 ± 5.9	45 ± 2	1.8
7/9	High	49	5.06	3	2	0.4 ± 0.1 (0.36)	192 ± 59.2	42 ± 3	5.5
	hCYP	68				0.5 ± 0.1 (0.45)	149 ± 39.0	44 ± 3	4.6
8/5	High	182	5.02	3	2	0.6 ± 0.1 (0.56)	109 ± 22.2	45 ± 3	4.0
	hCYP	279				0.7 ± 0.1 (0.56)	104 ± 21.7	46 ± 3	3.5
12/3	High	16	5.26	3	2	0.8 ± 0.2 (0.48)	88 ± 20.9	42 ± 3	2.8
	hCYP	17				1.0 ± 0.2 (0.71)	67 ± 10.1	58 ± 5	3.0
15/17	High	218	5.28	4	2/3	0.9 ± 0.1 (0.70)	81 ± 12.1	48 ± 3	2.8
	hCYP	339				0.7 ± 0.1 (0.67)	99 ± 16.4	50 ± 3	3.8
16	High	94	5.16	4	3	0.8 ± 0.1 (0.67)	90 ± 14.4	46 ± 4	3.0
	hCYP	138				1.0 ± 0.1 (0.86)	72 ± 6.9	56 ± 4	3.0
18	High	162	5.24	2	3	0.9 ± 0.1 (0.73)	79 ± 11.1	51 ± 3	3.0
	hCYP	252				0.7 ± 0.1 (0.70)	96 ± 14.8	50 ± 3	3.8
19	High	37	5.02	2	3	0.4 ± 0.1 (0.33)	155 ± 51.2	43 ± 4	5.7
	hCYP	46				0.8 ± 0.1 (0.73)	84 ± 11.9	56 ± 4	3.7
22	High	109	5.58	4	3	1.2 ± 0.1 (0.82)	56 ± 6.1	48 ± 3	2.1
	hCYP	177				1.1 ± 0.1 (0.88)	64 ± 5.5	51 ± 3	2.4
24/27	High	34	5.4	4	3	0.8 ± 0.1 (0.65)	91 ± 15.3	43 ± 3	2.8
	hCYP	47				0.9 ± 0.1 (0.79)	81 ± 9.8	53 ± 3	3.1
25	High	31	5.67	3	3	0.8 ± 0.2 (0.46)	87 ± 21.7	45 ± 3	3.0
	hCYP	51				0.7 ± 0.1 (0.70)	102 ± 15.6	48 ± 3	3.6
26	High	56	5.66	3	3	0.7 ± 0.1 (0.64)	105 ± 18.0	49 ± 3	3.7
	hCYP	95				0.5 ± 0.1 (0.60)	135 ± 25.9	50 ± 3	5.3
31/28	High	415	5.67	3	3	0.8 ± 0.1 (0.70)	91 ± 13.8	50 ± 3	3.3
	hCYP	678				0.8 ± 0.1 (0.80)	88 ± 10.3	52 ± 3	3.4
32	High	74	5.44	4	3	0.8 ± 0.1 (0.67)	85 ± 13.7	48 ± 3	3.2
	hCYP	121				0.6 ± 0.1 (0.62)	111 ± 20.7	50 ± 3	4.4
33/20/53	High	225	5.6	3/3/5	3/3/4	0.8 ± 0.1 (0.70)	91 ± 13.8	47 ± 3	3.1
	hCYP	351				0.8 ± 0.1 (0.80)	88 ± 10.3	53 ± 3	3.5
40	High	41	5.66	4	4	0.6 ± 0.1 (0.65)	107 ± 17.8	49 ± 3	4.3
	hCYP	62				0.6 ± 0.1 (0.79)	112 ± 13.5	54 ± 3	4.7
41/71	High	132	5.84	4	4	0.5 ± 0.1 (0.52)	147 ± 32.4	48 ± 3	5.0
	hCYP	200				0.4 ± 0.1 (0.57)	162 ± 32.9	53 ± 3	7.0
42	High	58	5.76	4	4	0.6 ± 0.1 (0.62)	123 ± 22.3	50 ± 3	4.4
	hCYP	90				0.5 ± 0.1 (0.62)	140 ± 25.8	54 ± 3	5.7
43	High	92	5.75	4	4	0.4 ± 0.1 (0.59)	155 ± 29.6	50 ± 3	6.6
	hCYP	140				0.4 ± 0.1 (0.51)	188 ± 43.6	53 ± 4	6.9
44	High	184	5.75	4	4	0.5 ± 0.1 (0.53)	146 ± 31.6	49 ± 3	5.2
	hCYP	265				0.4 ± 0.1 (0.51)	175 ± 40.2	54 ± 4	7.1
45	High	47	5.53	4	4	0.5 ± 0.1 (0.71)	128 ± 18.6	49 ± 3	5.2
	hCYP	68				0.6 ± 0.1 (0.67)	121 ± 20.1	56 ± 4	4.9
46	High	23	5.53	4	4	0.6 ± 0.1 (0.62)	112 ± 20.0	47 ± 3	4.1
	hCYP	33				0.6 ± 0.1 (0.71)	107 ± 16.1	52 ± 4	4.6
47/48	High	104	5.82	3	4	0.4 ± 0.1 (0.51)	160 ± 36.0	49 ± 3	6.5
	hCYP	161				0.4 ± 0.1 (0.51)	177 ± 40.8	54 ± 4	7.1
51	High	16	5.63	4	4	0.6 ± 0.1 (0.56)	126 ± 25.8	49 ± 3	4.3
	hCYP	28				0.5 ± 0.1 (0.50)	150 ± 35.5	50 ± 3	5.3
52/49	High	268	5.84	4/2	4	0.4 ± 0.1 (0.50)	158 ± 36.1	49 ± 3	6.5
	hCYP	378				0.4 ± 0.1 (0.45)	196 ± 51.0	54 ± 4	7.1
54/29	High	15	5.4	5/3	4/3	0.4 ± 0.1 (0.24)	190 ± 77.5	44 ± 4	5.8
	hCYP	17				0.7 ± 0.1 (0.66)	100 ± 16.9	56 ± 4	4.2
55	High	8	6.11	3	4	0.8 ± 0.1 (0.62)	91 ± 16.6	42 ± 4	2.8
	hCYP	12				0.4 ± 0.1 (0.55)	157 ± 33.2	49 ± 4	6.5
59	High	9	5.95	4	4	0.5 ± 0.1 (0.56)	132 ± 26.7	49 ± 3	5.2
	hCYP	16				0.5 ± 0.1 (0.64)	135 ± 23.7	50 ± 4	5.3
63	High	15	6.17	3	4	0.3 ± 0.1 (0.35)	219 ± 67.6	46 ± 3	8.1
	hCYP	25				0.4 ± 0.1 (0.54)	172 ± 37.2	48 ± 3	6.3
64	High	56	5.95	4	4	0.5 ± 0.1 (0.52)	140 ± 31.0	52 ± 3	5.5
	hCYP	88				0.4 ± 0.1 (0.57)	160 ± 32.9	55 ± 4	7.3
70	High	188	6.2	4	4	0.4 ± 0.1 (0.49)	167 ± 39.1	50 ± 3	6.6
	hCYP	275				0.3 ± 0.1 (0.42)	217 ± 59.7	54 ± 4	9.5
74	High	97	6.2	3	4	0.4 ± 0.1 (0.51)	172 ± 38.7	52 ± 3	6.9
	hCYP	158				0.3 ± 0.1 (0.42)	220 ± 60.4	52 ± 4	9.1
76/98	High	22	6.13	3/2	4/5	0.5 ± 0.1 (0.43)	153 ± 40.4	48 ± 3	5.1
	hCYP	25				0.4 ± 0.1 (0.56)	164 ± 34.5	56 ± 4	7.4
82/151	High	181	6.42	2/5	5/6	0.4 ± 0.1 (0.38)	174 ± 51.1	49 ± 3	6.5
	hCYP	228				0.4 ± 0.1 (0.46)	196 ± 50.3	56 ± 4	7.4

Table 4. Continued

PCB	Treatment	Food concn. (ng/g wet wt)	K_{ow}	Group[a]	No. Cl atoms	Dep rate (10^{-2})	Half-life (days)	Absorp. eff. (%)	BMF[b]
83	High	27	6.26	4	5	0.4 ± 0.1 (0.42)	172 ± 46.1	49 ± 3	6.5
	hCYP	37				0.3 ± 0.1 (0.43)	217 ± 58.7	54 ± 4	9.5
87	High	187	6.29	4	5	0.5 ± 0.1 (0.44)	154 ± 39.8	51 ± 3	5.4
	hCYP	244				0.4 ± 0.1 (0.46)	195 ± 49.9	58 ± 4	6.1
91	High	51	6.13	4	5	0.4 ± 0.1 (0.70)	166 ± 25.0	50 ± 3	6.6
	hCYP	72				0.3 ± 0.1 (0.40)	217 ± 62.6	53 ± 4	7.0
92/84	High	197	6.2	4/2	5	0.5 ± 0.1 (0.65)	135 ± 22.8	49 ± 3	5.2
	hCYP	248				0.5 ± 0.1 (0.63)	141 ± 25.3	56 ± 4	5.9
95/66	High	697	6.16	5/3	5/4	0.4 ± 0.1 (0.31)	164 ± 56.1	52 ± 3	6.9
	hCYP	985				0.3 ± 0.1 (0.39)	225 ± 66.6	54 ± 4	9.5
97	High	111	6.29	4	5	0.4 ± 0.1 (0.44)	163 ± 42.3	50 ± 3	6.6
	hCYP	149				0.4 ± 0.1 (0.49)	188 ± 45.4	55 ± 4	7.3
99	High	123	6.39	3	5	0.3 ± 0.1 (0.34)	203 ± 65.4	51 ± 3	9.0
	hCYP	167				0.3 ± 0.1 (0.34)	252 ± 83.0	54 ± 4	9.5
101	High	305	6.38	2	5	0.4 ± 0.1 (0.41)	172 ± 47.1	52 ± 3	6.9
	hCYP	412				0.3 ± 0.1 (0.34)	246 ± 81.2	53 ± 4	9.3
105	High	135	6.65	3	5	0.4 ± 0.1 (0.36)	181 ± 55.3	50 ± 2	6.6
	hCYP	177				0.3 ± 0.1 (0.44)	204 ± 54.7	59 ± 4	10.3
107/147	High	59	6.68	3/2	5/6	0.4 ± 0.1 (0.40)	194 ± 54.5	50 ± 3	6.6
	hCYP	73				0.2 ± 0.1 (0.30)	283 ± 79.1	58 ± 4	15.0
110	High	313	6.48	4	5	0.4 ± 0.1 (0.40)	171 ± 48.5	50 ± 3	6.6
	hCYP	400				0.3 ± 0.1 (0.44)	204 ± 54.1	57 ± 4	10.0
114	High	39	6.65	3	5	0.4 ± 0.1 (0.43)	164 ± 43.4	46 ± 2	6.1
	hCYP	49				0.4 ± 0.1 (0.45)	187 ± 48.1	56 ± 4	7.4
118	High	251	6.74	3	5	0.4 ± 0.1 (0.38)	180 ± 52.5	51 ± 3	6.7
	hCYP	324				0.3 ± 0.1 (0.37)	225 ± 69.5	57 ± 4	9.0
128	High	27	6.74	3	6	0.5 ± 0.1 (0.49)	146 ± 34.1	47 ± 2	5.0
	hCYP	33				0.3 ± 0.1 (0.41)	205 ± 58.5	56 ± 4	9.9
129/178	High	72	6.94	2/1	6/7	0.4 ± 0.1 (0.44)	156 ± 40.7	48 ± 2	6.3
	hCYP	92				0.4 ± 0.1 (0.52)	166 ± 37.5	57 ± 4	7.5
132	High	153	6.58	5	6	0.5 ± 0.1 (0.52)	136 ± 29.8	47 ± 2	5.0
	hCYP	185				0.5 ± 0.1 (0.59)	147 ± 29.0	58 ± 4	6.1
135	High	75	6.64	5	6	0.4 ± 0.1 (0.47)	159 ± 38.9	48 ± 3	6.3
	hCYP	91				0.4 ± 0.1 (0.43)	192 ± 51.7	58 ± 4	7.6
136	High	79	6.22	5	6	0.5 ± 0.1 (0.68)	132 ± 20.7	45 ± 3	4.7
	hCYP	96				0.5 ± 0.1 (0.59)	144 ± 28.2	54 ± 4	5.7
137	High	27	6.83	2	6	0.4 ± 0.1 (0.37)	186 ± 56.2	48 ± 3	6.3
	hCYP	36				0.4 ± 0.1 (0.48)	191 ± 47.3	56 ± 4	7.4
138	High	346	6.83	2	6	0.4 ± 0.1 (0.38)	182 ± 53.4	47 ± 2	6.2
	hCYP	427				0.3 ± 0.1 (0.30)	254 ± 90.7	55 ± 4	9.7
141/179	High	208	6.78	4/5	6/7	0.4 ± 0.1 (0.39)	171 ± 48.8	48 ± 2	6.3
	hCYP	259				0.4 ± 0.1 (0.43)	198 ± 53.2	57 ± 4	7.5
144	High	38	6.67	5	6	0.5 ∓ 0.1 (0.45)	137 ± 34.6	53 ± 2	5.6
	hCYP	49				0.4 ± 0.1 (0.48)	184 ± 45.3	53 ± 4	7.0
146	High	71	6.89	1	6	0.4 ± 0.1 (0.42)	166 ± 44.6	49 ± 2	6.5
	hCYP	90				0.3 ± 0.1 (0.37)	216 ± 66.1	57 ± 4	10.0
149/135	High	352	6.76	5/1	6	0.4 ± 0.1 (0.45)	162 ± 40.9	50 ± 3	6.6
	hCYP	437				0.3 ± 0.1 (0.43)	199 ± 54.0	56 ± 4	9.8
153	High	332	6.92	1	6	0.4 ± 0.1 (0.42)	177 ± 48.1	50 ± 3	6.6
	hCYP	423				0.3 ± 0.1 (0.37)	219 ± 68.1	57 ± 4	10.0
156	High	63	7.18	3	6	0.4 ± 0.1 (0.39)	163 ± 46.6	52 ± 2	6.9
	hCYP	82				0.4 ± 0.1 (0.45)	182 ± 47.1	60 ± 4	7.9
158	High	42	7.02	3	6	0.6 ± 0.1 (0.48)	124 ± 29.9	52 ± 3	4.6
	hCYP	65				0.3 ± 0.1 (0.27)	241 ± 93.6	50 ± 4	8.8
163	High	142	6.99	3	6	0.4 ± 0.1 (0.36)	170 ± 51.9	50 ± 2	6.6
	hCYP	182				0.3 ± 0.1 (0.41)	198 ± 55.8	59 ± 4	10.4
167	High	28	7.27	1	6	0.5 ± 0.1 (0.49)	134 ± 31.1	47 ± 2	5.0
	hCYP	36				0.5 ± 0.1 (0.53)	153 ± 34.0	55 ± 3	5.8
170/190	High	192	7.36	3	7	0.4 ± 0.1 (0.45)	164 ± 41.6	48 ± 2	6.3
	hCYP	241				0.4 ± 0.1 (0.43)	180 ± 49.3	60 ± 4	7.9
171	High	54	7.11	2	7	0.4 ± 0.1 (0.51)	156 ± 34.8	49 ± 2	6.5
	hCYP	69				0.4 ± 0.1 (0.46)	182 ± 46.2	58 ± 4	7.6
172	High	37	7.33	1	7	0.4 ± 0.1 (0.46)	165 ± 41.3	51 ± 2	6.7
	hCYP	49				0.4 ± 0.1 (0.45)	179 ± 46.6	58 ± 4	7.6
174	High	93	7.11	2	7	0.4 ± 0.1 (0.47)	160 ± 38.9	48 ± 2	6.3
	hCYP	119				0.4 ± 0.1 (0.41)	194 ± 55.1	58 ± 4	7.6
175	High	10	7.15	1	7	0.5 ± 0.1 (0.49)	154 ± 35.9	48 ± 3	5.1
	hCYP	20				0.4 ± 0.1 (0.54)	169 ± 37.0	52 ± 3	6.9
176/130	High	66	6.78	5/2	7/6	0.4 ± 0.1 (0.36)	184 ± 56.5	48 ± 3	6.3
	hCYP	82				0.5 ± 0.1 (0.54)	153 ± 33.4	51 ± 4	5.4

Table 4. Continued

PCB	Treatment	Food concn. (ng/g wet wt)	K_{ow}	Group[a]	No. Cl atoms	Dep rate (10^{-2})	Half-life (days)	Absorp. eff. (%)	BMF[b]
177	High	250	7.08	4	7	0.5 ± 0.1 (0.45)	147 ± 37.2	48 ± 2	5.1
	hCYP	268				0.4 ± 0.1 (0.41)	186 ± 52.4	56 ± 4	7.4
180/173	High	330	7.44	1	7	0.4 ± 0.1 (0.46)	162 ± 40.0	48 ± 2	6.3
	hCYP	415				0.4 ± 0.1 (0.41)	186 ± 52.5	58 ± 4	7.6
182	High	94	7.2	1	7	0.4 ± 0.1 (0.53)	159 ± 34.4	48 ± 2	6.3
	hCYP	122				0.4 ± 0.1 (0.46)	182 ± 46.8	56 ± 4	7.4
183	High	89	7.2	2	7	0.4 ± 0.1 (0.40)	165 ± 46.5	51 ± 3	6.7
	hCYP	112				0.3 ± 0.1 (0.41)	206 ± 57.9	60 ± 4	10.6
185	High	167	7.11	1	7	0.4 ± 0.1 (0.48)	156 ± 37.1	50 ± 3	6.6
	hCYP	212				0.4 ± 0.1 (0.44)	184 ± 48.5	56 ± 4	7.4
187	High	84	7.17	1	7	0.4 ± 0.1 (0.46)	159 ± 39.3	49 ± 2	6.5
	hCYP	110				0.4 ± 0.1 (0.43)	190 ± 51.7	56 ± 4	7.4
189	High	9	7.71	4	7	0.4 ± 0.1 (0.41)	176 ± 48.0	43 ± 2	5.7
	hCYP	11				0.4 ± 0.1 (0.46)	171 ± 43.7	53 ± 4	7.0
194	High	78	7.8	2	8	0.4 ± 0.1 (0.48)	158 ± 37.8	46 ± 2	6.1
	hCYP	100				0.4 ± 0.1 (0.41)	174 ± 49.1	56 ± 4	7.4
195	High	36	7.56	1	8	0.5 ± 0.1 (0.52)	143 ± 31.8	49 ± 2	5.2
	hCYP	45				0.4 ± 0.1 (0.47)	158 ± 39.8	57 ± 4	7.5
196/203	High	93	7.65	3	8	0.4 ± 0.1 (0.49)	156 ± 36.9	46 ± 2	6.1
	hCYP	119				0.4 ± 0.1 (0.45)	168 ± 43.9	57 ± 4	7.5
198	High	9	7.62	1	8	0.4 ± 0.1 (0.46)	158 ± 39.1	46 ± 1	6.1
	hCYP	9				0.4 ± 0.1 (0.46)	185 ± 47.1	54 ± 3	7.1
201	High	76	7.62	1	8	0.5 ± 0.1 (0.50)	153 ± 35.4	49 ± 2	5.2
	hCYP	99				0.4 ± 0.1 (0.43)	172 ± 46.6	57 ± 4	7.5
202/173	High	22	7.13	1/2	8/7	0.6 ± 0.1 (0.54)	110 ± 23.0	44 ± 2	3.9
	hCYP	27				0.5 ± 0.1 (0.57)	144 ± 29.3	51 ± 3	5.4
205	High	7	8	1	8	0.4 ± 0.1 (0.44)	166 ± 43.1	46 ± 2	6.1
	hCYP	9				0.4 ± 0.1 (0.42)	168 ± 47.0	51 ± 4	6.7
206	High	9	8.09	1	9	0.5 ± 0.1 (0.54)	140 ± 29.9	44 ± 2	4.6
	hCYP	11				0.5 ± 0.1 (0.60)	148 ± 28.5	53 ± 4	5.6
207	High	4	7.74	1	9	0.4 ± 0.1 (0.44)	162 ± 41.5	46 ± 2	6.1
	hCYP	6				0.4 ± 0.1 (0.46)	155 ± 39.8	49 ± 3	6.5
208	High	27	7.71	1	9	0.5 ± 0.1 (0.50)	139 ± 31.7	48 ± 2	5.1
	hCYP	36				0.5 ± 0.1 (0.45)	152 ± 39.7	53 ± 4	5.6
209	High	64	8.18	1	10	0.5 ± 0.1 (0.50)	127 ± 28.9	40 ± 2	4.2
	hCYP	248				0.5 ± 0.1 (0.44)	149 ± 39.5	43 ± 3	4.5

[a] Groups were determined using rules for grouping [5].
[b] The biomagnification factor (BMF) is calculated from the equation BMF = $\alpha F/k_d$.

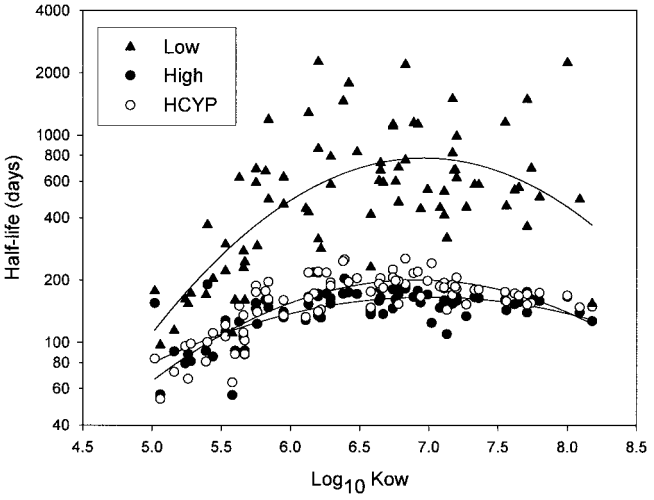

Fig. 3. Log half-life ($t_{1/2}$) of 92 polychlorinated biphenyl (PCB) congeners in juvenile rainbow trout versus log octanol–water partition coefficient (K_{ow}). Filled in circles represent PCB $t_{1/2}$s derived from the high-dose treatment. Open circles represent PCB $t_{1/2}$s derived from the high + CYP1A-inducing (hCYP) treatment. Triangles represent PCB $t_{1/2}$s derived from the low-dose treatment. The K_{ow} values of all PCBs were taken from Hawker and Connell [28].

phenyl) [8] and PCB 52 (2,2,5,5-tetrachlorobiphenyl) [33]. White et al. [8] found low concentrations (≤124 ng/g wet wt) 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. [33] found that rainbow trout fed fathead minnows dosed with 2,2,5,5-tetrachlorobiphenyl were able to absorb and metabolize this compound. Other studies have demonstrated conversion of ^{14}C radiolabeled 2,2′,5-trichlorobiphenyl to polar PCB compounds in green sunfish (*Lepomis cyanellus*) [34], goldfish (*Carassius auratus*) [35], and, to a lesser extent, bullhead (*Ictalurus natalis*) [35] but not in rainbow trout [34]; however, in these studies the metabolic products were not identified. Herbst et al. [36] were able to characterize these metabolites as phenols or methoxyphenols using chromatographic and mass spectrometric evidence. Melancon and Lech [7] also isolated a metabolite of 2,2′,5,5′-tetrachlorobiphenyl from the bile of rainbow trout and identified it as a conjugated phenol. Metabolism of PCB congeners in the above examples seems to be species-specific and the studies using trout suggest a limited ability to biotransform PCB congeners by this species.

None of the above studies, except Nichols et al. [33], ad-

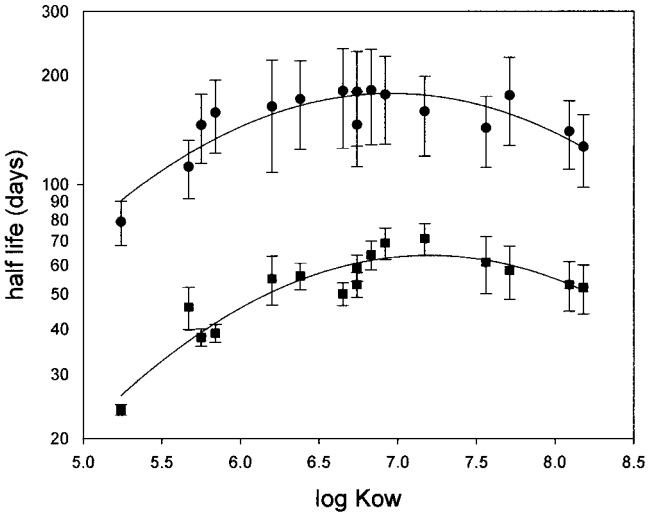

Fig. 4. Log half-life ($t_{1/2}$) of 16 recalcitrant polychlorinated biphenyl (PCB) congeners in juvenile rainbow trout versus log octanol–water partition coefficient (K_{ow}). Circles represent PCB $t_{1/2}$s derived from this experiment at 8°C. Squares represent PCB $t_{1/2}$s derived from a previous experiment at a water temperature of 12°C [23]. The second order regressions (solid lines) were derived from all 16 congeners in each experiment (log $t_{1/2}$[Buckman] = −2.59 + [1.39.log K_{ow}]−[0.1.log K_{ow}^2], r^2 = 0.78)(log $t_{1/2}$[Fisk] = −3.40 + [1.45.log K_{ow}]−[0.1.log K_{ow}^2], r^2 = 0.87) using the mean ± 1 standard error of three fish for each point. The K_{ow} congeners of all PCBs were reported previously [28].

dressed biotransformation of PCBs in fish dosed through dietary exposure, which is the most relevant pathway for fish to accumulate PCBs [28]. White et al. [8] intraperitoneally injected fish with PCB 77, which may result in different elimination mechanisms. A recent dietary accumulation study presented indirect evidence that fish can slowly metabolize PCB 126, but actual metabolites were not determined [13]. Also, the previous studies [7,8] analyzed for metabolites in the bile of fish and not in blood plasma, even though significant concentrations of OH-PCBs have been observed in this compartment in lake trout from the Great Lakes [10,11]. Hydroxylated PCBs in the plasma, which resemble hormones such as the thyroid hormone, are likely more biologically important to fish than the conjugated forms in the bile, which are then eliminated [8].

The lack of notable changes in PCB profiles and minimal amount of metabolites we detected in fish plasma suggest that concentrations of OH-PCBs found in the blood plasma of Great Lakes lake trout may be accumulated from sources other than biotransformation [10,11]. There also may be species-specific differences in biotransformation potential and perhaps lake trout are able to biotransform PCBs more readily than the rainbow trout used in our study. Further research is required to elucidate the toxicokinetics of OH-PCBs in fish and to better understand current field observations.

Temperature also may influence the activity of CYP1A-related enzymes and thus the elimination of xenobiotics by fish [30,37]. Various studies [38–39] on the influence of temperature on the 7-ethoxyresorufin-*O*-deethylase (EROD) activity (a measure of CYP1A activity) in fish livers indicate that EROD activity is correlated with temperature. These past studies would indicate that the CYP1A activity in our study at 8°C, may not have been active during the exposure and the first part of the depuration phase. An increase in biotransformation may be observed by lengthening the exposure period and/or increasing the temperature. This in turn, would increase the CYP1A activity and reduce the time to induce the enzymes. Although it has been suggested that temperature effects likely would be limited for more persistent substances such as PCBs and DDT [40], no studies to date have investigated the elimination of a large number of PCB congeners at various temperatures.

Thus, we show that at 8°C there is little or no biotransformation of PCBs by juvenile rainbow trout regardless of the addition of CYP-inducing congeners.

CONCLUSION

This study reports bioaccumulation parameters ($t_{1/2}$, absorption efficiency, and BMF) for 92 PCB congeners, and examined the biotransformation of PCBs and formation of OH-PCB metabolites in juvenile rainbow trout at 8°C. Half-lives ($t_{1/2}$s) of the PCBs analyzed ranged from 53 to 283 d. All congeners were assimilated in trout between the 40 and 60% range, and BMF data suggests that all congeners have the potential to biomagnify in fish. Depuration relationships (log $t_{1/2}$–log K_{ow} relationships) of this study were consistent between two treatments (high and hCYP inducing) as well as having nearly identical slopes to the Fisk et al. [18] experiment confirming the strength of the log $t_{1/2}$ to log K_{ow} relationship. Temperature may be a key factor in elimination of PCBs by juvenile rainbow trout because $t_{1/2}$s of PCB congeners at 8°C were approximately twice as long as $t_{1/2}$s of PCB congeners in fish held at 12°C [18]. We show that biotransformation of PCBs in juvenile rainbow trout held at 8°C is minimal or extremely slow even in fish exposed to CYP1A-inducing PCB congeners, suggesting that fish may accumulate a significant amount of OH-PCB metabolites from sources other than biotransformation from parent PCB congeners and/or are produced over a longer period of time than assessed in this study at low temperatures. Research studies on the biotransformation potential of PCBs at higher temperatures still are required to determine the role of temperature in cytochrome P450 enzyme kinetics. This, in turn, may affect the ability of fish to metabolize PCBs to their hydroxylated form.

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