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Bioaccumulation and biotransformation of chlorinated and brominated organohalogens and metabolites in a deepwater sculpin (Myoxocephalus thompsoni) food chain from Lake Huron.

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Bioaccumulation and Biotransformation of Chlorinated and Brominated Organohalogens and Metabolites in a Deepwater Sculpin (*Myoxocephalus thompsoni*) Food Chain from Lake Huron

By

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ABSTRACT

The benthic amphipod *diporeia hoyi (D. hoyi)*, deepwater sculpin (*Myoxocephalus thompsoni*) and burbot (*Lota lota*) have a predator-prey relationship common in the western Great Lakes. The study of persistent and bioaccumulative contaminants in these aquatic species has been limited to (legacy) chlorinated substances such as PCBs, where deepwater sculpin appears to possess an unusual metabolic capacity affecting PCB toxicokinetics. The present thesis reports on the first time identity of numerous chlorinated and brominated compounds in this Lake Huron food chain, i.e., legacy and emerging chlorinated (i.e., PCBs) and brominated (e.g., the flame retardants polybrominated diphenyl ether (PBDE)) contaminants. The depletion of PCB and PBDE congeners, presence of hydroxyl (OH) and/or methylsulphonyl ($MeSO_2$) containing analogues, bioaccumulation factors of > 1 in cases of both PCB and PBDE congeners and apparent metabolic products, and relationships to enzyme-mediated catalytic activity e.g., cytochrome P450 monooxygenase) suggested that deepwater sculpin and to a lesser extent burbot possess a metabolism capacity towards selected organohalogens. The present findings increase our understanding of the metabolism-related aspects of chlorinated and brominated toxicokinetics in the deepwater sculpin food chain from Lake Huron, and the presence of and exposure to new classes of xenobiotic compounds.

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DEDICATION

To the three most important women in my life: Marla, Nonna and Mom.

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LIST OF ABBREVIATIONS

BCPS: bis(4-chlorophenyl) sulfone
BFR: brominated flame retardant
CBz: chlorinated benzene
CHL: chlordane
CYP: cytochrome P450
DDT: dichlorodiphenyltrichloroethane
ECNI: electron capture negative ionization
GC: gas chromatograph
GLM: general linear model
HBCD: hexabromocyclododecane
HCH: hexachlorocyclohexane
K_{OW}: octanol-water partitioning coefficient
MeO: methoxylated
$MeSO_2$: methylsulfone
MLOQ: method limit of quantification
PCP: pentachlorophenol
S/N: signal-to-noise ratio
SIM: selected ion-monitoring
T_3: triiodothyronine
T_4: thyroxine
TH: thyroid hormone
TTR: transthyretin
NCI: negative chemical ionization
OC: organochlorine
OH: hydroxylated
4-OH-HpCS: 4-OH-heptachlorostyrene
PBB: polybrominated biphenyl
PBDE: polybrominated diphenyl ether
PCA: principal component analysis
PCB: polychlorinated biphenyl

CHAPTER I

GENERAL INTRODUCTION

1.1 The Lake Huron Aquatic Food Web and the Deepwater Sculpin (*Myoxocephalus thompsoni*)

Extensive research has occurred regarding Great Lakes food webs and trophic relationships although work in Lake Huron is lagging (Bronte *et al.* 2003; Dobiesz *et al.* 2005; Mills *et al.* 2003; Madenjian *et al.* 2002). *Diporeia hoyi* is abundant benthic amphipod that provides food for forage fish such as the deepwater sculpin in the Great Lakes (Dobiesz *et al.* 2005). Deepwater sculpin populations provide food and prey for predatory fish such as burbot (*Lota lota*), lake whitefish (*Coregonus clupeaformis*) and lake trout (Dobiesz *et al.* 2005). Relative ^{15}N stable isotope analysis has determined that *Diporeia hoyi*; deepwater sculpin and burbot (*Lota lota*) demonstrate ascending isotopic trophic levels in Lake Huron and Lake Michigan (Dobiesz *et al.* 2005; Stapleton *et al.* 2001). Although facing invasive ecological pressures, burbot and lake trout are the top fish predators in the aquatic food webs in Lake Huron (Dobiesz *et al.* 2005). Recent declines in abundance of *Diporeia hoyi* in Lake Michigan and Lake Huron affect populations of forage fish due to a lack of replaceable energy-rich food source (Dobiesz *et al.* 2005; Hondrop *et al.* 2005). Declines in forage fish food sources cause decreases in abundance of the top predators burbot and lake trout thus affecting lake trout fisheries rehabilitation in Lake Huron (Dobiesz *et al.* 2005).

Deepwater sculpin is of the same genus as the four-horned sculpin found in arctic regions. Deepwater sculpin are a forage fish species that feed benthically in deep cold lakes (usually ~5 °C or less) on aquatic invertebrates such as *diporeia hoyi* or opossum

shrimp (*Mysis relicta* and *Pontopereia*) in Lake Huron (Parker 1987). Deepwater sculpin are usually sampled at in the Great Lakes region at 120 to 150 meters but have been found as deep as 170 meters in Lake Superior (Parker 1987). In a food web context burbot are ecologically important, as they are top predators in the Great Lakes and predate on deepwater sculpin (Becker 1983). The burbot inhabit most waters in Alaska, Canada and the Northern United States.

Contaminant accumulation in aquatic biota is also in part due to the physical differences between each of the Great Lakes, which greatly influence atmospheric deposition (Luross *et al.* 2002). Lake Superior is cold and oligotrophic with the largest surface area (82100 km^2) whereas Lake Erie is warmer and more eutrophic with a smaller surface area (25700 km^2). Lake Huron lies between the two, as it is roughly 176 km wide and 229 m at its deepest point (Environment Canada). These differences in lakes result in Lake Superior being more likely to receive larger amounts of contaminants deposited on its surface when compared to Lake Erie (Eisenreich *et al.* 1981).

Concentrations of contaminants such as PCBs and other organochlorines in waters and fish from Lake Huron have declined from 1970-1999 and although contaminant loadings have declined, consumption advisories are still issued for certain species (Dobiesz *et al.* 2005). Temporal PBDE trends show that only Lake Superior is more pristine then Lake Huron, which point towards its greater coastline development.

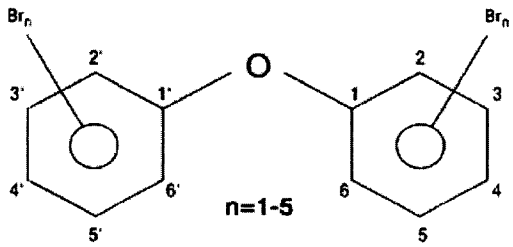
1.2 New and Emerging Contaminants (Brominated and Chlorinated) of Environmental Concern in Great Lakes Food Webs

The Great Lakes ecosystem is continuously exposed to atmospheric, urban, agricultural and industrial influxes of organic chemicals. For many years, there has been a specific focus on legacy organochlorine (OC) compounds in the environment, namely

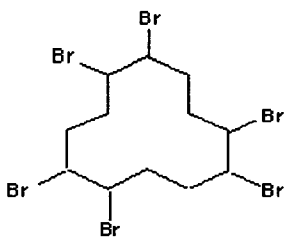
the polychlorinated biphenyls (PCBs) and OC pesticides and by-products, e.g. dichlorodiphenyltrichloroethanes (DDTs), hexachlorocyclohexanes (HCHs), chlordanes (CHLs), chlorinated benzenes (CBzs) and dieldrin. With respect to the Great Lakes, OC contaminants are transported over long distances by freshwater runoff (Kaiser *et al.* 1990) and atmospheric deposition (Bidleman *et al.* 1990; Larsson *et al.* 1990; Kucklick & Baker 1998). The persistence of PCBs in abiotic sources such as the atmosphere, water and sediment in the Great Lakes has been well documented (Baker *et al.* 1991; Jeremiason *et al.* 1994; Pearson *et al.* 1997) although concentrations in the Great Lakes water column, atmosphere and sediment have been declining (Jeremiason *et al.* 1994; Offenberg 1994). The primary source of PCBs in the Great Lakes atmosphere is from surface waters in the Great Lakes (Stapleton *et al.* 2001). Sediments act as a sink for PCBs and other hydrophobic contaminants, and recycle organochlorine contaminated sediments in benthic organisms (Lester *et al.* 1994; DePinto & Coull 1997).

Other OCs that have received much less or no attention includes e.g. bis (4-chlorophenyl) sulfone (BCPS) and pentachlorophenol (PCP) (Fig. 1.1). Limited documentation of these lipophilic and recalcitrant OCs have shown bioaccumulation to various degrees in organisms of aquatic food webs and ubiquitous occurrence in invertebrate, fish and/or avian species from different populations and locations throughout the Great Lakes and the Baltic (Li *et al.* 2003; Campbell *et al.* 2003; Valters *et al.* 1999; Olson & Bergman 1995). Pentachlorophenol or PCP is an organohalogen compound that used for wood preservation and can also be a metabolite of hexachlorobenzene (Renner 1988). PCP has been detected in abiotic samples from the Great Lakes including water and sediment (Quermarais *et al.* 1994). The hydrophobicity

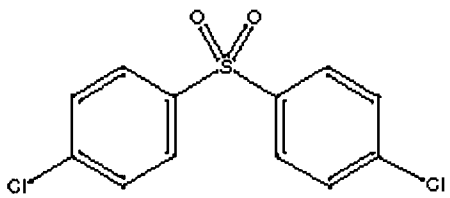
of PCP (Liber *et al.* 1997) inconsistent with its bioavailablity, bioconcentration and bioaccumulation in fish species including those from the Great Lakes, i.e. lake trout (*Salvelinus namaycush*) (Campbell *et al.* 2003) and several benthic- and pelagic-feeding fish species from the Detroit River (Li *et al.* 2003).



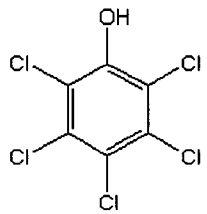
Polybrominated diphenyl ethers (PBDEs)



Hexabromocyclododecane (HBCD)



Bis (4-chlorophenyl) sulfone (BCPS)



Pentachlorophenol (PCP)

Figure 1.1. Generalized molecular structures of compounds and classes of selected chlorinated and brominated compounds investigated in the present study. Hydrogen atoms have been omitted from the structures for clarity.

However there is little understanding about the distribution and biotic concentrations of PCP in the Great Lakes (Li *et al.* 2003; Campbell *et al.* 2003; Quermarais *et al.* 1994; Poulton 1992).

Bis-4-chlorophenylsulfone or BCPS is used during the production of several high-temperature polymers and has also been used in several reactive dyes. Moreover it is a by-product of pesticide production (Olson & Bergman 1995). To our knowledge, there is no report of BCPS levels in North American biota, with the exception of one study that

qualitatively reported BCPS in egg homogenates from Great Lakes herring gulls (Letcher *et al.* 1995). Furthermore, limited studies have shown BCPS to be present in biota from Europe. Bis-4-chlorophenylsulfone (BCPS) was determined in perch (*Perca fluviatilis*) muscle from three locations off of the Latvian coastal area (Olson & Bergman 1995) as well as grey seal (*Halichoerus grypus*) blubber and egg from white-tailed sea eagle (*Haliaeetus albicilla*) from the Swedish Bothnian Seas and Baltic coasts (Olson *et al.* 1999). A recent study reported levels in glaucous gulls (*Larus hyperboreus*) from the Norwegian arctic (Verreault *et al.* 2005). Bis-4-chlorophenylsulfone (BCPS) concentrations in glaucous gulls were found to be comparable to levels of the most abundant PCB congeners (from 40 to 100 ng g^{-1} lipid) (Olson & Bergman 1995; Valters *et al.* 1999).

More recently, environmental concern has arisen as previously undetected brominated chemicals have been identified in various abiotic compartments (e.g. air, sediment and water) and tissues of Great Lakes biota. These new and emerging organohalogens include the brominated flame retardant (BFR) additives. The lipophilic BFRs that have received most environmental attention are the polybrominated diphenyl ethers (PBDEs), hexabromocyclododecane (HBCD) isomers and the polybrominated biphenyls (PBBs) (Fig. 1.1).

BFRs have shown potential for long-range transport and bioaccumulation in wildlife, and some have emerged as important classes of contaminants as tissue residues in a growing number of fish, seabird and marine mammal species worldwide, including the Great Lakes (Sellstrom *et al.* 1996, 1998; Anderson *et al.* 1999; Stapleton *et al.* 2004a; Tomy *et al.* 2004; Burreau *et al.* 1999; 2000). Although the commercial use of

PBBs and certain PBDE mixtures has been banned in several countries or are under increasing regulation, a global ban of these substances is nonexistent. There is as yet no ban or phase out contemplated for HBCD formulations (Alaee *et al.* 2003). Retrospective analyses of archived samples have reported the concentrations of certain BFRs (e.g. PBDEs) to increase rapidly over the last decades in Great Lakes biota such as lake trout (DeVault *et al.* 1996; Luross *et al.* 2002) and other aquatic food web components (Kucklick & Baker 1998).

With chemical properties similar to PCBs and increasing North American concentrations with time, PBDEs are an important emerging class of organohalogen contaminants in the Great Lakes region. PBDEs have been detected in air, water and sediment in the Great Lakes region (Strandberg *et al.* 2001; Gouin *et al.* 2002). BDE-47 (2,2',4,4'-tetrabromoDE) was the dominant congener detected in Great Lakes air followed by BDE–99 (2,2',4,4',5-pentabromoDE) and BDE–100 (2,2',4,4',6-pentabromoDE) (Strandberg *et al.* 2001). PBDE levels in water from the Great Lakes also have been sparingly studied. Lake Michigan samples found that determined concentrations of total PBDEs increasing from 0.031 to 0.158 ng L^{-1} from 1997 to 1999 and was comparable to PCB totals in water (Stapleton & Baker 2001). Concentrations (0.006 ng L^{-1}) were lower in Lake Ontario (Lucky *et al.* 2001) and comparable to samples from the San Francisco Valley (Oros *et al.* 2005). PBDE levels in sediment from the Great Lakes have been extensively studied in recent years. Concentrations of PBDEs in sediment from Lake Michigan, Lake Erie and Lake Superior were found to be 320 (Lake Michigan), 40 (Lake Erie) and 12 (Lake Superior) ng g^{-1} dry weight (Zhu & Hites 2005). Temporal trends of PBDEs in sediment cores showed concentrations to have doubling

times of 5 to 10 indicating exponential PBDE accumulation in which BDE-209 constituted 95 to 99% of the sum (Σ) PBDEs (Zhu & Hites 2005), which was consistent with previous findings in Lake Superior and Lake Ontario (Dodder *et al.* 2002; de Boer *et al.* 2000).

PBDE levels in fish from the Great Lakes watershed have been extensively studied in recent years. Chinook (*Oncorhynchus tschawytscha*) and coho salmon (*Oncorhynchus kisutch*) from Lake Michigan showed a dominance of BDE-47 and Σ-PBDE levels that varied between 773 and 8120 $\mu g\ kg^{-1}$ lipid (less than 10% of the Σ-PCB) (Manchester-Neesvig *et al.* 2001). Moreover, lake trout whole fish PBDE burdens from lakes Ontario, Superior, Huron and Erie were found to be 434, 392, 251 and 117 $\mu g\ kg^{-1}$ on a lipid weight basis (Luross *et al.* 2002) and lower then levels in common carp (*Cyprinus carpio*) (Σ-PBDE = 40.7 $\mu g\ kg^{-1}$ lipid) and large mouth bass (*Micropterus salmoides*) (Σ-PBDE = 163 $\mu g\ kg^{-1}$ lipid) from the Detroit River (Rice *et al.* 2002). Common carp samples from the Des Planes River in the same study were found to contain Σ-PBDE from two sites of 281 and 78.3 $\mu g\ kg^{-1}$ on a lipid weight basis.

HBCDs over the last 20 years have been incorporated into textiles, polystyrene, upholstered furniture, electrical equipment, insulation blocks, and in several parts of car interiors (de Wit 2002). HBCDs have low water solubility relative to PCBs as well as possibly an affinity for particles and sediments (IUCLID 1996). HBCDs may leach from product surfaces and result in environmental uptake into the lipophilic tissues of organisms (de Wit 2002). HBCD has been detected in air, water, sediment and biota from Europe and North America (Covaci *et al.* 2006). Data from Sweden, Finland and the United States is available documenting HBCD in air/atmosphere (Covaci *et al.* 2006; de

Wit 2002; de Wit *et al.* 2004; Remberger *et al.* 2004; Hoh & Hites 2005). Detection of HBCD in arctic regions suggests long-range atmospheric transport (de Wit *et al.* 2004; Law *et al.* 2006; Vorkamp *et al.* 2005). The persistence of HBCDs in the Great Lakes food webs has not been extensively studied. HBCD has been detected in sediment from the Detroit River (Marvin *et al.* 2006) as well as in soil samples near HBCD processing factories (Peterson *et al.* 2004). HBCD has also been detected in benthic invertebrates at lower levels then what has been detected in fish (Covaci *et al.* 2006). Concentrations reported in North American fish ranged from 3 to 80 ng g^{-1} lipid weight and found to be an order of magnitude lower than what was determined in European fish (Covaci *et al.* 2006). HBCD bio-isomerization from gamma to alpha isomer has also been demonstrated in lake trout (Law *et al.* 2004a; 2004b).

1.3 Bioaccumulation and Biotransformation of Chlorinated and Brominated Organohalogens in the Great Lakes Basin

Bioaccumulation of hydrophobic involves the transfer of contaminants from prey to predator trophic levels in a food chain. Biomagnification occurs when the concentration of a bioaccumulating contaminant is greater in the predator species in comparison to the prey species. Hydrophobic organohalogen contaminants such as PCBs, PBDEs and HBCD biomagnify up the food web. Bioaccumulation of PCBs in deepwater sculpin demonstrates strong congener pattern dominance of CB-99, -118, -153 and –180 (recalcitrant PCBs) (Bright *et al.* 1995; Stapleton *et al.* 2001). Bioaccumulation of PCBs in burbot has shown that highest accumulation occurs in liver, mainly due to its high lipid content (Paakkonen *et al.* 2005). PCB and PBDE contaminant levels have been studied in Lake Huron aquatic food web. Levels of PCBs in the Lake Huron water column were found to be the lowest of all the Great Lakes with the exception of Lake Superior and

contained an average of 130 pg L^{-1} PCBs (Anderson *et al.* 1999). PCBs were found to bioaccumulate the highest in lake trout from Lake Michigan then lake trout from Lake Huron or Lake Erie (Falk *et al.* 1999). Furthermore from 1976-1994 both juvenile forage and sport fish were found to have lower PCB levels and also show more of a decline in fish from Lake Huron then Lake Ontario (Scheider *et al.* 1998). PCB congeners 77/110, 66/95 and 118 in lake trout, walleye and whitefish from Lake Huron and Lake Michigan were found to have higher PCB bioaccumulation then fish from Lake Superior and this was thought because of their high alewife prey density (Gerstenberger & Dellinger 2002).

Fish are generally thought to have low capacity for cytochrome P450 monooxygenase activity, particularly CYP2B activity, which along with *meta-para*-chlorine unsubstituted PCBs are precursors for $MeSO_2$-PCB formation (Stapleton *et al.* 2001). Although $MeSO_2$-PCB metabolites have been detected in sardine (*Sardinops sagax*) and rainbow trout (*Oncorhynchus mykiss*) from Japan (Haraguchi *et al.* 1989) and in four-horned sculpin (*Myoxocephalus thompsoni*) from the Canadian arctic. In a previous Lake Michigan study of deepwater sculpin, $MeSO_2$-PCB formation was found and these findings potentially support findings of PCB metabolism in the deepwater sculpin (Stapleton *et al.* 2001).

Knowledge surrounding the bioaccumulation of chlorinated and brominated organohalogens in the Great Lakes is limited to legacy organohalogens such as PCBs and OC pesticides although recent studies have turned their focus to BFRs (Stapleton *et al.* 2001; Paakkonen *et al.* 2005; Zhu & Hites 2004; Stapleton & Baker 2003; Falk *et al.* 1999; Madenjian *et al.* 2002; Gerstenberger & Dellinger 2002; Scheider *et al.* 1998). PBDE bioaccumulation in Great Lakes sediment mirrors technical mixture compositions

and usage patterns (Zhu & Hites 2005; Stapleton & Baker 2003). PCB and PBDE bioaccumulation has been studied in Lake Michigan food webs (Stapleton & Baker 2003) however studies comparing bioaccumulation of PCBs and PBDEs in the Great Lakes are limited. PBDE bioaccumulation patterns in Lake Michigan demonstrated an anomaly regarding deepwater sculpin in comparison to lake trout; bloater and alewife, which showed deepwater sculpin to have very little accumulation of BDE-99, -100, -153 and –154 similar to carp (Stapleton *et al.* 2004a; 2004b). Temporal trends show a decline in PCB concentrations but exponential increases in PBDE and BFR concentrations in lake trout and walleye from 1980-2000 (Zhu & Hites 2004). Samples from Lake Huron in this study were found to be lower then lake trout and walleye samples from Lake Michigan and Lake Ontario, but higher then those from Lake Superior (Zhu & Hites 2004). Lake Huron also demonstrated a slight decrease in PBDE concentrations of both fish that was not demonstrated at the other lakes (Zhu & Hites 2004). PBDE levels in Lake Superior and Lake Huron may be mainly due to atmospheric transport, as well as the affects of downstream runoff because Lake Superior feeds into it (Luross *et al.* 2002). Tomy *et al.* (2004) also concluded that the bioaccumulation of PBDEs in lake trout was highly dependent on biotransformation via debromination metabolism (Tomy *et al.* 2004). Furthermore BDE levels in Great Lakes food webs display biomagnification from lower trophic organisms such as zooplankton and amphipods up to higher trophic level organisms including forage and predator fish (Stapleton & Baker 2003). Levels of HBCD in the Great Lakes are lower then levels in Europe by one order of magnitude and also have been shown to bioaccumulate in Lake Ontario food webs (Tomy *et al.* 2004).

Xenobiotic metabolism occurs through enzyme-mediated insertion of polar

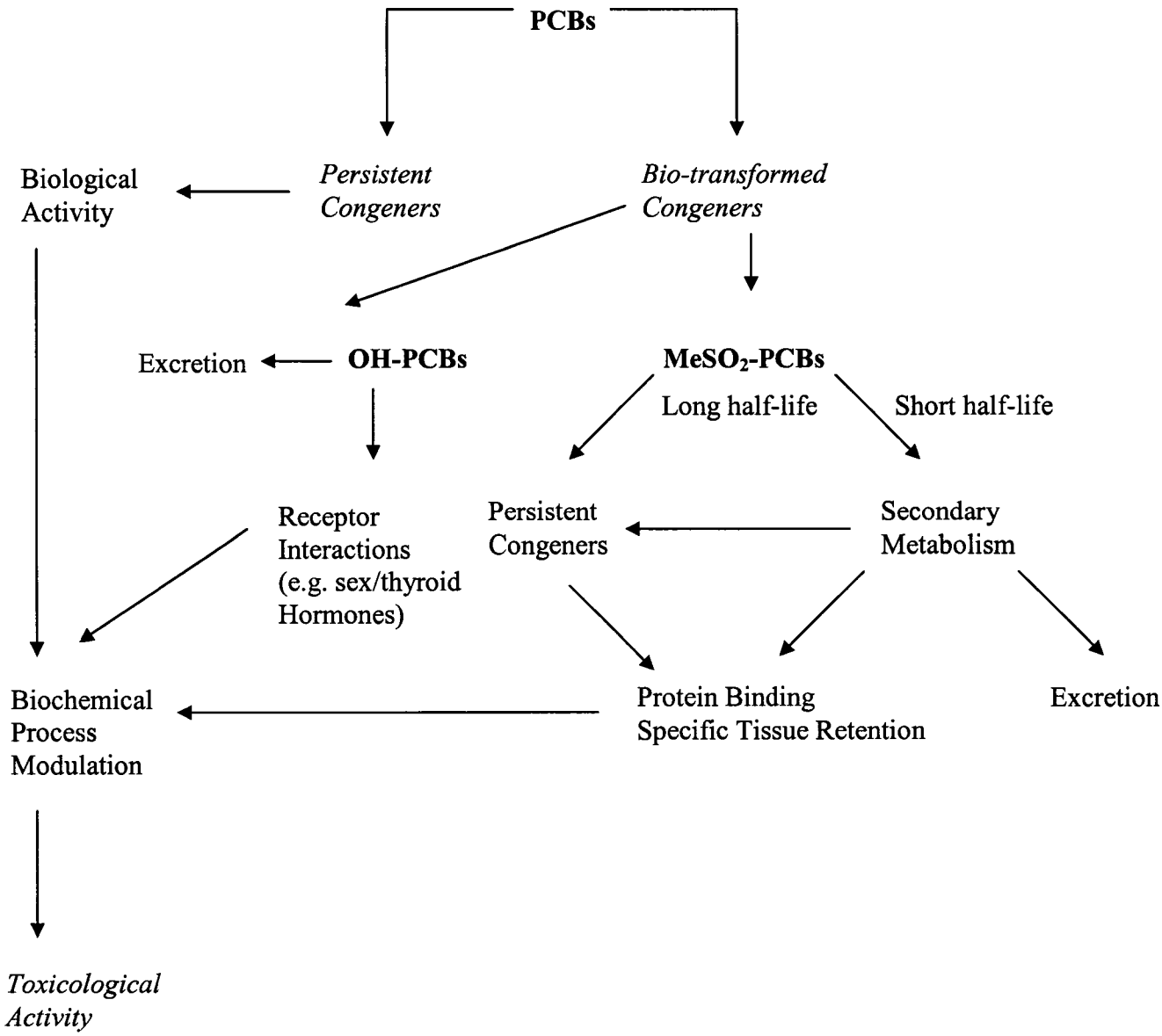


Figure 1.2. A schematic representation of factors affecting the biological fate and/or activity of PCBs and their metabolites taken from Letcher *et al.* (2000).

molecules such as oxygen or sulphur into a xenobiotic molecule. PCBs and PCB metabolites can be retained in biota due to their physico-chemical properties. Figure 1.2 demonstrates the relationships between PCBs, $MeSO_2$-PCBs and OH-PCBs and their excretion, retention and metabolism pathways. The biotransformation and recalcitrant nature of PCBs explain PCB accumulation patterns in biota. OH-PCBs, $MeSO_2$-PCBs as

well as OH-PBDEs and MeO-PBDEs have differing physico-chemical properties then their parent compounds (Letcher *et al.* 2000). Diet, reproductive condition, age and sex are factors influencing PCB toxicokinetics. The relatively long environmental half-lives of PCBs and PBDEs, their hydrophobicity and resistance to abiotic degradation makes them persistent in aquatic ecosystems (Harding *et al.* 1997; Kucklick & Baker 1998). The formations of these polar metabolites are an organism's way of clearing them, although several $MeSO_2$- and OH- residues are retained. Structural factors and biological half-lives determine whether a PCB or PBDE molecule is susceptible to metabolism or elimination (Letcher *et al.* 2000; Hakk & Letcher 2003).

The accumulation of organohalogens in vertebrates and their food webs to which they belong is influenced by enzyme-mediated (e.g. cytochrome P450 (CYP) monooxygenase) biotransformation processes, which increase the likelihood of detoxification and elimination from the organism via formation of more polar metabolites (Letcher *et al.* 2000). In addition to factors of variation related to ecosystem inputs and individual- and species-specific characteristics (e.g. sex, age, feeding ecology and migratory habits), CYP monooxygenase-mediated biotransformation of contaminants has important influence on the overall tissue organohalogen profiles and concentrations (Meerts *et al.* 2000; Letcher *et al.* 2000). CYPs are a large group of membrane-bound monooxygenases with a variety of functions such as synthesis/breakdown of endogenous hormones (e.g. reproductive steroid hormones) and biotransformation of organohalogens (Letcher *et al.* 2000).

With respect to PCBs metabolic susceptibility is due to chlorine substitution and position (Letcher *et al.* 2000; Safe 1990). Biological half-lives of PCB congeners are

determined by their susceptibility to metabolism. Metabolic susceptibility is determined by the number of adjacent hydrogen atoms on the biphenyl ring (Borlakoglu & Wilkins 1993). Three CYP sub-families (i.e. CYP 1, 2 and 3) are of particular importance with respect to the primary, or Phase I, metabolic pathways of halogenated aromatic contaminants, i.e. by direct insertion of a hydroxyl (OH)-group in the *meta*-position or via arene epoxide formation and subsequent ring opening with or without a 1,2-chlorine shift (e.g. CB101; Fig. 1.2). Phase I metabolites are subsequently metabolized via Phase II conjugation via glucuronidation, sulfonation and glutathionation, although competition with protective mechanisms such as protein binding may result in retention (Letcher *et al.* 2000).

Several metabolites of organohalogens such as the methylsulfone ($MeSO_2$ = CH_3SO_2) PCBs and *p, p'*-DDE, OH-PCBs and other mainly chlorinated phenolic compounds, are being detected in an increasing number of fish, reptile, mammal and bird species (Letcher *et al.* 2000 and references therein). CYP enzyme mediated PCB oxidation depends on the particular CYP enzyme subclass that mediates the reaction (Letcher *et al.* 2000). OH-PCBs are formed by direct insertion of a hydroxy group or through via formation of an arene oxide (Figure 1.2) that re-arranges to an OH-group utilizing an NIH or 1,2 shift (Haraguchi *et al.* 1989; Preston & Allen 1980). Due to their lipophilic properties being nearly similar to PCBs, the $MeSO_2$- PCBs/-*p,p'*-DDE have high bioaccumulative potential in lipid-rich tissues (e.g. liver), whereas OH-PCBs appear to be predominantly retained in blood via non-covalent binding to carrier proteins.

Structural analogues to the PBDEs, the methoxylated- (MeO) and OH-PBDEs, have also been reported in a few studies, and have been detected mainly in blood but also

adipose tissues and liver of fish, birds and mammals from the marine environment (Kierkegaard *et al.* 2004; Marsh *et al.* 2004; Sinkkonen *et al.* 2004; Teuten *et al.* 2005). Current understanding is that MeO-PBDEs detected in wildlife are mostly a consequence of accumulation via natural sources in marine environments (e.g. via formation in sponges and algae).

In contrast, OH-PBDEs can be of natural origin and/or metabolically-derived from the CYP-mediated degradation of precursor PBDEs of environmental importance (e.g. BDE47 and 49) (Hakk & Letcher 2003). It is also possible, although not documented hitherto in any species, that MeO-PBDE residues could be sourced, in part, via enzyme-mediated methylation of OH-PBDEs or direct methoxylation of PBDEs (Hakk & Letcher 2003). Recently, OH-PCB and OH-PBDE metabolites and naturally occuring products were detected in a wider range of marine mammals from the North American (Hoekstra *et al.* 2003; McKinney *et al.* 2006), Greenlandic (Sandala *et al.* 2004) and the Norwegian Arctic (Gabrielsen *et al.* 2004; Verreault *et al.* 2005; Wolkers *et al.* 2004). Other potential pathways for enzyme-mediated degradation of organobrominated compounds have also been shown. For example, metabolic debromination of highly brominated PBDEs (e.g. BDE183 and 209) was demonstrated in fish, leading to formation/enrichment of penta- and hexa-BDE congeners (Stapleton *et al.* 2004a; 2004b).

1.4 Toxicological Potential of Emerging Contaminants and Metabolites

In comparison to legacy OCs, there has been limited study in wildlife species, including fish, on the toxicokinetics and potential toxicodynamics of HBCD, BCPS and

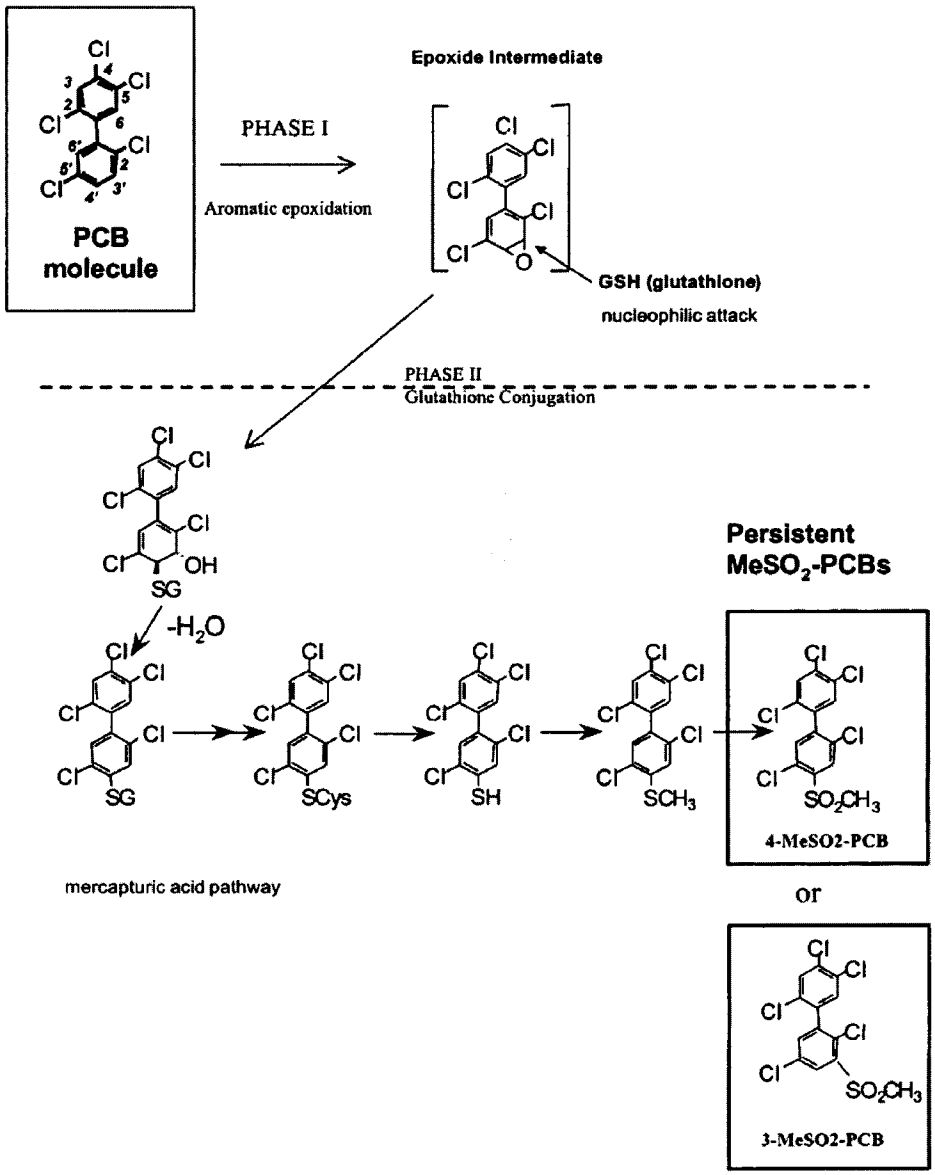


Figure 1.3. Simplified metabolic scheme for PCBs (exemplified for CB101) leading to formation and retention of persistent $MeSO_2$-PCB metabolites (adapted from Letcher *et al.* 2000 and Stapleton *et al.* 2001).

BFRs as well as metabolic products of precursor PCBs and PBDEs. Nonetheless, laboratory animal subjects exposed to these contaminants in long- and short-term dosage regimes have been useful surrogates for the study of health impacts in free-ranging animal populations exposed to anthropogenic substances. Briefly, biological effects mediated by HBCD contamination, such as nephrotoxicity, also were reported in

laboratory animals (Taylor *et al.* 2003). Although the toxicity of BCPS in wildlife remains unknown, exposure to various doses of BCPS in rodents has led to depressed growth rate and diverse hepatic effects (Poon *et al.* 1999).

Several comprehensive reviews have assessed the toxicological effects of BFRs, principally PBDEs, in exposed organisms with special emphasis on their endocrine disrupting properties in humans and wildlife (e.g. Vos *et al.* 2003; Legler & Brouwer 2003). The state of knowledge on the potential health risks associated with PBDE exposure suggests these compounds have the propensity to disrupt thyroid hormone (TH) dependant processes and cause neurological and developmental effects, and possibly cause cancer in laboratory animals (Vos *et al.* 2003; Legler & Brouwer 2003). Moreover, competitive bioassays *in vitro* have shown that TH-like OH-PBDEs bind to TTR with high affinity relative to thyroxine (T_4) (Meerts *et al.* 2000; Legler & Brouwer 2003). The OH-PBDEs have also been shown to be agonists *in vitro* of estrogen receptor-mediated gene expression in human embryonic kidney cells (Meerts *et al.* 2001). There is at present exceedingly little information about the biological effects and thus the toxicological potential of environmentally relevant MeO-PBDEs in laboratory animals and wildlife. Nevertheless, biological activity has been investigated for the compound 2'-MeO-BDE68, which has demonstrated antibacterial and anti-inflammatory activity in bacteria (Kuniyoshi *et al.* 1985).

The exposure to $MeSO_2$-PCBs, 3-$MeSO_2$-*p,p'*-DDE and OH-PCBs has been of important concern as a number of laboratory rodent studies have demonstrated toxicological potential including endocrine-related effects, CYP enzyme induction, cytotoxicity and competitive binding with the glucocorticoid receptors (Letcher *et al.*

2000). The OH-PCBs have received considerable attention with respect to environmental toxicology as a consequence of their endocrine-disrupting capacity via perturbation of thyroid hormone-dependant processes. For example, OH-PCBs have demonstrated a high competitive affinity *in vitro* for binding sites on thyroid hormone transport proteins (e.g. transthyretin (TTR)), conjugating enzymes and cell receptors (Letcher *et al.* 2000; Sandau 2000).

$MeSO_2$-PCB and OH-PCBs are potent inhibitors of the thyroid hormones and have estrogenic effects (Ulbrich & Stahlmann 2004). Tetra- and penta- $MeSO_2$-PCB congeners have been shown to decrease T_3 and T_4 levels in serum of rats (Kato *et al.* 1999), although $MeSO_2$-PCBs are more involved with protein binding issues (Letcher *et al.* 2000). OH-PCB metabolites with *meta-* or *para*-OH-groups have been shown to inhibit of TH homeostasis by interfering with enzymes responsible for sulfonation using phenol sulfotransferase family in *in vitro* rat studies (Schuur *et al.* 1998a; 1998b).

PCBs have been known to elicit toxic estrogenic and anti-estrogenic effects through the generation of OH-PCB metabolites (Layton *et al.* 2002). Estrogenic effects are demonstrated by their binding to estrogen receptors ERα and ERβ acting as agonists or antagonists (Schuur *et al.* 1998a; 1998b). 4-OH-2',4',6'-trichlorobiphenyl and 4-OH-2', 3',4',5'-tetrachlorobiphenyl has been known to stimulate transcriptional activity of both ERs and can induce difference receptor-ligand complex preferences (for estrogen response elements on DNA) and preferential binding of other cofactors (Connor *et al.* 1997; Kuiper *et al.* 1998; Hall *et al.* 2002). OH-PCBs also inhibit members of the OH-steroid sulfotransferase family with human estrogen sulfotransferase, which may possibly increase the availability of these particular endogenous estrogens (Kester *et al.* 2000).

1.5 Endocrine Disruption and Fish Hormonal (Thyroidal) Systems

It has been suggested, although yet to be confirmed, that sublethal exposure to a diversity of organohalogen contaminants and metabolically-derived products in avian species may elicit a variety of adverse developmental, behavioral, reproductive and bioenergetic effects by interfering, in part, with endogenous hormones and other cell messaging systems (i.e. endocrine disruption) (Dawson 2000; Burger *et al.* 2002). For instance, certain organohalogens were shown to interfere with the TH transport proteins and receptors, which can result in the perturbation of homeostasis of circulating or tissue-localized TH levels (Damstra *et al.* 2002). The major explanation evoked for the interaction between specific organohalogens and the endocrine system is their structural similarity with the endogenous hormones (e.g. thyroid hormones), with particularly high resemblance for certain metabolites such as the OH-PCBs/-PBDEs (Figure 1.3) (Letcher *et al.* 2000; Sandau 2000; Meerts *et al.* 2000, 2001; Legler & Brouwer 2003).

The mechanism of action of the thyroid hormones in fish has been described (Zhang & Lazar 2000). The thyroid hormones (T_3) are responsible for development, differentiation and metabolic balance (Lazar 1993). Thyroid hormones (THs) are small liposoluble hormones produced in thyroid follicles and have two bioactive forms, tetraiodothyronine (thyroxine, T_4) and triiodothyronine (T_3). The action of T_3 is mediated by nuclear T_3 receptors (TRs) that bind T_3 with high affinity (Lazar 1993). Disruption of TH transport by organohalogens such as OH-PCBs and OH-PBDEs have been previously studied (Brouwer & Van Den Berg 1986; Meerts *et al.* 2001). OH-PCBs have been shown to bind to plasma thyroid hormone transport protein and displace T_4 (Lans *et al.*1994).

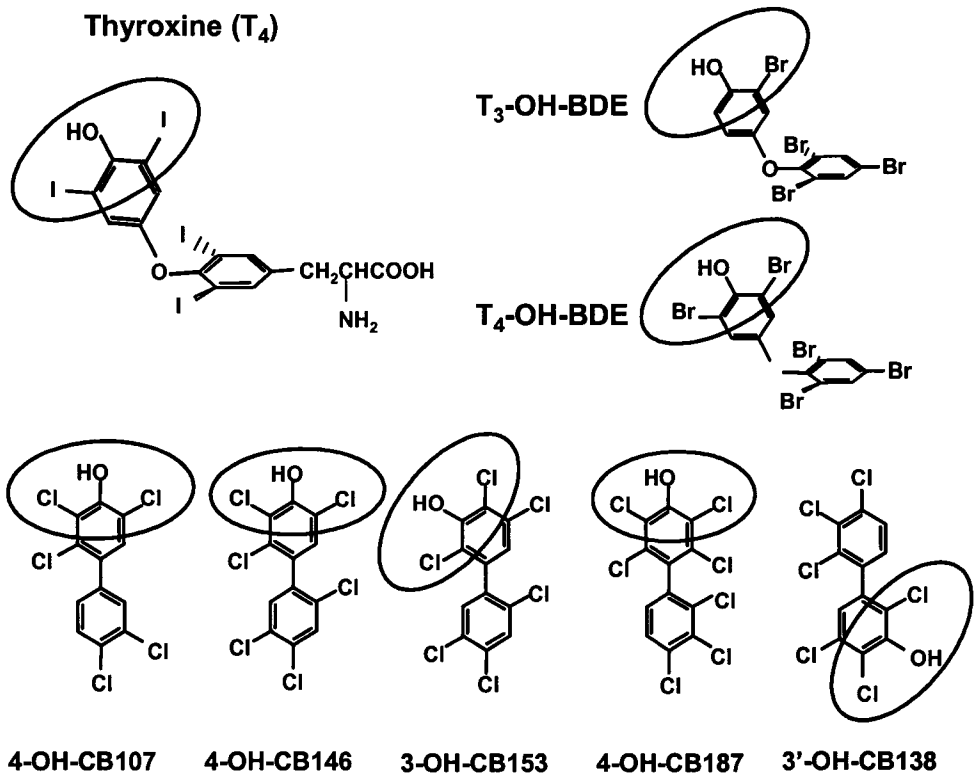


Figure 1.4. Molecular structures of thyroxine and major OH-PCB/-PBDE metabolites, and/or naturally occurring compounds of environmental relevance.

Recent progress in an understanding of the role of TH on fish development was studied through fish farming (Yamano 2005). Mother fish will deposit considerable amounts of thyroid hormones in developing oocytes. The T_3 to T_4 ratio in eggs and amount of thyroid hormones show species variation however the role of these hormones in eggs is unclear (Yamano 2005). THs have been known to promote larva juvenile metamorphosis in teleost fish and disorders in thyroid hormone in fish development could lead to deformity in juveniles (Yamano 2005). In addition, many environmental chemicals have potential disrupting TH function in developing fish embryos and juveniles.

1.6 Thesis Objectives

The primary objective of this thesis is to examine whether metabolism of chlorinated and brominated contaminants in the deepwater sculpin Lake Huron food chain with the hypothesis that in deepwater sculpin PCBs and PBDEs are metabolized to OH- and $MeSO_2$- contaminants and metabolites are accumulated but not produced in burbot. The first sub-objective is to identify and determine the composition of PCBs, PBDEs, their biotransformation products, and novel emerging compounds (BCPS, HBCDD, and PCP) in this Lake Huron food chain. This is an attempt to explain the presence of metabolites in this Lake Huron food chain.

The second sub-objective is to examine the bioaccumulation of PCBs and PBDEs and their biotransformation products in the different species and tissues of the deepwater sculpin Lake Huron food chain. This also includes examining catalytic assessment of cytochrome P450 and phase II enzyme activity in deepwater sculpin and burbot hepatic tissue using EROD and UDPGT bioassays. Metabolite residue patterns, bioaccumulation factors and catalytic activity between different trophic levels will attempt to confirm that the metabolite residues quantified in sub-objective 1 are formed in the deepwater sculpin by attempting to determine the role of bioaccumulation and catalytic activity as an accumulation route of these contaminants and metabolites. PCB and PBDE congener metabolite residues in different tissues and hepatic catalytic activity (where applicable) of both species of fish and *Diporeia hoyi* which will help to provide congener profiles, residue patterns and catalytic assessments that will help to indirectly confirm the ability of the deepwater sculpin to metabolize PCBs and PBDEs and thus provide an entrance for them to get into the Lake Huron Food chain.

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CHAPTER II

IDENTIFICATION AND COMPOSITION OF CHLORINATED AND BROMINATED CONTAMINANTS AND METABOLICALLY-DERIVED PRODUCTS IN THE DEEPWATER SCULPIN (*Myoxocephalus thompsoni*) AND ASSOCIATED FOOD CHAIN SPECIES FROM LAKE HURON

2.1 Introduction

The Great Lakes ecosystem continuously receives a wide variety of organic anthropogenic chemicals. As an example of "legacy" contaminants, it is well documented that PCBs are present in abiotic and biotic compartments of the Great Lakes watershed (Kaiser *et al.* 1990; Baker *et al.* 1991; Jeremiason *et al.* 1994; Pearson *et al.* 1997; Kucklick & Baker 1998; Jeremiason *et al.* 1994; Miller *et al.* 1992; DeVault *et al.* 1996; Stow 1995; Falk *et al.* 1999; Falk *et al.* 1999; Gerstenberger & Dellinger 2001). PCBs are persistent in the environment because of physico-chemical properties that facilitate bioconcentration, bioaccumulation and/or biomagnification in aquatic biota and their food webs and food chains (Harding *et al.* 1997; Kucklick & Baker 1988). As emerging brominated contaminants of environmental concern, the polybrominated diphenyl ether (PBDE) flame retardants (or brominated flame retardants (BFRs) in the Great Lakes watershed and aquatic ecosystem and have rapidly become established as ubiquitous organohalogen contaminants in the Great Lakes and worldwide environments (de Witt 2002). Octanol-water partition co-efficients (K_{ow}) log values of 5.0 to 8.0 and vapour pressure ranges makes them suitable candidates for classification as environmentally persistent (Eljarrat & Barcello 2004; Watanabe & Tatsukawa 1990; Tittelmier & Tomy 2000).

Structural analogues to PCBs and PBDEs, methylsulfonyl (CH_3SO_2- = $MeSO_2$-) PCBs, hydroxyl (OH-) PCBs and OH-PBDEs, which are generally degradation or metabolic products of PCBs or PBDEs, have been reported in aquatic vertebrates (e.g., fish) and their food webs from the Great Lakes in a very limited and recent number of studies (Li *et al.* 2003; Valters *et al.* 2005). Whole body homogenates of deepwater sculpin (*Myoxocephalus thompsoni*) and burbot (*Lota lota*) from Lake Michigan were found to contain quantifiable $MeSO_2$-PCBs (Stapleton *et al.* 2001), which was the first determination of $MeSO_2$-PCBs in aquatic biota from any of the Great Lakes. However, to our knowledge, there has yet to be a comprehensive and comparative study on the potential bioaccumulation or biomagnification of $MeSO_2$-PCBs, OH-PCBs or OH-PBDEs in a Great Lakes aquatic food chain. Other studies have also documented $MeSO_2$-PCB, OH-PCB or OH-PBDE congeners in fat and blood of aquatic vertebrate wildlife in European and Japanese marine waters (Letcher *et al.* 2000; Hakk & Letcher 2003). Levels of $MeSO_2$-PCBs were also reported in four composite liver samples of four-horn sculpins (*Myoxocephalus quadricornis*) from the Canadian high Arctic (Bright *et al.* 1995). Toxicological or biological effects have been documented for, e.g., endocrine and immunological, have been documented for several $MeSO_2$-PCB, OH-PCB or OH-PBDE congeners (Letcher *et al.* 2000; Kuniyoshi *et al.* 1985; Legler & Brouwer 2003; Meerts *et al.* 2001; Vos *et al.* 2003).

Bis-(4-chlorophenyl)sulfone (BCPS), pentachlorophenol (PCP) and HBCDs are organohalogens that have generally received little attention, or virtually nothing in the case of BCPS, as contaminants in the Great Lakes ecosystem. BCPS is commonly utilized as a pesticide (Tarasenko 1969) and a chemical applied to thermostable polymers

(Mark *et al.* 1988). BCPS has been reported in egg homogenates from herring gulls from Lake Ontario (Letcher *et al.* 1995) but there is no information in aquatic biota from the Great Lakes. Little is known about the toxicity of BCPS in wildlife (Poon *et al.* 1999). Technical mixtures of HBCDs (mainly the α-, β- and γ-HBCD isomers) are current-use, additive BFRs that are commonly utilized in textiles, polystyrene, upholstered furniture, electrical equipment, insulation blocks and in car upholstery (Covaci *et al.* 2006). Of the HBCD isomers, the α-isomer, and to a lesser extent the ß- and μ-isomers are found in wildlife tissues. Tomy *et al.* (2004) reported that for the prey fish species, the trends in α- and γ-HBCD levels were slimy sculpin > smelt > alewife and $\delta^{15}N$ stable isotope suggesting that HBCD biomagnifies in the Lake Ontario food web. Pentachlorophenol (PCP) is an organohalogen compound that has been used for wood preservation (Renner *et al.* 1988) and levels ranged from 60 to 3430 pg/g in benthic and pelagic fish from the Detroit River (Li *et al.* 2003). However, very little is known about the distribution and concentrations of PCP in the Great Lakes (Quermarais *et al.* 1994; Poulton 1992).

The benthic amphipod *diporeia hoyi* is an important prey for many fish in offshore areas of the Great Lakes (Hondorp *et al.* 2005). Deepwater sculpin also feed on *Diporeia hoyi* in Lake Huron. Deepwater sculpins are common in Lake Huron, Lake Superior and Lake Michigan, but some time ago populations have been extirpated from Lake Erie and Lake Ontario (Parker 1987). As reported for the Lake Michigan food web, deepwater sculpin are prey food for predatory fish species such as burbot (*Lota lota*) and lake trout (*Salvelinus namaycush*) (Stapleton *et al.* 2001).

Meta-para-chlorine-unsubstituted PCBs are known precursors for the formation of retained and persistent 3- and 4-$MeSO_2$-PCB formation, which is mediated by CYP2B-

and CYP3A-type isoenzymes (Letcher *et al.* 2000; Stapleton *et al.* 2001). Fish are generally thought to have low capacity for cytochrome P450 mono-oxygenase activity, particularly CYP2B activity (Stapleton *et al.*2001; Letcher *et al.* 2000; Li *et al.* 2003). However, $MeSO_2$-PCB metabolites have been detected in sardine (*Sardinops sagax*) and rainbow trout (*Oncorhynchus mykiss*) from Japan (Haraguchi *et al.* 1989) and in four-horned sculpin (same genus as deepwater sculpin) from the Canadian arctic (Bright *et al.* 1995). $MeSO_2$-PCB residues in deepwater sculpin from Lake Michigan (Stapleton *et al.* 2001) suggest that this species is capable of metabolically converting suitable PCB congener precursors into $MeSO_2$-PCB metabolites and potential OH-PCB and OH-PBDE metabolites. Studies by Stapleton *et al.* (2005a, 2005b and 2005c) have demonstrated that common carp are capable for debrominating PBDE congeners, but may also be capable of metabolism by oxidative pathways leading to OH-PBDE metabolites. The objective of this study is to identify, quantify and assess the relative composition of PCBs, PBDEs and emerging chlorinated and brominated contaminants (i.e., BCPS, HBCDs, PCP, OH-PCBs, $MeSO_2$-PCBs and OH-PBDEs) in liver, blood and/or whole body homogenate samples or pools of *Myoxocephalus thompsoni*, *Diporeia hoyi* and *Lota lota* from Goderich, Meaford and Meldrum Bay sampling sites in Lake Huron.

2.2 Material & Methods

2.2.1 Sample Collection

Fish and aquatic invertebrate samples were collected from Lake Huron in July 2003 (Goderich) and July 2004 (Meaford and Meldrum Bay) (Figure 2.1, Table 2.1). Blood was collected from burbot through a caudal vein puncture and immediately spun 1500 X *g* with sodium heparin solution (anticoagulant) to separate plasma. It was not

possible to remove blood from the sculpin samples as the individual fish were simply too small. After blood sampling, livers were removed from all fish species, and half was frozen at –20°C for contaminant analysis. *Diporeia hoyi* were collected and separated into two pools of approximately 20 g per pool, where each pool was composed of approximately 2000 individual *diporeia hoyi.* Samples were collected at three Lake Huron sites (Figure 2.1). For the present study, the sample collections were opportunistic and optimized within the sampling strategy of the annual Lake Huron fish and food web monitoring program administered by the Department of Fisheries and Ocean (DFO) Canada (M. Whittle, personal communication).

With respect to the Lake Huron sites (Figure 2.1), five individual burbot and five pools of five individual deepwater sculpin could be collected from an area off the shoreline from Goderich, Ontario. Samples collected inshore from Meaford in Georgian Bay in July of 2004 included 5 pools of 10 deepwater sculpin and 2 burbot. Samples obtained from Meldrum Bay in the very north of Lake Huron and collected in July 2004 included 2 pools of 10 deepwater sculpin and 10 burbot. Whole deepwater sculpin were homogenized into pools of 5 to 10 fish per pool. Individual burbot whole body was also homogenized in the same manner.

Table 2.1. Information on the *diporeia hoyi*, deepwater sculpin and burbot sample collections from Lake Huron locations (July 2003 and 2004).

Sample		Mass (g)	Length (mm)	Liver (% Lipid)	Whole Body (% Lipid)
Goderich					
Diporeia hoyi	(n=2 pools)	18.50			4.39
Deepwater sculpin	(n=5 pools of 5) individuals)	29.68 ± 9.71	136.64 ± 15.62	13.42 ± 6.25	3.62 ± 0.80
Burbot	(n=5 individuals)	1030.00 ± 286.18	502.40 ± 63.09	16.96 ± 10.08	0.70 ± 0.15
Meaford					
Deepwater sculpin	(n=5 pools of 10)	15.76 ± 3.00	113.74 ± 6.56	12.63 ± 5.85	2.57 ± 0.51

Burbot	(n=2 individuals)	987.00	474.50	43.23	2.38
Meldrum Bay					
Deepwater sculpin individuals)	(n=2 pools of 10)	14.39	114.45	17.53	0.82
Burbot	(n=10 individuals)	1064.15 ± 246.58	524.00 ± 36.42	48.27 ± 6.80	0.61 ± 0.25

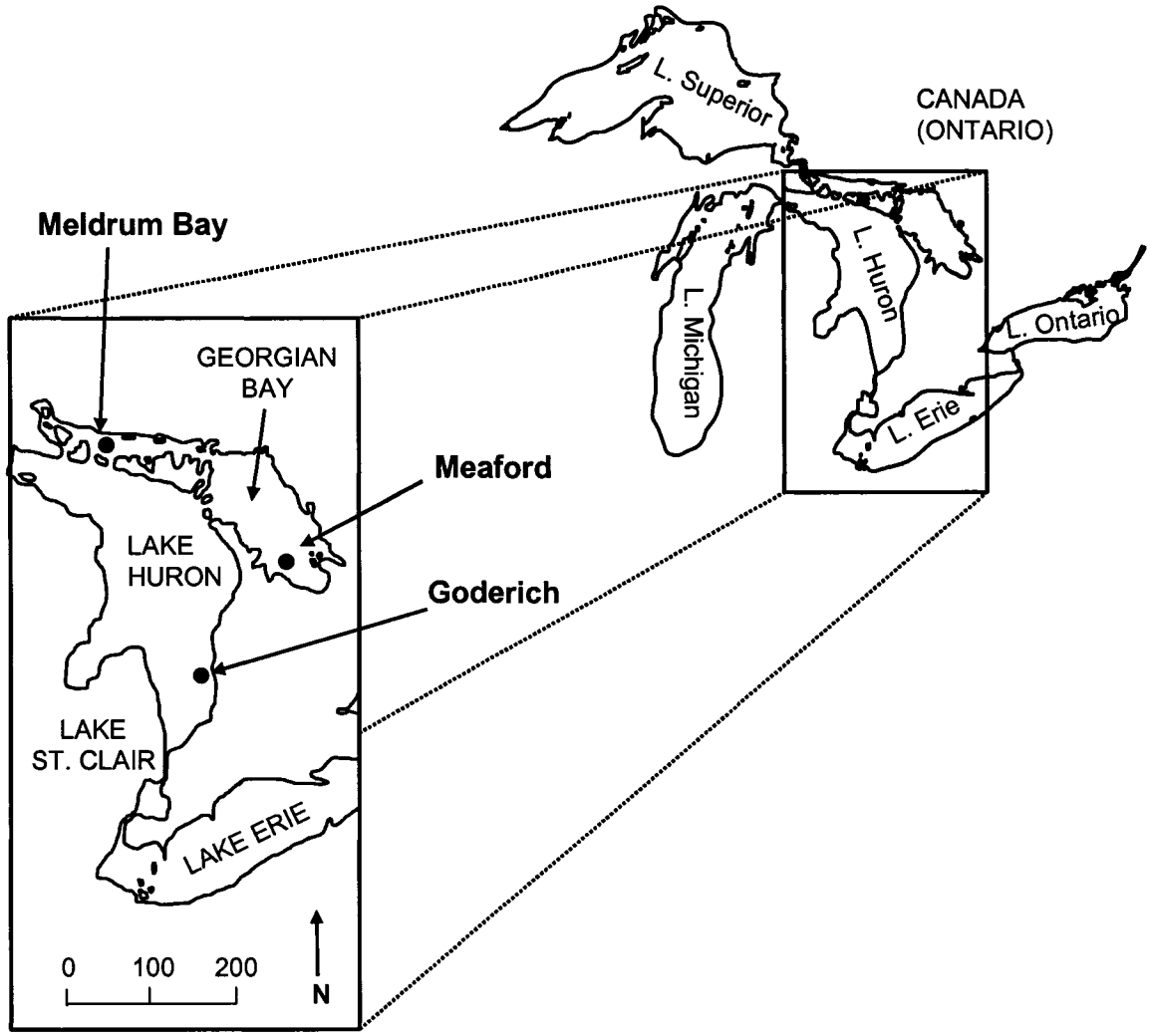


Figure 2.1. A map of the inshore sites on Lake Huron (July 2003 and July 2004) for *Diporeia hoyi*, deepwater sculpin and burbot sample collections.

2.2.2 Chemicals and Standards

The PCB and 4-OH-HpCS standards were supplied by the Canadian Wildlife Service, Environment Canada (Ottawa, ON, Canada). PBDE, α-HBCD and MeO-PCB standards were purchased from Wellington Laboratories (Guelph, ON, Canada). $MeSO_2$-

PCB, $MeSO_2$-DDE and MeO-PBDE standards were kindly provided by Åke Bergman and Göran Marsh (Stockholm University, Sweden). PCP was purchased from Accustandard (New Haven, CT, USA). The internal standards used were as follows: a mixture of 6 $^{13}C_{12}$-labeled PCB congeners (CB-28, -52, -118, -153, -180, and -194) for PCB analysis, a mixture of 3 $^{13}C_{12}$-labeled OC pesticides (1, 2, 4, 5-TeClBz, PnClBz and HBC) for OCs, and BDE-30 and -71 for PBDEs, α-HBCD and MeO-PBDEs. 3-$MeSO_2$-2-CH_3-2',3',4',5,5'-pentachlorobiphenyl was used as internal standard for $MeSO_2$-PCBs and $MeSO_2$-DDE, a mixture of 4 $^{13}C_{12}$-labeled OH-PCB congeners (4'-OH-CB120, 4'-OH-CB159, 4'-OH-CB172, 4'-OH-CB187) was used as internal standards for OH-PCBs, 4-OH-HpCS and PCP, 2'-OH-BDE28 as internal standard for OH-PBDEs. The authentic BCPS standard was purchased from Sigma-Aldrich. Standard addition spiking experiments were carried out and confirmed that the internal standards chosen for each class of contaminants equitably reflected the recovery of those analytes in the sample work-up. PCB numbering is abbreviated using the notation of Ballschmiter *et al.* (1992), and PBDEs are numbered analogously. PCB and PBDE metabolites are numbered by maintaining the same Ballschmitter *et al.* (1992) notation and denoting the position of the OH and/or $MeSO_2$-functional group on the biphenyl or diphenyl ether backbone, respectively, as a prefix to the abbreviated name (Maervoet *et al.* 2004). Chromatographic materials used for the analysis were as follows: Florisil (magnesium silicate, 60-100 mesh) purchased from Caledon Laboratories Ltd (Georgetown, ON, Canada), basic alumina (60-325 mesh) purchased from Fisher Scientific (Ottawa, ON, Canada) and silica gel (60-200 mesh, 150 Å) purchased from Sigma Aldrich (St Louis, MO, USA). All solvents and chemicals were at least of analytical-grade quality.

2.2.3 Contaminant Extraction

2.2.3.1 *Diporeia hoyi* Pools and Fish Homogenates and Livers

The extraction and clean-up of, e.g., polar bear fat tissue for PCBs, OCs, PBDEs and $MeSO_2$-PCBs has been described in detail previously, where adjustments were made for the determination of halogenated phenolic metabolites and compounds (Dietz *et al.* 2004; Sandala *et al.* 2004; Muir *et al.* 2006; Verreault *et al.* 2005a; Chu *et al.* 2003). More specifically, the extraction and clean up of liver tissue for all the chlorinated and brominated contaminant that have been described has been described previously (McKinney *et al.* 2006a; Guvenius *et al.* 2001, 2002; Kannan *et al.* 2005). Briefly, approximately 1 to 5 g of liver tissue and 15 g of whole body homogenate were extracted on sodium sulfate (Figure 2.2). This was then transferred to an extraction column and extracted with 100 mL of 50:50 n-hexane: DCM. The extraction mixture was spiked with 50 μL of the following standards; PCB internal standard (CB83 @ 346.5 pg/μL, CB122 @ 325.7 pg/μL), BDE-30 (428pg/μL), $MeSO_2$-PCB internal standard (196 pg/μL), $^{13}C_{12}$-4'-OH-CB120, $^{13}C_{12}$-4'-OH-CB159, $^{13}C_{12}$-4'-OH-CB172 and $^{13}C_{12}$-4'-OH-CB187 internal standards (100 pg/μL) and 2'-OH-BDE28 internal standard (211 pg/μL). The column extracted with 200 ml DCM/n-hexanes (1:1).

Lipids were removed by gel permeation chromatography (GPC). The organic layer was concentrated to ~0.5 ml and transferred to a KOH/silica column (1.5 grams, 33% KOH deactivated by w/w) and eluted with 50 ml 50:50 n-hexanes: DCM. All contaminants were extracted with MtBE/n-hexanes (1:1), and by KOH partitioning the HPCs were separated from the neutral contaminants. The organic fraction containing neutral contaminants was concentrated on a Florisil column (8.0 g, 1.2% H_2O deactivated

by w/w), and eluted with 75 ml of DCM/n-hexanes (1:1) (F_1) and 80 ml 7% methanol in DCM (F_2). F_1, containing PCBs OC pesticides and brominated flame-retardants, was

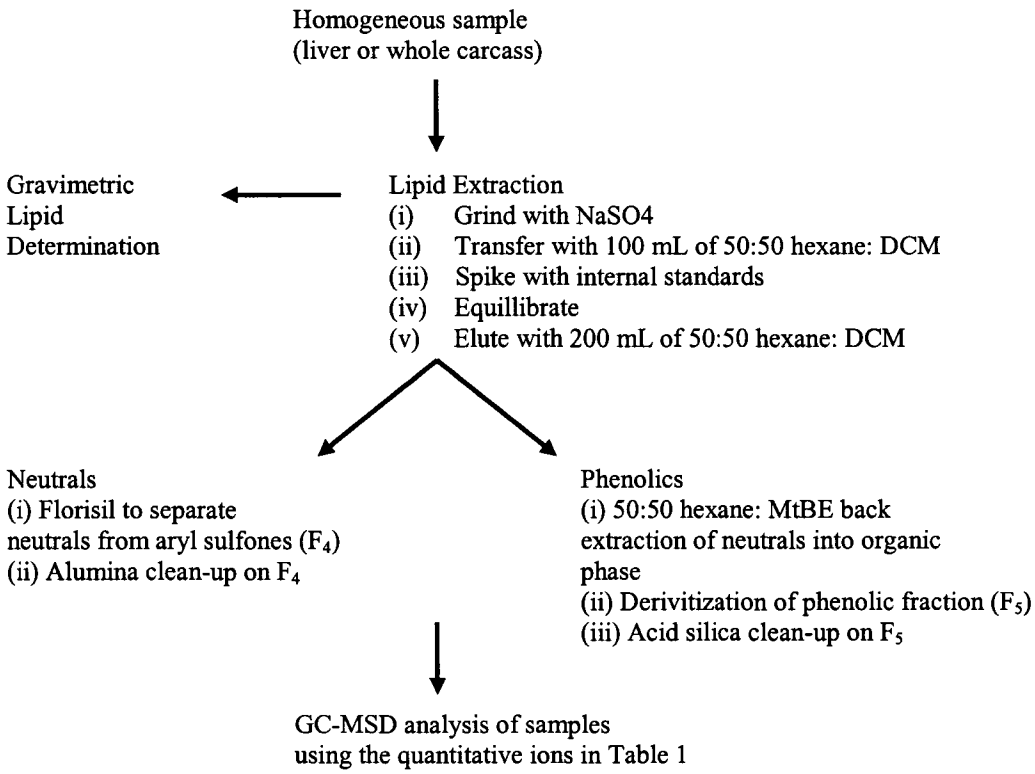


Figure 2.2. Schematic flow diagram of the extraction and clean-up methodology used to isolate chlorinated and brominated contaminants and by-products from *Diporeia hoyi* pools and whole body homogenate and liver samples form deepwater sculpin and burbot.

concentrated and solvent exchanged to 1 ml TMP in preparation for GC-MSD analysis. F_2, containing $MeSO_2$-PCBs/DDE, was concentrated to ~ 0.5 ml and loaded on a basic alumina column (3 grams, 2.3% H_2O deactivated by w/w) and eluted with 50 ml DCM/n-hexanes (1:1), of which the first 10 ml was discarded. The extract was concentrated and solvent exchanged to 100 µL TMP in preparation for GC-MSD analysis. The aqueous KOH fraction, containing all HPCs, was then acidified with concentrated H_2SO_4 to ~ pH 2. The reprotonated HPCs were extracted from the acidified aqueous fraction with MtBE/n-hexanes (1:1) dried over Na_2SO_4 and derivatized to their methoxy- analogues

using diazomethane. The methoxylated HPCs were purified on a silica column (3 g, 22% H_2SO_4 deactivated by w/w) and eluted with 50 ml 15% DCM in n-hexanes. The extract was concentrated and solvent exchanged to 100 μL TMP in preparation for GC-MSD analysis. The lipid content in blood was determined by a sulfo-phospho-vanilin reaction using olive oil-derived calibration curve as done by Verreault *et al.* (2005).

Whole body homogenates were analyzed for HPCs, and since none were quantifiable in this tissue type, the fraction was not taken and analyzed for them. The homogenates were analyzed on Florisil immediately after gel permeation chromatography (GPC). For blood samples, lipid levels were low and thus GPC was not required. Liver tissues did contain quantifiable HPCs and hence all three fractions were taken from the KOH partition step of the plasma clean-up (see section 2.2.3.2).

2.2.3.2 Plasma

The extraction and clean up of burbot plasma for all the contaminants has been described elsewhere (Sandala *et al.* 2004; Sjodin *et al.* 2001; Verreault *et al.* 2005b). Briefly, a plasma sample of approximately 2.5 to 5.0 g was spiked with the internal standards (50 μL) of each of the before mentioned internal standards with concentrations ranging from 100 to 500 pg/μL, followed by the addition of 1 mL 6M HCl and 3 mL 2-propanol. All contaminants were extracted with MtBE/n-hexanes (1:1), and by KOH partitioning the HPCs were separated from the neutral contaminants. The remaining sample work-up was identical as described for homogenates and liver samples. The lipid content in blood was determinant by a sulfo-phospho-vanilin reaction using olive oil-derived calibration curve.

2.2.4 Determination of organohalogens

All of the gas chromatography mass spectrometric detection (GC-MSD) analyses were preformed on an Agilent 6890 GC equipped with and Agilent 5793 MD detector and Agilent 7683 automated injector. Depending on the analyte or class, MSD was either in the electron impact (EI) or electron capture negative ionization (ECNI) mode.

2.2.4.1 PCBs

For the PCB analysis the GC was fitted with a fused silica DB-5 column [(5% phenyl) methylpolysiloxane, 30 m, 0.25 mm ID, 0.25 μm film thickness, J&W Scientific]. The temperature program for PCBs was 100°C (3 min), 20°C/min to 180°C, 2.5°C/min to 300°C for optimal chromatographic resolution of PCB congener-specific determination. Helium was used as carrier gas, the injector temperature was 250°C and the transfer line temperature to the MS was set at 280°C. The MS was set in electron impact (EI) ionization (positive) mode, with the ionization voltage set at 70 eV. The source and quadrupole temperature were 230°C and 150°C, respectively. Selected ion monitoring (SIM) of $[M]^-$ and $[M+2]^-$ was applied where the ions chosen were selected for PCB congener homolog groups, which provided the necessary mass spectral resolution to compensate for most chromatographic co-elutions of some PCB congeners. An external standard quantification approach was used for PCB concentration determination. The sum (Σ) PCB concentration was composed quantifiable concentrations of 51 individual or co-eluting congeners that were monitored: CB-28/31, -42, -44, -49, -52, -60, -64/71, -66/95, -70, -74, -84/101, -87, -97, -99, -105, -110, -118, -128, -129/178, -138, -141, -146, -149, -151, -153, -156/171/202, -158, -170/190, -172, -174, -177, -179, -180, -182/187, -183, -194, -195, -196/203, -200, -201, -206.

2.2.4.2 PBDEs, α-HBCD and MeO-PBDEs

For the PBDE, α-HBCD and MeO-PBDE analysis the GC was fitted with a fused silica DB-5 column [(5% phenyl) methylpolysiloxane, 15 m, 0.25 mm ID, 0.25 μm film thickness, J&W Scientific]. The temperature program was 90°C, 20°C/min to 310°C (15 min). Helium was used as carrier gas, the injector temperature was 250°C and the transfer line temperature to the MS was set at 280°C. The MS was set in the electron capture negative ionization (ECNI) mode, with an ionization voltage of 70 eV. The source and quadrupole temperature were 150°C and 106°C, respectively. Methane was used as collision gas. Using SIM, the isotopic bromine anions (*m/z* 79 and 81) were monitored. An internal standard quantification approach was used for the PBDE determination. The following congeners were monitored for in all tissue samples: BDE-17, 28, -47, -66, -85, -99, -100, -138, -153, -154, -183, -190, -209, BB-101 and -153 (coelutes with BDE-154), α-HBCD (i.e., accounts for the total of α-, β- and γ-HBCD isomers), 4'-MeO-BDE17, 6'-MeO-BDE17, 2'-MeO-BDE28, 4-MeO-BDE42, 3-MeO-BDE47, 5-MeO-BDE47, 6-MeO-BDE47, 4'-MeO-BDE49, 6'-MeO-BDE49, 2'-MeO-BDE68, 6-MeO-BDE85, 6-MeO-BDE90, 6-MeO-BDE99, 2-MeO-BDE123 and 6-MeO-BDE137.

2.2.4.3 $MeSO_2$-PCBs and $MeSO_2$-DDE

For the $MeSO_2$-PCBs and $MeSO_2$-DDE analysis the GC was fitted with a fused silica DB-5 column [(5% phenyl) methylpolysiloxane, 30 m, 0.25 mm ID, 0.25 μm film thickness, J&W Scientific]. The ramping program was 100°C (3 min), 20°C/min to 220°C (1 min), 3°C/min to 280°C (8 min). Helium was used as carrier gas, the injector temperature was 280°C and the transfer line temperature to the MS was set at 280°C. The MS was set in the ECNI mode with an ionization voltage of 70 eV. The source and

quadrupole temperature were 180°C and 150°C, respectively. Methane was used as collision gas. Using SIM the $[M]^-$ and $[M+2]^-$ ions were monitored for each chlorinated homolog group. An internal standard quantification approach was used for the $MeSO_2$-PCBs and $MeSO_2$-DDE determination. The following congeners were monitored for all tissue samples: 3'-$MeSO_2$-CB49, 4'-$MeSO_2$-CB49, 3-$MeSO_2$-CB52, 4-$MeSO_2$-CB52, 3-$MeSO_2$-CB64*, 4-$MeSO_2$-CB64, 3-$MeSO_2$-CB70, 4-$MeSO_2$-CB70, 3'-$MeSO_2$-CB87*, 4'-MeSO2-CB87, 3-$MeSO_2$-CB91*, 4-$MeSO_2$-CB91*, 3'-$MeSO_2$-CB101, 4'-$MeSO_2$-CB101, 3-$MeSO_2$-CB110, 4-MeSO2-CB110, 3'-$MeSO_2$-CB132, 4'-$MeSO_2$-CB132, 3'-$MeSO_2$-CB141*, 4'-$MeSO_2$-CB141*, 3-$MeSO_2$-CB149, 4-$MeSO_2$-CB149*, 3-$MeSO_2$-CB174*, 4-$MeSO_2$-CB174 and 3-$MeSO_2$-4,4'-DDE (There were no standards available for the marked (*) $MeSO_2$-PCB congeners. Relative response factors (RRF) of other congeners with the same number of chlorination were used).

2.2.4.4 OH-PCBs, 4-OH-HpCS and PCP

For the OH-PCB, 4-OH-HpCS and PCP (derivatized to MeO-analogues) analysis the GC was fitted with a fused silica DB-5 column [(5% phenyl) methylpolysiloxane, 30 m, 0.25 mm ID, 0.25 μm film thickness, J&W Scientific]. The ramping program was 80°C (1 min), 10°C/min to 250°C (5 min), 5°C/min to 300°C (5 min). Helium was used as carrier gas, the injector temperature was 280°C and the transfer line temperature to the MS was set at 300°C. The MS was set in the ECNI mode with an ionization voltage of 70 eV. The source and quadrupole temperature were 200°C and 150°C, respectively. Methane was used as collision gas. Using SIM the $[M]^-$, $[M+2]^-$ and $[M-15]^-$ ($[M-CH_3]^-$) ions of the MeO-containing derivatives of all OH-PCBs were monitored. An internal standard quantification approach was used for the OH-PCB, 4-OH-HpCS and PCP

determination. The MeO-PCB analogues of the following congeners were monitored for all the tissues: 4'-OH-CB79, 4-OH-CB97, 4'-OH-CB101/4-OH-CB134, 4-OH-CB107/4'-OH-CB108, 2'-OH-CB114, 3-OH-CB118, 4'-OH-CB120, 4'-OH-CB127, 4'-OH-CB130, 3'-OH-CB138, 4-OH-CB146, 4'-OH-CB159, 4-OH-CB162, 4-OH-CB163, 4'-OH-CB172, 4'-OH-CB177, 4-OH-CB178, 3'-OH-CB180, 3'-OH-CB182, 3'-OH-CB183, 3'-OH-CB184, 4-OH-CB187, 4-OH-CB193, 4'-OH-CB199, 4'-OH-CB200, 4'-OH-CB201, 4'-OH-CB202, 4,4'-diOH-CB202, 3'-OH-CB203/4'-OH-CB198, 4'-OH-CB208, 4-OH-HpCS and PCP.

2.2.4.5 OH-PBDEs

For the OH-PBDE analysis, the chromatographic and mass spectral parameters were the same as described for OH-PCBs. Using SIM the isotopic bromine anions (*m/z* 79 and 81) were monitored. An internal standard quantification approach was used for the OH-PBDE determination. The MeO-PCB analogues of the following congeners were monitored for all tissue samples: 6'-OH-BDE17, 6'-OH-BDE17, 6'-OH-BDE49, 2'-OH-BDE68, 6-OH-BDE47, 3-OH-BDE47, 5-OH-BDE47, 4'-OH-BDE49, 4-OH-BDE42, 6-OH-BDE90, 6-OH-BDE99, 2-OH-BDE123, 6-OH-BDE85, 6-OH-BDE137.

2.2.5 Quality Control and Assurance

The analytes were identified by comparison of their relative chromatographic retention times to that of authentic reference standards. The mean recoveries were based on the internal/recovery standards (Table 2.2). Since an external standard quantification approach was used, concentrations of PCBs and OC pesticides were only recovery corrected when recovery was <80%. Recoveries for all other contaminants were inherently recovery corrected using the internal standard quantification method.

Table 2.2. The mean recoveries for organohalogen classes of contaminants in Lake Huron samples.

Tissue Matrix	% Recovery ± SD
PCBs and OCs	
Diporeia hoyi	94
Deepwater sculpin homogenate	83 ± 7
Deepwater sculpin liver	85 ± 9
Burbot homogenate	76 ± 5
Burbot liver	95 ± 5
Burbot plasma	91 ± 6
PBDEs, HBCDs and MeO-PBDEs	
Diporeia hoyi	94
Deepwater sculpin homogenate	76 ± 12
Deepwater sculpin liver	86 ± 3
Burbot homogenate	84 ± 10
Burbot liver	91 ± 4
Burbot plasma	97 ± 3
$MeSO_2$-PCBs, 3-MeSO2-p,p'-DDE and BCPS	
Deepwater sculpin homogenate	83 ± 10
Deepwater sculpin liver	103 ± 4
Burbot homogenate	98 ± 20
Burbot liver	96 ± 3
Burbot plasma	106 ± 8
OH-PCBs, PCP and 4-OH-HpCS	
Deepwater sculpin liver	71 ± 12
Burbot liver	86 ± 8
Burbot plasma	94 ± 19
OH-PBDEs	
Deepwater sculpin liver	52 ± 11
Burbot liver	46 ± 11
Burbot plasma	47 ± 14

Quality assurance and quality control included laboratory method blanks, duplicate samples, matrix (IS) spikes, and calibration standard injections for each block of 5 samples to monitor changes in instrument sensitivity and analytical procedures that could affect the accuracy and precision of the quantitative analyte determinations. Method blank samples were analyzed to monitor for interferences and contamination and thus potentially result in an over-estimation of analyte concentrations. Method blanks showed

absence of background interference or contamination when analyzing for the organohalogens in question. The method limits of quantitation (MLOQs) for PCBs, OC pesticides and PBDEs were around 0.1 ng/g wet weight for all the tissues, for $MeSO_2$-PCBs, OH-PCB and OH-PBDE the MLOQs were around 0.05 ng/g wet weight for all the tissues. The MLOQs were based on a signal to noise (S/N) ratio of 10. Certified reference material (SRM 1588a, cod liver oil) from the National Institute for Standards and Technology was used as a check for the accuracy and reproducibility of the analytical method. Σ-PCB and Σ-OC pesticides (CHLs and DDTs) were within 5% and 8%, respectively of the consensus values of SRM 1945 reported by Schantz *et al.* (1995). Σ-PBDE was within 13% of the values reported by Kucklick *et al.* (2004) and Poster *et al.* (2004) on SRM 1588a. Analyte concentrations are shown on a wet weight basis as the HPCs determined have been shown not to be correlated with lipid, but rather are mainly protein-associated as indicated by there preference for blood (Verreault *et al.* 2005a, 2005b, 2005c). In the present study, and based on individual samples or pools, and where the numbers permitted, the lipid content and the sum concentrations of each of the HPC classes were not correlated (not shown).

2.2.6 Data Analysis

The use of descriptive statistics was not considered appropriate in this study to assess the significance of differences for organohalogen concentration in liver, whole body homogenate, plasma and/or pools, within the *diporeia hoyi*-deepwater sculpin-burbot food chain, as a function of each of the three Lake Huron sampling sites (Figure 2.1). The individual sample numbers were too low or absent. All sample sites were combined to yield a numerically appropriate set. Statistical analysis of variance in

organohalogen concentrations in liver tissue of both fish species was carried out using SPSS for Windows (Version 14.0, SPSS Inc., Chicago, Illinois, USA). Statistical significance was set at $p < 0.05$. Summary statistics were computed if analyte concentrations were quantifiable (i.e., above the MLOQ) in at least 50% of the samples. If this criterion was met, then for statistical purposes, concentrations <MLOQ was assigned a value between zero and half of the MLOQ.

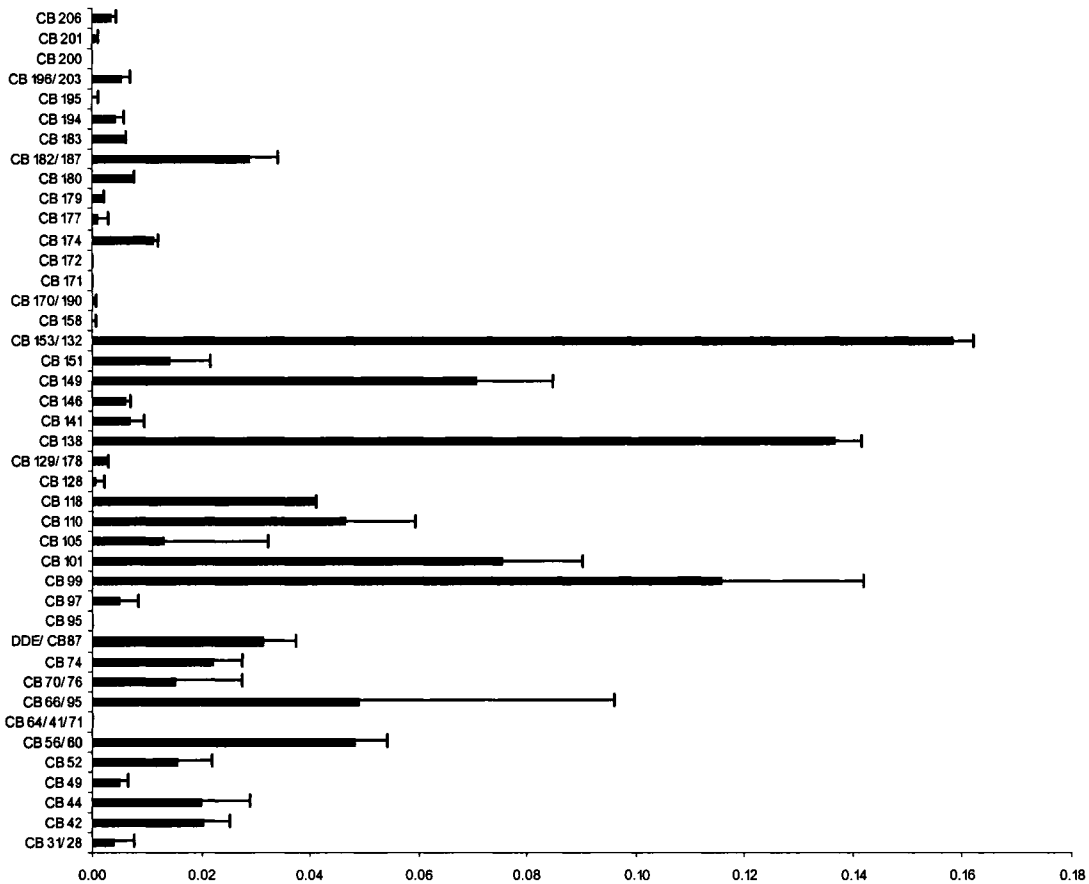
2.3 Results & Discussion

Trophic positions and relative ecological relationships between *Diporeia hoyi*, deepwater sculpin and burbot from the Great Lakes are known (Kitchell *et al.* 1994; Russell *et al.* 1999; Kitchell *et al.* 2000; Stapleton *et al.* 2001). Sample sites were treated as being representative of contamination in the food web from the area that they were taken (Figure 2.1). However as part of the characterization process, the intention of this chapter was to assess the site-specific commonalities of all chlorinated and brominated classes of contaminants and degradation products that were screened for in this study in *Diporeia hoyi* (pools), deepwater sculpin (Wojcik *et al.* 1986) (whole body and/or liver) and burbot (whole body, liver and/or plasma) which occupies the highest trophic position of this food chain (Fratt *et al.* 1997). That is, whether the three sampling locations can be used as a characterization of Lake Huron as a whole.

2.3.1 PCBs, $MeSO_2$-PCBs and OH-PCBs

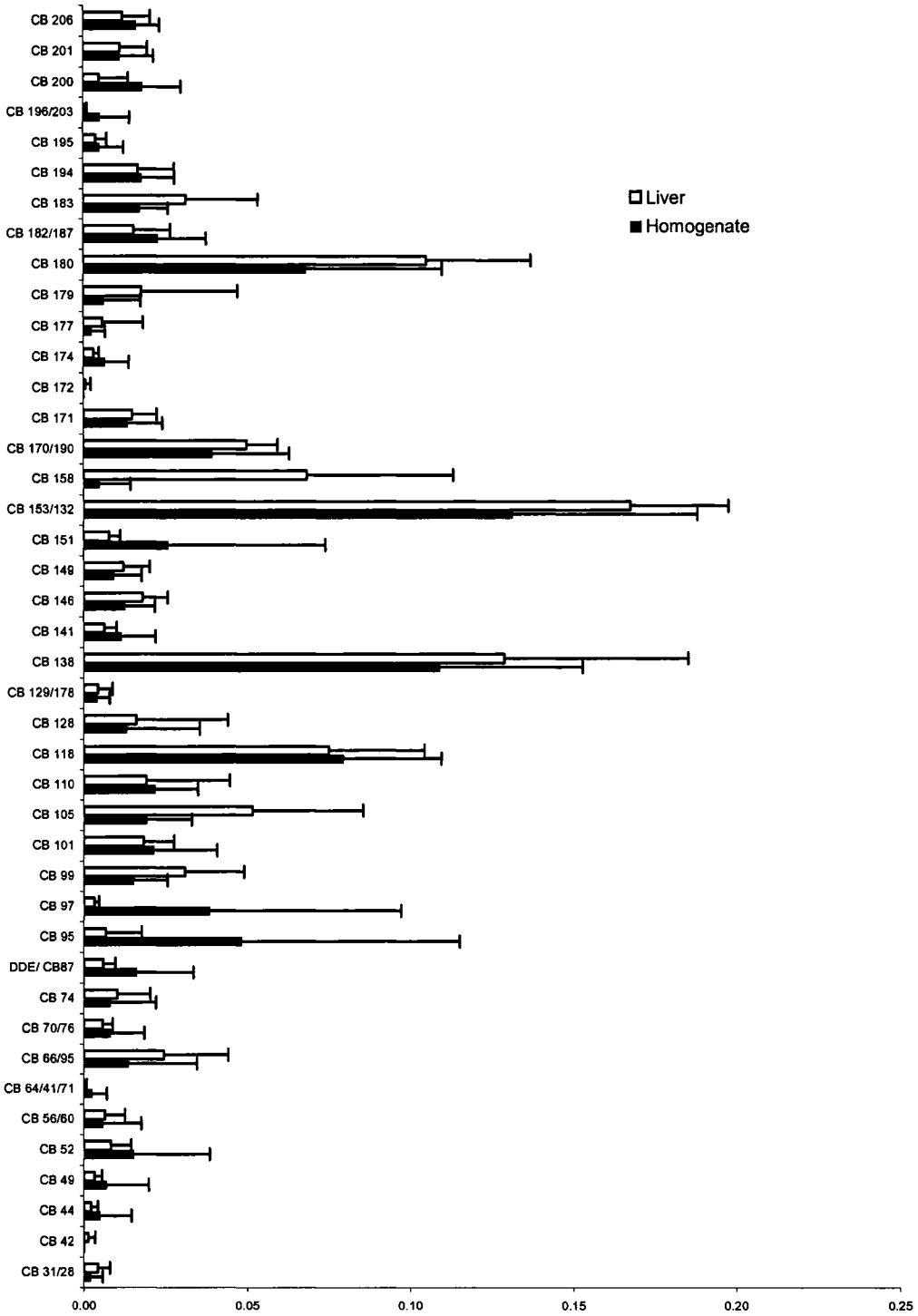
PCBs were identified and determined in samples form the deepwater sculpin food web (Figure 2.3). *Diporeia hoyi* could only be collected from the Goderich site in the south of Lake Huron (Table 2.1). Quantifiable PCB congeners in *diporeia* hoyi included the 2, 5-dichloro- or 2, 3, 6-trichloro-congeners CB-101, -110, and -149 as well as

recalcitrant congeners CB-99, -153, -118, -156, and -180. Although the CB-99, -153, -118, and -180 were present in deepwater sculpin and burbot from all three sites, the congener pattern showed that CB-101, -110 and -149 were depleted in the whole body and liver samples of these two fish relative to *diporeia hoyi.* The contrasting PCB congener patterns in these fish vs. invertebrate may suggest a metabolic capacity in deepwater sculpin and/or burbot toward congeners that were relatively depleted.

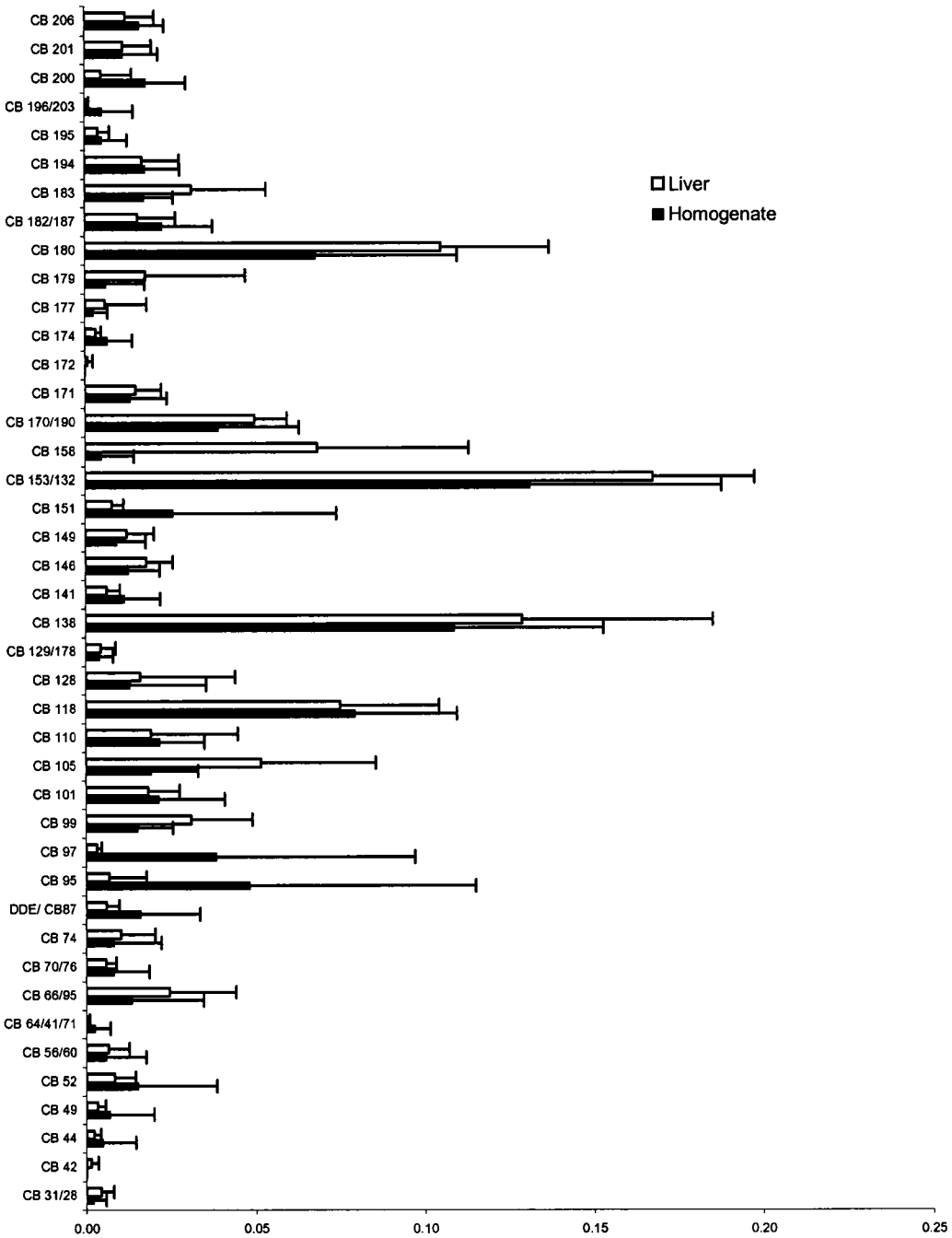


$[CB_X]$ / [Σ-PCB] Ratio

(a) *Diporeia hoyi*



(b) Deepwater sculpin



(c) Burbot

Figure 2.3. Representative PCB congener patterns for (a) *diporeia hoyi*, (b) deepwater sculpin and (c) burbot from Lake Huron. The congener patterns are shown as the ratio of the CB_X to Σ-PCB concentrations.

PCB congeners such as CB-101, -110, and -149 have been shown to be metabolic precursors of corresponding 3- and 4-$MeSO_2$-PCB metabolites, and have been identified in deepwater sculpin from Lake Michigan (Letcher *et al.* 2000; Stapleton *et al.* 2001). Recalcitrant PCBs in deepwater sculpin whole carcass and liver (Figure 2.3) from Lake Huron consistituted 40% and 24% of the Σ-PCB body burden that was comparable (47%) to what was previously determined in Lake Michigan deepwater sculpin homogenates (Stapleton *et al.* 2001). CB-105, -128 and -170 have *para*-chlorine atoms and no adjacent *meta-para*-chlorine unsubstituted phenyl rings. The congeners 99, 105, 118, 128, 153, 170 and 180 constituted 49% in homogenate and 33% of the Σ-PCB concentrations in deepwater sculpin liver, and were much lower than levels reported (from 68 to 92%) for these congeners in four-horned (*Myoxocephalus quadricornis*) and short-horned sculpin (*Myoxocephalus scorpius*) from the Canadian Arctic (Bright *et al.* 1995).

Table 2.3. Mean concentrations (ng/g lipid weight) and standard deviations of the most recalcitrant PCB congeners in deepwater sculpin and burbot liver from Lake Huron.

PCB Congener	Deepwater sculpin *Mean*	*SD*	Burbot *Mean*	*SD*
CB-99	433.9	552.0	166.5	190.4
CB-118	134.5*	109.0	328.8*	306.2
CB-153/132	409.1	146.7	826.9	746.3
CB-180	121.4**	150.3	503.4**	393.8

A student's t-test was applied to assess the significance (i.e., $*p < 0.05$ and $**p < 0.01$) between the mean concentrations for each congener.

Table 2.4. Mean concentrations (ng/g lipid weight) and standard deviations of the PCB congeners, which are known to be $MeSO_2$-PCB precursors, in deepwater sculpin and burbot liver from Lake Huron.

PCB Congener	Deepwater sculpin *Mean*	*SD*	Burbot *Mean*	*SD*
CB-49	43.1*	57.3	12.3*	12.6
CB-52	44.4	37.8	29.2	23.7
CB-64	27.0**	35.8	2.3**	2.3
CB-70	18.6	23.6	25.5	28.1

CB-74	76.0	94.9	8.7	48.1
CB-87	2.0**	6.8	28.7**	31.0
CB-101	9.5**	11.8	76.7**	68.8
CB-110	32.4	29.3	55.9	55.6
CB-149	2.8*	6.6	49.3*	59.2
CB-174	8.1	10.4	11.7	8.1

A one-tailed Student's t-test was applied to assess the significance (i.e., $*p < 0.05$ and $**p < 0.01$) between the mean concentrations for each congener.

The recalcitrant CB-99 and -153 congeners were not found in significantly higher concentrations in burbot versus deepwater sculpin liver (Table 2.3), which was consistent with deepwater sculpin versus burbot whole body homogenates from Lake Michigan (Stapleton *et al.* 2001). CB-87 and CB-101, which are PCB precursors of major 3- and 4-$MeSO_2$-PCB metabolites, were significantly lower in concentration deepwater sculpin relative to burbot liver (Table 2.4). These findings suggest that deepwater sculpin relative to burbot have a greater metabolic potential towards PCB congeners that are known precursors to $MeSO_2$-PCB metabolites.

$MeSO_2$-PCB metabolites were detectable and/or quantifiable in all fish homogenate and liver tissue but not detectable the pools of *diporeia hoyi*. Figure 2.4 shows a representative GC-MS (ECNI) mass chromatogram of the aryl sulfone fraction of a deepwater sculpin liver sample from Goderich. Deepwater sculpin from all three sites contained penta-, tetra- and hexa-chlorinated $MeSO_2$-PCB congeners similar to findings from Lake Michigan (Stapleton *et al.* 2001). In terms of concentrations, deepwater sculpin whole body homogenate and liver had similar congener patterns (Table 2.5). However, it appears there is a trend towards higher chlorinated $MeSO_2$-PCB congeners in the whole body homogenate relative to liver of deepwater sculpin.

The results of *t*-test deepwater sculpin versus burbot liver are presented in Table 2.5. Significant differences between species were observed for 13 of 22 $MeSO_2$-PCB

congeners and Σ-$MeSO_2$-PCB, however 3- and 4-$MeSO_2$-CB110 and 4-$MeSO_2$-CB149 were not significantly different. The 3-$MeSO_2$-CB132 congener demonstrated significantly higher concentrations in burbot ($p < 0.01$). The concentrations of 3- and 4-$MeSO_2$-CB101, 3- and 4- $MeSO_2$-CB110 and 3-$MeSO_2$-CB149 were not significantly different between deepwater sculpin and burbot liver from Lake Huron (Table 2.5) and from Lake Michigan (Stapleton *et al.* 2001). Accumulation of higher chlorinated congeners in whole carcass may be indicative of the longer half-lives of $MeSO_2$-PCB congeners that are more heavily chlorinated and thus generally have a slower rate of metabolism. Bioaccumulaion and biomagnification potential of PCBs versus $MeSO_2$-PCBs is discussed in greater detail in Chapter 3.

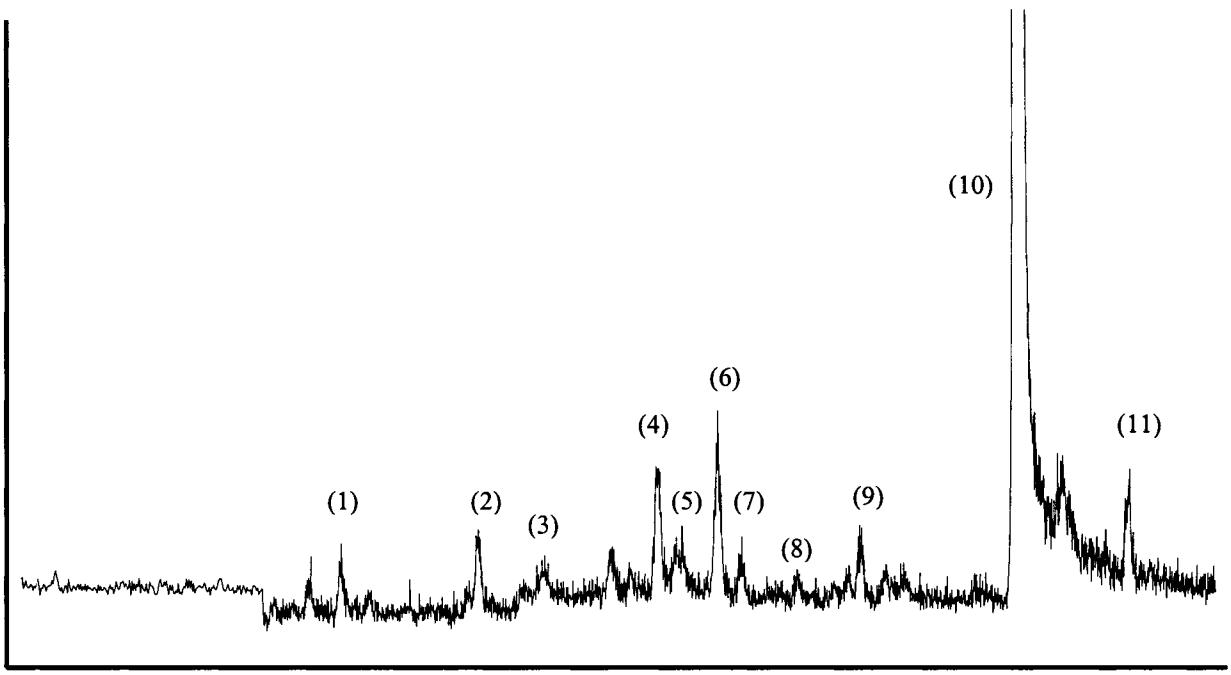


Figure 2.4. A representative GC-MS (ECNI) mass chromatogram (see section 2.2.4 for the ions monitored) for $MeSO_2$-PCB congeners in the liver of deepwater sculpin from Goderich. Peaks are the detectable and/or quantifiable $MeSO_2$-PCB congeners: (1) 4-$MeSO_2$-CB64; (2) 3-$MeSO_2$-CB70; (3) 4'-$MeSO_2$-CB101; (4) 3-$MeSO_2$-4, 4'-DDE; (5) 3-$MeSO_2$-CB110; (6) 3-$MeSO_2$-CB149; (7) 4'-$MeSO_2$-CB87; (8) 3'-$MeSO_2$-CB132; (9) 4'-$MeSO_2$-CB132; (10) $MeSO_2$-PCB IS; (11) 4'-$MeSO_2$-CB174.

Table 2.5. Mean concentrations (ng/g lipid weight) and standard deviations of $MeSO_2$-PCB congeners in deepwater sculpin and burbot liver from Lake Huron.

Congener	Deepwater sculpin *Mean*	*SD*	Burbot *Mean*	*SD*	┼t-value	┼┼Species Correlation
3-$MeSO_2$-CB49	0.8	0.6	2.8	3.4	-2.18*	0.39*
4-$MeSO_2$-CB49	0.8	0.2	2.5	2.6	-1.71*	0.39*
3-$MeSO_2$-CB52	0.5	0.5	2.0	2.4	-1.46*	0.37*
4-$MeSO_2$-CB52	0.2	0.3	0.5	0.1	-0.31	0.33
3-$MeSO_2$-CB64	0.1	0.3	0.1	0.3	-0.01	0.01
4-$MeSO_2$-CB64	0.1	0.3	0.5	0.6	-0.38*	0.35
3-$MeSO_2$-CB70	2.5	2.0	14.8	30.6	-12.35	0.26
4-$MeSO_2$-CB70	0.6	0.6	2.7	2.8	-2.19*	0.45*
3-$MeSO_2$-CB87	0.1	0.2	0.1	0.4	0.00	-0.06
4-$MeSO_2$-CB87	n.d.	n.d.	1.7	2.9	-1.74*	0.37*
3-$MeSO_2$-p,p'-DDE	n.d.	n.d.	0.7	1.8	-0.71	0.26
4-$MeSO_2$-CB91	0.7	1.0	n.d.	n.d.	0.66*	-0.46*
3-$MeSO_2$-CB101	3.7	3.4	19.6	30.5	-15.88*	0.33
4-$MeSO_2$-CB101	0.8	0.7	4.5	6.8	-3.68*	0.34
3-$MeSO_2$-CB110	0.2	0.4	3.2	6.3	-3.02	0.31
4-$MeSO_2$-CB110	1.4	1.4	18.5	56.5	-17.13	0.20
3-$MeSO_2$-CB132	0.1	0.2	3.4	4.0	-3.28*	0.48**
4-$MeSO_2$-CB132	1.3	1.4	6.2	7.5	-4.92*	0.40*
3-$MeSO_2$-CB149	1.8	2.6	15.0	18.4	-13.26*	0.43*
4-$MeSO_2$-CB149	0.5	0.6	1.7	3.6	-1.26	0.23
4-$MeSO_2$-CB174	3.0	3.9	17.9	21.0	-14.99*	0.42*
Σ-$MeSO_2$-PCB	18.9	6.7	118.7	142.0	-99.78*	0.42*

┼A one-tailed Student's t-test was applied to assess the significance (i.e., $*p < 0.05$) between the mean concentrations for each congener.
┼┼Correlations between $MeSO_2$-PCB concentrations in deepwater sculpin and burbot liver tissue

The only species from the deepwater sculpin food chain where plasma samples were possible were from burbot (Table 2.6). Similar to liver and whole body samples, regardless of the sampling site, burbot plasma had similar pattern of $MeSO_2$-PCB congeners, where penta- and to lesser extent tetra-$MeSO_2$-PCB congeners were more dominant, i.e., 3-$MeSO_2$-CB70 and 4-$MeSO_2$-CB49. The $MeSO_2$-PCB and PCB data suggests that $MeSO_2$-PCBs present in deepwater sculpin are formed in sculpin from accumulated PCBs, whereas in burbot $MeSO_2$-PCB may be present due to metabolic

formation from precursor PCBs and/or accumulation of $MeSO_2$-PCBs from the deepwater sculpin contribution to their diet.

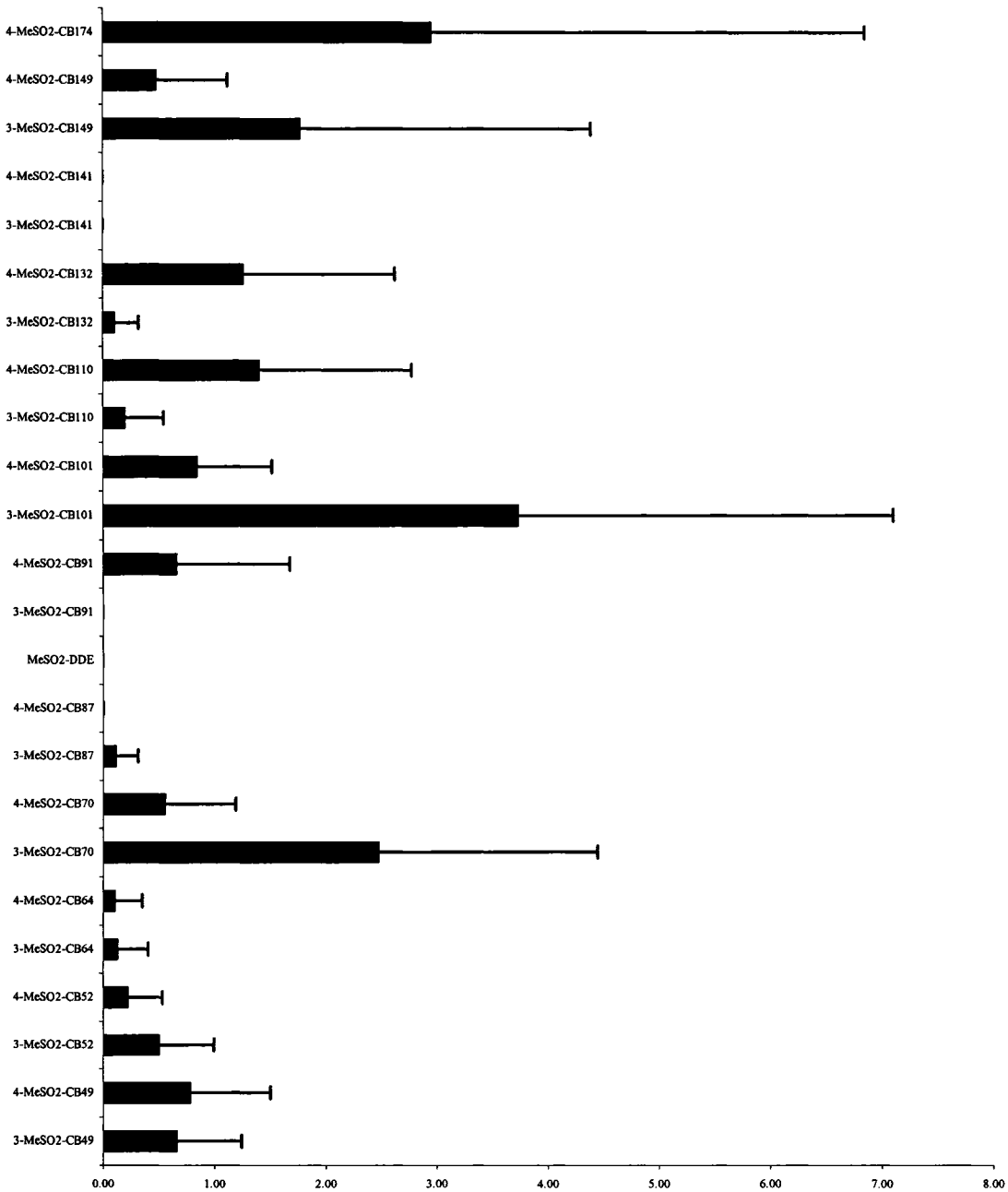
Table 2.6. $MeSO_2$-PCB (ng/g wet weight) for burbot plasma (n = 15) from Lake Huron

Congener	[$MeSO_2$-CB_X]	[$MeSO_2$-CB_X]/[Σ-$MeSO_2$-PCB]	[$MeSO_2$-CB_X]/ [CB_X]
4-$MeSO_2$-CB49	4.8 ± 2.8	0.8 ± 0.2	0.6
4-$MeSO_2$-CB87	0.1 ± 0.1	n.d.	
3-$MeSO_2$-CB101	0.0 ± 0.1	n.d.	0.0
4-$MeSO_2$-CB101	0.1 ± 0.2	0.0 ± 0.1	
4-$MeSO_2$-CB132	0.1 ± 0.1	n.d.	
3-$MeSO_2$-CB149	0.1 ± 0.1	n.d.	0.0
4-$MeSO_2$-CB174	0.2 ± 0.3	0.1 ± 0.1	
Σ-$MeSO_2$-PCB	5.2 ± 2.7		

OH-PCBs were detectable in the liver and/or plasma of both deepwater sculpin and burbot (Figure 2.6), but were not detectable in any whole body homogenate sample of deepwater sculpin or burbot or pool of *diporeia hoyi* (not shown). In contrast to PCBs and $MeSO_2$-PCBs, this indicates that OH-PCBs are metabolically formed in both deepwater sculpin and burbot, but have poor accumulative properties in either fish species, and thus are unlikely to have been accumulated in burbot, assuming that deepwater sculpin is a major dietary item. To our knowledge, this is the first report of OH-PCB determination in the liver or body homogenate of any fish species from the Great Lakes or North America. In the only known report, OH-PCBs were recently reported in the plasma of thirteen pelagic- and benthic-feeding fish species from the Detroit River (Li *et al.* 2003).

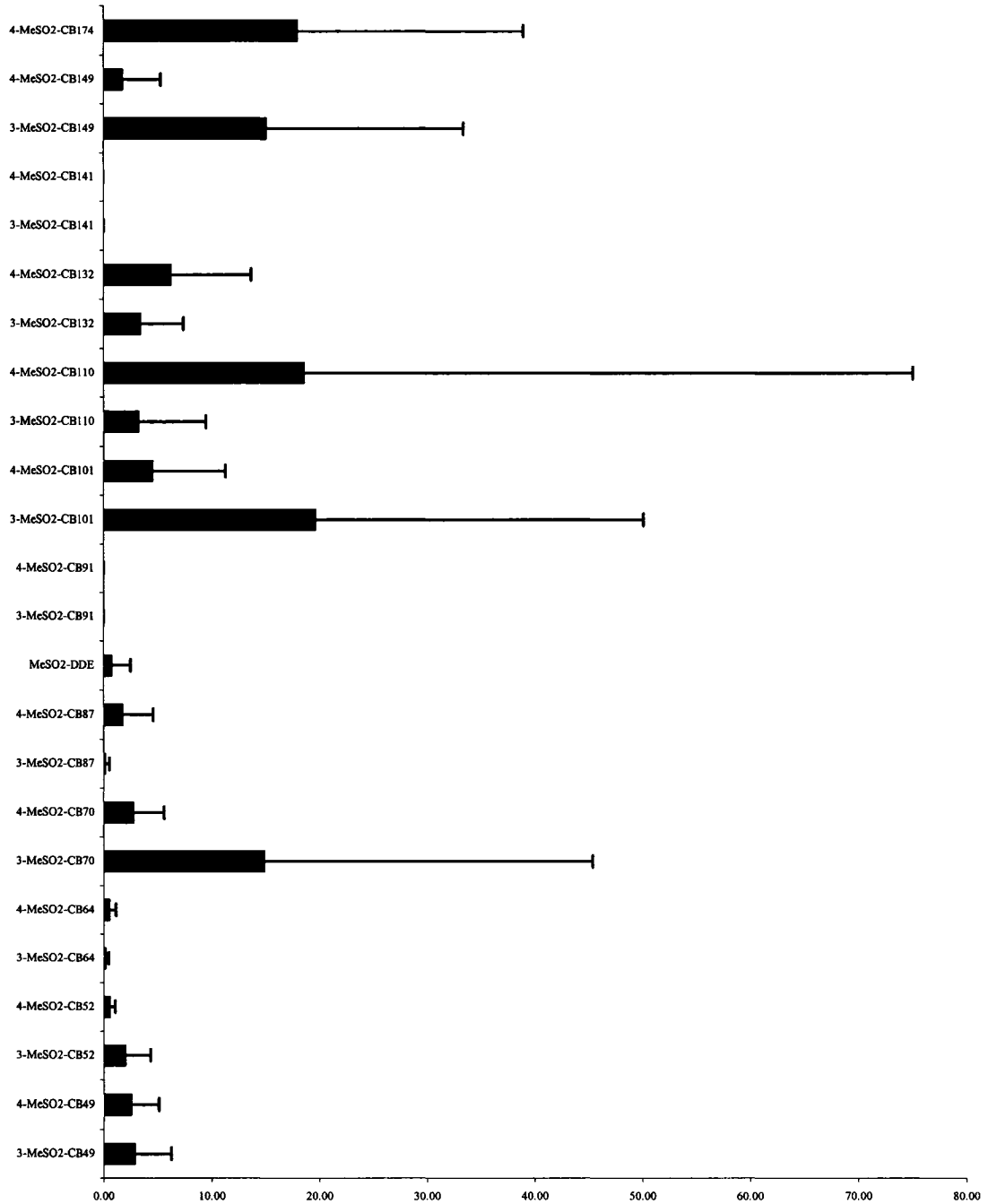
Figure 2.6 is a representative illustration of the OH-PCB congeners detected in the liver of deepwater sculpin. Although generally low in concentration, and in many cases less than the MLOQ, the OH-PCB congeners and congener patterns in deepwater sculpin liver and burbot plasma and liver were generally similar (Figure 2.7 and Table 2.7). The

trends showed that this was also true regardless of the three Lake Huron sites. Hexa- and hepta-chlorinated OH-PCB congeners were dominant.



[$MeSO_2$-CB_X] in ng g-1 lipid weight

(a) Deepwater sculpin liver from Goderich



[$MeSO_2$-CB_X] in ng g-1 lipid weight

(b) Burbot liver from Goderich

Figure 2.5. $MeSO_2$-PCB concentration levels in (a) deepwater sculpin (n = 12) and (b) burbot (n = 17) liver tissue from Lake Huron.

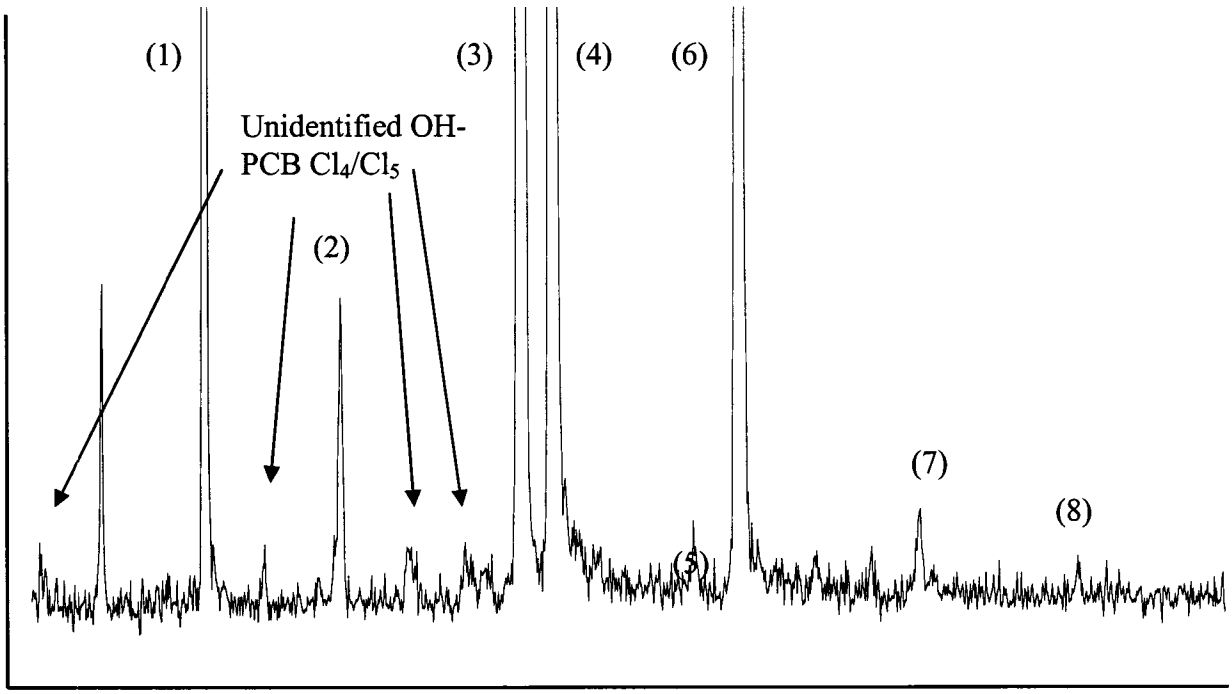
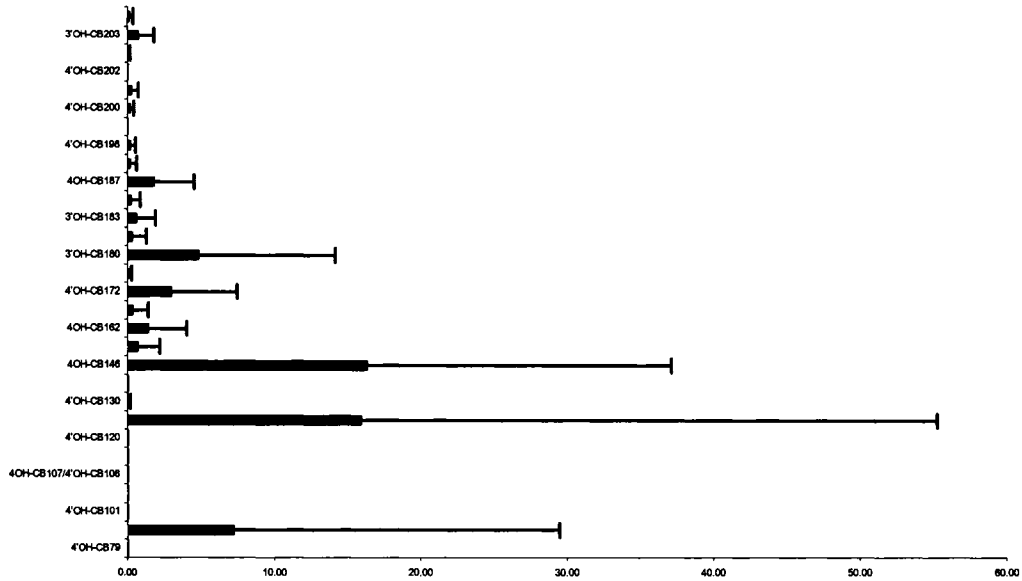


Figure 2.6. Representative GC-MS (ECNI) mass chromatogram (see Table 2 for ions monitored) for detectable OH-PCB congeners in the liver of deepwater sculpin from Goderich. Peaks are the OH-PCB congeners: (1) ^{13}C-4'-OH-CB120; (2) 3-OH-CB146; (3) ^{13}C-4'-OH-CB187; (4) ^{13}C-4'-OH-CB159; (5) 4'-OH-CB201; (6) ^{13}C-4'-OH-CB172; (7) 3'-OH-CB203; (8) 4'-OH-CB127.

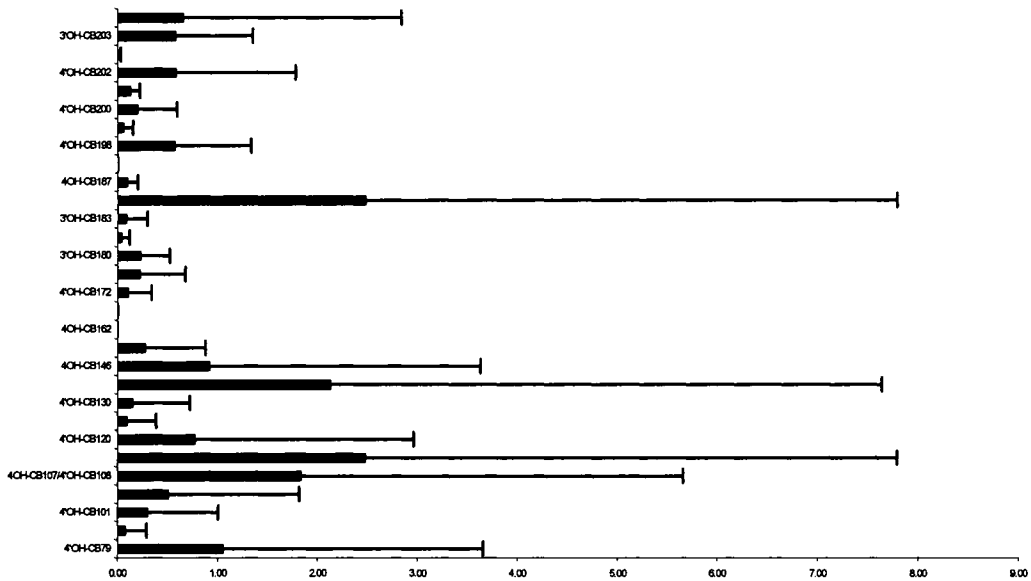
In fact, 4-OH-CB146, 4'-OH-CB172, 4-OH-CB187, 4-OH-CB97, 4'-OH-CB130, 4'-OH-CB159, 3'-OH-CB183 and 3'-OH-CB184 were the dominant OH-PCBs. The 4-OH-CB146, 4'-OH-CB172 and 4-OH- CB187 were most abundant and accounted for 18% ± 26% of the Σ-OH-PCB concentrations in all samples.

The liver and plasma samples of burbot from all three sites contained hexa- and hepta-chlorinated OH-PCB congeners. In general the liver and plasma concentrations were similar to the ΣOH-PCB concentrations in the liver of deepwater sculpin (Figure 2.7 and Table 2.7).



[OH-CB_X] in ng g^{-1} wet weight

(a) Deepwater sculpin liver tissue



[OH-CB_X] in ng g^{-1} wet weight

(b) Burbot liver tissue

Figure 2.7. OH-PCB concentration levels in (a) deepwater sculpin and (b) burbot liver from Lake Huron.

Regardless, there appeared to be congener pattern differences among samples from the three Lake Huron sites. Dominant OH-PCBs in liver, and to a lesser extent the plasma, of Goderich burbot included 4-OH-CB163, 4'-OH-CB79, 4-OH-CB107/108, 3'-OH-CB138, 4-OH-CB163 and 3'-OH-CB184. In contrast dominant OH-PCB metabolites detected from Meaford burbot liver included 4'-OH-CB201, 4'-OH-CB177 and 4-OH-CB146. Burbot liver from Meldrum Bay showed dominance by 4'-OH-CB208, 4'-OH-CB198 and 4'-OH-CB201. Many OH-PCB congeners were not detectable in the burbot plasma although the Σ-OH-PCB concentrations were similar to the livers.

This demonstrates the dominance of certain OH-PCB congeners regardless if in liver or plasma, and that in general the liver and plasma congener patterns are the same. What are intriguing are the site-specific differences in the OH-PCB congener patterns in the burbot liver. Unlike the PCBs and $MeSO_2$-PCBs, which are clearly more lipophilic, persistent and accumulative regardless of the tissue, the congener pattern but not the Σ-OH-PCB concentrations in burbot is sampling site-dependent. OH-PCBs may more sensitively reflect contaminant-mediated CYP450 enzyme induction of isoforms that mediated to conversion of PCBs to OH-PCBs in burbot. The type of CYP450s induced may be affecting the nature of the OH-PCB congeners metabolically generated. The OH-PCB congener patterns may also be influenced by site-specific capacities in burbot to retain (e.g., via competitive, non-covalent binding to thyroid transport protein that are important in fish such as thyroid albumin) and metabolically conjugate and perhaps eliminate OH-PCBs (Letcher *et al.* 2000). In any event, since we were not able to robustly assess the statistical significance in the differences in the apparent OH-PCB

congener patterns in burbot, more research is required to address these questions as a result of the present findings.

Table 2.7. OH-PCB (ng/g wet weight) in burbot plasma from Lake Huron.

Congener	[OH-PCB-X] ng/g wet weight
4-OH-CB146	0.1 ± 0.1
4'-OH-CB177	0.4 ± 0.3
3'-OH-CB183	0.3 ± 0.3
3'-OH-CB184	0.1 ± 0.2
4-OH-CB193	0.3 ± 0.5
4'-OH-CB201	0.2 ± 0.1
4,4'-diOH-CB202	0.2 ± 0.2
3'-OH-CB203	0.1 ± 0.1
Σ-OH-PCB	1.9 ± 1.7

2.3.2 Other Emerging Organochlorines

With the exception of one sample of burbot whole body from Meldrum Bay, the novel organohalogen BCPS was detected in all samples from Meaford and Meldrum Bay (Table 2.8). Levels of BCPS were lowest in burbot plasma, and were highest in deepwater sculpin liver tissue from both sites. Levels of BCPS in Lake Huron deepwater sculpin were found to be on the same order of magnitude as levels determined in Latvian perch (Olsson & Bergman 1999; Olsson *et al.* 1999; Valters *et al.* 1999); however the present Lake Huron burbot were lower. Burbot plasma samples were found to contain comparable levels of BCPS to what has been published in glaucous gull plasma from the Norwegian arctic (Verreault *et al.* 2005). Pentachlorophenol (PCP) was not detected in Goderich, however it was detected in all deepwater sculpin liver samples from Meaford (11.8 ± 11.2 ng/g lipid) and only in burbot liver and plasma from Meldrum Bay (14.6 ± 13.0 ng/g lipid weight in all liver samples, 0.1 ± 0.1 ng/g wet weight in only 2 of 11 plasma samples). Pentachlorophenol (PCP) concentrations increased in the downstream

Table 2.8. BCPS levels (ng/g lipid weight basis) in biota from Lake Huron.

	Goderich	*Meaford*	*Meldrum Bay*
Diporeia hoyi	131.4		
Deepwater sculpin			
Whole Body	25.4 ± 13.0	183.1 ± 142.3	79.0
Liver	9.9 ± 6.6	5543.0 ± 3357.2	180.5
Burbot			
Whole Body	0.6 ± 0.4	35.7	75.5 ± 172.9
Liver	5.6 ± 5.4	21.0	85.4 ± 117.0
Plasma	n.d.	20.5	9.8 ± 6.1

direction in Lake Huron as found in fish plasma from the Detroit River (Li *et al.* 2003). PCP in burbot plasma was comparable to levels in brown bullhead, lake sturgeon and channel catfish from the Detroit River (Li *et al.* 2002). 4-OH-HpCS was only detected in Goderich burbot liver samples (0.67 ± 0.43 ng/g wet weight, detected in all 6 samples) from Lake Huron. Levels of 4-OH-HpCS were found to be comparable to levels in benthic and pelagic fish from Lake Huron (Li *et al.* 2003).

2.3.3 Brominated flame retardants (PBDEs, HBCD and OH-PBDEs)

BDE congeners were detected in all *diporeia hoyi*, deepwater sculpin and burbot samples from the Lake Huron sites (Table 2.9). The dominant congener in all biotic samples was BDE-47, however in Deepwater sculpin to a larger degree. Tetra- and penta-brominated PBDE congeners in *diporeia hoyi* represented roughly 90 % of the Σ-PBDE concentrations, with the remainder consisting of BDE-154 and -153. *Diporeia hoyi* Σ-PBDE levels were higher than recent levels reported in Lake Erie, Lake Superior and Lake Michigan sediment (Zhu & Hites 2005) as well as Lake Huron sediment (Song *et al.* 2005). BDE congener patterns in *diporeia hoyi* were similar to those from Lake Huron sediment (Song *et al.* 2005), but illustrate an abiotic source of PBDEs for subsequent bioconcentration in the lower end of Lake Huron food web, which could be represented

by *diporeia hoyi*. PBDE bioaccumulation in the deepwater sculpin food chain will be discussed more thoroughly in Chapter 3.

Burbot liver and whole carcass demonstrated a preference for retention of penta- and hexa-brominated BDE congeners, constituting roughly 50% of the Σ-PBDE in all samples. PBDE levels in burbot were comparable to PBDE levels in lake trout from Lake Huron (Zhu & Hites 2004). In comparison to *diporeia hoyi* and burbot, deepwater sculpin tissue showed depletion of penta-brominated PBDE congeners, which only constituted roughly 5% of the Σ-PBDE burden. Studies have shown debromination of BDE-209 in carp (Stapleton *et al.* 2004). Depletion suggests potential debromination metabolism of higher brominanted PBDEs by the deepwater sculpin occurring in its liver tissue. Depletion occurred on a larger scale in liver tissue verses whole carcass and will be further discussed in chapter 3. High concentrations of BDE-47 and -154 coupled with the absence of BDE-209 in deepwater sculpin liver tissue may suggest debromination although further research is warranted.

Table 2.9. PBDE levels (ng/g lipid weight basis) in *diporeia hoyi*, deepwater sculpin and burbot tissue from Goderich in Lake Huron.

	D. hoyi (n = 2)	Deepwater sculpin (n = 5) Whole Body	Liver	Burbot (n = 5) Whole Body	Liver	Plasma*
BDE-17	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
BDE-28	n.d.	0.9 ± 2.0	n.d.	n.d.	n.d.	n.d.
BDE-47	55.81	179.3 ± 75.2	326.3 ± 145.5	120.0 ± 31.7	399.5 ± 133.5	1.3 ± 2.2
BDE-49	n.d.	n.d.	n.d.	n.d.	27.9 ± 18.8	n.d.
BDE-99	30.67	4.1 ± 4.1	n.d.	34.3 ± 32.7	150.3 ± 51.5	n.d.
BDE-100	15.84	3.0 ± 1.8	3.1 ± 4.4	31.2 ± 19.3	122.0 ± 44.5	n.d.
BDE-153	2.34	3.9 ± 0.4	2.4 ± 3.3	n.d.	34.3 ± 4.4	n.d.
BDE-154	6.18	19.6 ± 4.0	21.2 ± 12.9	11.0 ± 15.1	n.d.	n.d.
BDE-183	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Σ-PBDE	110.84	204.0 ± 79.0	338.5 ± 126.2	232.0 ± 109.7	814.2 ± 246.1	1.3 ± 2.2

PBDE (ng/g lipid weight) levels in tissue from Meaford in Lake Huron.

	Deepwater sculpin (n = 5) Whole Body	Liver	Burbot (n = 2) Whole Body	Liver	Plasma*
BDE-17	n.d.	n.d.	n.d.	n.d.	n.d.
BDE-28	7.5 ± 1.2	6.2 ± 3.6	n.d.	11.7	n.d.
BDE-47	365.1 ± 105.6	384.9 ± 128.6	257.2	569.5	3.8
BDE-49	n.d.	n.d.	n.d.	n.d.	n.d.
BDE-99	9.7 ± 2.9	12.4 ± 4.5	56.1	146.6	1.2
BDE-100	6.1 ± 2.8	0.7 ± 1.5	36.1	122.0	0.8
BDE-153	12.9 ± 2.6	13.9 ± 3.4	21.4	43.6	0.2
BDE-154	26.8 ± 7.5	29.3 ± 8.3	45.7	98.8	0.7
BDE-183	n.d.	n.d.	n.d.	1.8	n.d.
Σ-PBDE	538.5 ± 141.9	525.0 ± 143.2	581.6	1166.1	7.2

PBDE levels (ng/g lipid weight) in tissue from Meldrum Bay in Lake Huron.

	Deepwater sculpin (n = 2) Whole Body	Liver	Burbot (n = 10) Whole Body	Liver	Plasma*
BDE-17	n.d.	n.d.	37.5 ± 118.7	n.d.	n.d.
BDE-28	10.0	n.d.	2.0 ± 6.4	18.6 ± 9.4	n.d.
BDE-47	807.7	243.9	476.8 ± 270.4	1249.5 ± 632.0	10.9 ± 11.4
BDE-49	n.d.	n.d.	n.d.	n.d.	n.d.
BDE-99	146.8	49.8	111.8 ± 81.2	231.0 ± 156.0	2.1 ± 1.7
BDE-100	164.6	8.7	98.9 ± 59.8	276.8 ± 177.7	1.8 ± 1.9
BDE-153	39.1	18.0	29.6 ± 17.6	67.9 ± 34.3	0.6 ± 0.6
BDE-154	124.6	38.7	79.0 ± 36.8	236.3 ± 140.6	1.6 ± 1.6
BDE-183	n.d.	8.7	n.d.	0.7 ± 1.1	n.d.
Σ-PBDE	1292.8	506.7	939.4 ± 542.4	2545.9 ± 1379.1	21.1 ± 20.0

* Plasma data reported on ng/g wet weight basis.

The results of *t*-test and correlations for PBDE in deepwater sculpin and burbot liver tissue are presented in Table 2.10. Significant differences ($p < 0.05$) between species were observed for six out of nine PBDE congeners monitored. Congeners BDE-47, -99, -100, -153 and -154 in burbot liver were highly significantly greater ($p < 0.01$) than levels in deepwater sculpin liver. Furthermore the Σ-PBDE in burbot liver was also highly significantly ($p < 0.001$) greater than that for deepwater sculpin liver. The highly significant relationships

Table 2.10. Means, standard deviations, *t-values* and correlations for BDE congener concentrations (ng/g lipid weight) in deepwater sculpin and burbot liver tissue from Lake Huron.

Congener	Deepwater sculpin *Mean*	*SD*	Burbot *Mean*	*SD*	*t-value*	R^2
BDE-28	4.5	5.4	12.4	11.2	-2.50*	0.39*
BDE-47	383.8	178.4	919.5	636.5	-3.29**	0.39*
BDE-49	n.d.	n.d.	8.2	16.1	-2.10	0.37*
BDE-66	n.d.	n.d.	1.3	5.4	-1.00	0.33
BDE-99	29.8	58.6	197.3	127.9	-4.74**	0.01
BDE-100	21.9	49.6	213.1	156.9	-4.70**	0.35
BDE-153	14.3	16.9	55.1	31.0	-4.55**	0.26
BDE-154	37.8	34.8	174.2	132.2	-4.06**	0.45*
BDE-183	0.3	0.7	0.6	1.0	-0.93	-0.06
Σ-PBDE	492.5	312.7	1581.7	1070.3	-1.75**	0.42*

*$p < 0.05$ **$p < 0.001$

for congeners BDE-99, -100, -153 and -154 show that they are significantly higher ($p < 0.001$) levels in burbot liver then in deepwater sculpin and may suggest debromination metabolism (Stapleton *et al.* 2004) in deepwater sculpin liver tissue. Although BDE-47 is also highly significantly greater in burbot liver than in deepwater sculpin, further research is required to determine whether debromination metabolism is occurring.

HBCD was detected in samples from Goderich, Meaford and Meldrum Bay (Table 2.11). Moreover, HBCD was detected in all samples except for a deepwater sculpin liver and in all burbot plasma samples from Meaford. HBCD was not detected in sculpin homogenate samples, 4 of the 11 burbot homogenate samples and 6 of the 11 burbot plasma samples from Meldrum Bay. Determination of HBCD in Lake Huron fish were found to be at levels 10 fold lower than in Swedish pike (Sellstrom *et al.* 1998) but comparable to fish from Lake Winnipeg (Budakowski & Tomy 2003) as well as in Lake Ontario (Tomy *et al.* 2004). Detection of HBCD occurred at all three sites in Lake Huron, however it was barely detectable in samples from Goderich. HBCD may be a persistent,

bioaccumulative, anthropogenic compound from point sources that atmospherically transports itself to downstream sites such as Goderich (de Witt 2002). Statistical analyses of HBCD concentrations from Lake Huron are not shown, however burbot liver tissue was found to have a significantly higher concentration of total-(α)-HBCD then deepwater sculpin liver. The significant differences for total-(α)-HBCD concentrations show that HBCD may be biomagnifying from deepwater sculpin to burbot and will be further discussed in Chapter 3.

Table 2.11. HBCD levels (ng/g lipid weight) in biota from Lake Huron.

	Goderich	Meaford	Meldrum Bay
Diporeia hoyi	0.2		
Deepwater sculpin			
Whole Body	1.1 ± 0.7	82.9 ± 22.6	138.7
Liver	1.0 ± 1.0	10.6 ± 6.9	n.d.
Burbot			
Whole Body	n.d.	142.3	89.3 ± 65.3
Liver	2.3 ± 1.1	172.1	391.6 ± 167.1
Plasma	n.d.	n.d.	1.0 ± 1.3

OH-PBDEs were detected in the liver and/or plasma of deepwater sculpin and burbot from all Lake Huron sampling sites (Table 2.12). A representative mass chromatogram of OH-PBDEs in the liver of deepwater sculpin from Goderich in Lake Huron is shown in Figure 2.8. OH-PBDEs were below the MLOQ for whole body homogenates and *diporeia hoyi* pools, which suggests that like OH-PCBs, they are not bioaccumulative regardless of their source. This may also suggest that the OH-PBDEs in deepwater sculpin and burbot are PBDE metabolites assuming that *Diporeia hoyi* is representative of the deepwater sculpin diet and deepwater sculpin are representative of the burbot diet.

The OH-BDE congeners in deepwater sculpin liver and common to all Lake Huron sites were 4-OH-BDE42, 5-OH-BDE47 and 4'-OH-BDE49 (Table 2.12 and 2.13), where they comprised 59% ± 51% of the Σ-OH-BDE concentration regardless of the site. Like the situation for OH-PCBs, the Goderich site had more detectable OH-PBDEs in deepwater sculpin liver (Figure 2.8 and 2.9), but the Σ-OH-PBDE concentrations were essentially the same. This indicates that the more minor OH-PBDE congeners were simply more detectable in the Goderich samples. There is a virtual absence of studies of OH-PBDE is any aquatic species from the Great Lakes watershed including for fish. However, the present findings for Lake Huron fish are comparable to OH-PBDEs determined in the plasma of 13 species of pelagic- and benthic- feeding fish from the Detroit River (Valters *et al.* 2005). In this other study, the pattern of OH-BDE congeners was similar to the present fish, where 6-OH-BDE47 was an exceptionally dominant compound. In the case of the Detroit River that is the potential for OH-PBDE bioaccumulation from water (sourced possibly from wastewater treatment plants (WSTPs)) since Hua *et al.* (2005) showed that at least tri-brominated OH-BDEs were present in effluent from a major WSTP in Windsor, ON, and only in Detroit River surface waters. These results would suggest that the OH-PBDEs in the Lake Huron fish perhaps are more metabolically sourced.

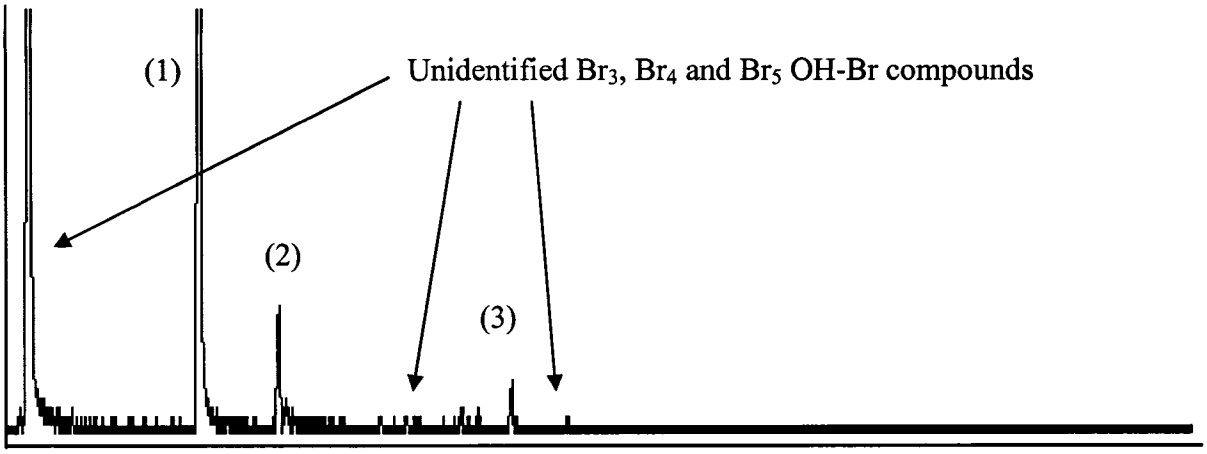
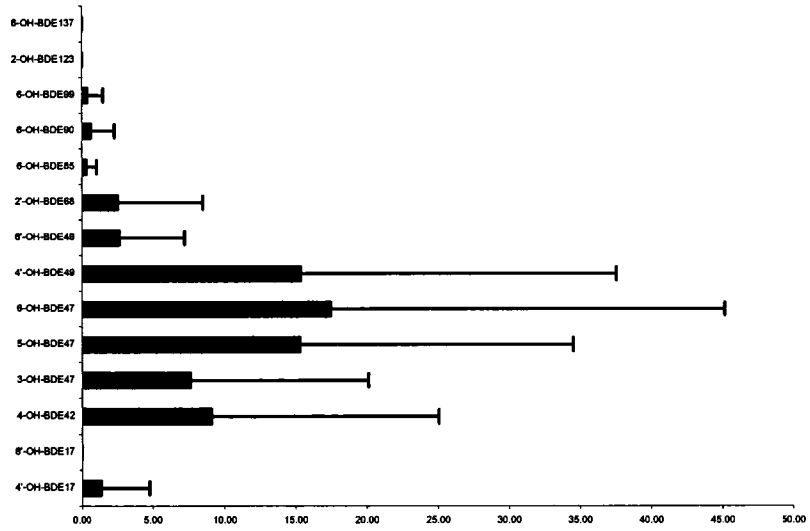
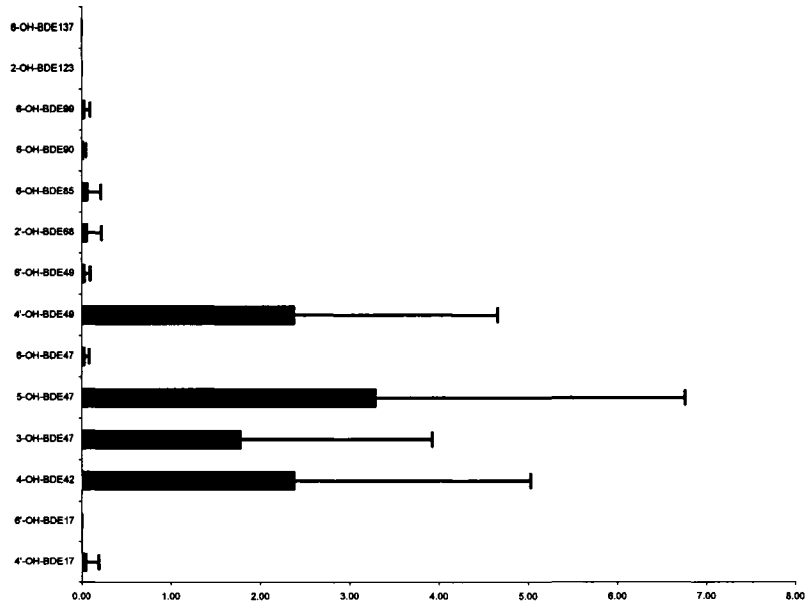


Figure 2.8. Representative GC-MS(ECNI) mass chromatogram (see Table 2.2 for ions monitored) for OH-PBDE congeners in the liver of deepwater sculpin from Goderich. Peaks are the OH-PBDE congeners: (1) 2'-OH-BDE28 Internal Standard (2) 2'-OH-BDE68; (3) 5-OH-BDE47



[OH-BDE_X] in ng g^{-1} wet weight

(a) Deepwater sculpin liver tissue



$[OH\text{-}BDE_X]$ in ng g^{-1} wet weight

(a) Burbot liver tissue

Figure 2.9. OH-BDE congener concentrations in (a) deepwater sculpin and (b) burbot liver from Lake Huron.

Table 2.12. OH-BDE congener concentrations (ng/g wet weight) in burbot plasma from Lake Huron.

Congener	[OH-PBDE-X] ng/g wet weight
4-OH-BDE42	4.6 ± 7.7
3-OH-BDE47	4.5 ± 4.7
5-OH-BDE47	2.4 ± 2.9
6-OH-BDE47	0.3 ± 0.5
4'-OH-BDE49	0.5 ± 1.2
6'-OH-BDE49	0.1 ± 0.3
2'-OH-BDE68	1.5 ± 1.6
6-OH-BDE90	0.3 ± 0.7
6-OH-BDE99	0.3 ± 0.7
2-OH-BDE123	2.8 ± 3.3
Σ-OH-PBDE	17.3 ± 11.3

Table 2.13. Means, standard deviations and *t-values* for OH-PBDEs (ng/g wet weight) in deepwater sculpin and burbot liver from Lake Huron

Congener	Deepwater sculpin	*SD*	Burbot *Mean*	*SD*	*t-value*	Correlations

	Mean					
4'-OH-BDE17	3.1	10.6	0.2	0.6	0.94	-0.21
4-OH-BDE42	5.9	14.9	1.9	2.7	0.92	-0.21
3-OH-BDE47	20.3	27.8	1.9	2.2	2.28*	-0.47*
5-OH-BDE47	20.1	20.9	3.7	3.7	2.70*	-0.52**
6-OH-BDE47	31.2	37.1	0.1	0.1	2.91*	-0.56**
4'-OH-BDE49	13.9	23.9	2.7	2.6	1.61	-0.35
6'-OH-BDE49	5.4	9.1	n.d.	n.d.	2.02	-0.42*
2'-OH-BDE68	1.6	5.4	0.1	0.2	0.97	-0.22
6-OH-BDE85	0.7	2.4	0.2	0.4	0.71	-0.20
6-OH-BDE90	1.5	5.2	n.d.	n.d.	0.97	-0.22
6-OH-BDE99	1.0	3.4	n.d.	n.d.	0.98	-0.22
Σ-OH-PBDE	104.5	107.1	10.76	7.1	3.03*	-0.57**

*$p < 0.05$ **$p < 0.01$

2.4 Conclusions and Implications

A number of legacy and previously unknown chlorinated and brominated organohalogens and possible degradation and metabolite products were identified and characterized in various tissues or pools of *Diporeia hoyi*, deepwater sculpin and burbot from several sites across Lake Huron. The identity and tissue- and site-specific comparisons of $MeSO_2$-PCB, OH-PCB and OH-PBDE congener patterns and levels suggests that their presence in this deepwater sculpin food chain could originate from previous degradation, bioconcentration and accumulation, and/or that they are metabolic products formed in Deepwater sculpin and/or burbot from accumulated PCB and PBDE congeners. For Lake Michigan fish and food webs the present results are consistent with the metabolic potential in this regard for deepwater sculpin and burbot as reported by Stapleton *et al.* (2001). Novel and emerging anthropogenic organohalogens such as BCPS, HBCD, PCP and 4-OH-HpCS are clearly present and accumulative in the deepwater sculpin food chain from Lake Huron. The determination of $MeSO_2$-PCB, OH-PCB and OH-PBDE metabolites in the deepwater sculpin food chain provides another class of contaminant concern to fish in Lake Huron. These newly characterized chlorinated and brominated contaminants emphasize the growing complexity of exposure

and potential toxicities to the aquatic food web of Lake Huron. Based on the characterization established in this chapter, in Chapter 3 we investigate in a more detailed fashion on the bioaccumulation, biotransformation and biomagnification of these organohalogens within this deepwater sculpin food chain.

2.5 References

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CHAPTER III

BIOACCUMULATION OF CHLORINATED AND BROMINATED CONTAMINANTS AND THIER BIOTRANSFORMATION PRODUCTS IN THE DEEPWATER SCULPIN (*Myoxocephalus thompsoni*) AND PREDATOR-PREY SPECIES FROM LAKE HURON

3.1 Introduction

The benthic amphipod *diporeia hoyi*, deepwater sculpin (*Myoxocephalus thompsoni*) and burbot (*Lota lota*) have a common predator-prey relationship within the aquatic food webs of Lakes Huron, Michigan and Superior (Russell *et al.* 1999; Kitchell *et al.* 1994; Kitchell *et al.* 2000; Owens *et al.* 1995; Paakkonen *et al.* 2005). Fratt *et. al* (1997) specifically showed that burbot is a predator of deepwater sculpin in Lake Michigan. Similar to bloater chub (*Coregonus hoyi*), in southeastern Lake Michigan and Lake Ontario it was previously reported that deepwater sculpin feed primarily on benthic amphipods (*Diporeia hoyi*) and opposum shrimp (*Mysis relicta*) (Wojcik *et al.* 1986; Owens *et al.* 1995). For Lake Michigan, Stapleton et al. (2001) recently exemplified via stable isotope analysis (i.e., $\delta^{15}N$) that *Diporeia hoyi*, deepwater sculpin and burbot occupy largely differing trophic positions in the Lake Michigan food web.

Bioaccumulation of PCBs, and to a lesser extent PBDEs, has been documented in species of Great Lakes fish and their food webs (Stapleton *et al.*, 2001; Paakkonen *et al.* 2005; Zhu & Hites 2004; Stapleton & Baker 2003; Falk *et al.* 1999; Madenjian *et al.* 2002; Gerstenberger & Dellinger 2002; Scheider *et al.* 1998). However, there is very limited PCB bioaccumulation information with respect to deepwater sculpin and relative to species within its Great Lakes food webs. For PBDEs, to our knowledge there is even

more limited information for Great Lakes fish with a few exceptions. For example, for lakes Michigan and Superior, Carlson and Swackhamer (2006) recently reported for several salmonid species PBDE and PCB levels. However, there are only three known reports of PBDEs in fish (mainly salmonids) from Lake Huron, although deepwater sculpin and burbot were not among the species studied (Chernyak *et al.* 2005; Zhu & Hites 2004; Luross *et al.* 2003). Size (growth), age, lipid content and trophic position influence bioaccumulation of PCBs and other organohalogens in fish and has been reported for e.g., in the deepwater sculpin foodweb from Lake Michigan (Stapleton *et al.* 2001).

The congener patterns and concentrations of PCB and PBDE residues in an organism's tissue in relation to their prey reveal insight into elimination factors, e.g., xenobiotic-metabolizing enzymes such as cytochrome P450 monoozygenases (CYP450s) which mediate oxidative biotransformation of a variety of organohalogens (Stapleton *et al.* 2001; Letcher, Klasson-Wehler & Bergman 2000; Hakk & Letcher 2003). Metabolic products of PCB or PBDE congeners that are retained and/or persistent in an organism may include $MeSO_2$-PCBs, OH-PCBs and OH-PBDEs. In Chapter 2 we determined for the first time that $MeSO_2$-PCB, OH-PCB and OH-PBDE classes of contaminants were present in the currently studied deepwater sculpin (liver) and burbot (liver and plasma) samples from Lake Huron. Furthermore, reports of these classes in the tissues of other aquatic biota from the Great Lakes are limited to various benthic- and pelagic-feeding fish species (plasma, liver and/or whole body composites) from the Detroit River (Li *et al.* 2003; Valters *et al.* 2005) and Lake Michigan (Stapleton *et al.* 2001; 2003), and Lake Ontario lake trout (plasma) (Campbell *et al.* 2003). However, we are not aware of studies

that examined the bioaccumulation of these contaminants in any predator-prey relationship in an aquatic food web in the Great Lakes.

PCB accumulation in abiotic sources in the Great Lakes including water and sediment has been well documented (Baker *et al.* 1991; Jeremiason *et al.* 1994; Pearson *et al.* 1997) and has stabilized (Jeremiason *et al.* 1994; Stow 1995). Hydrophobic contaminants such as PCBs and PBDEs partition from water to sediments, and are then bioavailable and subsequently bioaccumulate in invertebrate organisms (Lester *et al.* 1994; DePinto & Coull 1997). The specific mechanisms for PBDE absorption have not been as extensively studied (de Wit 2002). Of the new organohalogen classes of OH-PCBs, OH-PBDEs and $MeSO_2$-PCBs in Great Lakes fish species, the only attempt to address food chain or web bioaccumulation have been for $MeSO_2$-PCBs. Whole deepwater sculpin and burbot collected from Lake Michigan in 1997-1998 were analyzed and found to contain $MeSO_2$-PCBs (Stapleton *et al.* 2001). Based on corresponding precursor PCB congener patterns and the relationship in levels between burbot and its deepwater sculpin prey, Stapleton *et al.* (2001) suggested that deepwater sculpin likely metabolically forms $MeSO_2$-PCB metabolites from accumulated organochlorine biphenyl precursors, and burbot likely accumulate them from dietary prey and in particular from deepwater sculpin. In the only other known report on $MeSO_2$-PCBs in a sculpin species, Bright *et al.* (1995) reported very low ng/g (wet weight) levels of $MeSO_2$-PCBs in the liver of four-horned sculpin (*Myoxocephalus quadricornis*) collected in 1992-1993 from marine coastal areas of the Canadian high Arctic. Other chlorinated phenolic contaminants are known, such as the OH-trichlorinated diphenyl ether triclosan® and pentachlorophenol (PCP, Chapter 2), which are anthropogenically sourced, and uptake

and bioconcentration in fish has been reported for e.g., benthic- and pelagic-feeding species of fish from the Detroit River fish and in surface waters (Valters *et al.* 2005; Hua *et al.* 2006).

The objective of the present study is to investigate the bioaccumulation and potential for biomagification of classes of chlorinated and brominated contaminants and their metabolically associated degradation products (identified in Chapter 2) in the *diporeia hoyi*-deepwater sculpin-burbot food chain from Lake Huron. This is accomplished by the determination of contaminant class- and congener-specific determinations of bioaccumulation factors (BAFs), and in relation to hepatic microsomal CYP1A1 (Phase I) monooxygenase and uridine diphospho-glucuronosyl transferase (UDPGT) (Phase II conjugation) catalytic activities in burbot and deepwater sculpin.

3.2 Materials and Methods

3.2.1 Samples, Chemical Analyses and Bioaccumulation Factors

Sampling, contaminant residue sample work-up, analysis, quantification and data analysis methods are described in detail in Chapter 2.

Bioaccumulation factors (BAFs) were calculated utilizing whole carcass and liver tissue for PCBs, PBDEs, $MeSO_2$-PCBs, HBCD and BCPS. BAFs for OH-PCBs, OH-PBDEs, PCP and 4-OH-HpCS could only be determined in liver tissue. BAFs were calculated for Σ-PCB, Σ-PBDE, Σ-$MeSO_2$-PCB, Σ-OH-PCB and Σ-OH-PBDE as well as on a congener specific basis.

$$BAF\ (Compound\ X) = \frac{[Compound\ X]_{predator}}{[Compound\ X]_{prey}}$$

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BAFs were reported for each compound class based on the tissue that each compound accumulated in. Lipophilic compounds PCBs, PBDEs, $MeSO_2$-PCBs, HBCD and BCPS were calculated using lipid normalized concentrations, whereas OH-PCBs, OH-PBDEs, PCP and 4-OH-HpCS were calculated using wet weight concentrations. This was done to minimize the variability of lipid (Hebert & Kennleyside 1995).

3.2.2 Hepatic Microsomes and Protein Content

All glassware utilized for catalytic assays was cleaned and sterilized according to protocols outlined in Chapter 2. The homogenization buffer consisted of 8.71g of K_2HPO_4 (from Merck), 5.59g of KCl (from Sigma-Aldrich), 0.186g of Na_2EDTA (from Bio-Rad Laboratories) and 0.077g of DTT (Merck Company) dissolved in nano-pure water. The pH was adjusted to 7.4 using 5 M HCl with glycerol added to it to make a 1:4 glycerol: solution volume ratio. Total volume of the solution was brought to 500 mL with nano-pure water. Resuspension buffer consisted of the same solution as the homogenization buffer with the exception of KCl addition.

An approximately 2 g sample of liver tissue (stored at –80°C) was removed from the freezer and thawed on ice (~4°C). Roughly 6 mL of homogenization buffer was added to the liver tissue and liver was allowed to fully thaw. Liver tissue homogenized and centrifuged at 12 000 x g for 20 minutes at 4°C. The supernatant centrifuged a second time at 100 000 x g at 4°C for 60 minutes. After removing the supernatant, the pellet was resuspended in buffer and homogenized a second time. This was centrifuged and homogenized a final time. Microsomes were immediately frozen at -80°C until analysis. The Bradford Assay (Bradford 1976) was utilized to determine protein content of microsomes according to McKinney *et al.* (2004) using Bovine Serum Albumin (BSA) as

the standard protein solution. MRX Dynatech spectrophotometer with Biolinx™ version 2.20 Assay Management software was used to analyze 20 μL aliquots of microsomes. Measurement parameters included setting up six filters at 405 nm, 530 nm, 570 nm, 660 nm, 0 nm and 0 nm wavelengths. The test filter was set at 570 nm and absorbance of the spectrophotometer was set at 570 nm.

3.2.3 Ethoxyresorufin O-Deethylase (EROD) Assay

A volume of 275 μL of 1μM ethoxyresorufin, 50 μL of microsomes and 10 μL of 11mM NADPH solution to initiate the reaction were mixed together and Ethoxyresorufin-O-de-ethylase (EROD) activity was measured using a CytoFluor® Multi-Plate Reader (Series 4000) hardware using the CytoFluor Fluorescence Reader Version 4.1 software (from PerSeptive BioSystems).

3.2.4 Uridine Diphospho-Glucuronosyl Transferase (UDPGT) Assay

A volume of 195 μL of buffer solution, 5.00 μL of 7.3 mM 1-naphthylglucoronide (1-NG) solution and 200 μL of acetonitrile were mixed to make the recovery standard. The standard curve of 1-naphtholglucoronide ranged from 1.5625 μM to 50.0000 μM. The incubation mixture included 100 μL of buffer, 10 μL of 1-NG, 10 μL of Brij-58, 200 μL of fish microsomes and 10 μL of UDPGA. Pre-incubation of the reaction mixture occurred for 5 minutes. 10 μL of UDPGA co-factor was added to each tube and the reaction proceeded for exactly 25 minutes in a shaking water bath. Termination of the reaction occurred with 200 μL of cold acetonitrile solution. The tubes were left to cool on ice for 30 seconds and centrifuged for 10 minutes at 2000 rpm. The supernatant was removed and analyzed. Samples were analyzed within 10 hours of performing the reaction assay. Analysis occurred on an HPLC with a Jasco FP-920

fluorescence detector) at an excitation wavelength of 290 nm and an emission wavelength of 330 nm. The flow rate was initially set at 2.0 mL/minute and decreased to 1.5 mL/minute on the following gradient; 2.0 mL/minute from 0 to 11.3 minutes and at 15.6 minutes the gradient was set at 1.5 mL/minute.

3.2.5 Data Analysis

Statistical analysis of variance in contaminant BAFs and catalytic EROD and UDPGT activities in burbot and deepwater sculpin liver was carried out using SPSS for Windows (Version 14.0, SPSS Inc., Chicago, Illinois, USA). All hypotheses tested for analysis of variance between the tissues and species used a one- tailed Student's *t* test, where the maximum probability of a type I error was set to $p<0.05$ or $p<0.01$. Summary statistics were computed if BAFs (i.e., based on analyte concentrations) and enzyme activities were measurable in at least 50% of the samples. If this criterion was met, then for statistical purposes, concentrations <MLOQ was assigned a value between zero and half of the MLOQ. With the exception of HPC classes of contamiants, i.e., OH-PCBs, OH-PBDEs, PCP and 4-OH-HpCS, contaminants were associated with lipid content. Thus, for lipid-associated contaminants the variance caused by the effect of differences in individual extractable lipid content in tissues was minimized by using lipid-normalized concentrations (ng/g lipid wt).

3.3 Results and Discussion

3.3.1 Enzyme-Mediated Metabolic Potential

The identification and limited tissue composition (liver, plasma whole body and pools) in burbot, deepwater sculpin and/or *Diporeia hoyi* of the concentrations and congener patterns of PCBs, PBDEs, BCPS, total- (α)-HBCD, OH-PCBs, $MeSO_2$-PCBs 3-

$MeSO_2$-p,p'-DDE, and OH-PBDEs has been presented in Chapter 2. The concentrations and congeners patterns of the contaminants examined were assumed to be generally representative of the species in Lake Huron, as we were unable to obtain samples of *Diporeia hoyi* from the Meldrum Bay and Meaford site. For all liver, whole body and/or plasma samples from burbot or deepwater sculpin the concentrations and congener patterns all classes of contaminants showed similar patterns for all the sampling sites. Therefore, the site where the deepwater sculpin and burbot sample sets were most complete, the Goderich site, was chosen for the present assessment of bioaccumulation and potential biomagnification of the contaminants in the Lake Huron *diporeia hoyi*-deepwater sculpin-burbot food chain. Furthermore, Goderich was the only site where *diporeia hoyi* samples were obtainable, and the deepwater sculpin and burbot samples were most numerous and uniform in the number of individuals. That is, five individual burbot (whole body, liver and plasma) and five pools of five individual deepwater sculpin (whole body and liver) could be collected at the Goderich site (Figure 2.1 and Table 2.1).

Assessments of the hepatic EROD (CYP1A1) and UDPGT (glucuronide conjugation) catalytic activities in the present deepwater sculpin and burbot can give us some insight into the biotransformation potential to form and conjugate, respectively, PCB and PBDE metabolites. The mean EROD and UDPGT enzyme catalytic activities burbot and deepwater sculpin liver microsomes (Goderich) are shown in Table 3.1. EROD activity in Deepwater sculpin from Goderich was significantly ($p<0.01$) higher then that in burbot. EROD is used as a general indicator of CYP enzyme activity in fish (Eggens & Galgani 1992), and these results indicate that Lake Huron deepwater sculpin have marginal but significantly greater (hepatic) CYP capacity for potential CYP

enzyme-mediated metabolism for xenobiotics such as PCBs and PBDEs. Greater EROD (CYP1A1) activity in deepwater sculpin relative to burbot suggests that the former has a greater capacity to metabolize PCB congeners with *ortho-meta* chlorine-unsubstituted carbons, and via mediation by other CYP isoforms, potentially greater metabolic capacity for the metabolism of *meta-para* chlorine-unsubstituted PCB congeners (that are generally precursors of $MeSO_2$-PCBs) (Stapleton *et al.* 2001; Bright *et al.* 1995). Reports on the CYP catalytic enzyme activity is exceedingly rare for any species of sculpin from any population in the world. In one rare example, relative to the present deepwater sculpin from Lake Huron, hepatic EROD activity in freshwater longfin baikal sculpin (*Cottocomephorus inermis*) in the far eastern Russian Siberia were comparable and reported at 2 ± 7 pmol mg^{-1} min^{-1} (Stepanova *et al.* 1999).

Table 3.1. Mean ± standard deviations of EROD and UDPGT hepatic activities (pmol mg $protein^{-1}$ min^{-1}) for deepwater sculpin and burbot from Goderich in Lake Huron

	EROD ([R] pmol mg^{-1} min^{-1})	UDPGT (pmol mg^{-1} min^{-1})
Deepwater sculpin	7.344 ± 1.481**	70.81 ± 26.68
Burbot	5.356 ± 1.521	141.48 ± 25.83**

**$p < 0.01$

With respect to hepatic UDPGT activity, the present deepwater sculpin were significantly lower ($p<0.01$) than for burbot (Table 3.1). The lower (induced and/or constitutive) levels of hepatic UDPGT activity in deepwater sculpin versus burbot would suggest that the former has a diminished rate capacity to conjugate with glucuonic acid, and subsequently form glucuronides, with suitable contaminant substrates such as those that are hydroxylated, e.g., OH-PCBs, OH-PBDEs, PCP and 4-OH-HpCS. This would suggest that any halogenated phenolic contaminants that are formed and present in deepwater sculpin from Lake Huron would have a longer half-life with respect to

conjugation relative to burbot. If levels of hepatic UDPGT activity are elevated in burbot via contaminant-mediated induction relative to deepwater sculpin, this may also influence (increase) the half-life of endogenous substances such as hormones, i.e., via glucuronide conjugation, and thus elevated contaminant exposure have deleterious physiological ramifications in burbot (Xu *et al.* 2002).

The results of the differential and significant, catalytic Phase I and Phase II xenobiotic-metabolizing enzyme activities would suggest that the bioaccumulation of e.g., selected PCB and possibly PBDE congeners would be decreased, and known and apparent $MeSO_2$- and OH-containing metabolites would be increased in Lake Huron Deepwater sculpin relative to the burbot as predator. To further investigate this hypothesis, contaminant class- and congener-specific BAFs were examined in the *diporeia hoyi*- deepwater sculpin- burbot food chain from Lake Huron.

3.3.2 Bioaccumulation and Biotransformation

(i) PCBs, OH-PCBs and $MeSO_2$-PCBs

EROD and UDPGT enzyme activity suggested that deepwater sculpin versus burbot had a higher capacity to form and subsequently retain OH-PCB and $MeSO_2$-PCB metabolites. This would support the hypothesis that for the burbot-deepwater sculpin predator-prey-relationship, in general BAFs would be lower and perhaps <1 for PCBs and >1 for $MeSO_2$-PCBs and OH-PCBs. Table 3.2 lists the BAFs based on the samples from the Goderich site. That is, Σ-PCB and Σ-$MeSO_2$-PCB BAFs for *diporeia hoyi* (pool) to deepwater sculpin (whole body) and deepwater sculpin (whole body) to burbot (whole body). It was not possible to generate BAFs for *diporeia hoyi* to deepwater sculpin for

OH-PCBs and $MeSO_2$-PCBs, as congeners from either class were not quantifiable in *diporeia hoyi.*

Table 3.2. Mean bioaccumulation factors (BAFs) (± SD) for sum PCB, OH-PCB and $MeSO_2$-PCB concentrations in the deepwater sculpin food chain from Goderich.

	Diporeia hoyi to Deepwater sculpin		Deepwater sculpin to Burbot	
	Homogenate	Liver	Homogenate	Liver
†Σ-PCB	19.1 ± 11.6	20.8 ± 5.4	2.0 ± 3.3	1.4 ± 1.3
†Σ-BCPS	0.2 ± 0.1	0.08 ± 0.06	0.04 ± 0.03	120.6 ± 154.9
†Σ-$MeSO_2$-PCB	n.d.	n.d.	0.9 ± 0.8	3.9 ± 1.7
††Σ-OH-PCB	n.d.	n.d.	n.d.	1.3 ± 1.3

† PCB, BCPS & $MeSO_2$-PCB BAFs were calculated on a lipid weight basis.
†† OH-PCB BAFs were calculated on a wet weight basis.

Σ-PCBs were clearly biomagified from *diporeia hoyi* to deepwater sculpin with a BAF of about 20. PCBs have also been shown to bioaccumulate and biomagnify from *Diporeia hoyi* to deepwater sculpin from Lake Michigan (Stapleton *et al.* 2001; Kucklick & Baker 1998). Furthermore in Lake Huron and Lake Michigan, the primary food item of deepwater sculpin is *diporeia hoyi*, whereas for burbot the deepwater sculpin can make up a large or small proportion of the diet depending on which fish prey species are most available (Madenjian *et al.* 2002; Dobiesz *et al.* 2005). In contrast to *diporeia hoyi* to deepwater sculpin, the mean Σ-PCB BAF for deepwater sculpin to burbot was around unity regardless of whether the BAF was based on liver or whole body homogenate concentrations (Table 3.2). This would suggest that either deepwater sculpin is not completely representative of the burbot diet (Paakkonen *et al.* 2005; Dobiesz *et al.* 2005), and/or that burbot are capable of substantial metabolism/elimination of accumulated PCBs.

The BAF trends (>1) were similar to Σ-PCB BAFs on a congener-specific basis from *Diporeia hoyi* to deepwater sculpin (whole body homogenate or liver), where congeners highly recalcitrant in biota such as CB-153, -118, -138, –180 and -201 were

biomagnified (Table 3.3). This is consistent with BAFs (or biomagnification factors (BMFs)) reported in *Diporeia hoyi* to deepwater sculpin from the Detroit River in 1998 (Russell *et al.* 1999). In the present Lake Huron study, from deepwater sculpin to burbot the BAFs of more recalcitrant PCB congeners such as CB-153, -170/190, -180 and –201 were also consistent with biomagification.

Table 3.3. Mean bioaccumulation factors (BAFs) (± SD) for PCB congener concentrations in whole organism samples from the deepwater sculpin food chain from Goderich.

PCB Congener	Diporeia hoyi *to Deepwater sculpin*		*Deepwater sculpin to Burbot*	
	Mean	*SD*	*Mean*	*SD*
CB-99	9.3*	7.9	1.0	1.0
CB-118	45.8	42.1	3.7	1.9
CB-153	18.7*	15.6	1.9	2.2
CB-180	233.6	220.7	15.8	24.3
CB-49	188.5**	111.4	0.2	0.2
CB-52	16.2*	11.4	0.8	0.6
CB-64	0.0	0.0	0.1	0.0
CB-70	3.9*	2.3	1.0	1.1
CB-74	10.8	5.9	6.7	8.4
CB-87	26.7*	24.1	0.0	0.0
CB-101	1.6	1.3	5.0	3.2
CB-110	1.1	1.3	5.2*	2.8
CB-149	1.3	1.4	6.8	12.9
CB-174	3.0	6.6	0.8	0.6
CB-146	5.8	6.8	2.8	1.9
CB-172	0.0	0.0	0.0	0.0
CB-182	14.2	27.7	0.3	0.3
CB-183	79.5**	49.3	1.6	1.2
CB-200	0.0	0.0	1.9	1.3
CB-201	101.6**	62.6	0.4	0.7
CB-203	55.1*	48.0	1.1	1.0
Σ-PCB	19.1**	11.6	1.4	1.3

PCB congeners such as CB-49, -52, -64, -70, -87 and -101 are all known precursors to $MeSO_2$-PCB metabolites (Letcher al. 2000). The BAFs for these PCB congeners were all significantly higher for *Diporeia hoyi* to deepwater sculpin relative to deepwater sculpin to burbot. However, the *Diporeia hoyi* to deepwater sculpin and deepwater sculpin to burbot BAFs for these congeners tended to be much lower than for highly recalcitrant congeners such as CB-153 and -180. This would suggest that both

deepwater sculpin and burbot have a capacity to metabolize PCB congeners that are $MeSO_2$-PCB precursors. The reduction of $MeSO_2$-PCB precursor PCB congener in deepwater sculpin and burbot is supported by lower levels of these PCB congeners in previous findings on deepwater sculpin in Lake Michigan (Stapleton *et al.* 2001) and in four-horned sculpin from the Canadian Arctic (Bright *et al.* 1995).

Assuming *diporeia hoyi* is the primary prey of deepwater sculpin, the lack of $MeSO_2$-PCBs in *diporeia hoyi* pools suggested that all of the $MeSO_2$-PCBs found in deepwater sculpin are formed from PCB metabolism in deepwater sculpin. In contrast, the Σ-$MeSO_2$-PCB BAFs for deepwater sculpin to burbot, and based on whole body homogenates was ~ 1 and for liver it was ~ 3 (Table 3.2). Given that these BAFs are around or <1, and assuming that deepwater sculpin is the primary prey of burbot in Lake Huron, this suggests that $MeSO_2$-PCBs are marginally bioaccumulative and do not biomagnify, burbot possess a low capacity to metabolize PCBs to $MeSO_2$-PCBs, and/or burbot have a higher capacity to clear $MeSO_2$-PCBs than do deepwater sculpin. Regardless, the deepwater sculpin to burbot BAFs for $MeSO_2$-PCBs were higher in liver than for whole body, which indicates a storage preference for liver. This is further shown for $MeSO_2$-PCB congener-specific BAFs (Figure 3.1). This finding is consistent for the trend that $MeSO_2$-PCBs are preferentially localized in liver relative to other tissues as has been reported for a variety of avian and mammalian species (Letcher *et al.* 2000).

Based on congener-specific $MeSO_2$-PCB BAFs (Figure 3.1) using liver, there appeared to be an increasing trend with respect to the degree of chlorinated and possibly for 3-$MeSO_2$-substituted congeners being greater to 4-$MeSO_2$-containing congeners. Based on whole body homogenates for Lake Michigan deepwater sculpin and burbot,

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Stapleton *et al.* (2001) reported comparable $MeSO_2$-PCB congener BAFs of 0.01 to 0.22 as presently for Lake Huron (Figure 3.1). Also, Stapleton *et al.* (2001) found that in general higher BAFs for 3- versus 4-$MeSO_2$-substituted PCBs. It is interesting to compare $MeSO_2$-PCB BAFs in the present deepwater sculpin to burbot to that of other food chains, where such information is extremely limited. Previous studies involving polar bear (fat) and ringed seal (blubber) from the Canadian Arctic have shown BAFs for Σ-$MeSO_2$-PCB to be about 30 and comparable to the BAF for the highly recalcitrant PCB congener CB-153 (Letcher *et al.* 1998). As shown in Table 3.4, the deepwater sculpin to burbot BAFs for CB-153 versus Σ-$MeSO_2$-PCBs are not comparable indicating a similar tending towards biomagnification. However, these BAFs are considerably lower than that reported for ringed seal to polar bear (Letcher *et al.* 1998). This is due in large part to the polar bear feeding on ringed seal, which is at a very high trophic level in this marine ecosystem.

Table 3.4. Mean BAFs for CB-153 and sum-$MeSO_2$-PCBs in Lake Huron deepwater sculpin to burbot (liver).

	Goderich	Meaford	Meldrum Bay
CB-153	2.2	0.9	1.7
Σ-$MeSO_2$-PCB	4.4	0.4	4.7

*CB-153 & $MeSO_2$-PCB BAFs were calculated on a lipid weight basis.

Statistical analysis of correlations between deepwater sculpin to burbot $MeSO_2$-PCB BAFs (liver) and the EROD and UDPGT catalytic activities (not shown) showed no significant relationships. However, the BAFs were highly negatively correlated for congeners 3-$MeSO_2$-CB149 ($R^2 = -0.98$, $p < 0.001$) and 4-$MeSO_2$-CB149 ($R^2 = -0.98$, $p < 0.001$) with protein content. This is again consistent with preferential, protein-associated retention of $MeSO_2$-PCBs in the liver of biota (Letcher *et al.* 2000).

As shown in Figure 3.2 and Table 3.2, BAFs for Σ-OH-PCBs (Goderich) were

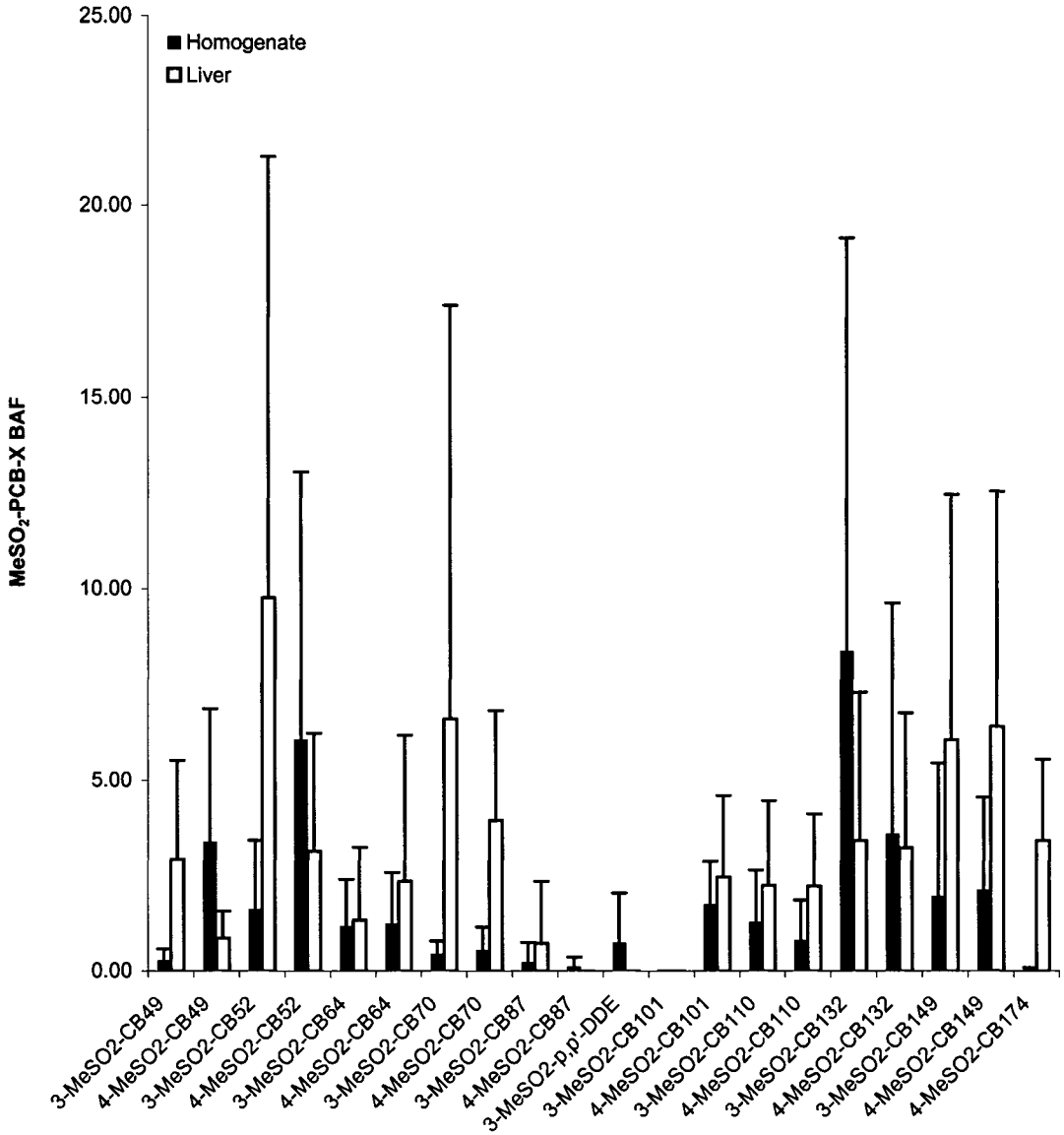

Figure 3.1. $MeSO_2$-PCB congener BAFs (±SD, error bars) for deepwater sculpin to burbot from Lake Huron. Plots are shown include BAFs generated on an ng/g lipid weight basis.

only possible for deepwater sculpin to burbot, and for Σ-OH-PCBs a BAF was only possible based on deepwater sculpin and burbot liver concentrations, as OH-PCBs were not detectable in whole body homogenates. The BAF for Σ-OH-PCBs of generally <1 (Table 3.2) indicates that OH-PCBs are not bioaccumulative from deepwater sculpin to

burbot, and would suggest that OH-PCBs found in both deepwater sculpin and burbot are formed from metabolic transformation of suitable PCB congeners within these organisms. Although there is a dearth of literature data, and although OH-PCBs have been shown to have log K_{OW} values that would tend to favour lipid accumulation, is unlikely that OH-PCBs have long enough biological half-lives and stability to accumulate in a predator-prey situation (Letcher *et al.* 2000).

The congener-specific BAFs for detected OH-PCBs in deepwater sculpin and burbot liver is shown in Figure 3.2, which was only possible for four congeners 4-OH-CB97, 4-OH-CB146, 4-OH-CB203 and 4'-OH-CB208 as these were quantifiable in both species (Chapter 2). Of the OH-PCB congeners in deepwater sculpin versus burbot (4-OH-CB97, 4-OH-CB146, 4-OH-CB203 and 4'-OH-CB208), no congener possesses *meta-para* chlorine-unsubstituted positions on the parent PCB phenyl rings. Only 4-OH-CB97 contains vicinal hydrogens, which were present in the *ortho-meta* positions. This suggests possibilities that OH-PCBs are formed via direct OH-insertion and metabolism in deepwater sculpin (Buckman *et al.* 2006; Campbell *et al.* 2003). The bioaccumulation of 4-OH-CB208 in burbot as well as the congeners detection in lake trout from Lake Superior, Lake Ontario and Lake Opeongo raises questions regarding the source of OH-PCBs are not expected to be formed by lake trout (Fisk *et al.* 2001). The 4-OH-CB146 in deepwater sculpin versus burbot may also be of concern as it shares similar structure to the thyroid hormones with the OH-group in the *para*-position (Letcher *et al.* 2000). Furthermore this congener has also been quantified in lake trout from Lake Superior, Lake Opeongo and Lake Ontario (Campbell *et al.* 2003; Fisk *et al.* 2001) and may also be

bioaccumulated through deepwater sculpin to lake trout in Lake Huron (Dobiesz *et al.* 2005) as well as Lake Michigan (Madenjian *et al.* 2002).

Despite the minimal number of congeners, OH-PCBs are clearly not biomagnified from deepwater sculpin to burbot as the BAFs are < 1. Significant statistical correlations (not shown) were not observed between BAFs (all < 1 from deepwater sculpin to burbot) generated for OH-PCBs and protein content, EROD activity and/or UDPGT activity, although negative relationships were observed for EROD and UDPGT activity for OH-CB97 and OH-CB208. This may indicate that oxidative PCB metabolism is likely occurring in both deepwater sculpin and burbot, and that any OH-PCBs generated in deepwater sculpin are not bioaccumulated in burbot. This is a plausible explanation since OH-PCBs are known to form from PCBs in fish. Buckman *et al.* (2005, 2006) demonstrated in PCB feeding studies with juvenile rainbow trout that this fish species is capable of PCB metabolism to OH-PCBs. Furthermore Li *et al.* (2003) reported OH-PCBs, and likely metabolites of accumulated PCBs, in the plasma of thirteen different species of benthic- and pelagic-feeding fish from the Detroit River. We may hypothesize therefore that deepwater sculpin from Lake Huron is definitely capable of metabolizing PCB congeners to both OH-PCB and $MeSO_2$-PCB metabolites since neither metabolite group was detectable in a major and representative prey (*diporeia hoyi*). However, OH-PCBs in burbot are also likely of metabolic origin from PCBs in burbot. However, the higher BAFs for $MeSO_2$-PCBs relative to OH-PCBs suggest that burbot can accumulate $MeSO_2$-PCBs from deepwater sculpin and/or metabolically form $MeSO_2$-PCBs from PCBs accumulated from deepwater sculpin.

BAF trends for chlorinated anthropogenic compounds BCPS (Table 3.2), PCP and

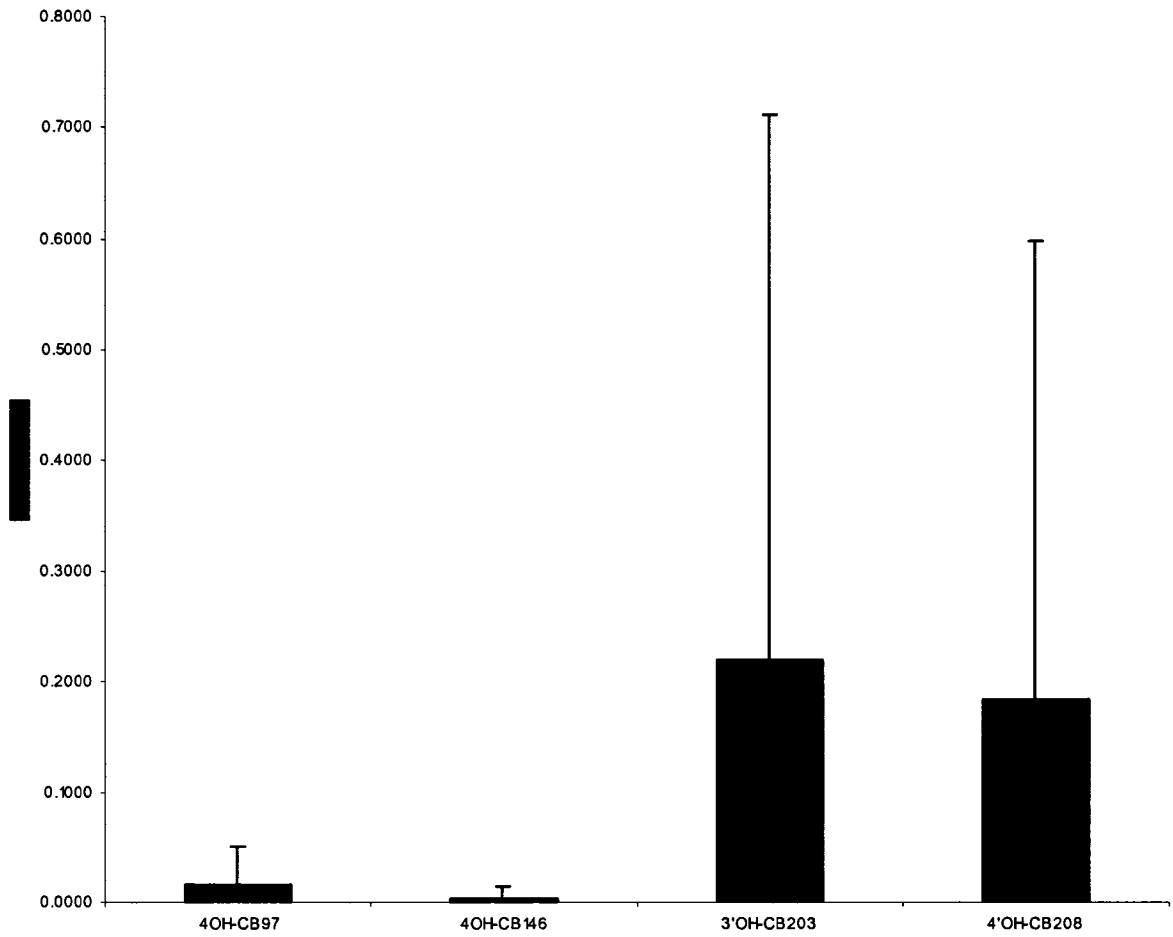

Figure 3.2. Congener-specific OH-PCB BAFs (mean ± SD, error bars) for deepwater sculpin and burbot from Lake Huron. BAFs were calculated based on wet weight contaminant concentrations.

4-OH-HpCS showed no significant differences ($p<0.05$) in BAFs or correlation of BAFs to catalytic activity from *diporeia hoyi* to deepwater sculpin and deepwater sculpin to burbot (not shown). BCPS was highly bioaccumulative and biomagnified in deepwater sculpin to burbot liver only (Table 3.2), which is consistent with the liver storage preference of $MeSO_2$-PCBs and 3-$MeSO_2$-p, p'-DDE.

(ii) PBDEs and OH-PBDEs

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Table 3.5 lists the BAFs of the brominated contaminants under study in the deepwater sculpin food chain from Lake Huron. The BAFs for Σ-PBDEs were around unity or <1 for either *diporeia hoyi* to deepwater sculpin or deepwater sculpin to burbot, and were comparable regardless of whether they were based on concentrations in liver or whole body homogenate. For *diporeia hoyi* to deepwater sculpin the BAFs for Σ-PBDEs were much lower than for Σ-PCBs (Table 3.2). One hypothesis is that in terms of PBDE exposure *diporeia hoyi* is not completely representative of the deepwater sculpin dietary regime. Alternately, deepwater sculpin may be capable of rapid and efficient metabolism or clearance of PBDEs accumulated from *diporeia hoyi*. In contrast, the deepwater sculpin to burbot BAFs for Σ-PBDEs and Σ-OH-PBDEs were comparable and similar to Σ-PCBs, Σ-OH-PCBs and Σ-$MeSO_2$-PCBs (Table 3.2).

On a congener-specific basis, BAFs for PBDE congeners are illustrated in Figure

Table 3.5. Mean bioaccummulation factors (BAFs) (±SD) for sum PBDEs, OH-PBDEs and total-(α)-HBCD in deepwater sculpin, *Diporeia hoyi* and burbot from Goderich in Lake Huron.

	Diporeia hoyi to Deepwater sculpin		Deepwater sculpin to Burbot	
	Homogenate	Liver	Homogenate	Liver
Σ-PBDE	1.6 ± 0.7	3.2 ± 1.2	1.4 ± 0.5	2.4 ± 2.9
Σ-OH-PBDE	n.d.	n.d.	n.d.	0.07 ± 0.05
total-(α)-HBCD	5.0 ± 3.2	4.6 ± 4.4	n.d.	0.2 ± 0.2

PBDE and HBCD BAFs were calculated on a lipid weight basis.
OH-PBDE BAFs were calculated on a wet weight basis.

3.3. BDE-47 demonstrated significantly greater BAFs ($p < 0.01$) from *diporeia hoyi* to deepwater sculpin then from deepwater sculpin to burbot (not shown). However no significant correlations were observed regarding any catalytic activity and BDE congener BAFs. Congener-specific BDE BAFs from the present Lake Huron deepwater sculpin to burbot (whole carcass or liver tissue based were similar) (Figure 3.3) were comparable to those found for pike from the River Viskan in Sweden (Sellstrom *et al.* 1998b) in which

BAFs for BDE–47 (6.6 to 19), BDE-99 (17) and BDE–100 (4.6 to 36) were determined. Levels in zebrafish that were chironimid larvae fed (treated with several BDE congeners) demonstrated highest accumulation for BDE-47, with –28, -100, -153 and –154 (Andersson *et al.* 1999). BDE-99 was not found to accumulate in this previous study, which is in agreement with the *diporeia hoyi* to deepwater sculpin results in the present study. In fact our results are also consistent with the results of Stapleton *et al.* (2004) who reported non-detectable levels of BDE-99 in deepwater sculpin (whole body homogenates) from Lake Michigan. Stapleton & Baker (2004) suggest that deepwater sculpin might be capable of metabolic debromination of BDE-99. Accumulation of BDE-99 compared to the Σ-PBDE occurred in high amounts in *diporeia hoyi* (27.65%) but very little in deepwater sculpin (3.88%) and burbot (11.50%). No BDE-99 was observed in deepwater sculpin liver tissue, but found to relate to lipid content and trophic level in burbot, which may be due to its top predatory position (Paakkonen *et al.* 2005).

Individual BDE congener BAFs were also found to be comparable to BAFs generated for lake trout relative to spiked food in a captive fish study (Tomy *et al.* 2004), however they were orders of magnitude lower than BMFs generated for Baltic Sea guillemot and grey seal (Sellestrom 1996). The low BDE-47 biomagnification in the carcass was found to not be comparable to levels in herring, sprat and salmon from the Baltic (Burreau *et al.* 1999; Burreau *et al.* 2000b). The lack of BDE-47 biomagnification may be attributed to debromination and oxidative metabolism (Stapleton *et al.* 2004) occurring in deepwater sculpin.

Similar to Σ-OH-PCB BAFs (Table 3.2), the mean BAF for Σ-OH-PBDEs (Table 3.5 was about unity or <1. The BAF for Σ-OH-PBDEs of generally <1 indicates that OH-PBDEs are not very bioaccumulative from deepwater sculpin to burbot, and would

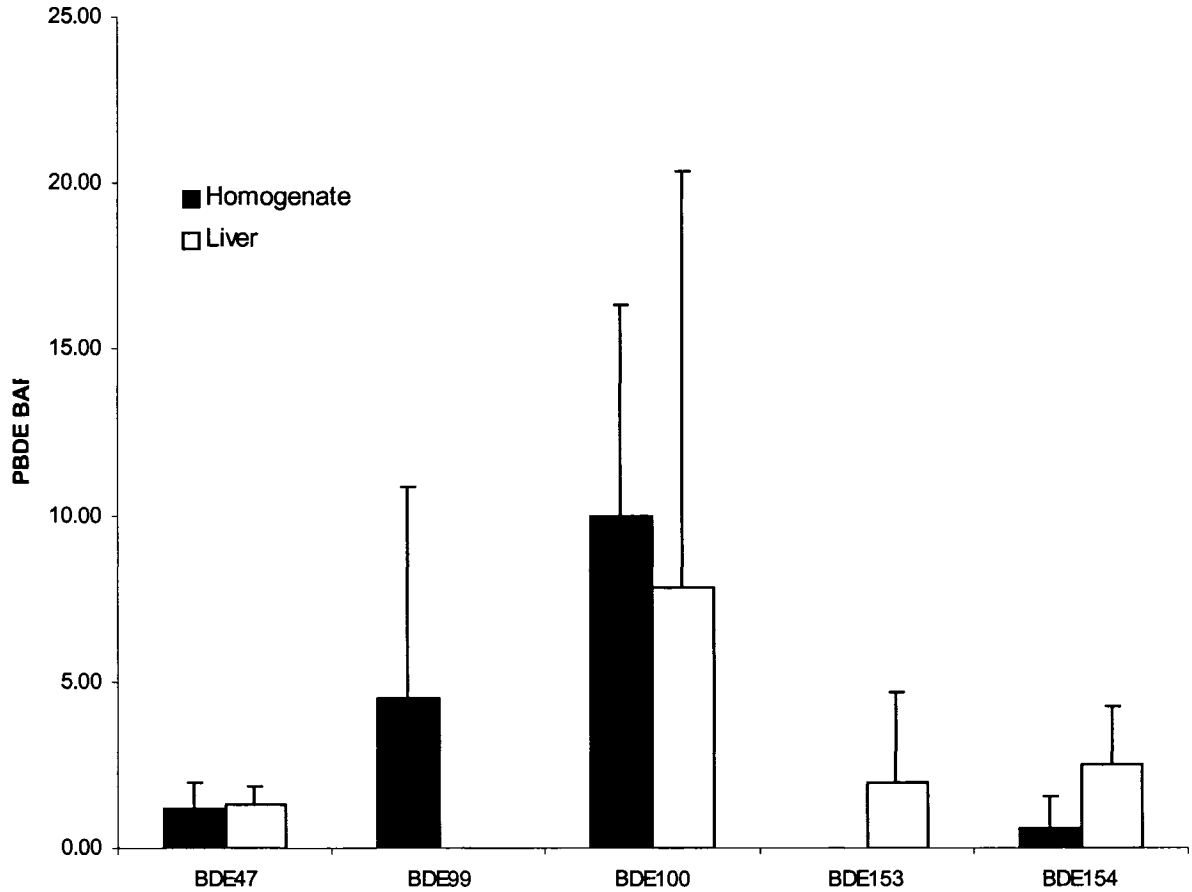

Figure 3.3. Mean BAFs (±SD, error bars) (liver and whole body based) for BDE congeners in deepwater sculpin and burbot from Lake Huron.

suggest that OH-PBDEs found in both deepwater sculpin and burbot could be formed from metabolic transformation of suitable BDE congeners within these organisms. Although there is a dearth of literature data, OH-PBDEs have been reported in the plasma

of Detroit River fish, but are not likely bio-concentrated from water via gill uptake (Valters *et al.* 2005; Hua *et al.* 2005), but occur from PBDE metabolism.

Figure 3.4 shows congener specific BAFs of detected OH-BDE congeners. As described in Chapter 2, OH-BDE congeners were found in both deepwater sculpin and burbot liver tissue. Trends in BAFs showed that those for OH-BDE congeners or Σ-OH-PBDEs were comparable to OH-PCB congeners and Σ-OH-PCBs (Table 3.2, Figure 3.2), with the exception of 5-OH-BDE47, which has a mean BAF <1. In a previous PBDE metabolism study with captive northern pike, ^{14}C-labeled BDE-47 was administered to brown trout and fed to pike (Burreau *et al.* 2000). Selective retention of six metabolites were observed in each tissue studied in a repeat of the experiments of Burreau *et al.* (2000), where Kierkegaard *et al.* (2001) identified 2-OH-2',4,4',6-tetraBDE and 2-OH-2',3,4,4'-tetraBDE metabolites in BDE-47 treated pike. Congener BDE-47 was deemed to be easily accumulated in pike and not readily metabolized because of the high lipophilic nature of BDE–47.

In the present study, for Deepwater sculpin and burbot liver, EROD activity showed a significant negative relationship ($p < 0.05$, -0.90) and UDPGT a positive relationship ($p > 0.05$, 0.40) with the BAFs for Σ-OH-PBDE. This would suggest that there are different oxidative metabolic pathways at work for $MeSO_2$-PCBs and OH-PCBs/OH-PBDEs. Other previous studies that have examined the metabolic formation of OH-PBDEs have been restricted to a study in rat microsomes and in dosed pike (Meerts *et al.* 2000; Burreau *et al.* 2000; Hakk & Letcher 2003).

With a BAF of ~5, total-(α) HBCD appeared to be biomagnified from *diporeia hoyi* to deepwater sculpin (Table 3.5). However, with a mean BAF <1 there was no

apparent total-(α) HBCD biomagnification from deepwater sculpin to burbot. Assumptions are made of course that these are the primary predator-prey relationships for this food chain. The mean BAF for *diporeia hoyi* to deepwater sculpin from Lake Huron was comparable to that of α–HBCD (the major HBCD isomer) in *diporeia hoyi* to slimy

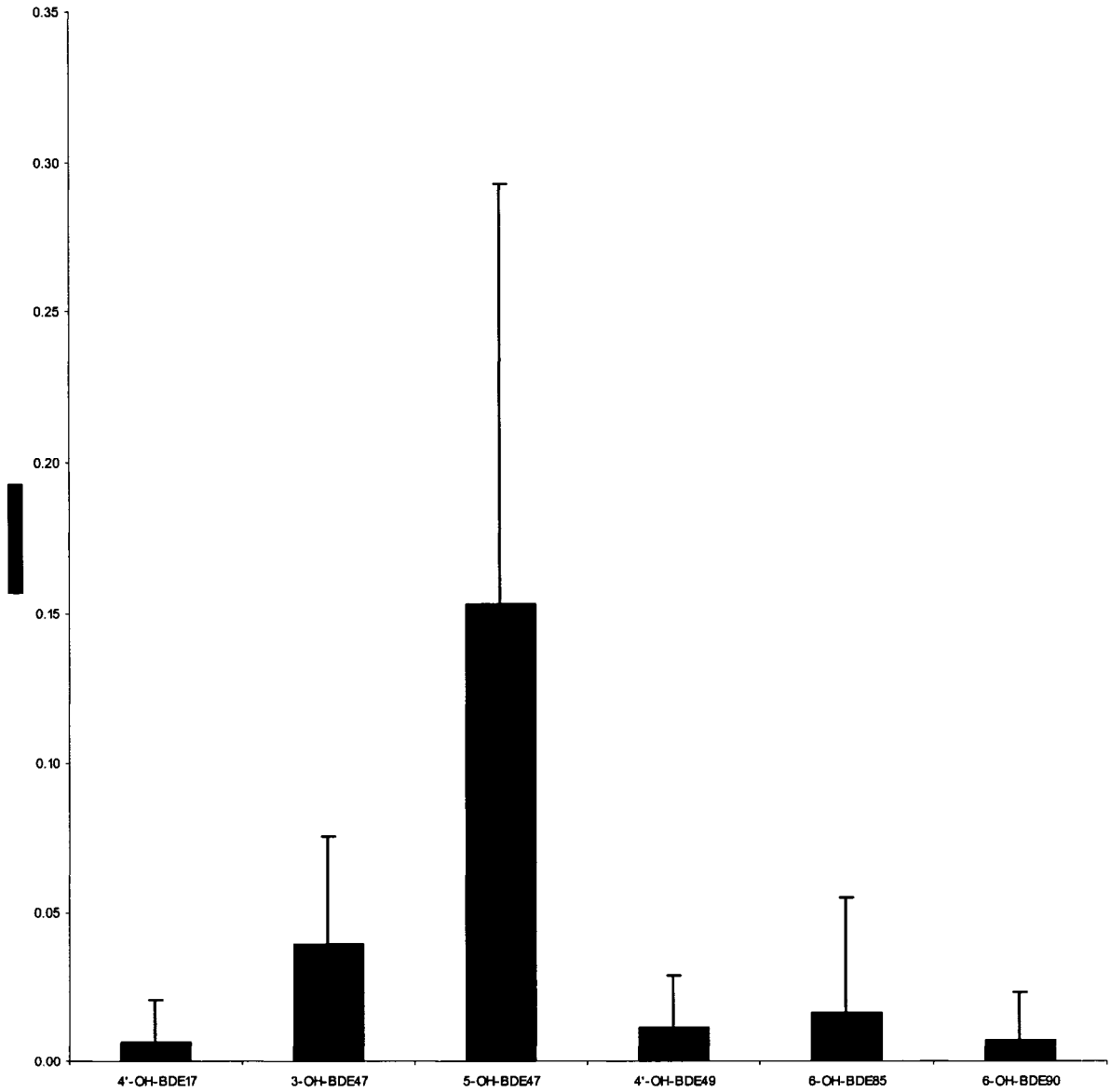

Figure 3.4. Mean, congener-specific BAFs (±SD, error bars) for OH-BDEs for deepwater sculpin to burbot (liver) from Lake Huron.

sculpin *(Cottus cognatus)* (mean BAF = 3.5) from Lake Ontario (Tomy *et al.* 2004). Also similar to the present deepwater sculpin to burbot BAF, Tomy et al. (2004) reported a slimy sculpin to lake trout (Lake Ontario) mean BAF 1.1 for α–HBCD. Although a clear conclusion could not be derived, Tomy *et al.* (2004) suggested that future research should be pursued to examine the bioaccumulation parameters of individual HBCD isomers in fish to try and understand the behavior and fate of isomers, and to test the possibility of biotransformation and isomerization. It is possible that the present deepwater sculpin and burbot can debrominated HBCDs, and metabolize HBCDs via CYP enzyme mediation to OH-HBCDs (Covaci *et al.* 2006).

3.4 Conclusions

The present study examined the bioaccumulation and biotransformation of PCBs and several classes of legacy and emerging, chlorinated and brominated contaminants in the *diporeia hoyi*-deepwater sculpin-burbot food chain from Lake Huron. The present study was pursued as PCB and PBDE precursor accumulation patterns in previous reports suggested that the deepwater sculpin is an anomaly among Lake Michigan fish in that it appeared to be capable of a higher rate of enzyme-mediated depletion of certain congeners (Letcher *et al.* 2000; Stapleton *et al.* 2001; Stapleton & Baker 2003).

Several main conclusions can be drawn regarding the contaminant bioaccumulation in relation to catalytic, hepatic CYP1A1 (Phase I) and UDPGT (conjugative Phase II) enzyme activity in deepwater sculpin and burbot. Firstly, significantly different CYP1A1 and UDPGT activities suggested that the bioaccumulation of e.g., selected PCB and PBDE congeners would be decreased, and

known and apparent $MeSO_2$- and OH-containing metabolites would be increased in Lake Huron deepwater sculpin relative to the burbot as predator.

We assessed BAFs among various legacy and emerging, chlorinated and brominated compounds in the *diporeia hoyi*-deepwater sculpin-burbot predator-prey relationship. Based on our findings, and in relation to catalytic enzyme activities, we hypothesize that deepwater sculpin from Lake Huron are capable of metabolizing PCB congeners to both OH-PCB and $MeSO_2$-PCB metabolites since neither metabolite group was detectable in a major and representative prey (*diporeia hoyi*). OH-PCBs in burbot are also likely of metabolic origin from PCBs in burbot. The higher BAFs for $MeSO_2$-PCBs relative to OH-PCBs suggest that burbot can accumulate $MeSO_2$-PCBs from deepwater sculpin and/or possibly metabolically form $MeSO_2$-PCBs from PCBs accumulated from deepwater sculpin. Similar to OH-PCBs, OH-PBDE BAFs indicate that OH-PBDEs are not very bioaccumulative from deepwater sculpin to burbot, and would suggest that OH-PBDEs found in both deepwater sculpin and burbot could be formed from metabolic transformation of suitable BDE congeners within these organisms. The low BAF for total-(α)-HBCD between fish species also suggests that metabolism may also be occuring in both species; however further research will be required to verify this.

The halogenated phenolics, OH-PCBs, OH-PBDEs, PCP and 4-OH-HpCS, were found to be non-detectable in whole body homogenate of both species, which would indicate that they are not bioaccumulative relative to PCBs, PBDEs, HBCDs and $MeSO_2$-PCBs. Significant catalytic differences between deepwater sculpin and burbot suggest that two-phase metabolism may be involved in deepwater sculpin and direct insertion may be the biotransformation process in burbot. Moreover the biomagnification of

$MeSO_2$-PCBs (in particular higher chlorinated congeners) from deepwater sculpin to burbot and lack thereof for HPCs (OH-PCBs, OH-PBDEs, PCP and 4-OH-HpCS) suggests that both fish species possess oxidative metabolism capacities.

Finally, the bioaccumulation of these chlorinated and brominated biotransformation products and novel organohalogen compounds (BCPS and HBCDs) presents new contaminant concerns in Great Lakes aquatic food webs. The bioaccumulation of $MeSO_2$-PCBs in burbot would suggest that other predators such as lake trout feeding on deepwater sculpin (Dobiesz *et al.* 2005; Bronte *et al.* 2003; Madenjian *et al.* 2003) would also accumulate sulfones. Furthermore, the high BCPS BAFs from *Diporeia hoyi* to deepwater sculpin and deepwater sculpin to burbot indicates that BCPS is bioavailable and bio- concentrates in benthos before biomagnifying itself through fish food webs.

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CHAPTER IV

GENERAL CONCLUSIONS AND FUTURE DIRECTIONS

4.1 Conclusions

In this thesis, the identity, composition and bioaccumulation was elucidated of legacy and emerging, chlorinated and brominated contaminants and their metabolic/degradation by-products in the deepwater sculpin food chain from Lake Huron. Several main conclusions can be drawn. Firstly, the non-detection of any oxidative metabolites in *Diporeia hoyi* suggests that these contaminants are metabolically formed in deepwater sculpin and/or burbot. Secondly, chlorinated and brominated parent compound accumulation patterns suggest that deepwater sculpin from Lake Huron are consistent with e.g., Lake Michigan in that the species possessed unusual metabolic capacities in the degradation of selected PCB and PBDE congeners, and the formation of OH- and $MeSO_2$-containing metabolites that may or may not be accumulating between deepwater sculpin and burbot.

PCB and PBDE parent compound patterns in deepwater sculpin are different then those in *Diporeia hoyi* or burbot that reflect different oxidative enzyme systems are at work while also demonstrating parent compound metabolic depletion. *Diporeia hoyi* are abundant in Lake Huron and a main food source for deepwater sculpin thus depleted $MeSO_2$-PCB precursor congeners and increased dominance of recalcitrant PCBs in deepwater sculpin and subsequent lack there of in *Diporeia hoyi* and/or burbot indicates deepwater sculpin's ability for biotransformation of PCBs/PBDEs. Furthermore, the lack of BDE-99 in deepwater sculpin but presence in burbot and *Diporeia hoyi* may be indicative of BDE congener debromination metabolism in deepwater sculpin and burbot.

Moreover, the low HBCD bioaccumulation between fish species suggests that HBCD metabolism may also be occuring in both species.

The biomagnification of $MeSO_2$-PCB metabolites and lack thereof for all HPC's suggests that oxidative metabolism is occuring in both species and that deepwater sculpin possess both of these pathways. Metabolic formation of $MeSO_2$-PCBs most likely only occurs in deepwater sculpin and accumulated to burbot. The major metabolic pathway of metabolite formation in deepwater sculpin seems to be the mercaptic acid pathway. Higher accumulation of $MeSO_2$-PCBs in liver of burbot relative to whole carcass further confirms that its source of sulfones. Furthermore, the detection of HPCs in both deepwater sculpin and burbot and subsequent lack of bioaccumulation suggests that these metabolites are formed in both fish species. Lack of detection in whole carcass and the low levels in liver tissue indicate that HPCs are not as bioaccumulative as parent compounds and $MeSO_2$-PCBs.

The dynamics of these chlorinated and brominated metabolites and novel organohalogen compounds (BCPS, HBCD, PCP and 4-OH-HpCS) presents new contaminant concerns in Great Lakes aquatic food webs. The bioavailablity of these contaminants and metabolites in Lake Huron also will bring about potential fish toxicological implications. The ecological role of deepwater sculpin as a forage fish in the Great Lakes may bring about secondary contaminant channels to other piscivorous fish species at higher trophic levels. The finding of detectable and biomagnified concentrations of sulfones in burbot who act as food for top bird predators and scavengers may also pose concerns among terrestrial ecosystems. Contamination of lake

trout and salmon fisheries may also bring about a human health concerns in relation to fish consumption.

4.2 Future Direction

There were several possible directions of research emerged from this thesis. They are as follows:

1. In vitro metabolism of PCB and PBDE congeners in Deepwater sculpin and Burbot

The *in vitro* metabolism of particular PCB and PBDE congeners in deepwater sculpin and burbot would have strongly confirmed formation of these metabolically derived biotransformation products. Investigation of the differing accumulation patterns of PCBs and PBDEs in this food chain would strengthen conclusions surrounding the deepwater sculpin and its metabolic capacity. Not only will relations between recalcitrant and precursor PCB congeners be more clearly defined, but also further examination into de-bromination metabolism would be accomplished. The absence of BDE-99 in deepwater sculpin liver tissue but presence and bioaccumulation in burbot is highly suggestive of metabolism similar to previous *in vitro* findings in carp, however without *in vitro* confirmation that this is occuring in Lake Huron deepwater sculpin. Thus *in vitro* analysis of these congeners will determine toxicological elimination patterns among parent congeners.

2. Full Catalytic Assessment of Hepatic Cytochrome P450s in Deepwater sculpin and Burbot in Comparison to Other Aquatic Species in its Food Web

Assessment and characterization of hepatic cytochrome P450 enzyme subfamilies in deepwater sculpin and burbot in relation to other organisms in its food web would be

beneficial in determining metabolic activity hotspots (presumed to be in the deepwater sculpin) throughout the food web. Catalytic activity data from our study is limited in interpretation because it is represented on a relative basis. Without any comparison to other foodweb members, data compared to other locations is not indicative of what is going on in the Lake Huron foodweb. Immuno-quantification and characterization of CYP enzymes found to be active in both deepwater sculpin and burbot will clarify enzymatic pathways and interspecies differences.

3. The Assessment of Persistent Legacy and Novel Chlorinated and Brominated Organohalogen Contaminants and their Biotransformation Products in the Lake Huron Aquatic Food Webs

Firstly moving upwards in trophic position, more work must be done in determining organohalogen biotransformation product levels in abiotic sources from Lake Huron as well as benthic invertebrates. Although *Diporeia hoyi* is the highest energy and most abundant macro-invertebrate, declines in its abundance makes other invertebrates such as *Mysis relicta* contaminant sources for the deepwater sculpin. Secondly, it would be advantageous to track metabolites throughout this food web in order to determine which fish species potentially bio-accumulate $MeSO_2$-PCBs in Lake Huron. Furthermore, knowledge of composition of predator fish diet would be advantageous in determining the distribution of $MeSO_2$-PCBs throughout the foodweb. Moreover, the detection of tetrabromo-bis-phenol-A in the phenolic fraction during initial screening should be continued. Finally, ^{15}N stable isotope analysis throughout the foodweb would help to better understand the ecodynamics of these contaminants in Lake

Huron, which has different ecological pressures then Lake Michigan or Lake Superior in which several stable isotopic analysis have been performed.

4. LC-MS Determination of the Different HBCD Isomers in Deepwater sculpin and Burbot

The lack of HBCD biomagnifications from deepwater sculpin to burbot also suggests may point towards deepwater sculpin metabolism of the μ-isomer and explain the high concentrations of α-isomer. Further LC-MS analysis of deepwater sculpin and burbot samples for the HBCD stereo isomers may reveal whether deepwater sculpin can metabolically transform μ-HBCD to α-HBCD and also determine BAF's to burbot, potentially determining whether the introduction of HBCD metabolites into the Lake Huron food web.

5. Genetic Similarities/Differences Between Enzyme Systems of Deepwater sculpin and Other Sculpin's Present in the Great Lakes System

Previous work surrounding the accumulation patterns of PCBs in other species of sculpin demonstrated that intra-genus differences might be involved. It would be interesting to see how similar the sculpin species are genetically, especially with respect to their cytochrome P450 and other xenobiotic metabolizing enzyme families. With deepwater sculpin being the freshwater cousin of the four-horned sculpin found in marine environments similarities between these species and their cytochrome P450 enzyme system would be expected and different to that of the slimy sculpin.

VITA AUCTORIS

Gianfranco Scipione was born in 1980 in Windsor, Ontario. He graduated from St. Thomas of Villanova Secondary School in 1999. From there he went on to the University of Windsor Ontario where he obtained a B.Sc. in Chemistry and Biochemistry in 2003. He is currently a candidate for the Master's degree in Environmental Science at the University of Windsor's Great Lakes Institute for Environmental Research and hopes to graduate in Fall 2006 as well as an M.B.A./L.L.B. candidate at the University of Windsor.