A SURVEY OF HALOGENATED PHENOLIC CONTAMINANTS AND METABOLITES IN THE PLASMA OF THREE BENTHIC FISH SPECIES FROM DETROIT RIVER POPULATIONS

Hongxia Li, Erin Bennett, Ken Drouillard, Doug Haffner and Robert J. Letcher

Great Lakes Institute (GLI), University of Windsor, Windsor, Ontario, Canada N9B 3P4

Introduction

The Detroit River is a channel connecting Lake Huron to Lake Erie via Lake St. Clair. The sediments and aquatic biota (zooplankton, benthic invertebrates and fish) of the Detroit River contain high concentrations of persistent polyhalogenated contaminants such as polychlorinated biphenyls (PCBs) and organochlorine (OC) pesticides (e.g., DDTs, chlorobenzenes and chlordanes) [1-5]. Octachlorostyrene (OCS) is also a significant contaminant in sediment and aquatic biota from the Huron-Erie corridor due to recent and historical industrial inputs into the St. Clair River [2-5]. Benthic and pelagic fish species are exposed to these xenobiotics via dietary uptake and/or chemical accumulation by partitioning from water. For contaminants with high K_{ow} values (log K_{ow} > 6.3) accumulation in Detroit River benthic fish (e.g., freshwater drum (*Aplodinotus grunniens*), shorthead redhorse (*Moxostoma macrolepidotum*), stonecat (*Noturus flavus*) and rock bass (*Ambloplites rupestris*)), was shown to be mainly a function of trophic interactions[3]. Furthermore, benthic feeding fish are more contaminated than pelagic feeding fish, even though some of the pelagic fish examined were piscivores, suggesting sediment ingestion act as an important exposure pathway to Detroit River biota. In these benthic fish (sampled in 1991), muscle concentrations of OCS were reported between 1000 and 10000 ppb (lipid wt. basis), and sum PCB concentrations > 4000 ppb (lipid wt. basis) [3,4].

Benthic fish from the Detroit River such as brown bullhead (*Ameiurus nebulosus*) have been shown to rapidly metabolize polycyclic aromatic hydrocarbons (PAHs). However, the rate of PCB metabolism in fish is considerably lower than for PAHs and is more congener selective. Immunochemical and catalytic assays have demonstrated that cytochrome P450 monooxygenase (CYP) activity is inducible in fish, whereas CYP2B-type enzyme activity is generally low and non-inducible [6]. Benthic fish species from the Detroit River possess significant levels of *meta-para*, chlorine-unsubstituted PCB congeners [3], which are ideal substrates for CYP2B-type enzymes, and precursors for persistent aryl methyl sulfone PCB ($MeSO_2$-PCB) metabolites [7]. Polyhalogenated aromatic hydrocarbons (PHAHs), which include PCBs and OCS, have been shown to be biotransformed to retained or persistent metabolites, in some cases with appreciable biological half-lives [7]. Persistent $MeSO_2$-PCB and retained hydroxylated PCB (OH-PCB) have been reported in a growing number of species, and have been shown to elicit biological and toxicological effects in exposed organisms, and *in vitro* cell systems, including estrogenic and thyroidogenic endocrine effects [7]. The deepwater sculpin (*Myoxocelphalus thompsoni*) from Lake Michigan, was recently found to be capable of forming $MeSO_2$-PCB metabolites from *meta-para*-PCB congeners [8]. More than 120 phenol-type organohalogen substances, including OH-PCBs, OH-polybrominated diphenyl ethers (OH-PBDEs) and pentachlorophenol (PCP), were recently detected in the blood, muscle and eggs of Baltic salmon (*Salmo salar*), although a majority of these compounds were not identified [9]. In general, very little is known regarding PHAH metabolism and/or accumulation of PHAH phenolic compounds and metabolites in fish. The present studies surveyed PHAH metabolism, by determination of PCBs, OCs, $MeSO_2$-PCB metabolites, and OH-PCB

metabolites and other halogenated phenolic compounds in plasma of common carp (*Cyprinus carpio*), white sucker (*Catostomus commersoni*) and brown bullhead populations from the Detroit River.

Materials and Methods

Fish were collected and blood taken from individuals captured during August and September of 2001. The plasma was isolated by centrifugation and stored frozen at -20 °C until chemical analysis. The plasma from 3 or 4 individual fish from each species or collection site (Table 1) required pooling to permit adequate detection and quantification of PHAHs, especially phenolic PHAHs. Approximately 3.0 to 4.0 grams of the plasma pools were extracted and four contaminant fractions were separated, i.e., PCBs, organochlorine pesticides (OCs), $MeSO_2$-PHAHs and OH-PHAHs. The procedures of Sandau *et al.* [9], Hovander *et al.* [10] and Letcher *et al.* [7] were used with modifications. All four fractions were analyzed using gas chromatography/electron capture detection (GC/ECD). GC/electron impact mass spectrometry (GC/MS(EI)) and/or GC/MS with electron capture negative ionization (GC/MS(ECNI)) was used to identify the structure or isomer of OH-containing compounds in plasma. An external standard quantification approach was used for PCBs (40 congeners including coelutions) and OCs (*p,p'*-DDT, *p,p'*-DDE, *p,p'*-DDD, a-HCH, b-HCH, g-HCH, oxychlordane, *trans*-chlordane, *cis*-chlordane, *cis*-nonachlor, heptachlor epoxide and OCS). CB-83 and CB-122 were used as PCB and OC internal standards. GC/ECD quantification for the $MeSO_2$- and OH-containing compounds were based on a $MeSO_2$-internal standard (3-$MeSO_2$-2-Me-2',3',4,5,5'-pentachlorobiphenyl) and OH-PCB-internal standards (4-OH-CB72), respectively. ECD responses and retention times were compared to authentic standards (i.e., 16 $MeSO_2$-PCBs, 3-$MeSO_2$-*p,p'*-DDE, 14 OH-PCBs and 4-OH-heptachlorostyrene (4-OH-HpCS) and pentachlorophenol (PCP)). Extraction efficiencies for PCBs, OCs and aryl sulfones were >90% while the OH-PCB internal standard was >95%.

Results and Discussion

Table 1 lists PHAH concentrations and the identified concentrations of phenolic PHAH metabolites and compounds measured in Detroit River fish plasma. Despite variations in species and sampling location, the Σ-PCB, S-chlordane and Σ-DDT concentrations were generally similar in the plasma of carp, white sucker and bullhead. $MeSO_2$-PCB concentrations are not reported, even though an apparent congener pattern was observed, because the levels were near the limit of detection (<0.002 ppm (w.w.)).

All fish species examined were capable of forming a number of OH-PCBs, as well as 4-OH-HpCS (Table 1). PCP was also detected. Plasma OH-PCBs are dependent on the relative activity of Phase I CYP enzyme, Phase II conjugation enzymes and thyroid hormone transport protein in the blood [7]. The presence of OH-PCBs and 4-OH-HpCS at varying levels indicates that these processes are active in these fish species, i.e. PCB and OCS metabolism, and OH-PCB and 4-OH-HpCS retention or conjugation (and subsequent excretion). However, the Σ-OH-PCB / Σ-PCB ratio ranged from 0.005 to about 0.030, and was similar to the approximate six-fold difference in the Σ-PCB levels (Table 1). This ratio suggests that the OH-PCB forming capacity of these benthic fish is low and OH-PCB conjugation activity high, despite the relative proportionality to the levels of Σ-PCBs. The Σ-OH-PCB / Σ-PCB ratio was also similar to the 0.002 ratio found for ringed seal plasma from the Canadian Arctic [7].

Variable amounts of penta- to hepta-chlorinated OH-PCBs were observed in the fish plasma (Table 1), while octa- and nona-OH-PCB isomers were detected only in white sucker and carp plasma (comprising 30 % to 60 % of the Σ-OH-PCB). The presence of penta- to nona-chlorinated OH-PCBs are consistent with the range of chlorinated metabolites reported in human, polar bear, seal and bird plasma samples [7]. Judging by the greater number of detectable OH-PCBs in white sucker and carp, it

Table 1. Concentrations of PCBs, OCs and phenolic compounds and metabolites in the plasma of three benthic fish species from the Detroit River. Concentrations are in ng/g (wet weight basis).

Sample	White Sucker	Common Carp #1	Common Carp #2	Brown Bullhead #1	Brown Bullhead #2
Capture Site	Celeron Island	Grosse Isle	Grosse Isle	Hennipen Marsh	Peche Island
4'-OH-CB101	-	-	0.050	0.064	0.228
4'-OH-CB121	0.092	0.126	0.362	0.086	0.073
4-OH-CB112	0.728	0.150	0.492	0.165	-
4-OH-CB107 [a]	0.112	0.131	0.132	0.129	0.236
4-OH-CB165	0.027	0.028	-	-	0.036
3'-OH-CB138	0.010	1.111	-	-	-
4'-OH-CB130	0.009	0.031	-	-	-
4-OH-CB187 [a]	0.117	0.047	0.013	-	0.048
4'-OH-CB159	0.019	0.010	-	-	0.049
3'-OH-CB180 [a]	0.014	0.084	0.008	-	-
4-OH-CB193	0.038	0.053	0.021	0.019	-
OH-Cl_8-CB [a, b]	0.172	0.505	0.084	-	-
OH-Cl_9-CB [a, b]	1.456	0.653	0.379	-	-
PCP [a, b]	3.427	0.262	0.766	0.046	0.141
4-OH-HpCS [a, b]	0.080	0.181	0.024	0.016	-
ΣPCB	103.869	193.588	60.46	38.392	80.526
Σ chlordane	2.828	3.952	1.374	0.887	1.170
ΣDDTs	10.442	26.204	3.95	2.311	8.658
OCS	0.012	0.013	0.045	0.008	0.036
ΣOH-PCB	2.794	2.911	1.541	0.463	0.442
ΣOH-PCB/ΣPCB	0.027	0.015	0.026	0.012	0.005
4-OH-HpCS /OCS	6.675	13.923	0.533	2.000	N/A

a: confirmed by GC/MS(ECNI) in white Sucker plasma.
b: confirmed by GC/MS(ECNI) in common carp plasma.

appeared that these more highly PHAH-exposed individuals had a higher capacity to form OH-PCB metabolites. This may reflect a higher CYP enzyme activity, and thus metabolic capacity to form OH-PCBs, or the uptake patterns of PCB congeners. Regardless, the uniqueness of the formation of OH-Cl8-CB and OH-Cl9-CB congeners appears to unique to white sucker and carp. In the plasma of sucker, PCP, OH-Cl_9-CB, 4-OH-CB112, 4-OH-HpCS were dominant compared to the lower levels of 3'-OH-CB138 and 4'-OH-CB130. In the rat, 4-OH-2,3,5,3',4'-pentaCB dominates, whereas the higher chlorinated OH-PCBs are only minor components. In human plasma, the hepta- and hexa-chlorinated OH-PCBs are much more abundant compared to the rat [12]. OCS (a byproduct of electrolysis industries) is a significant contaminant in sediment and aquatic biota in the Huron-Erie corridor [2-5]. 4-OH-HpCS, an apparent metabolite of OCS, was recently identified in Canadian arctic marine mammals [7,10]. The range of 4-OH-HpCS to OCS ratios was from 6.67 to 13.92 in sucker and carp, while it was much lower or not detected in bullhead (Table 1). Again, this may be a function of diet or metabolic capacity.

The 4-OH-HpCS / OCS ratio ranged from 5.19 to 216 and averaged 0.260 in polar bear and ringed plasma, respectively.

To our knowledge, this study is the first report on phenolic PCB and PHAH metabolites in benthic freshwater fish. It is likely to assume that these phenolic metabolites do not accumulate via the diet, since these phenolic metabolites are not known to be accumulative and are unlikely formed via PCB metabolism in lower, dietary trophic levels (e.g., zooplankton, benthic invertebrates). Both OH-PCBs and 4-OH-HpCS are known to possess estrogenic and/or thyroidogenic modulation potential [7,10]. Knowledge regarding the PCB and OC biotransformation and PHAH metabolite formation is important for assessing contaminant fate and the potential health impacts on fish and their aquatic ecosystems.

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