Environ. Sci. Technol. **2003**, *37,* 1720−1725

Hydroxylated PCBs and Other Chlorinated Phenolic Compounds in Lake Trout (*Salvelinus namaycush*) Blood Plasma from the Great Lakes Region

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Recently, there has been an increase in studies focusing on an emerging class of organic contaminants, hydroxylated PCBs (OH-PCBs) and chlorinated phenolic compounds (CPCs) in the environment, particularly in northern regions of Europe and Canada. Since information for fish from the Great Lakes are scarce, we determined the blood plasma concentrations of OH-PCB congeners, pentachlorophenol (PCP), 2,3,4,5-tetrachlorophenol (TCP), and 4-hydroxyheptachlorostyrene (4-OH-HpCS) for lake trout (*Savelinus namaycush*) collected from two of the Great Lakes, Lake Ontario and Lake Superior, and two regional lakes, Lake Champlain and Lake Opeongo. PCP was the dominant CPC in lake trout (105−658 pg/g of plasma). Detectable concentrations of 2,3,4,5-TCP and 4-OH-HpCS were found in all lake trout (2.6−101 and 0.4−27 pg/g, respectively). Highest concentrations were found in trout from Lake Ontario and Lake Superior. Sixteen OH-PCBs were quantified, with 4-OH-CB187 having the highest concentration in all samples (10−173 pg/g of plasma). Unexpectedly, highly chlorinated OH-PCBs such as 4′-OH-CB199 (mean 21.4 and 74.4 pg/g), 4,4′-diOH-CB202 (18.3 and 27.7 pg/g), and 4′-OH-CB208 (24.5 and 34.7 pg/g) were found in lake trout from Lake Ontario and Lake Superior, respectively. Future studies to delineate the sources and impacts of CPCs in the Great Lakes catchment are needed.

Introduction

The widespread environmental persistence of polychlorinated biphenyls (PCBs) is well-known, particularly for the Laurentian Great Lakes (*1*). PCB concentrations in Great Lakes fish have been declining since early 1980s, with the lowest ∑PCB concentrations found in fish from Lake Superior and the highest from the lower Great Lakes (*1*). Even so, PCBs continue to be a concern for human and wildlife health, in part because they can be metabolized to other classes of persistent and toxic contaminants by biota (*2*, *3*). These metabolites include hydroxylated polychlorinated biphenyls (OH-PCBs) (*2*). The major route of OH-PCB formation in biota is oxidation via the cytochrome P450 (CYP) enzyme system (*2*). The end result of the CYP-mediated metabolism is dependent upon the chlorine arrangement and the type of CYP enzyme involved (*4*).

There are other chlorinated phenolic compounds (CPCs) including pentachlorophenol (PCP), which is not only used for wood preservation and other biocide use but is also a metabolite of hexachlorobenzene (HCB) (*5*). Tetrachlorophenol (TCP) congeners, rarely identified in fish, are an industrial byproduct often found in pulp mill effluents and may also be metabolites of penta- and tetrachlorobenzenes. In addition, 4-hydroxyheptachlorostyrene (4-OH-HpCS) was recently identified in polar bears and humans and is thought to be a metabolite of octachlorostyrene (OCS) (*6*−*8*). The abiotic and biotic transformation of PCBs to OH-PCBs and the distribution, concentrations, and health effects of CPCs are poorly understood, particularly in fish and other aquatic biota (*2*).

OH-PCBs and other CPCs are found predominantly in blood (*9*), which is known to retain organic metabolites and organic compounds that bind to proteins (*10*). In a study of Baltic Sea salmon (*Salmo salar*), a large number of CPCs, several of which were OH-PCBs as identified by mass spectrometry (data not published), were found in the fish blood and muscle (*11*). OH-PCBs have also been quantified in blood of albatrosses (*Diomedea* spp.) (*12*), white-tailed sea eagles (*Haliaeetus albicilla*) (*13*), polar bears (*Ursus maritimus*) (*14*, *15*), and humans (*8*, *16*). In these studies, OH-PCBs found in blood samples collected from humans and grey seals (*Halichoerus grypus*) were dominated by a few congeners, including 4-OH-CB187 (4-hydroxy-2,2′,3,4′,5,5′,6-heptachlorobiphenyl), 4-OH-CB146 (4-hydroxy-2,2′,3,4′,5,5′-hexachlorobiphenyl), and 4′-OH-CB109 (4′-hydroxy-2,3,3′,4′,5-pentachlorobiphenyl) (*8*, *9*). In blood plasma collected from polar bears, important congeners included 4-OH-CB187, 4-OH-CB146, and 4-OH-CB193 (4-hydroxy-2,3,3′,4′,5,5′,6-heptachlorobiphenyl) (*14*). Concentrations of ∑OH-PCB in the blood of grey seals, polar bears, and humans have been in the same range as some of the most persistent individual PCB congeners (*9*, *14*, *15*).

There is mounting evidence that some OH-PCBs may have a greater potential to disrupt biological systems than their parent compound. OH-PCBs can compete with thyroxine for binding sites on transthyretin (TTR), one of the three main thyroid hormone transport proteins in mammals (*17*). This is because the chemical structures of some OH-PCBs with a para OH group and adjacent chlorine atoms, particularly 4′-OH-CB109, 4-OH-CB146, and 4-OH-CB187, share a similar structure to the thyroid hormones (T3 and T4), which have a para OH with adjacent iodine atoms. PCP and 4-OH-HpCS also have strong affinities for TTR (*8*, *18*), and will be considered with OH-PCBs in this study. Problems with the thyroid gland, mainly observed as large goiters and thyroid hyperplasia, have been observed in Great Lakes salmonids for more than 30 yr (*19*), and there is still significant evidence for thyroid hyperplasia in salmonids of the Great Lakes (Brown, S., National Research Institute, Environment Canada, unpublished data). Although a number of explanations have been suggested, including toxicity associated with

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10.1021/es026225m CCC: $25.00 © 2003 American Chemical Society
Published on Web 03/26/2003

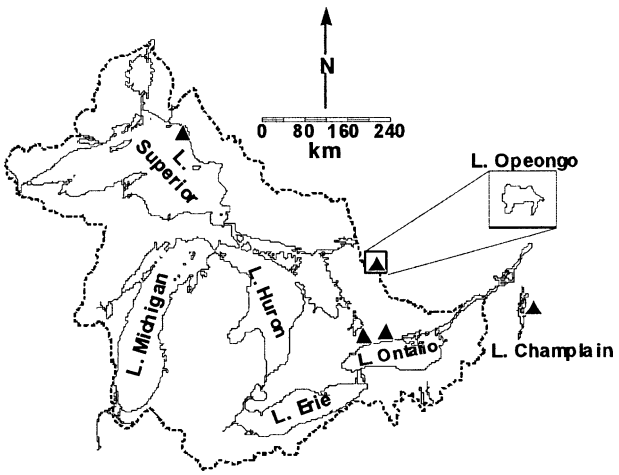

FIGURE 1. Map of sampling sites (▲) on the Great Lakes and Lakes Opeongo and Champlain. The dotted line indicates the boundary of the Great Lakes basin.

organochlorine contaminants (*19*), a definitive cause has not been found.

In vivo and in vitro laboratory assays indicate that several OH-PCB congeners have the potential to distrupt endocrine and metallothionein processes in fish (*20*−*22*). For example, 4′-OH-CB30 (4′-hydroxy-2,4,6-trichlorobiphenyl) has been shown to inhibit metallothionein transcription in hepatic tissue of Arctic char *Salvelinus alpinus*, thereby increasing liver susceptibility to metal toxicity in this fish species (*20*). Exposing juvenile rainbow trout (*Oncorhyncus mykiss*) to 4′-OH-CB30 and 4′-OH-CB61 (4′-hydroxy-2,3′,4′,5′-pentachlorobiphenyl) resulted in the production of the vitellogenin, an egg yolk protein precursor (*21*). Vitellogenin synthesis and estrogen-dependent cycles were also altered in a culture of rainbow trout hepatocyte cells after exposure to a number of OH-PCBs (*22*).

The relatively high levels of PCBs (*1*, *23*) and concern about the presence of thyroid hyperplasia (*19*) in Great Lakes salmonids along with the endocrine-disrupting potential (especially thyroid) of OH-PCBs and CPCs makes the lack of such data an important gap. In this study, we collected lake trout blood samples from two Great Lakes (Ontario and Superior) and two smaller lakes (Champlain and Opeongo) in southern Ontario and northeastern United States (Figure 1). Lakes Ontario and Champlain are located in densely populated regions with multiple industrial and regional sources of contaminants. Lakes Superior and Opeongo are more remote lakes, with the atmospheric deposition of organic contaminants being the most important predominant source.

Methods

Fish and Blood Collection. Sampling methods were similar on all lakes and took place between May and August in 2000 and 2001. The lakes sampled, approximate latitude/longitude coordinates, and year of collection are provided in Table 1. In Lake Ontario, lake trout were collected from near Port Credit and Cobourg ON; the Lake Superior fish were collected from near Marathon, ON; and the Lake Champlain fish were collected from near Burlington, VT (Figure 1). Overnight bottom sets of nylon gillnet with stretched mesh sizes ranging from 7.5 to 11.5 cm (equivalent to commercial mesh sizes of 3.0−4.5 in.) were made in 30−40 m of water. Total lengths of selected fish were restricted to between 50 and 100 cm; an exception was a 153-cm-long lake trout from Lake Ontario in 2000. Whole blood was collected only from fresh fish by cardiac puncture or caudal vein using 10-mL Vacutainers (Fisher Scientific) containing dried sodium heparin, a standard anti-clotting compound. Filled Vacutainers were stored either on ice or refrigerated at 4 °C prior to centrifuging. Within 30 min of collection, the filled Vacutainers were centrifuged (approximately 5000 rpm for 5 min), and the plasma was decanted into 5 mL cryovials and frozen until analyzed.

PCB numbers follow the IUPAC numbering scheme, while OH-PCBs follow the proposed scheme by Letcher et al. (*2*). To have sufficient volume (>4 mL) for CPC analyses, some blood plasma samples were pooled. Blood plasma samples from two individual lake trout from Lake Opeongo were pooled to form a single composite sample. Likewise, two composite samples were obtained from four lake trout from Lake Ontario in 2001 in addition to nine fish blood plasma samples analyzed individually. Two composite blood plasma samples were created from five lake trout from Lake Superior in addition to two individual samples. While it is not possible to statistically compare the single composite Lake Opeongo sample to these from other lakes, we include it to provide comparisons among Great Lakes and the smaller lakes. Correlations were analyzed using the Pearson Correlation Coefficient function in SYSTAT software (version 10, SPSS Corporation) (see Tables S1 and S2 in Supporting Information).

OH-PCB, Chlorinated Phenolic, and Organochlorine Analysis. Plasma collected in 2000 ($n = 10$) and 2001 ($n = 15$) were extracted and analyzed at the National Wildlife Research Centre (NWRC), Hull, PQ, and National Water Research Institute (NWRI), Burlington, ON, respectively. The methods were developed from those detailed in Sandau et al. (*6*, *8*). The methods used for extraction and cleanup were nearly identical between the two locations. The analysis and quantitation methods varied between the locations. A series of eight OH-PCBs were analyzed at NWRI and were purchased from Wellington Laboratories. The NWRC had 14 CPC standards for quantitation, and these standards were purchased from Wellington Laboratories (Guelph, ON, Canada) or were donated by Åke Bergman (University of Stockholm, Sweden). Additional analyses of the NWRI samples were not possible because the low volume used (~50 mL) was not sufficient to allow a second analysis at NWRC.

Briefly, plasma samples (4−6 mL) were spiked with $^{13}C_{12}$-labeled standards, mixed, and allowed to equilibrate. Samples analyzed at NWRC used the following recovery standards: [^{13}C]pentachlorophenol, [^{13}C]OH-CB159, [^{13}C]tetraClBz, [^{13}C]-PnClBz, [^{13}C]HClBz, [^{13}C]-β-HCH, [^{13}C]DDE, and [^{13}C]PCBs 28, 52, 118, 153, 180, and 194. Samples analyzed at NWRI used the following recovery standards: [^{13}C]OH-CB120, [^{13}C]-OH-CB187, and nonlabeled PCBs 30 and 204. Proteins were denatured using 2-propanol/HCl, and contaminants were extracted using a conventional liquid/liquid extraction technique (MTBE/hexane). Lipids were determined in the NWRI samples by gravimetric methods using 1/10th of the extract and did not vary significantly among samples. Lipids were not determined in the NWRC samples. The combined organic phase was reduced in volume and partitioned with KOH (0.5 M). The neutral compounds (PCBs, DDTs, etc.) were removed in the organic phases while the acidic compounds (OH-PCBs) were ionized and isolated in the aqueous phase. The aqueous phase was washed with hexane, and the hexane was added to the organic phase fraction. The washed aqueous phase was then acidified and back-extracted with hexane/MTBE, derivatized with diazomethane, and cleaned up on silica/sulfuric acid column (22% H_2SO_4, 5 g). This will be referred to as the CPC fraction. The organic phase went through two cleanup steps, a Florisil (8 g, 1.2% deactivated) and Silica gel (3 g, 22%) column cleanup. Organic and CPC fractions were reduced to a final volume of approximately 100 μL, and a performance standard was added (aqueous phase: 4′-Me-4-MeO-CB112 for NWRC samples

TABLE 1. Mean Concentrations ± SD of Organochlorine and Chlorinated Phenolic Compounds for Lake Trout from Lakes Ontario, Champlain, Opeongo, and Superior Along with the Appropriate Geographical Coordinates of Sampling Sites, Year of Sampling, and Size/Ages of Fish[a]

	Lake Ontario	Lake Champlain	Lake Ontario	Lake Opeongo	Lake Superior
longitude	43°33′−43°58′	44°29′	43°33′	45°42′	48°45′
latitude	79°35′−78°10′	73°16′	79°35′	78°23′	86°26′
year	2001	2001	2000	2000	2000
sample size	11	4	5	1	4
TL (cm)	68.3 ± 8.4 (56−80.5)	62.9 ± 4.9 (57−68)	82.2 ± 39.8 (57−153)	61	63.0 ± 24.7 (40−96)
weight (g)	3593 ± 1326 (1575−5704)	2647 ± 459 (2009−3104)	4322 ± 3071 (1797−9653)	2422	1859 ± 1079 (596−3063)
age (yr)	7.6 ± 2.4 (4−12)	7.8 ± 0.5 (7−8)	7.8 ± 5.2 (5−17)	12	7.3 ± 3.0 (4−11)
% lipid	0.27 ± 0.08 (0.19−0.41)	0.20 ± 0.04 (0.16−0.26)	na	na	na
p,p′-DDE	25.9 ± 9.2 (10.2−37.7)	8.9 ± 3.7 (6.0−13.7)	41.6 ± 22.0 (23.2−75.1)	8.0	11.9 ± 5.6 (5.2−17.8)
HCB	645 ± 277 (51−1125)	151 ± 42 (97−197)	1270 ± 309 (892−1639)	403	653 ± 204 (488−951)
CB28	0.51 ± 0.26 (0.20−1.02)	1.06 ± 0.62 (0.41−1.88)	7.07 ± 8.04 (0.85−19.07)	0.41	3.81 ± 5.93 (nd-12.60)
CB52	1.73 ± 0.48 (1.24−2.57)	1.478 ± 0.50 (0.98−2.09)	6.30 ± 4.12 (2.69−11.88)	0.98	2.79 ± 3.33 (0.17−7.67)
CB99	3.86 ± 1.19 (2.33−5.58)	1.69 ± 0.700 (1.06−2.54)	13.434 ± 4.323 (6.98−17.56)	1.96	3.32 ± 2.95 (0.67−7.51)
CB105	3.78 ± 1.19 (1.72−5.22)	1.67 ± 0.70 (1.05−2.47)	7.96 ± 2.99 (4.35−10.9)	0.76	2.66 ± 2.27 (0.55−5.82)
CB153	9.70 ± 2.60 (6.4−13.1)	4.10 ± 1.80 (2.6−6.4)	28.1 ± 11.3 (15.8−42.7)	5.3	32.5 ± 43.8 (0.4−97.5)
CB138	10.95 ± 3.07 (7.30−15.45)	4.91 ± 2.13 (3.10−7.59)	29.35 ± 13.84 (16.2−48.03)	4.79	23.40 ± 24.12 (4.60−58.31)
CB187	7.9 ± 3.3 (nd−11.4)	2.3 ± 1.1 (1.4−3.7)	11.4 ± 6.2 (4.2−18.7)	0.2	8.8 ± 9.2 (0.8−21.6)
CB199	0.09 ± 0.010 (nd−0.26	0.08 ± 0.04 (0.03−0.13)	11.2 ± 15.0 (0.69−34.0)	nd	17.8 ± 34.9 (nd−70.1)
CB202	0.09 ± 0.13 (nd−0.43)	0.07 ± 0.10 (0.005−0.22)	1.40 ± 1.33 (nd−2.77)	nd	0.35 ± 0.71 (nd−1.41)
∑PCB[b]	136 ± 54 (86−257)	63 ± 22 (43−87)	511 ± 445 (37−1246)	23.6	101 ± 112 (83−234)
2,3,4,5-TCP	na	na	40.5 ± 36.6 (4.4−101)	19.7	10.2 ± 11.0 (2.6−26.1)
PCP	na	na	357 ± 206 (105−658)	504.2	244 ± 142 (126−451)
4′OH-HpCS	na	na	23.6 ± 2.4 (20.3−27.0)	2.4	0.6 ± 0.2 (0.4−0.9)
4′-OH-CB50	na	na	12.5 ± 17.1 (nd−42.7)	9.8	4.2 ± 5.1 (nd−10.2)
4-OH-CB93	na	na	14.4 ± 11.5 (nd−29.0)	6.1	24.6 ± 9.3 (15.1−36.9)
3-OH-CB118	14.3 ± 11.7 (0.0−39.7)	nd	na	na	na
4′-OH-CB107 + 4-OH-109	na	na	4.9 ± 2.7 (1.7−8.0)	0.3	1.5 ± 0.5 (0.7−1.9)
4′-OH-CB120	na	na	6.7 ± 5.1 (1.5−13.8)	<0.01	7.4 ± 5.6 (2.3−13.2)
4′-OH-CB121	na	na	3.8 ± 3.4 (nd−9.3)	<0.01	16.3 ± 21.2 (15.1−36.9)
4-OH-CB146	1.1 ± 3.6 (0.0−12.0)	<0.01	na	na	na
3′-OH-CB138	5.0 ± 6.4 (nd−20.2)	1.2 ± 2.4 (nd−4.9)	na	na	na
4′-OH-CB130	6.8 ± 9.6 (nd−24.2)	2.5 ± 5.0 (nd−10.0)	na	na	na
4-OH-CB187	75.5 ± 34.2 (50.9−173.1)	17.8 ± 10.8 (9.5−32.9)	104.5 ± 45.6 (37.1−160)	14.7	61.0 ± 33.5 (38.4−111)
3′-OH-CB180	5.3 ± 7.2 (nd−23.2)	1.9 ± 2.2 (0.0−4.0)	5.1 ± 2.2 (3.2−8.8)	nd	4.8 ± 2.0 (2.4−7.4)
4′-OH-CB172	31.8 ± 20.1 (14.0−80.8)	10.5 ± 3.3 (6.7−13.9)	na	na	na
4-OH-CB193	na	na	5.9 ± 1.6 (3.9−7.6)	nd	4.1 ± 3.5 (1.6−9.2)
4′-OH-CB199	na	na	74.4 ± 23.5 (50.8−107.2)	26.9	21.4 ± 15.0 (0.8−34.4)
4,4′-diOH-CB202	na	na	27.7 ± 29.2 (nd−76.2)	nd	18.3 ± 19.0 (4.9−46.4)
4′-OH-CB208	na	na	24.5 ± 30.9 (3.7−76.2)	nd	34.7 ± 69.0 (nd−138)

[a] Some fish blood plasma samples are pooled, and the sample size listed here indicates the number of samples, both composite and individual, processed for organic analyses (see Methods for details). Concentrations are in pg/g wet weight except for PCB and DDT congeners, which are in ng/g wet weight. Analytical standards used to quantify chlorinated phenolic compounds and OH-PCBs differed between years. na, not analyzed; nd, not detected (<0.2 pg/g). [b] ∑PCB is the sum of CB 1, 3, 4+10, 5+8, 6, 12+13, 14, 15, 16+32, 17, 18, 19, 21, 22+51, 24+27, 25, 26, 28, 29, 31, 33+20, 40, 41+64+71, 42+49, 44, 45, 47, 48, 52, 53, 56+60, 63, 65, 66,. 70, 74, 76, 77+110, 81, 82, 83, 84, 85, 87, 89+92, 90, 91, 95, 97, 99, 100, 101, 103, 105, 107+147, 110, 114+134+143, 118+149, 119, 124+135+144, 128, 129, 130, 131, 132, 134, 137, 136, 138+163, 141, 146, 151, 153, 156+171, 157, 158, 166, 167, 169, 170, 171, 172, 174, 175, 176, 177, 178, 179, 180, 182+187, 185, 189, 190, 191, 193, 194, 195, 196+203, 197, 198, 199, and 201.

and CB166 for NWRI; organic phase: [^{13}C]CB138 for NWRC and CB30/CB204 for NWRI.

GC−MS and ECD Analysis. All OH-PCBs were analyzed by GC−electron capture negative ionization (low resolution) mass spectrometry GC−ECNI−MS using DB-5 columns ([5% phenyl]methylpolysiloxane, 30 m × 0.25 mm i.d. × 0.25 μm film thickness, J&W Scientific, Folsom, CA) and helium carrier gas. ECNIMS was performed using methane (99.99% pure) as the reagent gas. At NWRC, analyses were performed on a Hewlett-Packard (Atlanta, GA) 5890A series II gas chromatograph equipped with an HP 7673A automatic injector and a Hewlett-Packard 5988A gas chromatograph-mass spectrometer. At NWRI, analyses were conducted using a GC−ECNI−MS using an Agilent 6890 gas chromatograph equipped with an Agilent 7673 automatic injector and an Agilent 5973 GC−MS. PCB congeners and organochlorine pesticides were determined by high-resolution capillary GC with electron capture detection using a Hewlett-Packard 6890 GC equipped with a 30 m × 0.25 mm, 0.25 μm film thickness DB-5 column programmed at 15 °C/min to 150 °C and 3 °C/min to 265 °C. Carrier gas was H_2 (about 1 mL/min), and makeup gas was N_2 (40 mL/min) was used for separation. PCB congeners and OC pesticides were quantified by GC-ECD using a series of authentic external standards.

The percent recoveries of the organic phase (i.e., organochlorines) recovery standards were 77.1 ± 5.0 (mean ± 1 SE) and 80.2 ± 3.0 for NWRC and NWRI, respectively. The percent recoveries of the aqueous phase (i.e., CPCs and OH-PCBs) recovery standards were 87.3 ± 6.5 (mean ± 1 SE) and 84.4 ± 4.4 for NWRC and NWRI, respectively. In light of consistent recoveries no correction were made to the concentration data.

Results and Discussion

Lake trout from Lake Ontario and Lake Superior had the higher mean concentrations of 4-OH-CB187, 3′-OH-CB180, *p,p′*-DDE, ∑PCBs, and most PCB congeners (Table 1). This is consistent to other organochlorine contaminant studies for lake trout and other fish species, which have determined that Great Lakes fish usually have higher concentrations than the other regional inland lakes (*23*).

PCP and 2,3,4,5-TCP concentrations were similar in lake trout from Lake Ontario, Lake Superior, and Lake Opeongo (Table 1). PCP was a major CPC congener, with highest concentrations (105−658 pg/g) in most lake trout plasma samples (Table 1). High PCP concentrations has also been observed in plasma for humans and Baltic Sea salmon (*6*, *7*, *11*). Lake trout PCP concentrations are within the ranges measured (60−3430 pg/g) for several fish species from the Detroit River (*24*). In lake trout, 2,3,4,5-TCP (3−101 pg/g) was also an important CPC congener. However, relatively little is known about the distribution and concentrations of PCP and 2,3,4,5-TCP in the Great Lakes (*25*, *26*). The Great Lakes region, with its high density of industry, such as the wood preserving plants on Lake Ontario and the pulp mills around Lake Superior, likely has had a relatively high input of PCP and TCP congeners in the recent past (*26*). Accordingly, some Lake Ontario regions such as the Bay of Quinte and St. Lawrence River have measurable TCP and PCP concentrations in water and sediment (*25*, *26*). PCP and TCP compounds are known to dissipate from water rapidly, but limnocorral experiments have shown that the majority of chlorophenols will remain in water column even after 40−60 days (*27*), which would indicate that PCP and TCP congeners would remain bioavailable to lake trout in regions of higher concentrations such as Lake Ontario.

Concentrations of 4-OH-HpCS was highest in lake trout from Lake Ontario (20.3−27.0 pg/g; Table 1). The 4-OH-HpCS concentrations in lake trout are somewhat lower than seen for most Detroit River fish species, including black crappie (*Pomoxis nigromaculatus*; 310 pg/g) and largemouth bass (*Micropterus salmoides*; mean, 60 pg/g) (*24*). Published values for 4-OH-HpCS has been reported for polar bears and ringed seals (*6*), and those concentrations (9.1 and 0.062 ng/g of plasma respectively) were higher than seen in lake trout (0.40−27 pg/g of plasma). However, concentrations in lake trout were similar to those found in human umbilical cord plasma in Quebec (5−34 pg/g of plasma) (*7*). OCS has been hypothesized to be the parent compound of 4-OH-HpCS (*6*), and this may be supported by the similarity of trends for 4-OH-HpCS concentrations in lake trout and OCS measured for fish in other studies. For example, fish from Lake Ontario collected between 1974 and 1981 had higher OCS concentrations (35−281 ng/g wet weight) than those from Lake Superior (<1 ng/g) (*28*). In turn, lake trout collected from Lake Superior (1.5 ± 1.1 ng/g, n = 75) in 1998 had higher concentrations than those from Lake Opeongo (0.3 ± 0.2, n = 14) and Lake Champlain (0.3 ± 0.5, n = 21) (Muir, D. C. G., National Research Institute, Environment Canada, unpublished data). OCS is usually considered to be a minor environmental contaminant in biota but was widely distributed in the fish and sediment of the lower Great Lakes in the 1970s and 1980s (*28*, *29*). In Lake Ontario, the source of OCS was hypothesized to be the waste product of electrolytic chlorine production (*29*). The ratios of 4-OH-HpCS to CB153 in blood plasma in lake trout in this study (1.8×10^{-5} to 8.0×10^{-4}) are quite low as compared to polar bears and ringed seals (0.71and 0.02, respectively), supporting very slow CYP-mediated metabolism in lake trout (*3*, *30*).

4-OH-CB187 was the most abundant OH-PCB congener measured (Table 1) and also has been found to be a major congener in blood plasma samples from most species examined so far, including albatross in the north Pacific (*12*), polar bears from the Canadian Arctic (*14*), and white-tailed sea eagle nestlings and humans from Baltic Sea region, Faroe Islands, and the Canadian Arctic (*8*, *9*, *13*, *31*−*33*). 4-OH-CB187 was also a major congener in 13 fish species from contaminated Detroit River (*24*). In short, 4-OH-PCB187 is an important environmental hydroxylated PCB congener in diverse vertebrate species from different ecosystems.

The next important set of OH-PCB congeners for the lake trout from Lake Ontario and Lake Superior were the highly chlorinated congeners (4′-OH-CB199, 4′-OH-CB208, and 4,4′-diOH-CB202). The presence of these highly chlorinated OH-PCBs was somewhat unexpected in the lake trout, as this species and fish in general are believed to have limited ability to biotransform highly chlorinated PCB congeners (*3*, *30*). This would suggest that these chemicals were bioaccumulated and not formed in the fish through biotransformation. Likewise, OH-PCBs with 8 and 9 chlorines have been exclusively detected in benthic-feeding white sucker (*Catostomus commersoni*) and common carp (*Cyprinus carpio*) from the Detroit River, which was also attributed to dietary uptake of previously metabolized PCB-contaminated food items (*24*). 4,4′-diOH-CB202 has been found at fairly high concentrations in polar bears, which can efficiently biotransform PCBs, although it was not one of the overall dominant contaminant congeners (*15*). In a study of white-tailed sea eagle nestlings, a peak in the GC−ECNI−MS chromatographs of blood plasma extracts indicated the presence of an octachlorobiphenyl congener with two OH groups (*13*), which is consistent with 4,4′-diOH-CB202. The hydroxylation kinetics of the octa- and nonachlorophenyls, either abiotic or biotic, and the toxicokinetics of the highly chlorinated OH-PCBs are still unknown.

After the octa- and nonachlorophenylols, the tetra- to heptachlorophenylols were the next important set of OH-PCB congeners (Table 1). The elevated 4-OH-CB193, 4′-OH-CB172, and 4′-OH-CB130 concentrations in lake trout may not be unique to this species. In Detroit River, these congeners

were also important in several pelagic fish species (*24*). In polar bears, 4-OH-CB193 is an important OH-PCB congener ranking after 4-OH-CB187, while 4′-OH-CB172 and 4′-OH-CB130 are also present at relatively high concentrations (*14*). OH-PCB congeners with the OH position in the meta position are not often found at high concentrations in plasma samples from other species (*2*, *8*, *14*), so it is interesting to note that 3-OH-CB118, 3′-OH-CB138, and 3′-OH-CB180 are important OH-PCB congeners for lake trout from Lake Ontario. Similarly, 3′-OH-CB138 and 3′-OH-CB180 also have been measured for several fish species from the Detroit River, with 3′-OH-CB138 being particularly important (*24*).

Lake trout from Lake Champlain did not have detectable concentrations of 4-OH-CB146, while those from Lake Ontario had concentrations ranging from below detection limit (estimated at 0.2 pg/g based on 3× S/N) to 12 pg/g (Table 1). Another commonly measured congener, 4′-OH-CB107/109, was present at relatively low concentrations in all analyzed lake trout. This is unlike other blood plasma studies for humans and birds, which all reported relatively high concentrations of 4-OH-CB146 and 4′-OH-CB109 in human and bird samples (*8*, *12*). The precursors of 4-OH-CB146 are CB138 and CB153 (*2*). These are important PCB congeners in lake trout in this study (Table 1) and elsewhere (*34*) as well as birds and humans (*8*, *12*). The low concentrations of 4-OH-CB146 in lake trout, despite the presence of CB138 and CB153, suggests that this hydroxylated PCB congener is being biotransformed by fish less quickly than for mammals and birds.

It should be noted that there are likely many more OH-PCBs and CPCs present in these lake trout. A large number of compounds were present in the plasma but could not be quantified due a lack of appropriate standards. As well, ECNIMS has a bias toward higher chlorinated compounds. Lower chlorinated compounds (e.g., tri- and tetra-OH-CB congeners) are more difficult to detect or quantify using this method. This is because electron capture produces intense signals for highly chlorinated PCBs but produces a smaller signal for the lower chlorinated PCBs. This is a result of changing electron capture cross sections with chlorine content (*35*). Although some lower chlorinated congeners appeared to be present in these fish (e.g., tri- and tetra-OH-CBs), they could not be validated or quantified using this measurement technique. The number of individual CPCs observed in the lake trout was similar to those observed in North American mammalian and bird blood (*14*) and Baltic Sea salmon blood samples (*11*).

Total concentrations of the analyzed OH-PCBs were low relative to ∑PCBs (<0.1%). The ratios of 4-OH-CB187 to CB153, which are correlated ($r^2 = 0.548$, $p < 0.001$; Figure 2), in lake trout consistently range from 0.003 to 0.01, which are much lower than seen in albatrosses (from 0.3 to 1.1) (*12*), white-tailed sea eagle nestlings (from 0.1 to 0.3) (*13*), humans (from 0.1 to 0.43) (*8*), and polar bears (from ≈1 to 12) (*15*). The ratios of 3′-OH-CB180 and CB153 were even lower (≤0.002), although those were also correlated ($r^2 = 0.310$, $p < 0.025$; Figure 2). It is generally believed that birds and terrestrial mammals have much greater metabolic capacity than fish (*36*), so OH-PCB:PCB ratios (and 4-OH-HpCS:OCS ratios) should be relatively low in fish as compared to mammals and birds. For example, a review comparing several PCB metabolism studies demonstrated that rats (*Rattus* spp.) metabolize coplanar CB77 (3,3′,4,4′-tetrachlorobiphenyl) at a more rapid rate than marine scup (*Stenotomus chrysops*) and catfish (*Ictalurus punctatus*) (*4*). Although CB 77 is probably metabolized by CYP 1A, which is inducible and potentially active in all vertebrates including fish, it does suggest that fish have inferior biotransformation ability compared with birds and mammals. However, the comparison of different types of organisms (i.e., fish vs birds and mammals) is confounded by the lack of knowledge on the source and persistence of OH-PCBs and other CPCs (particularly the highly chlorinated compounds with chlorine molecules in the ortho position).

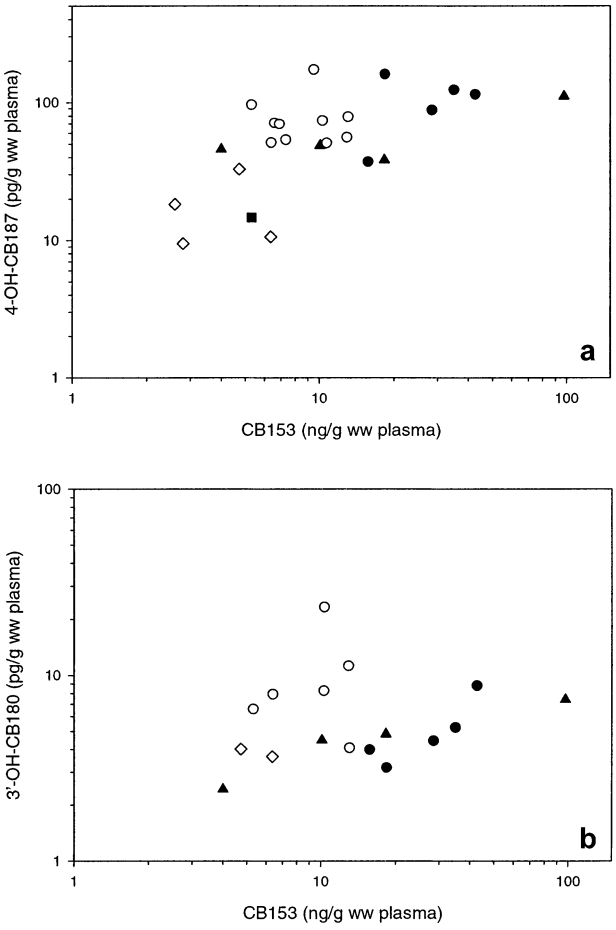

FIGURE 2. Lake trout blood plasma 4-OH-CB187 (a) and 3′-OH-CB180 (b) vs CB153 concentrations. Symbols indicate Lake Ontario 2001 (●), Lake Ontario 1999 (○), Lake Superior (▲), Lake Opeongo (■), and Lake Champlain (◇).

It is known that some fish species may biotransform lower-chlorinated PCBs. For example, the marine scup injected intraperitoneally with CB77 had the metabolites 2-OH-CB77, 5-OH-CB77, 6-OH-CB77, 4-OH-3,3,4′,5-tetrachlorobiphenyl, a diOH-tetrachlorobiphenyl, and a diOH-trichlorobiphenyl measured in their bile samples (*37*). The rates of metabolism, even for the metabolites with the OH in the para position, was found to be very slow in the scup making it difficult for the investigators to measure the actual rate of metabolism (*37*). As such, the metabolism of higher-chlorinated non-coplanar PCBs would likely to be even slower. The deepwater sculpin (*Myoxocephalus thompsoni*) from Lake Michigan has demonstrated capacity to metabolize PCBs to methylsulfonyl-PCBs ($MeSO_2$-PCBs), which also are mediated by cytochrome P450 iso-enzymes (*3*). This may be unique to sculpin, as in the same study, a food web effect was demonstrated where lake trout and bloater chub (*Coregonus hoyi*) had no measurable $MeSO_2$-PCBs in their tissue, but burbot (*Lota lota*), a predator of deepwater sculpin, had measurable $MeSO_2$PCB concentrations (*3*). Lake trout has been demonstrated to lack P4501A-like and P4502B-like alterations of PCB congeners (*30*), which may support the likelihood of bioaccumulation of OH-PCBs from the environment as opposed to in situ metabolic alternations of PCBs. At the moment, no information exists for biotransformation of the highly recalcitrant PCBs to OH-PCBs nor bioaccumulation

of OH-PCBs in lake trout or many other salmonids.

PCB and OH-PCB concentrations in lake trout generally correlate well with known PCB trends in sediment and water. For example, in deep-water core studies, surface sediments from Lake Superior have approximately 7−9 ng/g dw (*38*); from L. Ontario, 100 ng/g dw (*38*); from Burlington Harbor of Lake Champlain, from <100 to 500 ng/g dw (*39*); and from Lake Opeongo, 10 ng/g dw (Muir, D. C. G., unpublished data). It may be that there is also greater OH-PCB and CPC formation in the more contaminated lakes and subsequent bioaccumulation by the lake trout. It is known that the higher-chlorinated PCBs have slow abiotic OH-radical transformation rates relative to the lower-chlorinated PCBs, especially in turbid environments (*40*), but steady-state kinetic modeling suggest that the half-lives of PCBs in lakes (prior to hydroxylation with the OH radical) range between 10 and 100 days (*41*). In addition, there are many potential direct inputs of OH-PCBs into the food webs of the Great Lakes, including municipal and agricultural waste effluents containing mammalian metabolites. OH-PCBs have been detected in the faeces of grey seals and guillemots (*Uria algae*) from the Baltic Sea (*42*), and given the higher metabolism rates of mammals and birds relative to fish (*4*), waste effluents may represent a source of previously metabolized PCBs and CPCs to aquatic food webs. However, the presence of OH-PCBs, PCP, and 2,3,4,5-TCP in lake trout from remote Lake Opeongo may point to some metabolic capacity to hydroxylate PCBs and/or an important atmospheric source of highly chlorinated OH-PCBs to the lake. In any case, the presence of OH-PCBs and other CPCs in lake trout from the Great Lakes represents a potential environmental and health risk in the catchments. The sources, concentrations, distribution, and physiological effects of CPCs on fish and mammals urgently need to be delineated in order to be able to adequately assess the impacts on the lake ecosystems and the people living around the Great Lakes.

Acknowledgments

The authors thank Andrea Buckman, Colin Darling, Stephanie Brooks (Environment Canada), and Sue Newson (Canadian Wildlife Services) for their analytical expertise at National Water Research Institute. Mike Keir (Great Lakes Laboratory for Fisheries and Aquatic Sciences, Department of Fisheries and Oceans) assisted with the blood plasma sampling in the field. Dr. Courtney Sandau provided valuable insight and CPC standards. Funding for this project was provided by the Great Lakes Sustainability Fund (D.M.W.) and the Great Lakes 20/20 Fund (A.T.F. and D.C.G.M.).

Supporting Information Available

Two correlation matrix tables. This material is available free of charge via the Internet at http://pubs.acs.org.

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Received for review October 9, 2002. Revised manuscript received January 30, 2003. Accepted February 11, 2003.

ES026225M