ORGANOHALOGENATED PERSISTENT ORGANIC POLLUTANTS IN AMERICAN EEL (*ANGUILLA ROSTRATA*) CAPTURED IN EASTERN CANADA

by

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Abstract

Recruitment of American eels (*Anguilla rostrata*) to Lake Ontario has declined rapidly over the past few decades. The commercial yellow eel fishery in Lake Ontario was closed in 2004 due to a lack of eel abundance. Researchers have been attempting to ascertain the reasons for the decline, although thus far, without definitive answers.

In this thesis, the question of chemical contamination is addressed as it relates to female eel spawner quality. Spatial concentration trends of halogenated persistent organic pollutants (POPs) are described in eels collected from across eastern Canada, as well as temporal concentration trends in eels collected from a historically important area of northeastern Lake Ontario, Canada. Chlorinated POPs in eels, namely, organochlorinated pesticides, polychlorinated biphenyls, polychlorinated dibenzo-*p*-dioxins and dibenzofurans are all significantly less than historic values which peaked in the 1960-70s. Measured concentrations of chlorinated POPs in eels from Lake Ontario have decreased by up to 3-fold over the past three decades, and exceeded toxicity thresholds historically for surrogate species (European eel and lake trout). Thus, chlorinated POPs may have had an effect on spawner quality. Concentrations of legacy POPs in eels were dependent on their origin, with eels from highly urbanized and industrialized areas having significantly higher concentrations than eels captured in less developed regions. Similar trends were observed for polybrominated diphenyl ethers and chlorinated norbornene flame retardants. A number of emerging brominated compounds were also measured in these eels by non-target analysis including bromophenols, bromobenzenes, and bromoanisoles. This thesis demonstrates that eels are an ideal species to investigate local sources of pollution, and provide chemical data that may be used in the future, when more toxicity information is available for eels, to assess the health risks posed by accumulated chemical contaminants.

Preface

Beginning in November 2007, Dr. Peter Hodson, Department of Biology and School of Environment Science, Queen's University, and a group of about 20 scientists from several Canadian universities and government agencies, addressed the issue whether chemical contaminants are contributing to the recruitment failure of American eel. This research was funded mainly through a Natural Sciences and Engineering Research Council of Canada strategic grant. For this research study, I was, in part, responsible for providing the chemical characterization of all the eels sampled. This thesis begins with a review of chemical contaminants likely to accumulate in American eels, before focusing on the results of the chemistry related aspects of the NSERC project, considering four main null hypotheses: first, that there are no temporal trends in concentrations of persistent organic pollutants in eels or dioxin-like toxicity; second, that there are no geographical differences in concentrations of persistent organic pollutants in adult eels; third, that concentrations of persistent organic pollutants in adult eel do not exceed published thresholds for toxicity to eels or surrogate species; and lastly, that there are no other compounds of concern that accumulate in eels. The chemical characterization of contaminants in eels evolves throughout this thesis, beginning with legacy compounds that have well established analytical methods to new or emerging compounds, which require some method development, to unknown compound identification using novel analytical techniques for non-target and post-target analysis.

Acknowledgements

It is a pleasure to thank my promoters, Dr. Mehran Alaee and Dr. R. Stephen Brown, whose guidance and support from not merely a scientific perceptive has been invaluable. I am grateful for Dr. Peter Hodson, who allowed me to become a part of this project, and for all the hours spent answering my e-mails and editing whatever I sent his way. I would like to thank the remaining member of my committee, Dr. Philip Jessop, for his time and suggestions throughout my graduate studies.

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To the other two members of the musketeers, Patrick Cashin and Elize Ceschia, thank you for your friendship and encouragement, especially leading up to my comprehensive exam. I only wish that I could have returned the favour!

I owe my deepest gratitude to my family; first and foremost, to my parents for their unconditional love and support throughout the years. You always told me that I could accomplish anything I wanted to, and I guess this is proof. To my Aunt Eileen and Uncle Lamar, you have been there throughout my undergraduate and graduate studies, whether it was taking me out for a birthday dinner when the rest of my family was miles away, or driving me up north for holidays; I am very grateful. Finally, and most importantly, I thank my wife, Jeanine, who has supported me both emotionally and financially. We have had quite the journey together thus far, and I look forward to many more years to come.

Co-Authorship

This manuscript style thesis contains a number of chapters (2-5) that have been submitted or accepted for publication in peer-reviewed journals as research articles. The submitted chapters were revised based on suggestions and comments from the following core co-authors: Dr. Peter V Hodson, Dr. Michel Lebeuf, Dr. Mehran Alaee and Dr. R Stephen Brown, as well as from the other co-authors listed in the manuscripts below.

List of manuscripts submitted or accepted for publication

Byer, J. D., Lebeuf, M., Trottier, S., Raach, M., Alaee, M., Brown, R. S., Casselman, J., and Hodson, P. V. (2013) Trends of POPs (1988-2008) and potential effects on American eel (*Anguilla rostrata*) from Lake Ontario, Canada. J Great Lakes Res. *Submitted*.

Byer, J.D., Lebeuf, M., Alaee, M., Brown, R.S., Trottier, S., Backus, S., Keir, M., Casselman, J., and Hodson, P. V. (2013) Spatial trends of organochlorinated pesticides, polychlorinated biphenyls, and polybrominated diphenyl ethers in Atlantic anguillid eels. Chemosphere. 90, (5), 1719-1728.

Byer, J.D., Alaee, M., Brown, R.S., Lebeuf, M., Backus, S., Keir, M., Pacepavicius, G., Casselman, J., Olivera, K., Belpaire, C., Verreault, G., and Hodson, P.V. (2013) Spatial trends of dioxin-like compounds in Atlantic eels. Chemosphere. 91, (10), 1439-1446.

Byer, J.D., Lebeuf, M., Alaee, M., Brown, R.S., Backus, S., Pacepavicius, G., Marvin, C.H., MacInnis, G., Casselman, J., and Hodson, P. V. (2013) Historical concentrations (1988-2008) of emerging halogenated flame retardants in American eel captured in Eastern Lake Ontario. Environ Sci Technol. *Submitted*.

Statement of Originality

I hereby certify that all of the work described within this thesis is the original work of the author. Any published (or unpublished) ideas and/or techniques from the work of others are fully acknowledged in accordance with the standard referencing practices.

Jonathan D Byer

May, 2013

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List of Abbreviations

ABS:	Acrylonitrile butadiene styrene
AhR:	Aryl hydrocarbon receptor
ANOVA:	Analysis of variance
ATE:	Allyl 2,4,6-tribromophenyl ether
BAF:	Bioaccumulation factor
BATE:	2-Bromoallyl 2,4,6-tribromophenyl ether
BB-153:	2,2',4,4',5,5'-Hexabromobiphenyl
BCF:	Bioconcentration factor
BE:	Canal Dessel-Schoten, Belgium
BFR:	Brominated flame retardant
BSAF:	Biota-sediment accumulation factor
BTBPE:	1,2-bis(2,4,6-tribromophenoxy)ethane
CFR:	Chlorinated flame retardant
CHL:	Compounds related to chlordane
CP:	Chlordene Plus
CRM:	Certified reference material
DBDPE:	Decabromodiphenyl ethane
DDD:	1,1-Dichloro-2,2-bis(p-chlorophenyl)-ethane
DDE:	2,2-Bis(p-chlorophenyl)-1,1-dichloroethylene
DDT:	1,1,1-Trichloro-2,2-di(4-chlorophenyl)ethane
Dec:	Dechlorane
DFO-ISB:	Department of Fisheries and Oceans, Inspection Service Branch
DLC:	Dioxin-like compound

dl-PCB:	Dioxin-like polychlorinated biphenyl
DP:	Dechlorane Plus
DPMA:	1,5-Dechlorane Plus mono-adduct
DPTE:	2,3-Dibromopropyl-2,4,6-tribromophenylether
ECNI:	Electron capture negative ionization
EI:	Electron ionization
FR:	Flame retardants
FWHM:	Full width at half maximum
GC:	Gas chromatography
HBB:	Hexabromobenzene
HBCD:	Hexabromocyclododecane
HCB:	Hexachlorobenzene
HCDBCO:	(1R,2R,5R,6R,9S,10S)-5,6-dibromo-1,10,11,12,13,13 hexachloro-tricyclo[8,2,1,0(2,9)]-tridec-11-ene
HCH:	Hexachlorocyclohexane
HFR:	Halogenated flame retardant
HRMS:	High resolution mass spectrometry
KAM:	Kamouraska, Quebec
Kow:	Octanol-Water Partition Coefficient
LC:	Liquid chromatography
LO:	Lake Ontario
LOD:	Limit of detection
LOQ:	Limit of quantitation
MOE:	Ontario Ministry of Environment

mo-PCB: Mono-ortho polychlorinated biphenyl

MS: Mass spectrometry

MSD: Mass selective detector

NB: Miramichi River, New Brunswick

no-PCB: Non-ortho polychlorinated biphenyl

NRC: Research Council of Canada

NS: Margaree River, Nova Scotia

NY: Hudson River, New York

OBIND: 4,5,6,7-Tetrabromo-1,1,3-trimethyl-3-(2,3,4,5-tetrabromophenyl)-indane

OCP: Organochlorine pesticide

PBB: Polybrominated biphenyl

PBBz: Pentabromobenzene

PBDD: Polybrominated dibenzo-*p*-dioxin

PBDE: Polybrominated diphenyl ether

PBDF: Polybrominated dibenzofuran

PBEB: Pentabromoethylbenzene

PBT: Pentabromotoluene

PC: Principal component

PCA: Principal component analysis

PCBs: Polychlorinated biphenyl

PCDD: Polychlorinated dibenzo-*p*-dioxin

PCDF: Polychlorinated dibenzofuran

PCN: Polychlorinated naphthalenes

PFTBA: Perfluorotributylamine

POP:	Persistent organic pollutant
pTBX:	2,3,5,6-Tetrabromo-p-xylene
PVC:	Polyvinyl chloride
RCF:	Le Cren's relative condition factor
REACH:	Registration, Evaluation, Authorisation, and Restriction of Chemical substances
RO:	Rivière Ouelle
RSO:	Rivière du Sud-Ouest
SIM:	Selected ion-monitoring
SLR:	St. Lawrence River
TBB:	2-ethylhexyl-2,3,4,5-tetrabromobenzoate or EHTeBB
TBBPA:	Tetrabromobisphenol-A
TBP:	2,4,6-Tribromophenol
TBPH:	Bis(2-ethyl-1-hexyl)tetrabromophthalate or BEHTBP
TCDD:	2,3,7,8-Tetrachlorodibenzo-*p*-dioxin
TCP:	Class of tris(4-chlorophenyl)methanol and tris(4-chlorophenyl)methane
TCPM:	Tris(4-chlorophenyl)methanol
TCPMe:	Tris(4-chlorophenyl)methane
TEF:	Toxic equivalency factor
TEQ:	Toxic equivalent
ToF-MS:	Time-of-flight mass spectrometry
WS:	Water solubility
ww:	Wet weight

Chapter 1
Introduction

1.1 The predicament of eels

American eel (*Anguilla rostrata*) are not exactly a poster child for threatened species in Canada, with their snakelike body covered in mucous, and their far offshore migration. Most Canadians are probably unaware of their existence, unless they have ordered *unagi* from a sushi restaurant or were involved with the now all but defunct eel fisheries in Canada. In truth, eels are not typically considered to be a part of the Canadian culture today, which disregards the long history of eel fishery subsistence by Aboriginal peoples and early European settlers recorded back to the 1600s.

Freshwater eel (*Anguilla sp.*) have complex life cycles, and because of their far offshore migration and the fact that spawning has not yet been observed directly, except for Japanese eel [1, 2], they are poorly understood. Nonetheless, eels are known to develop through five principal life cycle stages, leptocephalus, glass eel, pigmented eel or elver, yellow eel, and silver eel stages [3, 4].

Eel stocks worldwide have experienced dramatic and unsustainable declines in recent years throughout most of their geographic distribution. This is most evident for three relatively well monitored anguillid species: Japanese eel (*Anguilla japonica*), European eel (*A. anguilla*), and American eel (*A. rostrata*) (Figure 1-1), which have seen a reduction in recruitment by up to 99 percent over the past few decades [5-7].

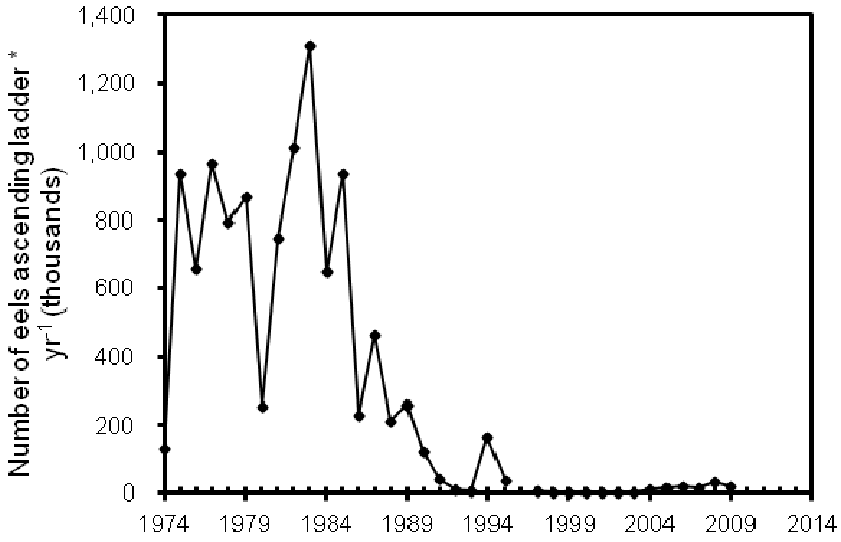


Figure 1- 1. Total number of juvenile yellow American eels ascending the eel ladder at the Moses-Saunders Hydroelectric Dam, Cornwall, Ontario; no counts were available for 1996. Plotted using data from the Ontario Ministry of Natural Resources [8].

European eel have been listed as 'critically endangered' [9] and Japanese eel are considered 'vulnerable' by the International Union for Conservation of Nature. American eel were recommended as a species of 'special concern' by the Committee on the Status of Endangered Wildlife in Canada in 2006, and upgraded to 'threatened' in 2012 [3, 10]. Accordingly, there has been an increasing amount of eel related research, particularly for European eel, as indicated by the number of publications available. There are reports which suggest that a variety of anthropogenic and environmental factors such as over-fishing [5, 11], pollution [12-14], habitat deterioration [15, 16], parasites and diseases [17-20], climatic/oceanic change [21-23], and changing predator-prey dynamics are contributing to reduced recruitment [24, 25]. However, despite these efforts, the exact reasons for the declines remain unidentified.

Since 1995, researchers have focused increasingly on spawner and recruit quality as the central link to eel decline. The reduction in migrating silver eel quality may be characterized as the impairment of an adult eel's ability to successfully migrate to the Sargasso Sea and spawn,

and the decreased viability of offspring attempting to return to continental waters, i.e. recruitment [26]. Eels have several physiological and ecological characteristics that make them susceptible to accumulating contaminants, including relatively high lipid content, longevity, diverse dietary habits, and the ability to inhabit a variety of aquatic environments [3]. These habitats include freshwater and estuarine ecosystems that are often the centre of industrial and urban development, and the recipient of chemical pollutants. In particular, hydrophobic persistent organic pollutants (POPs) accumulate to a significant extent in the fat tissue of eels [12, 27-31]. A number of studies have documented effects caused by hydrophobic contaminants in eels, such as impaired embryonic development [14, 32-36]. It has also been suggested by Belpaire et al. [27] that the accumulation of contaminants by eels may cause a reduction in their lipid content, resulting in impaired spawner quality [37]. However, a direct relationship between the reported effects and a population level response has not yet been established.

Legacy POPs such as organochlorine pesticides (OCPs), polychlorinated biphenyls (PCBs), polychlorinated naphthalenes (PCNs), and polychlorinated dibenzo-*p*-dioxins and dibenzofurans (PCDD/Fs) have been reported to be toxic and bioaccumulative in fish and wildlife, and as such pose a possible health risk to eels [38-43]. In particular, 17 PCDD/Fs and four of the dioxin-like PCBs (dl-PCBs) known as ‘dioxins’ are recognized to be highly toxic to fish and to share a common mode of action mediated by interaction with the aryl hydrocarbon receptor (AhR) protein [44, 45]. The significance of this mode of action to the population biology of fish was most notably highlighted by Cook et al. [46] who concluded that the complete extirpation of lake trout from Lake Ontario in the mid-1900s could be explained by AhR-mediated early life stage toxicity.

Environmental concentrations of many classes of chlorinated compounds, including the ones listed above, have been declining over the past three decades, mainly due to stricter

environmental regulations. These compounds could still play a role in the decline of the eel population because of the longevity of eels. Over the same time period, new contaminant classes have emerged such as organohalogenated flame retardants, several compounds of which are persistent, bioaccumulative, and associated with toxic effects in fish and wildlife. For example, polybrominated diphenyl ethers (PBDEs) have been produced at high volumes for the last thirty years, and have been banned recently (2006) by many governments worldwide [47, 48]. Furthermore, in 2010 Howard and Muir [49] identified 610 chemicals used in commerce that have the potential to be persistent and bioaccumulative. Consequently, if POPs are affecting the quality of spawning eels, the potential causes are vast and ever changing.

American eel are the only known *Anguillidae* in North America [2] and will be the species of interest in this thesis. However, information not readily available for American eel will be inferred, where possible, from European eel studies due to their physiological similarities and overlapping spawning sites, illustrated by the occurrence of hybrids [50, 51]. They also have a shared characteristic of exposure to dioxin-like compounds and other contaminants.

1.2 Population status and management

Recruitment of American eel to Lake Ontario has diminished dramatically, with more than a 98% decline from 1982-1992 (Figure 1-1). The St. Lawrence River and Lake Ontario eel stock, the group of eels inhabiting Lake Ontario, the St. Lawrence River, and their tributaries, was once thought to represent about 5% of the entire American eel species, and about 19% of the fecund eel population [7, 11]. The term "stock" within the concept of panmixia has been defined previously for American eels (Castonguay et al., 1994a). Silver eels migrating from this region are large in size, have high fecundity, and are exclusively female; they have the highest potential egg production per exiting migrant anywhere in their distribution range [3]. Thus, extinction of

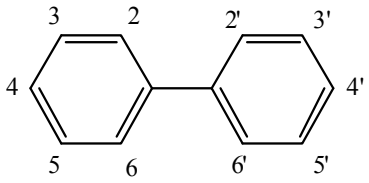
this stock could reflect and augment a severe species-wide decline in recruitment. However, management of this stock is very complex as American eel inhabit many ecosystem types and jurisdictions, including international waters.

Although the American eel population is critically endangered, management strategies are difficult to synchronize. For example, in Canada, the Province of Ontario has closed its commercial yellow eel fishery due to low eel abundance [52], yet the Atlantic coast provinces continue to have open harvest because eels are still present. The different approaches to eel management may have dramatic implications for the eel population as a whole. For instance, if the panmixia theory is correct, a population decline at the periphery of its range should shrink the distribution toward the center [53, 54], i.e., from the areas of lowest population density (farthest distance) to the areas of highest population density. This could explain why eel populations towards the center of the range appear abundant, and encourages exploitation at unsustainable rates. Alternatively, if eel populations are genetically different, fisheries management becomes essential to ensure that endangered females from upstream ecosystems are not captured during their migration through areas where eel are not endangered. Regardless, species-wide management should be conducted to ensure the eel's continued existence, similar to recommendations for the European eel [26, 54, 55]. The European plan recommends identifying areas where spawner quality is high with low contaminant burdens so that these areas may be protected. The strategy also includes the possibility of stocking these identified areas with eels from contaminated areas to reduce their body burden of contaminants prior to spawning. In Canada, the exact opposite is occurring. Ontario Power Generation and the Ontario Ministry of Natural Resources have been stocking glass eels from the Maritimes into the upper St. Lawrence and Lake Ontario since 2006. This removes eels from areas where chemical contamination is low and places them in areas with high contaminant concentrations and increases the risk of

contaminant related effects on reproduction. Stocking glass eels from coastal to inland waters may also spread infection of the parasitic nematode *Anguillicola crassus*, having been introduced to North America through Europe and its native Japan. The parasite infects the swim-bladder and reduces swimming capacities [57, 58]. Stocking does not consider many other factors such as the implication for sex determination in these transported eels, highlighting the need for a more comprehensive understanding of eel biology to support a more comprehensive management strategy in Canada and North America.

1.3 Polychlorinated biphenyls

Starting in the 1920s in North America, PCBs ($\mathbf{C_{12}H_{10-x}Cl_x}$) were produced commercially for use as dielectric and coolant fluids in electrical devices and many other applications (Figure 1-2). PCBs are viscous liquids with low water solubilities (<0.1 μg/L) and low vapour pressures. They were produced by the direct chlorination of biphenyl as mixtures because the synthesis of individual congeners is too difficult to be practical. For commercial applications, technical mixtures were produced, and defined by their chlorine content in percentage by weight. Depending on the duration of the chlorination process, different technical mixtures can be obtained based on desired properties. Various brands of commercial PCB mixtures were produced around the world, including Aroclor (USA), Clophen (Germany), Phenoclor (France), Fenclor (Italy), Sovol (former USSR), Chlorofen (Poland), and Kanechlor (Japan). An estimated 1.2 million metric tonnes of PCB mixtures were produced from these sources, believed to be most of the world's production [59, 60]. Due to their persistence in the environment, toxicity, and potential to bioaccumulate, PCBs were banned from production and open use in the 1970s by many countries and were included in the Stockholm Convention on Persistent Organic Pollutants in 2001 [60, 61].



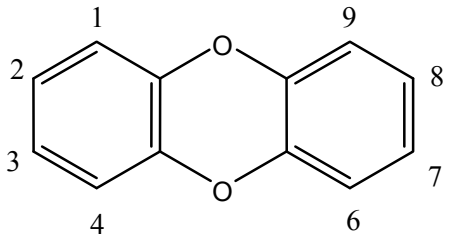
biphenyl

Figure 1- 2. General structure and numbering of biphenyl. Polychlorinated aromatic compounds contain chlorine substituents in place of hydrogen on the aromatic ring(s) according to the numbering system illustrated.

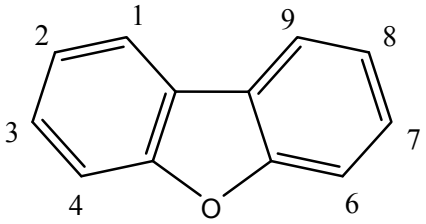
1.4 Dioxin-like compounds

1.4.1 Characteristics of PCDDs, PCDFs, and dioxin-like PCBs

Numerous reviews on polychlorinated dibenzo-*p*-dioxins (PCDDs) (**$C_{12}H_{8-x}Cl_xO_2$**), polychlorinated dibenzofurans (PCDFs) (**$C_{12}H_{8-x}Cl_xO$**), and polychlorinated biphenyls (PCBs) are available in the literature [38, 39, 62-64]. PCDD/Fs and dioxin-like PCBs (dl-PCBs) are a series of almost planar aromatic halogenated compounds with similar physical-chemical properties [60, 65, 66]. For each class, the resulting number of congeners, including positional isomers, is quite large; there are a possible 75 PCDD, 135 PCDF, and 209 PCB congeners. There are 17 PCDD/Fs that are 2,3,7,8-substituted, and 12 non-ortho and mono-ortho substituted PCBs, often referred to as "dioxins", that comprise the World Health Organizations list of dioxins and dioxin-like substances (Figure 1-3).



dibenzo-*p*-dioxin



dibenzofuran

Figure 1- 3. General structure and numbering for dibenzo-*p*-dioxin and dibenzofuran

All PCDD/Fs are organic solids with high melting points (tetra-octaCD range from 175-330°C). Higher chlorinated PCDD/Fs and PCBs have low vapour pressures (< 1.0 x 10^{-3} Pa) and water solubilities (< 1.0 x 10^{-4} mg/L), and log K_{ow} > 4 [67]. Dioxins have a tendency to sorb strongly to surfaces of particulate matter. As the degree of chlorination increases, melting point and hydrophobicity increase, and vapour pressure and water solubility decrease. They represent a significant class of environmental pollutants because of their persistence and tendency to bioaccumulate due to their hydrophobic/lipophilic nature and resistance to metabolism. Furthermore, several dioxins cause dermal toxicity, immunotoxicity, carcinogenicity, teratogenicity, and cardiovascular and endrocrine disruption [38].

Minor quantities of dioxins are formed naturally from processes such as forest fires and volcanic eruptions [60, 68]. However, the majority of recent dioxin production is attributed to byproducts of industrial processes, including (1) chemical reactions (the production of chlorophenols, chlorophenoxy herbicides, and PCBs); (2) low temperature thermal reactions (incineration of municipal, hospital, organochlorine materials and hazardous waste); (3) metal production and metal manufacturing (secondary copper smelting); and (4) pulp bleaching with elemental chlorine [65].

1.4.2 Environmental fate

Reviews of the environmental fate of PCDD/Fs and PCBs have been detailed elsewhere [64, 69-71]. In short, PCDD/Fs and PCBs exist in environmental and biological samples as complex mixtures of various congeners. The number and position of the chlorine atoms as well as the structure of the aromatic rings help to govern their fate. Physical properties such as hydrophobicity and solubility for individual congeners determine their absorption. Highly chlorinated isomers tend to be more resistant to metabolism, which is required for excretion through urine and feces [72]. PCDD/Fs are typically present in biota at pg/g (wet weight basis)

concentrations, and PCBs from ng/g to µg/g ww. Exposure routes are through water, air, soil and sediment. Differences in composition are linked to sources and physical-chemical properties of individual congeners [38, 60].

1.4.3 Chemical characterization

A review by Reiner et al. [73] covers advances in analytical methodologies and instrumentation for PCDD/F and dl-PCB analysis from the 1970s through to the 2000s. In summary, the most utilized method at present involves gas chromatography - isotope dilution - high resolution mass spectrometry (GC-ID-HRMS). This technique implements ^{13}C-labelled internal standards for a high degree of precision and accuracy for congener-specific determination, and is considered the gold-standard for dioxin analysis [29, 31, 74, 76]. Specific congeners have been measured at ppt and ppq concentrations in sample matrices. Background contamination is a limiting factor for lower detection limits. Multidimensional orthogonal methods (GC x GC) or (LC x GC) may be used to reduce detection limits and cost in the future [73, 76].

1.4.4 Toxic equivalency factors (TEFs)

The complex nature of PCDD, PCDF, and dl-PCB mixtures makes risk evaluation difficult. As a result, the TEF concept was introduced to simplify risk assessment for decision-makers. Experimental data on the relative potencies (REPs) of PCDDs, PCDFs, and dl-PCBs for fish, mammals and birds have been collected and assessed to determine TEF values, which indicate an order-of-magnitude estimate of the toxicity of a compound relative to 2,3,7,8-TCDD, the most toxic congener (TEF = 1). Two comprehensive reviews have established TEF values for PCDD/Fs and dl-PCBs [38, 39]; however, much of this research focuses on mammalian TEFs. Fish-specific TEF values rely heavily on lake trout REPs and the appropriateness of these TEF values for eels is unknown. Fish-specific TEFs have not been reviewed by the WHO since 1998.

TEF values, in combination with chemical residue analysis data and assuming toxicity of PCDD/Fs and dl-PCBs is additive, can be used to calculate concentrations in toxic equivalents (TEQs). TEQs can be calculated for various environmental samples including soil, water, sediment, and tissue using the following equation:

$$TEQ = \Sigma_{n1}[PCDD_i \times TEF_i] + \Sigma_{n2}[PCDF_i \times TEF_i] + \Sigma_{n3}[dlPCB_i \times TEF_i] + \ldots \quad \{1\}$$

where the sum of each measured individual $PCDD_i$, $PCDF_i$, and $dlPCB_i$ congener concentration multiplied by its respective TEF yields the total TEQ [38]. The resultant TEQ values are then used to compare or evaluate risk against established guidelines for mammals, fish, or birds. For example, the European Commission has established toxicity threshold guidelines for human consumption of fish (4 pg PCDD/F TEQ/g ww) and for total PCDD/Fs and dl-PCBs in European eel (12 pg TEQ/g ww) [77, 78]; Canada does not have a similar guideline.

1.4.5 Bioaccumulation and biomagnification of dioxin-like contaminants in eels

Dioxin-like contaminants biomagnify up food chains, reaching greatest concentrations at higher trophic levels in piscivorous and fatty fish like eel [79-82]. Several comprehensive reviews on the persistence, bioavailability, bioaccumulation, and biomagnification of dioxin-like compounds in aquatic ecosystems are available in the literature [69, 83-85].

As eels grow and transform from elvers to silver eels, their trophic position changes as their diet shifts to larger prey. They also become higher in fat content, but their growth rate decreases with age. Larsson et al. [86] found that elvers have a significantly lower fat content than yellow eels, which have a lower fat content than silver eels. Likewise, concentrations of hydrophobic contaminants increase all the way through their life stages. A study by Tapie et al. [87] showed that legacy POPs including PCB concentrations increase significantly from glass eels to silver eels. Migrating eels stop feeding at the onset of the silver stage, thus, the yellow eel

stage represents the period over which the majority of chemical uptake occurs. As benthic predators, yellow eels often reside in contaminated sediments where accumulation of contaminants through their gills, skin and dietary intake may occur (Figure 1-4) [88]. Eels are quite robust, surviving in degraded water conditions and often inhabiting environments polluted with mixtures of contaminants. They are also very effective at accumulating hydrophobic contaminants because of their relatively high fat content (> 20% at maturity). Contaminant concentrations are lower in fast-growing fish than in slow-growing fish such as eels due to dilution of contaminants into a larger mass (biodilution), which is predominantly seen in highly biomagnified compounds such as PCBs [89].

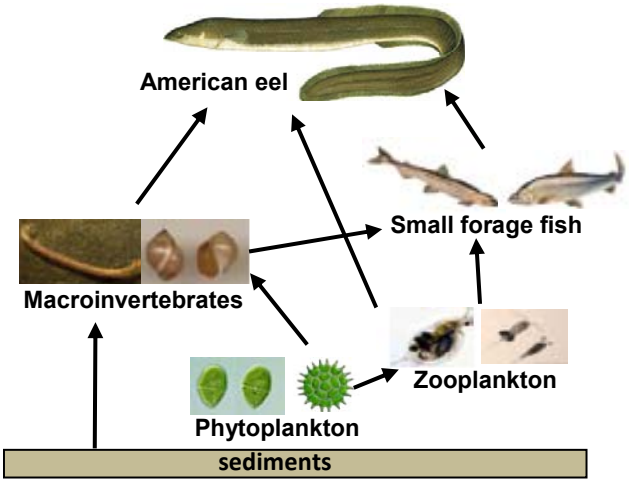


Figure 1- 4. Food web dynamics of Lake Ontario in which bioaccumulation of POPs have been observed. American eel are a benthic top predator. Adapted from NOAA, Great Lakes Environmental Research Lab [90]. www.glerl.noaa.gov.

A series of papers on the bioaccumulation of PCBs, PCDDs and PCDFs has shown that the concentrations of contaminants in sediment are related to concentrations in European eels. For PCBs, biota-sediment accumulation factor (BSAF) values and biomagnification increase with the

degree of chlorination. However, lower BSAF values and low bioaccumulation were evident for higher chlorinated PCDDs and PCDFs [91-93]. Variations observed for PCBs and PCDD/Fs in eel may occur due to differences in bioavailability/uptake, which is controlled by the physico-chemical properties of the substance, and food web dynamics. Because eels bioaccumulate hydrophobic contaminants, yellow eel are being used as a bio-indicator and PCBs as a biomarker for pollution monitoring [94].

1.4.6 Polychlorinated naphthalenes

Polychlorinated naphthalenes (PCNs) are halogenated planar aromatic compounds with similar physico-chemical properties to PCDD/Fs and PCBs (Figure 1-5). PCNs were produced as technical mixtures, also similar to PCBs; in Europe they were called Nibren waxes made by Bayer (Germany), Seekay (UK), Clonacire (France), Cerifal (Italy), and Woskol (Poland). In the United States, the largest volume PCN products were called Halowax (produced by Koppers).

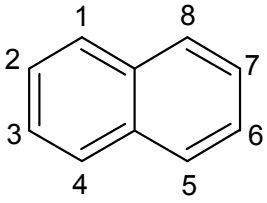


Figure 1- 5. General structure and numbering of naphthalene; chlorine substituted for hydrogen in positions 1 to 8 yield 75 total congeners $C_{10}H_{8-x}Cl_x$.

PCNs were used mainly in electrical devices, but major PCN production came to an end by the early 1980s, although a few companies still supply PCNs commercially [66]. They were first measured in the Great Lakes in the early 1980s, and are typically present in biota from pg/g to ng/g ww, similar to non-ortho PCBs [41]. Experimental data on individual PCN congener toxicity is limited. Only about one third of the 75 PCN congeners have been investigated for dioxin-like toxicity, and the TEF values were three to six orders-of-magnitude less than 2,3,7,8-TCDD [95-99]. Nonetheless, PCNs can contribute significantly to total TEQs [41].

1.4.7 Bromo-chloro dibenzodioxins and dibenzofurans

Polybrominated dibenzo-*p*-dioxins and dibenzofurans (PBDD/Fs) and mixed bromo-chloro dioxins and furans (PXDD/Fs), are understudied compared to chlorinated dioxins. The number of possible PBDD/Fs are the same as for PCDD/Fs, totaling 75 PBDD and 135 PBDF congeners, with 17 possible 2,3,7,8-substituted congeners. In contrast, there are considerably more PXDD/Fs, with a total of 4600 congeners and about 1000 possible 2,3,7,8-substituted congeners. Brominated and bromo-chloro dioxin and furan mixtures are formed in a similar way as chlorinated dioxins and furans, through thermal, chemical, photochemical, and biological processes [100]. Thermal and chemical formation during combustion in the presence of bromine and chlorine sources, and chemical manufacturing of polybrominated diphenyl ethers (PBDEs) are likely responsible for the majority of PBDD/Fs and PXDD/Fs produced.

Bromo and bromo-chloro-2,3,7,8-substituted PBDD/F and PXDD/F compounds have the ability to interact with the AhR, and cause equivalent, or in some cases higher, biological responses than their chlorinated analogues [96, 101-103]. Despite their similar properties to PCDD/Fs, PBDD/Fs and PXDD/Fs remain unregulated internationally, and TEF values have not been established. Understanding the toxicological effects of PBDD/Fs and PXDD/Fs may be important because of the increased production of organic brominated compounds, largely brominated flame retardant chemicals, since the 1980s. Thus, we can expect that concentrations of PBDD/Fs and PXDD/Fs in the environment to have increased since then.

1.5 Organochlorine pesticides

1.5.1 DDT, DDE, and DDD

DDT, 1,1,1-trichloro-2,2-di(4-chlorophenyl)ethane, is a pesticide first produced in 1939 before its widespread use during World War II to control malaria, typhus, and other insect-borne diseases. Its structure is shown in Figure 1-6. Technical-grade DDT contains only 65-80% of the active

ingredient (4,4'-DDT), 15-21% of the inactive 2,4'-DDT, 4% of 4,4'-DDD, about 1.5% of 1-(*p*-chlorophenyl)-2,2,2-trichloroethanol, along with up to 10 other compounds [104, 105]. About 1.8 million tonnes of DDT has been produced to date. DDT and its metabolites, DDE (2,2-bis(p-chlorophenyl)-1,1-dichloroethylene) and DDD (1,1-dichloro-2,2-bis(p-chlorophenyl)-ethane), were discovered to be highly persistent and bioaccumulative in the 1960s. They are endocrine disruptors and probable human carcinogens [104], and have been linked to bird egg-shell thinning [106]. DDT was banned in the early 1970s by most developed countries and was listed under the Stockholm Convention on Persistent Organic Pollutants. However, DDT is still used in many developing countries for disease prevention [61].

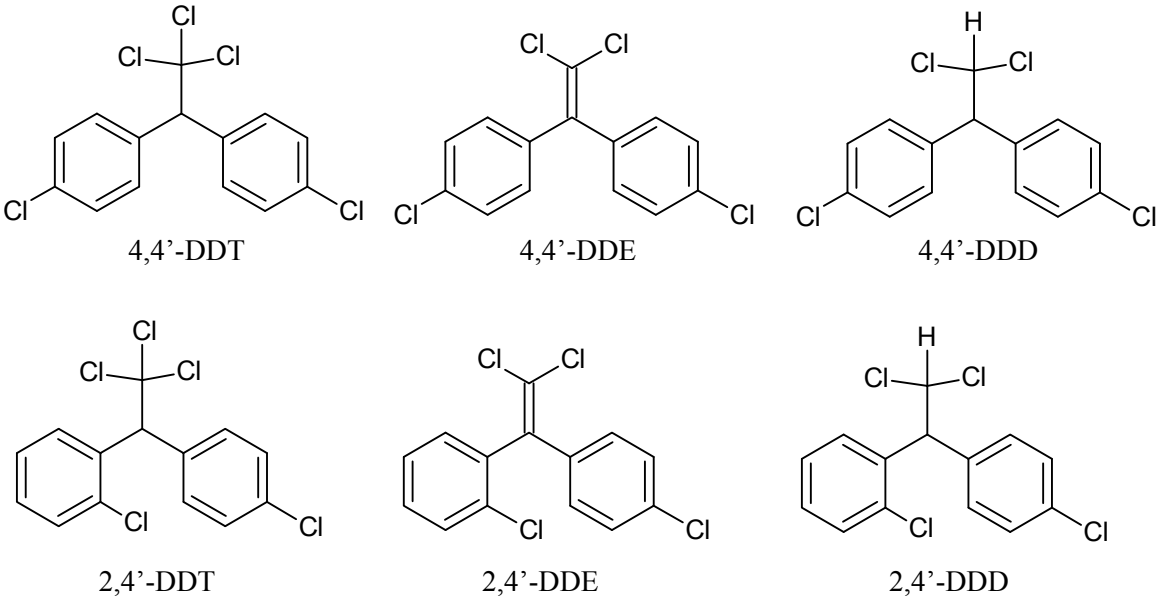
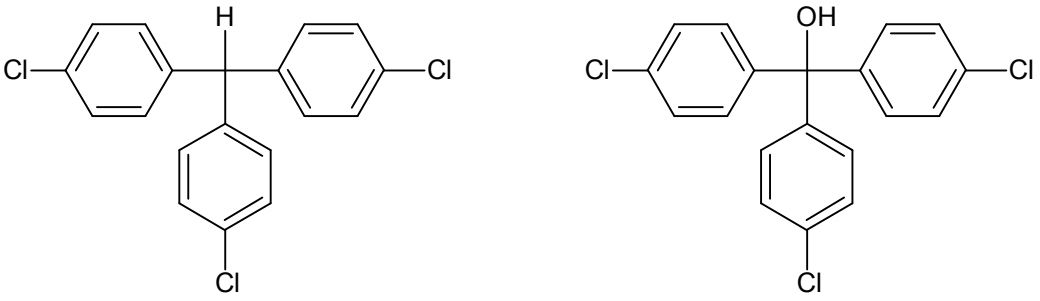


Figure 1- 6. Chemical structure of DDT and its metabolites.

1.5.2 Tris(4-chlorophenyl)methanol and methane

Tris(4-chlorophenyl)methanol (TCPM) and its alleged precursor tris(4-chlorophenyl)methane (TCPMe) (see Figure 1-7) are global contaminants that are structurally related to DDT [107, 108]. Production and usage information for TCPM and TCPMe are limited, but they are anthropogenic

compounds used in the production of dyes and are impurities in the production of DDT [108, 109]. TCPM and TCPMe have a log K_{ow} of 6.0 and 6.5, respectively, and are persistent, biomagnifying in food webs up to 100-fold. They undergo long-range atmospheric transport and have been measured in remote northern environments. Limited toxicity information is available for TCPM and TCPMe [107, 108], but TCPM acts as an androgen receptor agonist [106].



Tris(4-chlorophenyl) methane (TCPMe) Tris(4-chlorophenyl) methanol (TCPM)

Figure 1- 7. Structure of tris(4-chlorophenyl)methanol and methane

1.5.3 Hexachlorocyclohexane

Hexachlorocyclohexane (HCH) was produced globally in two main forms: technical-grade HCH and Lindane. It was used as an agricultural insecticide on food crops and pharmaceutically for the treatment of lice and scabies. There are eight structural isomers, five of which are present in technical-grade HCH produced by the photochlorination of benzene: 60-70% α-HCH, 5-12% β-HCH,10-15% γ-HCH, 6-10% δ-HCH, and 3-4% ε-HCH (Figure 1-8). Lindane, however, is almost 99% γ-HCH which is the most active insecticidal form [111]. HCH is relatively persistent, fairly volatile, and undergoes long range transport, resulting in it being measured in biota worldwide. It is carcinogenic to humans and causes related toxic effects in wildlife. Specifically α-HCH, β-HCH, and Lindane were added to the new POPs list under the Stockholm Convention in 2009 [48].

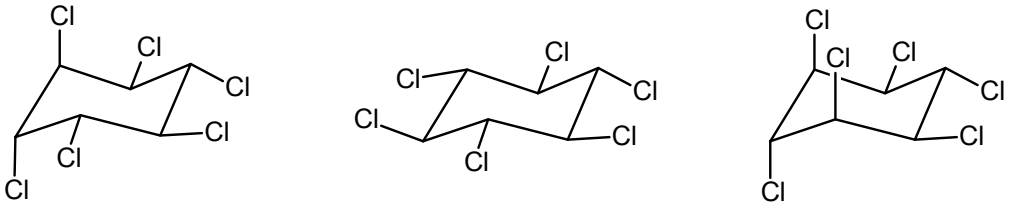


Figure 1- 8. Structure of three most common hexachlorocyclohexane isomers.

1.5.4 Hexachlorobenzene

Hexachlorobenzene (HCB) is a white crystalline solid that was introduced as a fungicide in 1945 for the treatment of crop seeds. It is not very soluble in water (<0.006 mg/L at 25ºC), has a log K_{ow} of 5.73, and a vapour pressure of 1.45 x 10^{-6} kPa. HCB was banned in the mid-1960s in North America and added to the initial list of POPs under the Stockholm Convention because of its high persistence, bioaccumulation potential, and toxicity as a carcinogen [61, 112].

1.5.5 Cyclodienes

A large number of pesticides are derived from a Diels-Alder reaction with hexachlorocyclopentadiene to form adducts including: heptachlor, *cis*- and *trans*-chlordane, *cis*- and *trans*-nonachlor, and aldrin. Hexachlorocyclopentadiene is also the precursor for mirex (Figure 1-9).

Heptachlor is a white powder when pure that was used primarily in the 1960s and 1970s to kill termites and as an agricultural insecticide. It was phased out or banned in most countries in the 1980s, but it is restricted to controlling fire ants in industrial electrical junction boxes like power transformers. *Heptachlor epoxide* is also a white powder that is generated in the environment when heptachlor mixes with oxygen, or through metabolism by bacteria and animals. Heptachlor epoxide is more commonly measured in the environment than heptachlor [61, 113].

Chlordane is a white solid that was used to control a wide range of insects on a range of agricultural crops. The two chlordane isomers *cis*- and *trans*-chlordane as well as *cis*- and *trans*-nonachlor are present in technical chlordane. *Oxychlordane* is formed in the environment similarly to heptachlor epoxide. Chlordane is persistent with a half-life in soil up to one year. It is very bioaccumulative, toxic to wildlife, and is indicated as a possible human carcinogen [61, 114].

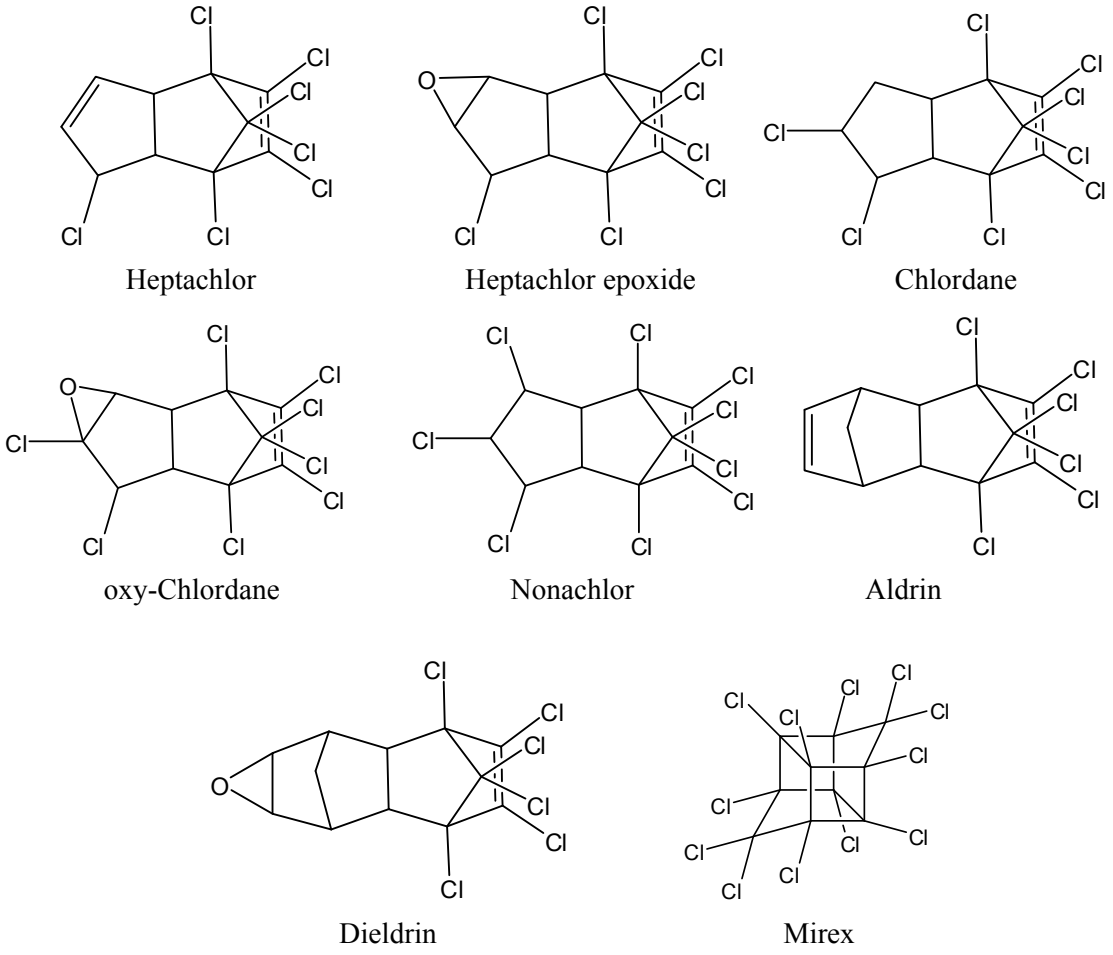


Figure 1- 9. Various pesticides derived from hexachlorocyclopentadiene. All of these compounds are on the list of the "dirty dozen" POPs under the Stockholm Convention [61].

Aldrin and *dieldrin* are white powders with similar structures that were used as broad-spectrum insecticides to control pests. Like the other cyclodienes, aldrin and dieldrin were used extensively in the1960-70s, before being restricted and banned in the late-1980s. Aldrin is readily converted to dieldrin in the environment, so dieldrin levels are much higher than from dieldrin use alone. Dieldrin is very persistent with a soil half-life of approximately five years. They are both bioaccumulative and toxic to fish and wildlife [61, 115].

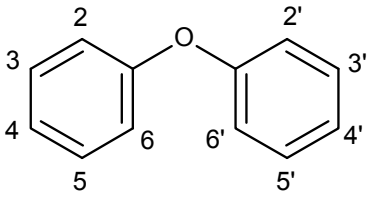
Mirex is a white crystalline solid that was used from 1959 to 1972 to control termites and fire ants, and also as an additive flame retardant in rubbers, plastics, and textiles. Mirex is also known by the trade name Dechlorane. Mirex is extremely persistent with a half-life of up to 10 years in soil, and is very bioaccumulative and biomagnitive. It is toxic to aquatic organisms and is a possible human carcinogen, although it is moderately toxic compared to the cyclodienes [61, 116].

1.6 Historic high production volume brominated flame retardants

Around the world, fires are a major source of property damage, injury, and loss of life. As a result, flame retardant chemicals (FRs) are added or applied to materials to increase the fire resistance of products [117]. Halogens are effective in capturing free radicals (I>Br>Cl>F) that are produced during the combustion process and stopping the propagation of the flame. Thus, organohalogenated compounds have been designed to act as a storage and delivery system of halogens to products. However, only organochlorine and organobromine compounds have been found to be suitable, with brominated flame retardants (BFRs) being the most popular choice due to their higher trapping efficiency and lower decomposing temperature [117-119].

1.6.1 Polybrominated diphenyl ethers

Polybrominated diphenyl ethers (PBDEs) are additive flame retardants, and until recently, represented the highest production group of any BFR. The structure and numbering of PBDE congeners is given in Figure 1-10, and follows Ballschmiter and Zell nomenclature [119].



diphenyl ether

Figure 1- 10. Chemical structure of PBDEs; total of 209 congeners $\mathbf{C_{12}H_{10-x}Br_xO}$.

PBDEs are produced in three technical mixtures, penta-BDE, octa-BDE, and deca-BDE, classified by their average bromine content [120]. In 2001, the global market demand for penta-, octa-, and deca-BDE was 7500, 3790, and 56100 metric tonnes, respectively. PBDEs are an environmental concern having been measured in almost every environmental matrix including air/dust, water, sediment, soil, sludge, biota, and even human serum [121-125]. The penta-BDE and octa-BDE formulations have undergone a series of voluntary phase-outs and bans worldwide in 2006; however, deca-BDE is still being produced, but is scheduled for voluntary phase-out by 2013 [47]. The penta-BDE and octa-BDE commercial mixtures were listed as new POPs under the Stockholm Convention in 2009 [48].

1.6.2 Polybrominated biphenyls

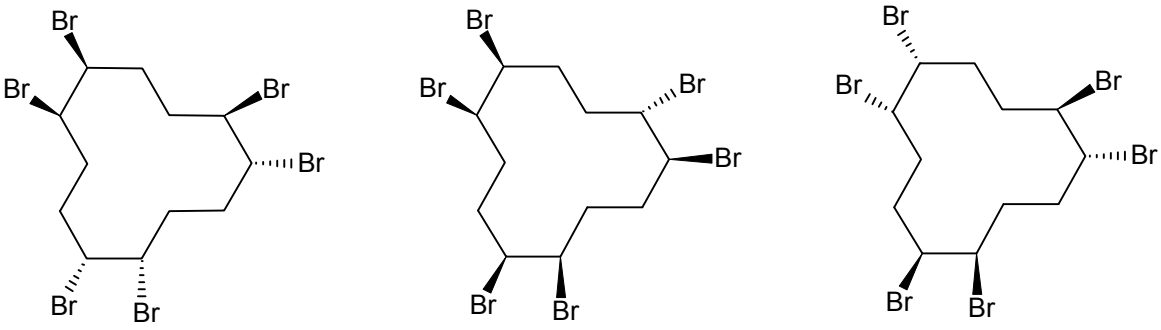
Polybrominated biphenyls (PBBs) are colourless to off-white solids that have bromine substituted for hydrogen in biphenyl ($\mathbf{C_{12}H_{10-x}Br_x}$), analogous to PCBs. They have low vapour pressures and are insoluble in water. PBBs have been used as additive flame retardants since the 1970s in electrical devices, textiles, and plastics and are the main component in FireMaster BP-6 and FF-1, although they are no longer produced in most countries. The most well-known incident involving PBB occurred in 1973 when several thousand pounds of PBBs were accidently mixed with live-

stock feed and distributed to farms in Michigan. The consequences were devastating to animals given the feed, several showing heptatoxicity and other toxic effects [127].

Hexabromobiphenyl (BB-153) is the dominant congener in the FireMaster BP-6 and FF-1 flame retardants and is very persistent in the environment, highly bioaccumulative in biota, and is classified as a possible human carcinogen, in addition to having other toxic related effects. Thus, it was listed as one of the nine new POPs under the Stockholm Convention in 2009 [48].

1.6.3 Hexabromocyclododecane

Hexabromocyclododecane (HBCD) is a colourless solid used as a flame retardant (Figure 1-11). Its main application is in expanded and extruded polystyrene that is used as thermal insulating foam in buildings and construction. HBCD has secondary applications in textiles and high impact polystyrene [128, 129]. In 2001 it was the third highest production flame retardant on the global market at 16 700 metric tonnes [130]. There are four technical mixtures of HBCD produced that contain up to 16 possible diastereomers, but the dominant isomers are alpha- (10-13%), beta- (1-12%), and gamma-HBCD (75-89%) [117].



alpha–HBCD beta–HBCD gamma–HBCD

Figure 1- 11. Structure of the most abundant hexabromocyclododecane isomers ($C_{12}H_{18}Br_6$).

HBCD is persistent in the environment with a log K_{ow} of 5.6, and is considered to be bioaccumulative and known to have toxic effects on wildlife. It is currently being considered by

the Stockholm Convention on POPs under Annex A as a new POP, with an exception for use in expanded and extruded polystyrene because it remains the only suitable BFR for this application [131].

1.6.4 Tetrabromobisphenol A

Tetrabromobisphenol A (TBBPA) is a crystalline solid with the chemical formula $\mathbf{C_{15}H_{12}Br_4O_2}$ (Figure 1-12). TBBPA is the highest production flame retardant on the global market by a wide margin at 199,700 metric tonnes in 2001, representing 58.7% of the total BFRs produced. TBBPA is used either in a reactive or additive form. Its main application utilizes the reactive form where it is incorporated covalently into epoxy resins for electric circuit boards. It is used additively for other polymer applications such as acrylonitrile butadiene styrene [130].

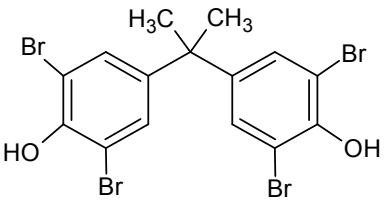


Figure 1- 12. Structure of tetrabromobisphenol A

TBBPA is considered to be persistent in the environment with a predicted log K_{ow} between 5.9 and 7.2. It has been measured in a variety of environmental matrices including air, sediment, waste water and biota, and biomagnifies in food webs.

TBBPA has been subjected to a European Commission risk assessment study that suggested it be classified as very toxic to aquatic organisms with no classification for human health [131]. For daphnia and fish, the LC_{50} for TBBPA is < 1 mg/L. The bioaccumulation factor is > 100 in fish. It is also in the pre-registration phase under REACH, the European Community Regulation on chemicals and their safe use and handling.

1.7 Halogenated norbornene flame retardants

Dechlorane (Dec) -602, -603, -604, and Dechlorane Plus (also called Dechlorane-605) are additive flame retardants that replaced non-agricultural mirex after it was banned in the 1970s. They are synthesized by the Diels-Alder reaction of hexachlorocyclopentadiene with various cyclic compounds to form adducts [133]. Chlordene Plus (CP) is structurally related to Dechloranes [134]. A study by Shen et al. [135] analyzed a number of cyclodiene pesticides for nonbornene flame retardants, and found that Dechlorane-602, -604, and DP were not present as byproducts. Dec-603 was detected in aldrin and dieldrin, and CP was measured in chlordene and chlordane. It was suggested, therefore, that Dec-603 and CP are present in the environment as the result of currently banned pesticides; whereas, Dec-602, -604, and DP are produced for commerce. Their chemical structures are illustrated in Figure 1-13.

Dec-602 is used in thermoplastics, polyvinyl chloride (PVC), and high voltage electrical cabling. Dec-604 is used for the same applications as Dec-602, in addition to silicon greases. Dec-602 has been reported to be the most bioaccumulative of the mirex replacements with Dec-603, Dec-604, and DP having considerably lower bioaccumulation potential and biota sediment accumulation factors (BSAFs) [133, 136, 137]. Limited information is available about the toxicity of Dec-602, -603, -604, and CP.

Dechlorane Plus (DP) is by far the most studied of the norbornene flame retardants ($\mathbf{C_{18}H_{12}Cl_{12}}$). It was first synthesized in the 1960s, but only recently became a high production volume chemical used mainly in electrical coatings and connectors, as well as in plastic roofing materials. Technical DP contains two isomers, *syn*- and *anti*-DP. It was first discovered in the environment around the Great Lakes near the OxyChem production facility in Niagara Falls, NY, in 2006 [133]. Since then, a second facility was discovered in Huai'in, China (Anpon) [138]. Two reviews of DP are available in the literature [133, 139]. DP persists in the environment, and has

been measured in air, sediment, soil, and biota. The 1,5-DP mono adduct and 1,3-DP mono adduct, byproducts from the production of DP, as well as dechlorinated *anti*-[DP-Cl] and *anti*-[DP-2Cl] have also been measured in the environment. DP is not very bioaccumulative, with a BSAF less than one, and a predicted log K_{ow} between 9.3 and 11.3. DP will undergo long-range atmospheric transport; however, it does not appear to biomagnify in food webs. Limited toxicity data are available, but early indications are that DP is not very toxic to fish and wildlife. A recent human risk assessment near a DP production facility and e-waste facility in China, the most contaminated region with DP in the world to date, revealed that the risk to human health from DP is low [140].

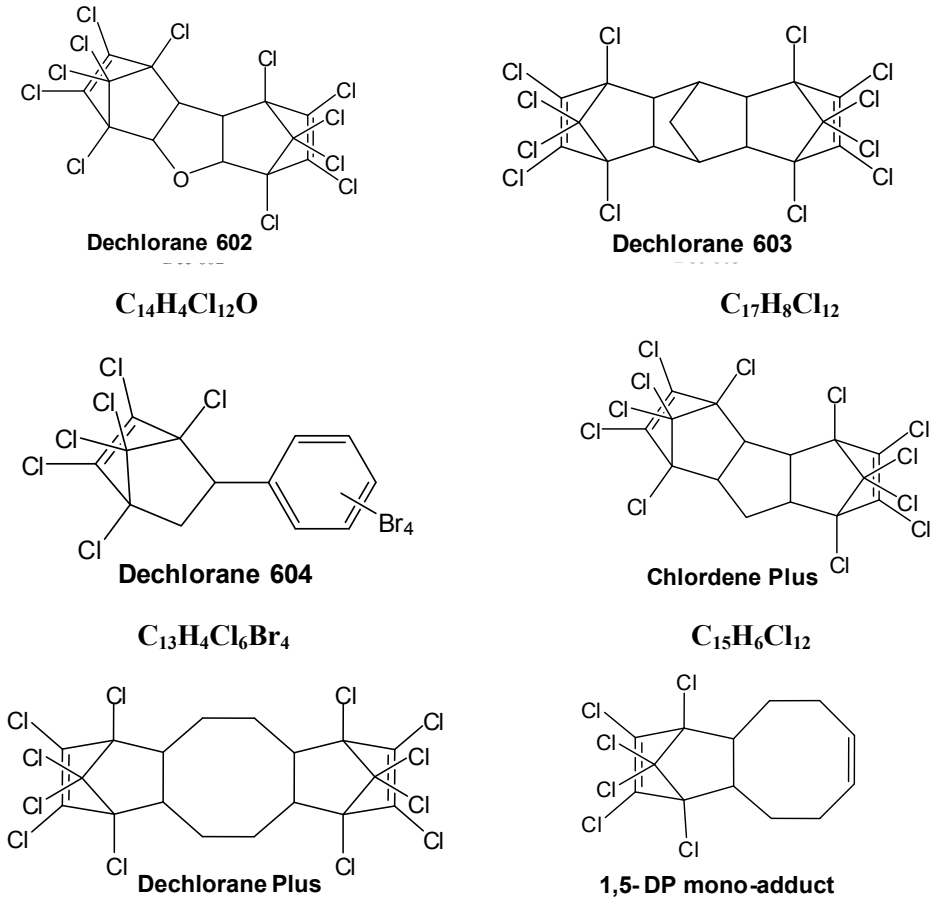


Figure 1- 13. Chemical structure of select norbornene flame retardants.

1.8 New and emerging brominated flame retardants

With the ban of penta- and octa-BDE, potential restrictions on HBCD and TBBPA, and the phase-out of deca-BDE, new replacement flame retardants are needed to meet the market demand. And the global demand for flame retardants is continuing to rise, as indicated by a Freedonia Group study which stated an increase in demand of 6.1% annually from 1.7 million tonnes in 2009 to 2.2 million tonnes in 2014 [141]. Furthermore, 1,2-bis(2,4,6-tribromophenoxy)ethane (BTBPE) has been suggested to replace octa-BDE [142]; Firemaster 550, which contains 2-ethylhexyl-2,3,4,5-tetrabromobenzoate (TBB) and bis(2-ethyl-1-hexyl)tetrabromophthalate (TBPH), has been suggested to replace penta-BDE [143]; decabromodiphenyl ethane (DBDPE) is expected to replace dec-BDE [144]. Table 1-1 shows a list of some new bromobenzene based flame retardants along with some of their physical property and usage information. Most bromobenzene-based flame retardants are incorporated into polymers and plastics. A number of the BFRs listed below have been measured in the environment [145-147], and all except TBPH, OBIND, and DBDPE are predicted to bioaccumulate based on their log K_{ow}. However, the predicted log K_{ow} does not preclude the occurrence of their breakdown products in the environment, which may bioaccumulate and persist similar to what has been observed for decaBDE [148, 149].

Toxicity data are very limited for most new BFRs; however, environmental concentrations are needed to provide environmental relevance. The properties that make BFRs effective flame retardants also make their chemical analysis by classical techniques difficult. Consequently, most studies that measure BFRs use injection port temperatures <300°C and short gas chromatographic columns (<15 m) with thin film coatings (0.10μm) to avoid decomposition or debromination [150, 151].

Table 1- 1. Chemical structure, properties, and uses for emerging brominated flame retardants.

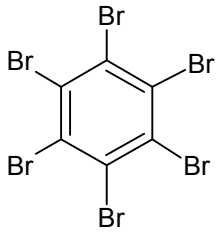
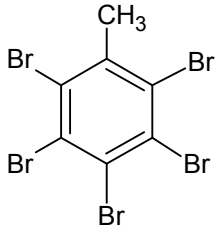
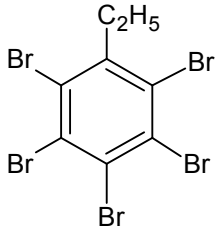
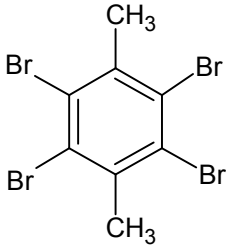
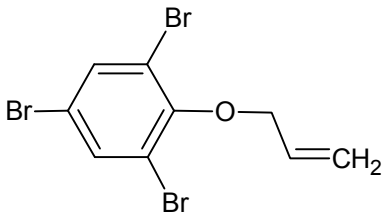
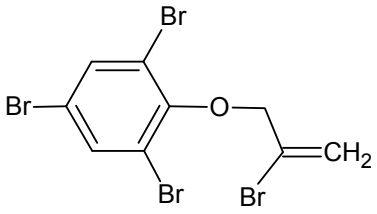
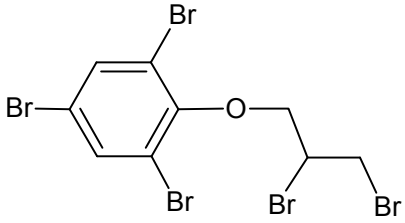
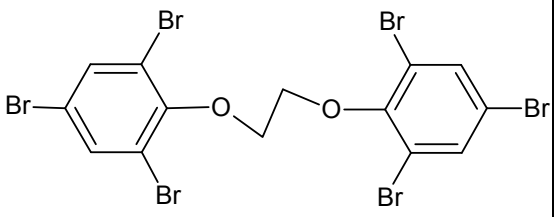
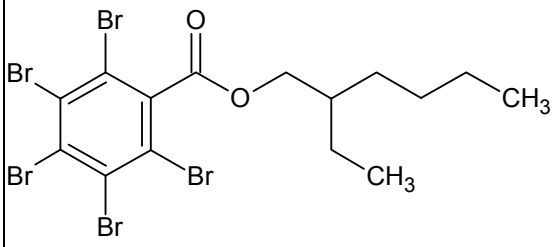
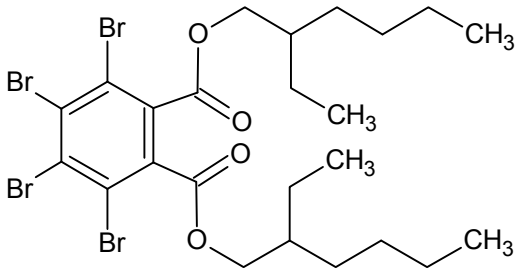
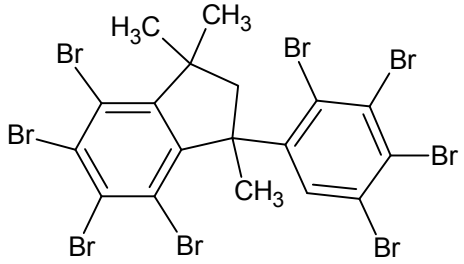
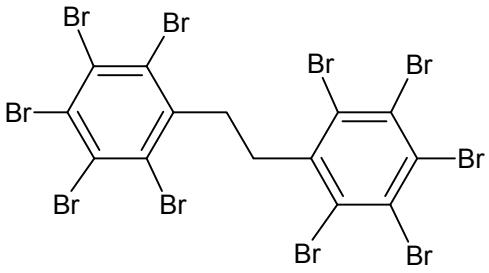
Chemical Structure	Chemical Properties [152]	Uses [150-152]
 Hexabromobenzene	HBB C_6Br_6 MW = 551.49 g/mol S_{water} = 0.00218 mg/L (0.00016)[a] mg/L Log K_{ow} = 7.33 (6.07) HLC = 2.85 Pa·m^3/mol	polymers, plastics, textiles, wood, and paper
 Pentabromotoluene	PBT $C_7H_3Br_5$ MW = 486.62 g/mol S_{water} = 0.0009351 mg/L Log K_{ow} = 6.99 HLC = 7.31 Pa·m^3/mol	polymers such as polyester; latex, textiles, rubbers and plastics
 Pentabromoethylbenzene	PBEB $C_8H_5Br_5$ MW = 500.65 g/mol S_{water} = 0.0002895 mg/L Log K_{ow} = 7.48 HLC = 11.3 Pa·m^3/mol	acrylonitrile butadiene styrene (ABS), a variety of polymers, textiles, and rubbers
 2,3,5,6-tetrabromo-p-xylene	pTBX $C_8H_6Br_4$ MW = 421.75 g/mol S_{water} = 0.004717 mg/L Log K_{ow} = 6.65 HLC = 18.8 Pa·m^3/mol	plastics, polymers such as polyesters and polyurethanes

Table 1-1. Chemical structure, properties, and usage information for BFRs continued...

Chemical Structure	Chemical Properties	Uses
 allyl 2,4,6-tribromophenyl ether	ATE $C_9H_7Br_3O$ MW = 370.87 g/mol S_{water} = 0.07793 mg/L Log K_{ow} = 5.59 HLC = 44.1 Pa·m^3/mol	polymers such as polyester, polyethylene, polypropylene, and polystyrene
 2-bromoallyl 2,4,6-tribromophenyl ether	BATE $C_9H_6Br_4O$ MW = 449.76 g/mol S_{water} = 0.01162 mg/L Log K_{ow} = 5.98 HLC = 53.4 Pa·m^3/mol	high-impact plastic
 2,3-dibromopropyl 2,4,6-tribromophenyl ether	DPTE $C_9H_7Br_5O$ MW = 530.68 g/mol S_{water} = 0.001752 mg/L Log K_{ow} = 6.34 HLC = 0.0216 Pa·m^3/mol	polypropylene
 1,2-bis(2,4,6-tribromophenoxy)ethane	BTBPE $C_{14}H_8Br_6O_2$ MW = 687.64 g/mol S_{water} = 6.551e-7 mg/L (0.2) mg/L Log K_{ow} = 9.15 HLC = 0.0431 Pa·m^3/mol	high-impact polystyrene, ABS, thermoplastics, and electrical coatings in electronics

Chemical Structure	Chemical Properties	Uses
 2-ethylhexyl-2,3,4,5-tetrabromobenzoate	TBB or EHTeBB $C_{15}H_{17}Br_5O_2$ MW = 628.82 g/mol S_{water} = 6.055e-7 mg/L Log K_{ow} = 9.64 HLC = 0.0298 Pa·m^3/mol	PVC, neoprene, polyurethane foam, electrical insulation and coatings
 bis(2-ethyl-1-hexyl)tetrabromophthalate	TBPH or BEHTBP $C_{24}H_{34}Br_4O_4$ MW = 706.15 g/mol S_{water} = 1.98e-9 mg/L Log K_{ow} = 11.95 HLC = 0.0312 Pa·m^3/mol	polyurethane foam, PVC, textile backings, and electrical insulation and coatings
 octabromotrimethylphenylindane	OBIND $C_{18}H_{12}Br_8$ MW = 867.53 g/mol S_{water} = 1.957e-11 mg/L Log K_{ow} = 13.03 HLC = 0.0467 Pa·m^3/mol	high-impact polystyrene, ABS, polyethylene, and other polymers
 decabromodiphenylethane	DBDPE $C_{14}H_4Br_{10}$ MW = 971.23 g/mol S_{water} = 1.16e-12 mg/L Log K_{ow} = 13.64 HLC = 0.00298 Pa·m^3/mol	electrical wires and cables, high-impact polystyrene, ABS plastic, resins, and textile back-coating

[a]Numbers in brackets are experimental values; all others are calculated or estimated using EPI Suite [153]. MW = molecular weight; S_{water} = water solubility; K_{ow} = octanol-water partitioning coefficent; HLC = Henry's Law Constant.

1.9 Thesis body outline

The work described in this thesis is organized according to the following chapters (2-6), which address four null hypotheses.

1. There are no temporal trends in concentrations of persistent organic pollutants in eels or dioxin-like toxicity.
    i. Chapter 2 will detail historic concentrations of several classes of halogenated persistent organic pollutants in American eels captured from eastern Lake Ontario, Canada. Concentrations of PCDD/Fs and corresponding TEQs will be compared with toxicity thresholds in surrogate fish species. Historic trends for a variety of organochlorine pesticides and PCBs will also be detailed. Lastly, PBDE concentrations in eels from three time points were measured and compared to concentrations in other fish species in Lake Ontario.
2. There are no geographical differences in concentrations of persistent organic pollutants in adult eels.
    i. Chapter 3 describes geographical differences in concentrations of OCPs, PCBs, and PBDEs in eels captured from eastern Canada, the United States, and Belgium.
3. Concentrations of persistent organic pollutants in adult eel do not exceed published thresholds for toxicity to eels or surrogate species.
    i. Chapter 4 reports geographical differences in concentrations of PCDD/Fs, dioxin-like PCBs, and PCNs, as well as resultant TEQs in eels captured from eastern Canada, the United States, and Belgium. TEQs will be calculated for fish

and mammals, and compared to toxicity thresholds for surrogate fish species and to consumption guidelines, respectively.

4. There are no other compounds of concern that accumulate in eels.

    i. Chapter 5 provides concentrations of emerging chlorinated and brominated flame retardants in eels captured from eastern Lake Ontario, Canada, in 1988, 1998, and 2008.

    ii. Chapter 6 details a method for identifying new and emerging halogenated compounds in eels captured across eastern Canada by gas chromatography with detection by time-of-flight mass spectrometry.

All of the chemical analyses reported in this thesis were performed using whole fish homogenates prepared as described in the appendix.

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Chapter 2

Trends of POPs (1988-2008) and potential effects on American eel (*Anguilla rostrata*) from Lake Ontario, Canada

Abstract

High concentrations of persistent organic pollutants (POPs) have been reported in American eels (*Anguilla rostrata*) from Lake Ontario (LO), which could have played a role in the decline of their recruitment during the 1980-90s. To compare the history of contamination with the history of recruitment, three groups of ten large female eels captured in Eastern LO in 1988, 1998, and 2008 were analysed for POPs; namely, polychlorinated dibenzo-*p*-dioxins and dibenzofurans, polychlorinated biphenyls, and several organochlorine pesticides, as well as polybrominated diphenyl ethers. Mean concentrations of POPs were significantly lower in 2008, up to 3-fold, than in previous years. Contaminant data from this study were combined with POP concentrations in eels from LO reported by two monitoring programs and occasional studies to show for the first time that concentrations of POPs in American eels have declined exponentially since the early 1980s by an average of 9.1 ± 1.9 % per year. Toxic equivalent (TEQ) concentrations calculated using fish toxic equivalency factors from dioxin-like compounds (DLC) indicated that prior to 2000, LO eels accumulated TEQ concentrations above the threshold value for chronic toxicity (5 pg/g ww of TEQ determined for lake trout), assuming an efficient transfer of DLCs to their eggs. These results suggest that embryotoxicity of maternally-derived DLCs from LO eels could have contributed to a possible lack of reproductive success and the apparent decline in recruitment of juvenile eels to LO.

2.1 Introduction

Recruitment of juvenile American eels (*Anguilla rostrata*) to Lake Ontario (LO) declined drastically in the 1980-90s, which led the Committee on the Status of Endangered Wildlife in Canada to classify eels as a threatened species [1]. Although, many anthropogenic threats to eels have been proposed (e.g. habitat modifications, dams, fisheries, parasites), the primary cause of this decline is still not established. A few studies have shown that eels from LO are highly contaminated with persistent organic pollutants (POPs) [2-4], which raises the question of the possible role of chemical contamination in recruitment decline.

Only a few contamination data on eels from LO were reported in the open literature and there are no documented temporal trends of POPs [2-4]. However, concentrations of legacy POPs have been determined in LO eels as part of two monitoring programs (Ontario Ministry of Environment (MOE); Department of Fisheries and Oceans, Inspection Service Branch (DFO-ISB)), but these data were not widely diffused. Chemical data have also been reported in female silver eels collected along the St. Lawrence during their migration to the Sargasso Sea. Although various approaches have been applied to determine their origin, it cannot be certain that they grow specifically in LO [5-7].

Lake trout (*Salvelinus namaycush*) has been monitored extensively in LO to establish temporal trends for many legacy POPs, including polychlorinated dibenzo-*p*-dioxins and furans (PCDD/Fs) [8-10], as well as polychlorinated biphenyls (PCBs) and several organochlorine pesticides (OCPs) [10-15]. Increasing and then decreasing temporal trends of polybrominated diphenyl ethers (PBDEs), a class of chemicals more recently regulated in Canada, have also been reported in LO lake trout [13, 16-19]. Some of these studies were based on samples collected and data generated by the MOE and DFO-ISB monitoring programs.

Some POPs, especially the PCDD/Fs and PCBs with planar conformations (dioxin-like compounds or DLCs), are highly toxic to mammals, birds, and fish. Potential effects of DLCs on LO lake trout were reported by Cook et al. [20], who demonstrated that the complete extirpation of naturally produced trout in the mid-1960s could be explained by historic loadings of DLCs into LO. Cook's model predicted 100% lake trout mortality between 1940 and 1980 when DLC concentrations were at their highest. These findings suggest that eels, a top predator fish like lake trout, could have been affected similarly by high concentrations of POPs accumulated in their tissues. These POPs were likely transferred to eggs, possibly causing embryo-toxicity and impaired recruitment.

The objectives of the present research were to: (1) assess concentrations and patterns of POPs, including PCDD/Fs, PCBs, OCPs, and PBDEs, in American eels collected in LO in 1988, 1998, and 2008; (2) determine temporal trends of these POPs in LO eels by combining our data with those obtained from the MOE and DFO-ISB monitoring programs and occasional studies; (3) compare temporal trends of POPs in LO eels with those reported in lake trout from LO; and (4) determine temporal changes in toxic equivalent concentrations of DLCs in American eels and assess their potential effects on eel recruitment.

2.2 Methods

2.2.1 Sampling, condition, and age determination

Eels were captured in eastern LO by electrofishing in June or July from Main Duck Island (43°55.76'N 76°36.11'W) in 1988 and 1998 and from Prince Edward Bay (43°57.01'N 76°58.01'W) in 2008 (Figure 2-1). All eels were weighed (W), their length (L) measured, and their condition factor calculated (CF = [W (g)/L^3 (cm)] x 100). Le Cren's relative condition factor (RCF) was also calculated as recommended by [21]. The relative condition factor (k=W/W') compares the observed (W) and the expected (W') weight of each fish, which is calculated from

the length-weight regression ($W=aL^b$) obtained by considering all individuals. For each eel, the sagittal otoliths were removed for aging according to a validated method [22]. For each sampling year, the 10 eels were selected for chemical analysis based on their age, so that the average approximated 20 years. All selected eels were large yellow or silver (sexually maturing) females. A section representing about 10% of the eel's weight was taken posterior to the anus of each fish and some internal tissues (liver, section of ovary) were removed for complementary analyses prior to homogenisation.

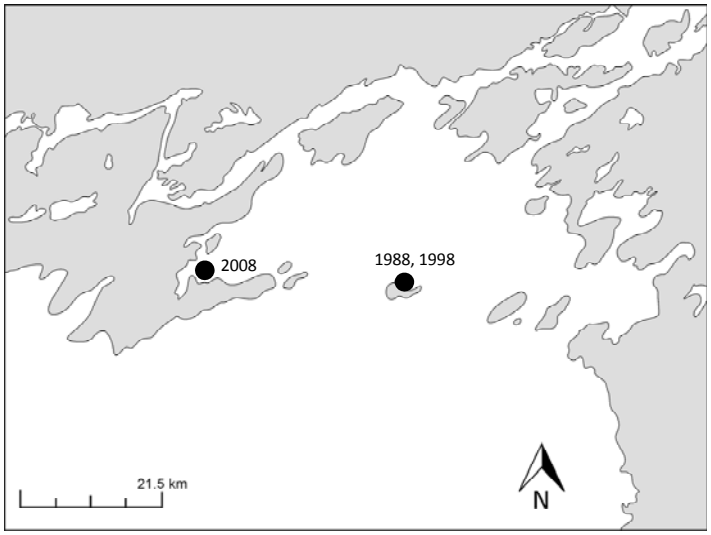

Figure 2- 1. Map of eastern Lake Ontario showing the sampling sites. Eels were collected from Main Duck Island, Ontario in 1988 and 1998, and from Prince Edward Bay in 2008.

2.2.2 Chemical analysis

The analytical methods used in this study were detailed previously in [23, 24]. Briefly, whole fish homogenates were prepared from the eels captured in LO. Eel tissue extracts used for chemical analysis were prepared from approximately 20 g of homogenate. Each sample was dried chemically with anhydrous sodium sulphate, spiked with 2,2',3,3',4,4',5-[$^{13}C_{12}$]-HeptaCB and 2,3,7,8-[$^{37}Cl_4$]-TCDD, and extracted with dichloromethane. The extract was split by weight into

four portions: 1) 10% for gravimetric lipid determination, 2) 40% as backup, 3) 25% for OCP, PCB, and PBDE analysis, and 4) 25% for PCDD/F and non-ortho (no)-PCB analysis.

The third fraction was spiked with a solution of eight [$^{13}C_{12}$]-PCB surrogates, four [$^{13}C_{12}$]-PBDEs, and 11 [$^{13}C_{12}$]-OCPs, (Wellington Laboratories, Guelph, ON). Sample clean-up removed lipid by gel permeation chromatography with Biobeads SX-3, and a 2-layered packed 5% deactivated silica-alumina column. The sample extracts were reduced in volume and spiked with two additional [$^{13}C_{12}$]-PCB instrument performance standards. Gas chromatography-mass spectrometry (GC-MS) was used to identify and quantify PCB, PBDE, and OCP concentrations using a ThermoQuest TraceGC equipped with a Finnigan PolarisQ ion trap, operated in electron ionization (EI) and MS/MS mode [25]. Forty-six singly and coeluting PCB congeners were measured (IUPAC numbers 8, 15, 18, 28/31, 33, 37, 40, 44, 49, 52, 66/70, 74, 87, 95, 99, 101, 105, 110, 114, 118, 123, 128, 138, 149, 151, 153, 156, 157, 167, 170, 171, 177, 180, 183, 187, 189, 191, 194, 195, 199, 205, 206, 208, 209). The OCPs analysed were α, β, δ, γ-hexachlorocyclohexanes (HCH), hexachlorobenzene (HCB), heptachlor, heptachlor epoxide (isomer B), oxychlordane, α and γ-chlordane, cis and trans nonachlor, tris(4-chlorophenyl) methanol (TCPM) and tris(4-chlorophenyl)methane (TCPMe), mirex, dieldrin, 1,1,1-trichloro-2,2-bis(4-chlorophenyl)ethane (4,4'-DDT), and its metabolites [1,1'-dichloro-2,2-bis(4-chlorophenyl)ethane (4,4'-DDD) and 1,1-dichloro-2,2-bis(4-chlorophenyl)ethene (4,4'-DDE)], as well as 1,1,1-trichloro-2-(4-chlorophenyl)-2-(2-chlorophenyl)ethane (2,4'-DDT) and its metabolites [1,1'-dichloro-2-(4-chlorophenyl)-2-(2-chlorophenyl)ethane (2,4'-DDD) and 1,1-dichloro-2-(4-chlorophenyl)-2-(2-chlorophenyl)ethene (2,4'-DDE)]. The 14 PBDE congeners quantified were (IUPAC numbers 17, 25, 28, 33, 47, 49, 66, 75, 99, 100, 153, 154, 155, 183). Highly brominated octa to deca-PBDEs were not quantifiable in eel samples, occurring at concentrations <0.1 ng/g ww.

The fourth fraction was spiked with a solution of 15 [$^{13}C_{12}$]-PCDD/F, 4 [$^{13}C_{12}$]-no-PCB purchased from Wellington Laboratories (Guelph, ON, Canada). Sample clean-up was identical to the third fraction followed by the elution of the extract through a Cosmosil 5PYE column by high performance liquid chromatography to isolate the no-PCBs and PCDD/Fs. One fraction contained the no-PCBs and the other contained the PCDD/Fs. The fractions were reduced in volume and spiked with additional [$^{13}C_{12}$]-PCDD and [$^{13}C_{12}$]-PCB instrument performance standards. Gas chromatography- high resolution mass spectrometry (GC-HRMS) analyses was carried out on a Micromass AutoSpec mass spectrometer (Micromass, Manchester, UK) connected to a Hewlett-Packard 6890 GC (Hewlett Packard, Palo Alta, CF, USA) equipped with a CTC A200S autosampler (Leap Technologies, Chapel Hill, NC, USA). GC-HRMS tuning was done using perfluorokerosene as a reference compound (10,000 resolution at 5% peak height definition) over the mass range of the PCDD/F and no-ortho PCB congeners.

Samples were analysed in batches of ten with one procedural blank, one spiked lab blank, one in-house reference material (L. Trout), and one certified reference material (CRM) (CARP-2, National Research Council of Canada). The average percent coefficient of variance for reference material replicates was 12 ± 8 for PCBs, 22 ± 16 for OCPs, and 17 ± 21 for PBDEs, which indicates a fairly high degree of reproducibility. The Carp-2 Certified Reference Material certified only 10 PCBs (IUPAC numbers 18, 28, 44, 52, 118, 128, 153, 180, 194, and 206), and provided reference values for other select PCBs (IUPAC numbers 8, 66/95, 101/90, 105, 138/163/164, 170/190, 187/182, 209) and OCPs (γ-chlordane, 2,4'-DDE, trans-nonachlor, dieldrin, 4,4'-DDE, 2,4'-DDE, and 4,4'-DDD). The certified PCB values had percent differences ranging from -1 to 49% (mean = 18%), and the reference values ranged from -56 to 43% (mean = 5%). PCB- 28, which coeluted with PCB-31 by our method, was included in the calculation. OCP reference values had percent differences that ranged between -43 and -1% (mean = -26%),

excluding γ-chlordane which was almost 2-fold higher by our method, and 4,4'-DDD coeluted with 2,4'-DDT. Reference values for PBDE congeners were not included in the CARP-2 CRM. The average recovery for spiked lab blanks was 98 ± 12% for PCBs, 97 ±15 % for OCPs and 110 ±17 % for PBDEs. Recoveries of internal standards were statistically similar for all three time periods analysed, so storage and aging effects of older samples did not affect recoveries. Individual compounds measurable in blanks occurred at concentrations much less than 1% of those in samples (e.g. HCB, p,p'-DDE, PCB-118, -153, and PBDE-47). Based on the QA/QC results, lower bound concentrations were determined using method blank subtracted values for data handling and statistical purposes.

Replicate samples had an average percent coefficient of variation of 22 ± 20 and 14 ± 16 for PCDD/Fs and no-PCBs, respectively. Reference material values were compared to this study with a paired t-test using mean values from three replicates for the nine PCDD/F congeners reported. All PCDD/F congeners were statistically similar to the reported values at a 95% CI. PCB-118 was the only other dioxin-like compound reported by the NRC in this material, and was statistically similar. Blank samples had concentrations below the LOQ for all 17 PCDD/F congeners > 95% of the time, with the remaining having concentrations of < 0.36 pg g^{-1} ww (OCDD). Concentrations of the non-ortho PCBs in blanks were below the LOQ < 5.0 pg g^{-1} ww. For statistical purposes, non-detectable concentrations were assigned a value of half the LOQ.

2.2.3 Stable isotope analysis

Subsamples of whole fish homogenates were freeze dried and pulverized to a fine powder using a ball mill grinder. Samples were washed successively with methanol, dichloromethane and hexane, and centrifuged. The lipid-free tissue was oven-dried, ground in a mortar, and stored in desiccation vials until analyzed. Approximately 0.3 mg of dried tissue was used in the simultaneous analysis of stable C and N isotopes on The Delta Plus Continuous Flow Stable

Isotope Ratio Mass Spectrometer (Thermo Finnigan / Bremen-Germany) coupled to a Carlo Erba Elemental Analyzer (CHNS-O EA1108 - Italy) at the Environmental Isotope Lab, University of Waterloo, ON, Canada. All results were expressed in conventional delta notation (δ) relative to Peedee Belemnite limestone for $\delta^{13}C$ and atmospheric nitrogen for $\delta^{15}N$. The analytical precision, assessed by the repeat analysis of approximately 15% of the samples, was 2% and 1% or less for $\delta^{15}N$ and $\delta^{13}C$, respectively.

2.2.4 Data from monitoring programs

POP concentrations have been determined in LO eels as part of two monitoring programs (Ontario Ministry of Environment (MOE); Department of Fisheries and Oceans, Inspection Service Branch (DFO-ISB)). The MOE database contains POPs data in eels collected in LO primarily in late summer and reported on six occasions between 1981 and 2004. Only eels weighing 0.5 kg or more and collected in the northeastern part of LO, including Prince Edward Bay, Main Duck Island, and the Bay of Quinte, were included, and represented up to 180 eels, depending on the analyte. All chemical analyses were performed on samples of muscle tissue homogenate. The DFO-ISB database contains contaminant data in eels collected each year between 1983-1988 and 1990-1991. Only eels weighing more than 0.5 kg from two areas located on the northeastern part of LO, near Bay of Quinte and Kingston were considered, representing up to 86 eels, depending on the analyte. All chemical analyses were performed on samples of whole fish homogenate.

2.2.5 Data treatment and statistical analysis

Contaminant data were presented on a wet-weight (ww) basis. The concentrations of POPs were compared among sampling years by analysis of variance (ANOVA) on log-10

transformed data to satisfy criteria of normality; no transformations were applied to biometric and stable isotope or contaminant pattern data. Bonferroni *post hoc* tests identified pairwise significant differences of contaminant concentrations between sampling years.

Temporal trends were also examined on a ww basis because lipid content was not available from all datasets included in our assessment. A first-order exponential decrease $[POP]_t = [POP]_{t=0}\, e^{-bt}$ described temporal trends for most contaminants in LO eels. Half-lives were calculated as -ln(2)/b. This approach was justified because temporal trends of POPs in lake trout have been described successfully by an exponential decrease model that enabled the calculation of the apparent loss rate of POP concentrations per year [12-14, 26-28].

$$\text{Rate Loss} = (1-e^{-b}) \times 100\%$$

2.3 Results and Discussion

2.3.1 Biometric data for eels

The mean age of the eels approximated 20 years and was not significantly different among sampling years (p=0.714) (Table 2-1). Lengths ranged from 81.5 to 127 cm and weights from 1.0 to 3.5 kg. At this age, length and weight, eels from LO are ready to initiate their spawning migration back to the Sargasso Sea [1]. There was no significant difference in mean weight (p=0.054) among sampling years, but eels collected in 2008 were significantly longer (p=0.001). As a result, the CF and RCF of eels collected in 2008 were significantly lower than in 1988 or 1998 (p=0.002 and 0.044, respectively), corresponding to a significantly lower mean lipid content (p=<0.001) (Table 2-1), and suggesting a decreasing condition with time. Desjardins et al. [2] reported length and weight of 30 eels collected in LO in 1982. The mean CF for these female eels was not significantly different than the CFs of eels collected in 1988 and 1998, but higher than those collected in 2008. Ryan et al. [4] reported a mean lipid content of 36.6 ± 4.5 %

in six eels collected in LO in 1980, 20 % greater than the highest values reported in Table 2-1, suggesting a consistent decreasing trend over 28 years. Similar temporal declines of RCF and lipid content were reported for yellow European eel (*Anguilla anguilla*) from Belgium over eight years (1996-2004), and of lipid content of eels from The Netherlands over 27 years (1977-2004) [29]. The trends reported in Europe were confounded by a mixture of sexes [30], whereas the eels from LO were exclusively female [1] (Table 2-1). Belpaire et al. [29] and De Boer [30] identified several possible causes of declining condition, including pollution, disease, and global environment change, factors that also affect American eels.

Table 2- 1. Number of eels, mean age, length, weight, condition factor, lipid content, $\delta^{15}N$ and $\delta^{13}C$ in eels collected in Lake Ontario in 1988, 1998 and 2008. Different letters indicate a significant difference (ANOVA) between sampling years.

	1988	1998	2008
Number	10	10	10
Sex	Female	Female	Female
Age (yr)	20.4 ± 2.4 [1]	19.5 ± 2.0	20.0 ± 2.9
Length (cm)	94.6 ± 7.0[a]	86.9 ± 5.1[a]	106.2 ± 14.5[b]
Weight (kg)	2.1 ± 0.4	1.6 ± 0.5	2.2 ± 0.8
Condition factor (g/cm^3)	0.24 ± 0.02[a]	0.24 ± 0.02[a]	0.18 ± 0.03[b]
Le Cren's relative condition factor	1.10 ± 0.12[a]	1.03 ± 0.22[a, b]	0.91 ± 0.14[b]
Lipid content (%)	30.7 ± 2.4[a]	28.9 ± 2.0[a]	23.2 ± 2.9[b]
$\delta^{15}N$ (‰)	16.1 ± 0.5[a]	16.2 ± 0.6[a]	15.2 ± 0.4[b]
$\delta^{13}C$ (‰)	- 23.2 ± 0.9[a]	- 22.9 ± 1.4[a]	- 18.8 ± 1.6[b]

[1]standard deviation

There was also a corresponding significant change in mean $\delta^{15}N$ and $\delta^{13}C$ between eels collected in 2008 and those collected in 1988 or 1998 ($p \leq 0.001$; Table 2-1). Changes in the LO environment have been reported by Dietrich et al. [31], especially in the food web structure of eastern LO, leading to a change in lake trout diets. A change in food web structure was consistent with temporal changes in stable isotope composition ($\delta^{15}N$ and $\delta^{13}C$) of lake trout from LO collected between 1979 and 2004. To our knowledge, changes in the diet of eels from LO have not been studied. While the decreasing trend of $\delta^{15}N$ in eels between 1998 and 2008 (Table 2-1) was similar to that of lake trout between 1998 and 2004, $\delta^{13}C$ values of eels increased, opposite to the trend in lake trout [18]. This difference may reflect the benthic character of eels compared to the pelagic lake trout. According to Hodell and Schelske [32], $\delta^{13}C$ trends in LO sediment cores are particularly variable over time.

2.3.2 Contamination of eels by POPs

Contamination of eels collected in 1988, 1998 and 2008 from LO was characterised for ΣPCDD/Fs, ΣPCBs, several OCPs and ΣPBDEs (Table 2-2). Regardless of the sampling year, ΣPCBs predominated in eels, reaching concentrations up to 1901 ng/g ww in an individual collected in 1988. Concentrations of ΣPCBs represented on average 64% of the POP contamination (sum of all classes) measured in this study; the concentrations of ΣPCBs combined with those of OCPs, explained 96% of the POP contamination of eels. While the 17 individual PCDD/Fs comprised 13% of the 128 individual POPs measured, their contribution to POP concentrations was very low, less than 0.002%. On average, ΣPBDEs contributed about 4% of the POP contamination in eels. The proportion of each individual class of POPs relative to the sum of POPs was not significantly different among sampling years (Figure 2-2A), suggesting that no important inputs or disappearances of a given class of POPs occurred within that time period. The

organochlorine pesticides (OCPs) comprised of several sub classes, namely ΣDDTs, ΣCHLs, ΣHCHs and ΣTCPs along with individual compounds such as HCB, Dieldrin, and Mirex. The main contribution to OCPs came from ΣDDTs and ΣCHLs; when combined, they represented >70% of this class of POPs. Mirex concentrations were relatively high in eels from LO, exceeding 10% of OCP concentrations. This was not too surprising since point sources of Mirex located on the shore of the Niagara and Oswego Rivers drained into and contaminated LO in the 1960-70s [33]. The proportion of OCPs represented by some sub-classes or individual compounds stayed constant with time, whereas ΣDDT increased, and ΣCHL, ΣHCH, and Mirex decreased significantly in 2008 relative to 1988 and 1998 (Figure 2-2B). This change in OCP general pattern with time could result from a variation of inputs to LO or the transformation of certain OCPs in LO or/and within the eels.

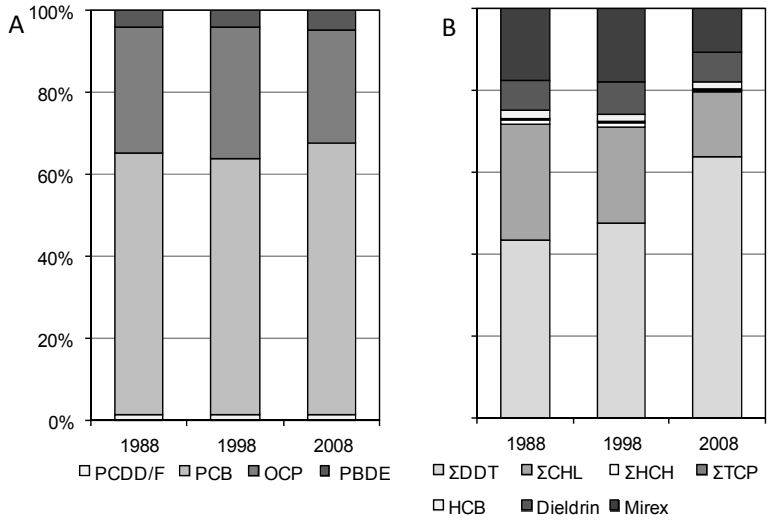

Figure 2- 2. Proportion of major classes (A) among all POPs or (B) OCPs for each sampling year.

Table 2- 2. Mean concentrations[1] of ΣPCDD/Fs, ΣPCBs, ΣDDTs, ΣCHLs, ΣHCHs, ΣTCPs, HCB, Dieldrin, Mirex and ΣPBDEs in eels collected in Lake Ontario in 1988, 1998 and 2008. Different letters indicate significant difference (ANOVA) between sampling years. N=10/year.

	1988	1998	2008
ΣPCDD/Fs	26.0 ± 8.0 [2, a]	22.4 ± 4.6 [a]	7.4 ± 3.6 [b]
ΣPCBs	1262 ± 288 [a]	1028 ± 250 [a]	364 ± 181 [b]
ΣDDTs	265 ± 83 [a]	245 ± 48 [a]	102 ± 54 [b]
ΣCHLs	169 ± 51 [a]	125 ± 38 [a]	27 ± 18 [b]
ΣHCHs	4.7 ± 0.8 [a]	4.6 ± 0.4 [a]	0.6 ± 0.1 [b]
ΣTCPs	2.0 ± 0.5 [a]	1.5 ± 0.5 [a]	0.4 ± 0.2 [b]
HCB	12.6 ± 3.6 [a]	10.0 ± 2.9 [a]	2.6 ± 1.3 [b]
Mirex	105 ± 38 [a]	93 ± 30 [a]	18 ± 12 [b]
Dieldrin	42 ± 20 [a]	40 ± 14 [a]	11 ± 5.0 [b]
ΣPBDEs	75 ± 8.7 [a]	66 ± 23 [a]	24 ± 17 [b]

[1]ΣPCDD/Fs are in pg/g ww otherwise in ng/g ww, [2]standard deviation

2.3.3 Concentrations, patterns and trends

PCBs

Concentrations of ΣPCBs in the 30 eels from LO ranged from 163 to 1901 ng/g ww (Table 2-2). Eels collected in 2008 were significantly less contaminated than those collected in 1988 and 1998. Most PCB congeners were detected in all samples, except for a few low chlorinated ones (CB 18, 37 and 81) that had concentrations above the LOQ in only some samples; the concentrations of CB 15, 33 and 40 were systematically below the LOQ. The four no-PCB congeners, CB 77, 81, 126 and 169, were quantified by high resolution MS which

allowed an LOQ about two orders of magnitude lower than the LOQ by low resolution ion trap. The most predominant congeners were two hexa-CBs (CB138 and 153) with an average combined contribution of about 30% of ΣPCBs.

The proportions of the different homolog groups relative to ΣPCBs were calculated for each sampling year. As expected, the hexa homolog group predominated followed by penta and hepta homolog groups (Figure 2-3A). These three groups together represent almost 90% of the measured PCB contamination. Except for the octa-deca homolog group, which had a significantly higher relative contribution in 2008, the different homolog groups had a similar contribution relative to ΣPCBs among the sampling years.

Among the PCB congeners considered in this study, each dioxin-like PCB congener (dl-PCBs; toxic effects similar to those of 2,3,7,8-TCDD) was analysed individually. The proportions of the four no-PCBs and eight mo-PCBs were about 0.05% and 14% of ΣPCBs, respectively. The dl-PCB patterns in eels captured in LO were dominated by PCB-118 and to a lesser extent by PCB-105. These two congeners contributed on average > 80% to the total dl-PCBs in LO eels. Ryan et al. [4] reported a mean PCB concentration of 4900 ± 2780 ng/g ww (ranging from 2120 to 7680 ng/g ww) in eels from LO collected in 1980 (Figure 2-4). The mean concentration of ΣPCBs was about 4 times higher than in eels collected in LO in 1988. For the MOE monitoring data, the most contaminated eel was collected in 1981 with a PCB concentration of 8269 ng/g ww. The MOE reported mean values of 3867 and 897 ng/g ww for 1986 and 1993 respectively. The mean PCB concentration in eels collected in 1988, 1998, and 2008 in the present study agreed well with the PCB concentrations reported by the MOE, and with an exponential regression relating concentration to the year sampled (Figure 2-4). For the DFO-SB monitoring data, the most contaminated eel was collected at the beginning of the program in 1983 and had a PCB concentration of 6240 ng /g ww. The mean concentration of ΣPCBs in eels collected in

1988 was 1318 ng/g ww, a value almost identical to the 1262 ng/g ww obtained in the present study. In 1982, Desjardins et al. [2, 3] collected 25 eels in LO, weighing more than 0.5 kg, during the mid-summer and reported PCB, as *Aroclor* 1254, mean concentrations of 6682 ± 2686 ng/g ww . This average value is in the same range of those reported by others (Ryan et al, [4], MOE, DFO) in the same period. Temporal trends of PCBs reported in Figure 2-4 combine the data from the studies described above where eels were collected in LO. Although these data were generated by various laboratories, there was a distinct and statistically significant exponential trend over the time period examined (R^2=0.82). This trend represents a decrease of about 9.7% per year.

DDTs

Eels collected in 2008 were significantly less contaminated with ΣDDT than eels collected in previous years (Table 2-2), but no significant differences were observed between eels collected in 1988 and 1998. Among the six compounds comprising ΣDDTs, 4,4'-DDE was by far the predominant constituent, representing about 70 % of the ΣDDTs regardless of the sampling year (Figure 2-3B). The high ratio of 4,4'-DDE relative to ΣDDTs in eels suggests that the source of these compounds in LO is not recent, considering that 4,4'-DDE is a degradation product of technical DDT [34].

DDT data for eels collected in LO between 1981 and 2004 were obtained from the MOE monitoring program where ΣDDT concentrations (sum of 4,4'-DDE, 4,4'-DDD, 4,4'-DDT and 2,4'-DDE) were reported in 180 eels (Figure 2-4). Mean concentrations of ΣDDT in eels collected in1988 and 1998 by the present study appear to be lower and higher, respectively, than the mean concentrations reported by MOE in eels collected in adjacent years (Table 2-2). Data for DDT + metabolites in 86 eels from eastern LO were obtained through the DFO-ISB monitoring program between 1983 and 1991. The most contaminated eel was collected at the beginning of the program in 1984 and had a ΣDDTs concentration of 1720 ng/g ww. The mean concentration

of eels collected in 1988 was 417 ng/g ww, a value almost two times higher than the 265 ng/g ww obtained in the present study.

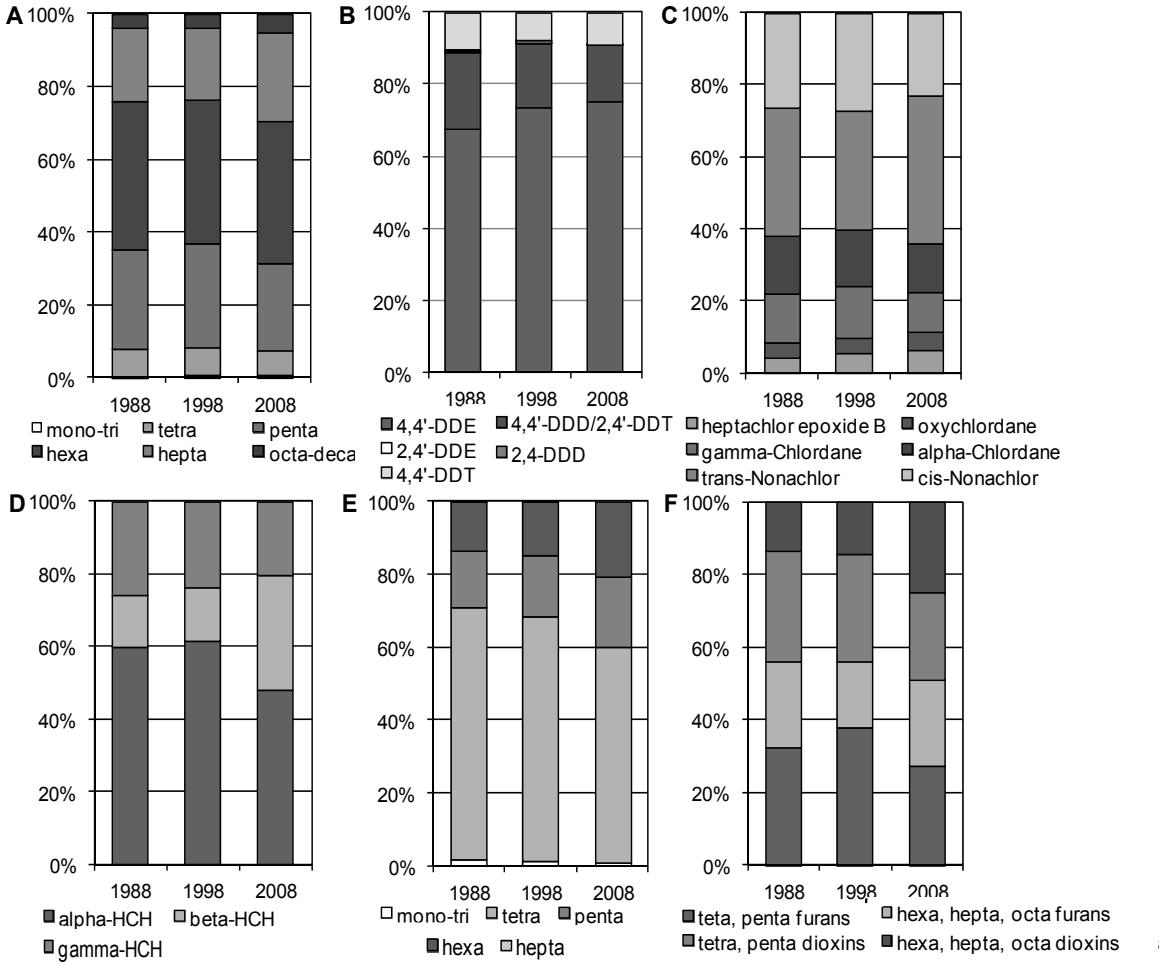

Figure 2- 3. Proportion of homolog groups of (A) PCBs, (B) DDTs, (C) CHLs, (D) HCH, (E) PBDEs, and (F) PCDD/Fs for each sampling year from the present study; N = 10/year.

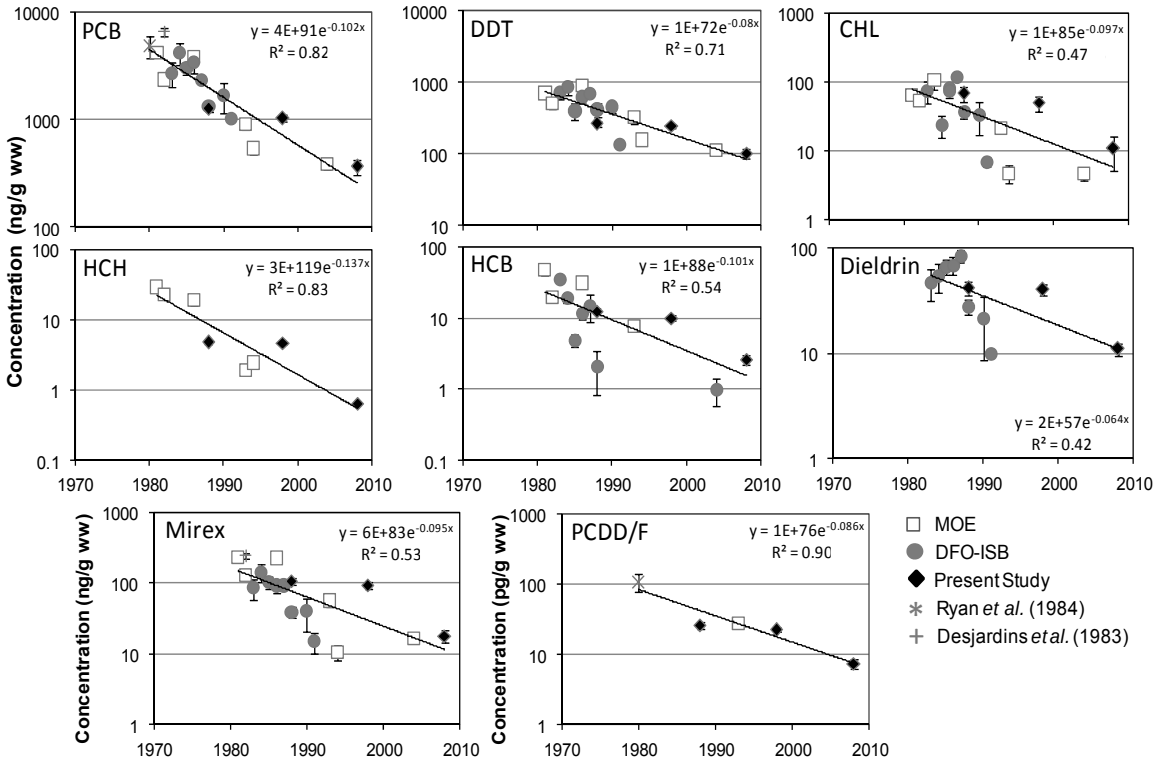

Figure 2- 4. Temporal trend of ΣPCBs, ΣDDTs, ΣCHLs, ΣHCHs, HCB, Dieldrin, Mirex (ng/g ww), and ΣPCDD/Fs (pg/g ww) in eels from Lake Ontario, error bars represent standard errors. Slopes were used to calculate half-lives ($t_{½}$ ± 1 standard error) for each compound class. Data were derived from the present study, Ryan et al. [4], Desjardins et al. [2,3], the Department of Fisheries and Oceans Canada (DFO-ISB), and the Ontario Ministry of the Environment (MOE). CHL data does not include nonachlor because the monitoring datasets did not measure nonachlor. Biological details for the present study are shown in Table 2-1.

The temporal trend of ΣDDT in LO eels shown in Figure 2-4 is based on data from this study, and the MOE and DFO-ISB monitoring programs. Despite the fact that the ΣDDT data come from various laboratories, and sometimes with different related compounds such as the 2,4' structural isomers, the trend described an exponential decrease over time (R^2=0.71). This trend represents a loss rate of 7.7% per year.

CHLs

Among the seven compounds related to technical chlordane and its transformation products examined in this study, heptachlor was systematically below the detection limit. As a result, six compounds were included in the ΣCHL (Table 2-2). Heptachlor is not only a minor component of technical chlordane, representing at most 10% of the mixture, but the main component of another pesticide produced as technical heptachlor [35]. The predominance of the breakdown product, heptachlor epoxide, in eel samples examined in the present study (Figure 2-3C) suggested historic applications of either technical chlordane or technical heptachlor in the immediate environment of LO eels. The sum of trans and cis-nonachlor isomers represented the highest proportion of ΣCHL, about 60 %, whereas the sum oxychlordane and heptachlor epoxide represented less than 10%. The relative proportion of each compound was not significantly different among the three sampling years (Figure 2-3C). These results suggested that sources of chlordane in LO eels have not changed over the period examined. On the other hand, concentrations of ΣCHL in eels from LO collected in 1988 and 1998 were not significantly different, but were higher than in eels collected in 2008 (Table 2-2). This indicated a significant decreasing trend for those compounds in LO eels.

Compounds in eels from LO related to technical chlordane were also reported by other studies, but none reported nonachlor isomers. For the sake of comparison, these isomers were excluded from the temporal trend results reported in Figure 2-4. Temporal trend data included those from this study and from the DFO-ISB and MOE monitoring programs. Based on the proportion of the different CHL constituents (Figure 2-3C), the MOE data were augmented by 25% to compensate for oxychlordane and heptachlor epoxide not being measured. Although this adjustment improved the relationship (R^2), it did not change the exponential decreasing trend characterized by the loss rate of 9.2 % per year.

HCHs

Only three of the four HCH isomers examined in this study were detected; δ-HCH was systematically below the LOD of the analytical method used. As a result, reported ΣHCH comprised α, β and γ (lindane) isomers (Table 2-2). The proportion of α-HCH was significantly higher in 1988 and 1998 than in 2008, whereas the opposite was observed for β-HCH. On the other hand, the proportion of γ-HCH showed a statistically significant decreasing proportion with time (Figure 2-3D). This is in agreement with the current-use of HCH in North-America, which is limited to quasi-pure γ-HCH in pharmaceutical applications [36]. According to the ATSDR review on HCH isomers, β-HCH is more effectively bioaccumulated than γ-HCH, which may explain the change in pattern observed in LO eels. This is further supported by the significantly lower ΣHCH concentrations in LO eels collected in 2008 compared to those collected in 1988 and 1998 (Table 2-2). The decreasing proportion and concentrations of γ-HCH suggested negligible recent inputs of HCHs in LO.

Data on ΣHCH in LO eels were acquired through the MOE monitoring program. Combining the two data sets revealed a clear exponential decrease with time ($R^2 = 0.83$), with a fast apparent loss rate estimated at 12.8 % per year (Figure 2-4). The DFO-ISB monitoring program focused only on γ-HCH, but all concentrations were below detection limits of their analytical method (5 ng/g ww). To the best of our knowledge, temporal trends of HCH concentrations in lake trout have never been published. Therefore, this study is the first one to report HCH temporal trends in a top predator fish from LO.

TCPs

The ΣTCPs comprise two related compounds, TCPM and its presumed precursor TCPMe [37, 38]. The origin of TCPMe is not well established, but recent chlorine isotope analyses have confirmed its anthropogenic source [39]. Both TCP compounds were consistently detected in LO

eels, but TCPM was by far the predominant form, representing 85-90 % of ΣTCPs. There were no significant changes in the proportions of the TCP constituents in eels among the sampling years. On the other hand, concentrations of ΣTCPs in eels from LO collected in 1988 and 1998 were not significantly different, but were higher than in eels collected in 2008 (Table 2-2). This indicated a significant decreasing trend for those compounds in eels from LO.

Results of ΣTCPs in eels from LO are limited to those reported in this study and did not reveal a significant exponential decrease with time (p=0.21). Considering the very limited data available, these results and the model used to interpret the data must be considered as preliminary results. To the best of our knowledge, these chemicals have never been reported in lake trout or any other fish from LO.

HCB, Mirex and Dieldrin

Individual persistent organic pollutants, namely HCB, Mirex and Dieldrin, were detected consistently in eels from LO, and concentrations were lower in 2008 than in 1998 and 1988 (Table 2-2). High concentrations of Mirex in eels from LO reflect local sources [33, 40]. Data on HCB, Mirex, and Dieldrin were also reported in eels collected in LO through the DFO-ISB and MOE monitoring programs and Desjardins et al. [2, 3]. When combined with these past data, there were significant exponential declines in HCB and Mirex in LO eels, with a decrease estimated at 9.6 and 9.1 % per year, respectively, during this almost 30 year period (Figure 2-4). Despite highly variable Dieldrin concentrations, a significant decreasing trend was observed that was characterized by a loss rate estimated at 6.2 % per year (Figure 2-4).

PBDEs

To the best of our knowledge, no previous study reported PBDE concentrations in recently collected (2008) eels and in eels collected more than 20 years ago (1988). Eels collected in 2008 had significantly lower PBDE concentrations compared to eels collected in 1988 or 1998

(Table 2-2). The decrease in concentrations was also associated with a significant change in congener patterns. The PBDE pattern was dominated historically by tetra-PBDEs, but the proportion of tetra-PBDEs decreased significantly in 2008 to the advantage of penta- and hexa-PBDEs (Figure 2-3E). Concentrations of highly brominated congeners (octa, nona and deca) were near or below detection limits. Considering that tetra- and penta-PBDEs originate from the same commercial formulation [41], opposite changes of proportion over time between these two homolog groups was likely the result of differential degradation or (bio)transformation. On the other hand, the presence of BDE-154 in LO eels, a debromination product from highly brominated congeners, suggest that BDE-154 was either produced by eels from highly brominated congeners or bioaccumulated from their environment, likely through their diet.

While PBDE concentrations in 2008 were 1/3 of those in 1988, no attempt was made to describe any temporal trends because data on PBDEs in LO eels were limited. On the other hand, Luross et al. [17] reported exponentially increasing PBDE concentrations in lake trout from LO. Thereafter, Zhu and Hites [19] reported that ΣPBDE concentrations in LO lake trout had leveled off in the middle of 1990s. These observations were supported by Batterman et al. [16] and Carlson et al. [13], whereas Ismail et al. [18] reported for the first time a decreasing trend of PBDEs in LO lake trout, beginning around 1995. If the temporal trend of PBDEs in LO eels is similar to the one in lake trout, then maximum PBDE concentrations would have occurred between 1988 and 1998 in LO eels. Hence, the apparent trend of decreasing PBDE concentrations in LO eels between 1998 and 2008 is likely representative.

PCDD/Fs

Concentrations of ΣPCDD/Fs in eels collected in LO in 1988, 1998 and 2008 ranged from 3.82 to 43.0 pg/g ww (Table 2-2). On average, eels collected in 2008 were significantly less contaminated, about three times, than those collected in 1988 or 1998. Three congeners, 2,3,7,8-

TCDF, 2,3,4,7,8-PeCDF and 2,3,7,8-TCDD, were detected in all eel samples, whereas 1,2,3,4,7,8,9-HpCDF was never detected. Nine of 17 PCDD/F congeners with chlorine atoms in 2,3,7,8 positions were detected in > 90% of the samples, but two congeners, 2,3,4,7,8-PeCDF and 2,3,7,8-TCDD, represented about 50% of ΣPCDD/F concentrations in all samples. The relative contribution of less (tetra-penta) and more substituted (hexa, hepta and octa) dioxin and furan congeners was not significantly different between 1988 and 1998. In 2008, however, the proportion of the less substituted furans and dioxins decreased, whereas the more substituted dioxins increased (Figure 2-3F). Differences in concentrations and patterns of PCDD/Fs among sampling years were not affected by replacing 'non-detected' with half of the concentrations of the LOQ.

Limited data were available in the literature for PCDD/Fs in American eels from LO. Ryan et al. [4] reported 2,3,7,8-TCDD in five of six American eels captured in LO in 1980, with a mean of 19.8 pg/g ww (range = 6.4 to 38.5 pg/g ww). In comparison to eels collected from LO in 1988, the closest time period investigated in this study, the mean concentration of 2,3,7,8-TCDD was four times lower at 5.7 pg/g ww (range = 4.2 to 6.9). The PCDD/F data in eels collected in 1993 were obtained from a monitoring program conducted by MOE, and represented 10 eels collected in the northeastern part of LO in late summer. The MOE measured the 17 congeners substituted in the 2,3,7,8 positions and reported a mean value of 28.0 ± 11.8 pg/g ww, in good agreement with the mean concentration in eels collected in 1988 and 1998 reported in the present study (Table 2-2).

An exponential decreasing trend was obtained by combining ΣPCDD/Fs data from this study with data from the MOE and Ryan et al. [4], despite only a few studies reporting PCDD/F concentrations in eels from LO. Because Ryan et al. [4]reported only 2,3,7,8 TCDD, ΣPCDD/Fs was estimated using the mean ratio of 5.5 (± 1.3) between ΣPCDD/Fs / 2,3,7,8 TCDD calculated

in eels from this study and the MOE program. This approach is justified by the constant ratio observed between 2,3,7,8 TCDD and other PCDD/F constituents in lake trout from LO with time [42]. The resulting trend was characterized by a loss rate estimated at 8.2 % per year (Figure 2-4).

2.3.4 Comparison of temporal trends for lake trout from LO

Because of similarities in habitat and trophic status, LO lake trout provide a good perspective on contaminant concentrations in eels. Borgmann and Whittle [15] reported temporal trends of PCB in four year old lake trout collected in eastern LO from 1977 to 1988 as part of the monitoring program conducted by DFO-ISB. Huestis et al. [43] also reported contaminant concentrations in lake trout collected from the same site and same age class as Borgmann and Whittle [15], but for a longer time period (1977-1993). The monitoring program conducted by the MOE also included lake trout from LO (1976-2006) [9, 42]. A third monitoring program managed by the US-EPA has also focused on lake trout from LO near Oswego [11, 14]. Carlson et al. [13] extended the dataset to range from 1977 to 2002. Overall, the long-term temporal trends were described by an exponential decrease for ΣPCBs, ΣDDTs, ΣCHLs, HCB, Mirex, and Dieldrin in lake trout from LO, and similar to those observed in eels from LO (Table 2-3). The differences between lake trout and eels in the rate of change of POPs concentrations might reflect differences in fish ecology (diet and habitat), physiology (capacity for metabolizing compounds), or within- or among-year error in chemical analyses.

Table 2- 3. Rate loss and half-life comparisons between eels and lake trout captured in Lake Ontario for select classes of compounds.

	Rate loss eels[†] (% per year)	Rate loss l. trout[‡] (% per year)	Half-life eels[†] (years)	Half-life l. trout[‡] (years)	Range[‡] (year)
PCB	9.7	6.7-9.5	6.8	6.9-10.0	1976-2006
DDT	7.7	3.7-8.3	8.6	8.0-18.4	1977-2002
CHL	9.2	2.7-8.6	7.2	7.7-25.3	1977-2002
HCH	12.8	N/A	5.1	N/A	N/A
HCB	9.6	9.3	6.9	7.1	1977-2002
Mirex	9.1	6.7	7.3	10.0	1977-2002
Dieldrin	6.2	8.4	10.8	7.9	1979-1998
PCDD/F	8.2	7.7	8.0	8.7	1979-2004

[†]The present study; [‡]Borgmann and Whittle [15], Huestis et al. [43], Bhavsar et al. [42], De Vault et al.[14], Hickey et al. [27], Carlson et al. [13], and Gewurtz et al. [9].

2.3.5 Variables affecting temporal trends

Many variables are known to influence temporal trends of toxic chemicals in biota. The accumulation of POPs by fish could differ between sexes, but eels from LO and the upper St. Lawrence are recognised to be virtually all female [1], and were determined histologically to be all female in the present study. Thus, all new chemical data reported in the present study represented one sex only, but the sex of eels in past studies was not reported by the MOE or DFO. Another variable that can affect the accumulation of POPs is the age of the fish. All LO eels selected in the present study averaged about 20 years old (Table 2-1), and were close to sexual maturity and migration out of the lake. Eel ages were not available for the other datasets but

length and/or weight were reported. To limit the age range, weight was used as a proxy for age, and only fish weighing 0.5 kg or more were considered. Evidently, variability in temporal trend data may be in part explained by the relatively large range of age for the eels examined. The sampling site in LO may also be important to consider, since LO is a large lake offering a variety of habitats characterised by different degree of contamination [44]. Borgmann and Whittle [15] reported the effects of sampling sites in LO on the concentrations of several POPs in lake trout (e.g. PCB, Mirex). They observed that the western basin of LO is much more contaminanted with POPs than the eastern basin. In the present study, all eels were captured in the eastern part of LO for the three time periods investigated (Figure 2-1). To be comparable, only sites from the eastern part of LO were considered from MOE and DFO-ISB datasets. Eels examined by Desjardins et al. [2, 3] and Ryan et al. [4] and reported in this study were from LO, but could not be associated to any specific sites or sectors.

Differences in carbon stable isotope ratio ($\delta^{13}C$) among sampling years indicate a sharp increase from 1998 to 2008 (Table 2-1), suggesting different carbon sources and a shift of the diet of eels to different prey or prey from different origins, despite eel collections from similar areas of LO. Changes in $\delta^{13}C$ among collection years could also reflect a change in carbon input or productivity of the eastern part of LO. Similar significant differences were observed for $\delta^{15}N$ and lipid content in eels collected in 1988 and 1998 compared to those collected in 2008 (Table 2-1). Without additional information, it is not possible to firmly discriminate between: 1) change of conditions with time in eastern LO, such as a change in the diet of eels, or 2) sampling of a group of eels from a different origin, including residents of adjacent rivers. The consistency of data within each collection year and the good fit of the new contaminant data with those of other datasets suggest, however, that eels collected in 2008 are representative of eastern LO. Because the focus of the present study was to examine temporal changes of contamination in eels from LO

and not changes that have occurred in the LO ecosystem, no attempt was made to adjust contaminant data for lipid content or $\delta^{15}N$, a proxy of trophic position; although it can be relevant in certain conditions [45, 46].

Another variable that may be influential was the tissue selected for chemical analyses. In this study, all of our analyses were of whole eel homogenates, but for some monitoring program datasets, POPs analyses were restricted to muscle tissue (edible portion). Previously, we found no significant differences in POP concentrations between eel muscle and whole fish homogenate within the limits of analytical variability for all classes of POPs analysed [47]. This indicates that tissue selection and muscle proportion in the sample had no effect on the results. The analytical method could have been a crucial variable when data from different sources were combined. Data reported in the early 1980s used different methodologies than those used today, and quality controls are now much more systematic and extensive. Combining data from different datasets using different methods and, as detailed above, often different congeners or isomers for a given class of POP add to the variability of temporal trends. Nevertheless, despite all of these variables and uncertainties, temporal trends of POPs in LO eels reported in the present study were very significant for most classes of POPs or specific contaminants.

2.3.6 Potential effects of toxic chemicals on eel recruitment

Fish and wildlife exposed to POPs are at risk of a variety of toxic effects. Among the most toxic POPs are the PCDD/Fs and PCBs congeners with chlorine substituted in meta and para positions and having a planar conformation. These compounds act additively on the aryl-hydrocarbon receptor (AhR) and have a common receptor-mediated mechanism of toxicity [48]. These DLCs have been reported in various matrices of LO including fish [49, 50]. Their relatively low concentrations in eels cannot be neglected because of their high toxicity (Table 2-2). Cook et al. [20] demonstrated that the complete elimination of natural reproduction by lake

trout in the mid-1960s could be explained by historic loadings of DLCs into LO. The present findings suggest that eels, a top predator like lake trout in LO, could have also been affected by high concentrations of DLCs accumulated in their tissues. These DLCs were most likely transferred to eggs, possibly causing embryo-toxicity and impaired recruitment. This hypothesis has been explored in European eel (*Anguilla anguilla*) from Belgium where DLC concentrations were considered to be detrimental to their reproduction in the majority of the sites investigated [51]. Concentrations of DLCs are commonly expressed in toxic equivalents (TEQs) calculated using World Health Organization Toxicity Equivalency Factor (WHO-TEF) method for fish and mammals [48]. Because this study focused on potential health effects on eels from LO rather than on their consumers, TEQs were calculated using fish-specific TEFs for PCDD/Fs and dl-PCBs (Table 2-4). The PCDD/Fs contributed about 80% of ΣTEQs, and proportional contributions have not changed significantly over the years. The ΣTEQs in eels were significantly higher in 1988 and 1998 than in 2008 by 4- to 5-fold ($p < 0.001$).

Limited DLC data were available for American eel, and most datasets were incomplete due to the analytical limitations of the time. Using mean concentrations, ΣPCDD/Fs were estimated in eels captured in LO in 1980 [4] and by the MOE in 1993. TEQs were subsequently estimated from the PCDD/F data using the mean ratio TEQs:ΣPCDD/Fs calculated from the eels examined in the present study (Tables 2-2 and 2-3). Similarly, TEQs for PCBs (mono + non-ortho congeners) were calculated from ΣPCBs reported in eels captured in LO in 1980 and 1993 (MOE) using the mean ratio TEQs:ΣPCBs calculated from the eels examined in this study (Tables 2-2 and 2-3). This approach was validated for fish from the Great Lakes [52]. Estimated mean ΣTEQ concentrations were 59.5 ± 35.6 and 14.4 ± 6.9 pg/g ww for eel from LO collected in 1980 and 1993, respectively. When the estimated TEQs in eels collected in LO are combined, there is an

exponential decrease over the last 30 years characterized by a loss rate of 8.7 % per year (Figure 2-5).

Table 2- 4. Mean toxic equivalent concentrations (pg/g ww) of PCDD/Fs-TEQ, no-ortho PCBs-TEQ, mono-ortho PCBs-TEQ and ΣTEQ in eels collected in Lake Ontario in 1988, 1998 and 2008. Different letters indicate significant difference (ANOVA) between sampling years. N=10/year.

	1988	1998	2008
PCDD/F-TEQ	12.0 ± 2.3 [1, a]	10.9 ± 2.5 [a]	2.8 ± 1.4 [b]
no-ortho PCB-TEQ	0.9 ± 0.2 [a]	0.7 ± 0.2 [a]	0.2 ± 0.1 [b]
mono-ortho PCB-TEQ	2.4 ± 0.4 [a]	1.9 ± 0.6 [a]	0.5 ± 0.2 [b]
ΣTEQ	15.3 ± 2.8 [a]	13.5 ± 3.1 [a]	3.5 ± 1.7 [b]

[1]standard deviation

To assess the effects of DLC on eel recruitment, ideally one would determine the concentration of DLC-TEQs in a spawning eel's eggs and compare this concentration to a mortality threshold. A population level effect could be determined by assessing the percentage of eel eggs exceeding the threshold. As detailed in Byer et al. [23], concentrations of DLC contaminants in wild eel eggs during spawning have never been measured. However, concentrations of DLCs in tissues of migrating silvering eels were revealed to be highest in gonads with gonad/maternal concentration ratios averaging about 1.4 for PCBs [7]. Calculating egg TEQs using this multiplication factor, mean concentrations ranged from 4.9 to 83.2 pg TEQ/g ww egg. Reliable DLC mortality thresholds have not been established for eels but early life stage mortality guidelines are available for lake trout [20]. Assuming these DLC mortality thresholds are applicable to eels, the sublethal effects guideline of 5 pg TEQ/ g ww egg was exceeded by

more than half of the most recently collected eels (Table 2-4). From the TEQ temporal trend estimated in eel eggs (Figure 2-5, dotted line), about half of the eels collected in late 1980s exceeded the early life mortality threshold of 30 pg TEQ /g ww egg. The mean TEQ concentration in eels collected in 1980 was more than two times higher than the mortality threshold. These results indicate a high risk of eel recruitment failure in the 1980s. Also, sediment core records and Cook's model for lake trout eggs predict a maximum of TEQs in the late 1960s, about 5 times higher than the 1980s. Considering that recruits take several years (7-10) to reach LO, the decline in recruitment of American eels to LO corresponds to the time period where eels were highly exposed to DLCs.

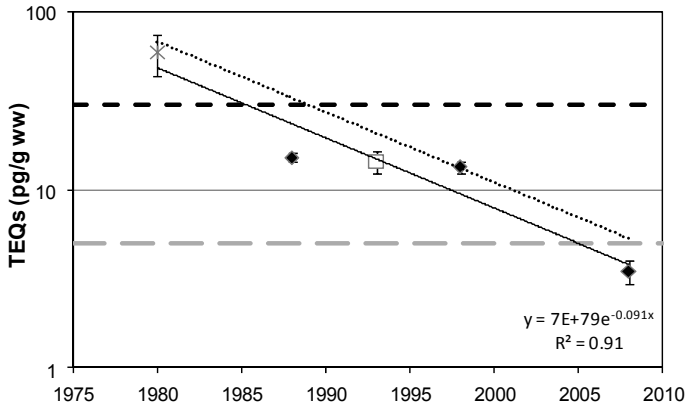

Figure 2- 5. Temporal trend of TEQ (pg/g ww) in eels (dark line) and predicted in eel eggs (dotted line) from Lake Ontario. Error bars represent standard errors and N=10/year. Threshold values of 5 pg/g ww egg for sublethal effects and 30 pg/g ww egg for early life mortality defined for lake trout (*Salvelinus namaycush*) are presented as dashed lines. Data were derived from (♦) the present study, (∗) Ryan et al. [4], and (□) the Ontario Ministry of the Environment (MOE). Biological details for the present study are shown in Table 2-1.

2.4 Conclusion

The present study is the first to report long-term temporal trends of POPs in American eel, and trends of HCH and TCPs in LO fish. In general, legacy POP concentrations in eels collected in LO have decreased exponentially over the last 30 years. Long-term loss rates averaged 9.1 % per year and were similar to those reported in lake trout from LO. A risk assessment of dioxin-like compounds in LO eels after converting to TEQs showed that concentrations were two times higher in the early 1980s than the threshold value (30 pg/g ww of TEQ determined for lake trout) related to early life stage mortality. These novel results suggest that in the years prior to 2000, embryo toxic effects of DLCs in LO eels impaired their reproductive success and contributed to the population decline.

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Chapter 3

Spatial trends of organochlorinated pesticides, polychlorinated biphenyls, and polybrominated diphenyl ethers in Atlantic *anguillid* eels

Abstract

The bioaccumulation of lipophilic contaminants such as polychlorinated biphenyls (PCBs), organochlorine pesticides (OCPs), and polybrominated diphenyl ethers (PBDEs) can result in a reduction in fitness and spawner quality in eels and may be a factor in *Anguillid sp*. population declines. Contaminant concentrations in eels have been studied extensively in Europe, but data for American eels are severely lacking. Concentrations of PCBs, OCPs, and PBDEs were determined in American eel from eastern Canada and New York, USA, along with European eel from Belgium. Principal component analysis revealed that eels captured in the St. Lawrence estuary were a mixture of upstream migrants from the St. Lawrence River watershed, and fish captured in local tributaries. Contaminant concentrations were dependent on origin, related to the local environment, and were lower than historic values. In Canada, concentrations of OCPs and PCBs in eel tissues were below the Canadian human consumption guidelines for contaminants in fish, indicating that the current risk to consumers is low. However, concentrations of PCBs, total DDT, and mirex in eels from L. Ontario and the upper St. Lawrence R. were above Great Lakes guidelines for the protection of piscivorous predators. Concentrations of penta-BDE homologs exceeded the Canadian guideline for environmental quality in over half of the eels in this study, but concentrations of the other homolog groups were below the guideline.

3.1 Introduction

American (*Anguilla rostrata*) and European eel (*Anguilla anguilla*), have similar but distinctive spawning locations in the Sargasso Sea [1]. Their leptocephali migrate with ocean currents to the Atlantic coast of North America (*A. rostrata*) or Europe (*A. anguilla*), and upon entry into continental waters, metamorphose to glass eels before becoming pigmented and migrating upstream as elvers. The final juvenile phase is the yellow eel stage and the longest at 10 to 20+ years [2]. Yellow eels transform into silver eels and mature sexually during their return migration to the Sargasso Sea where they spawn and die. Most sexually differentiated eels in Canadian waters are female (> 95%) and are exclusively female in Lake Ontario and the upper St. Lawrence River [3]. In contrast, there are lower percentages of females in the Maritimes, e.g. in the Saint John River, New Brunswick, 93% of 970 eels sampled were female, and in the East River, Nova Scotia, 45% were females [3, 4]. Eels are long-lived, subject to commercial fisheries, and reproduce only once during their life cycle, making their populations vulnerable to decline. Atlantic eels, in addition to other *Anguillid sp.*, have experienced dramatic and well documented reductions in recruitment and abundance [5]. Many hypotheses have been suggested to explain the declines ranging from anthropogenic to environmental factors [1], but to date, researchers have failed to establish the most significant cause of their decline.

In Europe, there has been a recent emphasis on spawner quality as the central link to eel decline. The reduction in quality of migrating silver eel may be characterized as the impairment of the adult eels' ability to successfully migrate to the Sargasso Sea and spawn, and/or the viability of offspring attempting to return to continental waters, i.e. recruitment [6].

Eels have several ecological and physiological characteristics that make them susceptible to accumulating contaminants, including relatively high lipid content, long life expectancy, diverse dietary habits, and the ability to inhabit a variety of aquatic environments, including those near

urban and industrial development [4]. In particular, halogenated persistent organic pollutants (POPs) accumulate to a significant extent in the fat tissue of eels [7-12]. A number of studies have documented the affects of lipophilic contaminants in eels such as impaired embryonic development [13-18]. It has also been suggested by Belpaire et al. [19] that the accumulation of contaminants is linked to a reduction in lipid content in eels, resulting in impaired spawner quality. However, a direct relationship between the reported effects and a population level response has not yet been established.

Eels can inhabit freshwater, estuarine, and marine environments, and these dissimilar environments have differing food webs, which can have an effect on contaminant exposure. Carbon and nitrogen stable isotope analyses are useful tools for examining the trophic dynamics of an aquatic ecosystem. The $^{13}C/^{12}C$ and $^{15}N/^{14}N$ ratios are often expressed in delta values in parts per thousand ($\delta^{13}C$ and $\delta^{15}N$) difference from a standard. The $\delta^{13}C$ reflects the primary production source responsible for energy flow in an ecosystem. Large differences in $\delta^{13}C$ are an indication of different food sources or a different food web based on different primary producers. The $\delta^{15}N$ corresponds to trophic position as ^{15}N is transferred to predators from prey, and increases up the food web [20]. Biplots of $\delta^{13}C$ and $\delta^{15}N$ have been used to differentiate eels based on aquatic environment and food web structure [21].

Eels are harvested in almost every phase of their life-cycle, and consumed as fresh, jellied, marinated or smoked eel. In 1997, about 800 t of eels were harvested in Canada, with a market value over $6.0 million. American eel were one of the top three commercially-fished species in Lake Ontario prior to the closure of the yellow eel fishery in 2004, because of low abundances and contaminant advisories [4, 22]. In Canada, elvers are exported from the east Coast to Asia, and yellow and silver eels are shipped from Quebec and the Maritimes to western Europe [3, 23].

Our objectives were to determine if there are differences in chemical contaminant concentrations among eels collected from across eastern Canada, and to compare the concentrations in eels with human consumption and environmental quality guidelines as well as for the protection of piscivorous predators. Concentrations of synthetic POPs, organochlorinated pesticides (OCPs), polychlorinated biphenyls (PCBs), and polybrominated diphenyl ethers (PBDEs) were measured in large yellow eels and silver eels from Canada, as well as in eels from the USA and Belgium, used as positive controls. The data were used to assess the current state of legacy POPs contamination in eels. It was not possible to perform a risk assessment of eel health posed by these contaminants, because guidelines for fish health are not available for the classes of compounds studied.

3.2 Experimental Details

3.2.1. Sample collection

Eels were collected in 2007 and 2008 throughout eastern Canada, and in 2009 from the USA and Belgium (Figure 3-1). Whole fish were stored frozen at -80 °C at the Fish Contaminants Laboratory of Environment Canada (EC) in Burlington, ON. Before homogenization, several tissues were dissected from each carcass for other analyses, including the liver, and small sections of muscle (approx. 10% by weight) and gonad; sagittal otoliths were removed for age determination. Whole fish homogenates were prepared in accordance with standard lab practices, sub-divided into 50 and 100 g solvent rinsed jars and stored in EC's National Biological Tissue Archive at -80 °C [24].

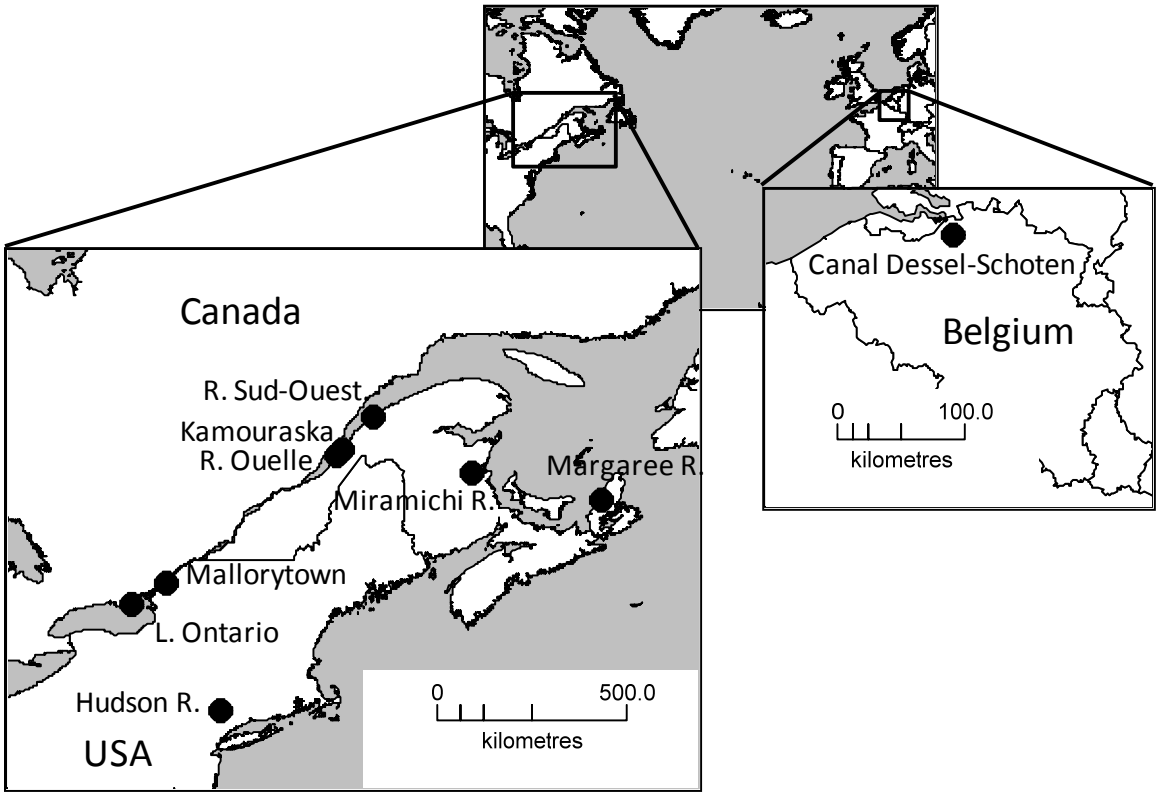

Figure 3- 1. Eel capture locations in eastern Canada, New York (USA), and Belgium.

3.2.2. Age determination

Age was determined from transverse sections of sagittal otoliths and acetate replicas, deciphered by a Calcified Structure Age-Growth data Extraction Software (CSAGES) and a standardized Calcified Structure Age Interpretation System (CSAIS) [25]. Interpretation procedures followed [26], using techniques validated from tag-recapture and tetracycline fluorescent labeling.

3.2.3. Histological analysis

Frozen gonad tissue was excised from the middle of the left gonad and fixed in 10% phosphate-buffered formalin. Fixed tissue was embedded in paraffin and 5 µm sections were stained with

hematoxylin and eosin [27]. Maturity of oocytes was graded from 1 to 4 with the following morphological criteria: stage 1: no cytoplasmic vacuoles; stage 2: small number of large cytoplasmic vacuoles; stage 3: large cytoplasmic vacuoles forming a complete circle in peripheral cytoplasm and covering less than 50% of the cytoplasm; stage 4: vitellogenic oocytes with large and small vacuoles covering more than 50% of the cytoplasm [28]. The most advanced and the predominant stages (>50 %) of oocyte maturity were determined on each section by examining approximately 200 randomly selected oocytes with a microscope at 160 X. The diameters (greatest length through the nucleus) of 10 oocytes from the most advanced stage of maturation were measured at 100 or 200 X with an image analyzer system (Image-Pro Express 6.0, Media Cybernetics, Inc., Silver Spring, MD, USA). We used the following criteria to distinguish silver migrant eels from resident yellow eels: median oocyte diameter > 0.13 mm and the predominant oocyte stage ≥ 3. These criteria were developed by Cottrill et al. [29] and represent the lower limits observed in known migrant eels captured from Quebec City and Kamouraska, QC. Eels that did not meet one of these two criteria were classified as 'intermediate' eels. Because gonad sections were frozen prior to histological processing, the morphological distinction between stage 2 and 3 oocytes was sometimes lost.

3.2.4. Chemical analysis

Eel tissue extracts were prepared for chemical analysis from approximately 20 g of homogenate. Each sample was dried chemically with anhydrous sodium sulphate, spiked with 2,2',3,3',4,4',5-[$^{13}C_{12}$]-HeptaCB and 2,3,7,8-[$^{37}Cl_4$]-TCDD, and extracted with dichloromethane. The extract was split by weight into four portions: 1) 10% for gravimetric lipid determination, 2) 40% as backup, 3) 25% for OCP, PCB, and PBDE analysis, and 4) 25% for PCDD/F, PCN, and dl-PCB analysis [30]. The third fraction was spiked with a solution of eight [$^{13}C_{12}$]-PCB surrogates, four [$^{13}C_{12}$]-PBDEs, and 11 [^{13}C]-OCPs (Wellington Laboratories, Guelph, ON).

Sample clean-up consisted of lipid removal using gel permeation chromatography with Biobeads SX-3, and a 2-layered packed 5% deactivated silica-alumina column [31]. Gas chromatography-mass spectrometry (GC-MS) was used to identify and quantify PCB, PBDE, and OCP concentrations with a ThermoQuest TraceGC equipped with a Finnigan PolarisQ ion trap, operated in electron ionization (EI) and MS/MS mode [31]. Forty-six singly and coeluting PCB congeners were measured (IUPAC numbers 8, 15, 18, 28/31, 33, 37, 40, 44, 49, 52, 66/70, 74, 87, 95, 99, 101, 105, 110, 114, 118, 123, 128, 138, 149, 151, 153, 156, 157, 167, 170, 171, 177, 180, 183, 187, 189, 191, 194, 195, 199, 205, 206, 208, 209). The OCPs analysed were α, β, δ, γ-hexachlorocyclohexanes (HCH), hexachlorobenzene (HCB), heptachlor, heptachlor epoxide (isomer B), oxychlordane, α and γ-chlordane, *cis* and *trans*-nonachlor, tris(4-chlorophenyl)methanol (TCPM) and tris(4-chlorophenyl)methane (TCPMe), mirex, dieldrin, 1,1,1-trichloro-2,2-bis(4-chlorophenyl)ethane (*p,p'*-DDT), and its metabolites [1,1'-dichloro-2,2-bis(4-chlorophenyl)ethane (*p,p'*-DDD) and 1,1-dichloro-2,2-bis(4-chlorophenyl)ethene (*p,p'*-DDE)], as well as 1,1,1-trichloro-2-(4-chlorophenyl)-2-(2-chlorophenyl)ethane (*o,p'*-DDT) and its metabolites [1,1'-dichloro-2-(4-chlorophenyl)-2-(2-chlorophenyl)ethane (*o,p'*-DDD) and 1,1-dichloro-2-(4-chlorophenyl)-2-(2-chlorophenyl)ethene (*o,p'*-DDE)]. The 14 PBDE congeners quantified were (IUPAC numbers 17, 25, 28, 33, 47, 49, 66, 75, 99, 100, 153, 154, 155, 183). The highly brominated octa to deca-PBDEs were not quantifiable in most samples, with concentrations < 0.1 ng g^{-1} ww.

3.2.5. Quality assurance and quality control

Samples were analysed by capture site with one silica method blank, one spiked blank, one in-house reference material (L. Trout), and one certified reference material (CARP-2; National Research Council of Canada, Ottawa, ON). Replicate eel samples, as well as reference material

replicates resulted in an average percent coefficient of variance of 16 for PCBs, 20 for OCPs, and 24 for PBDEs, which indicates a fairly high degree of reproducibility.

The Carp-2 Certified Reference Material certified only 10 PCBs (IUPAC numbers 18, 28, 44, 52, 118, 128, 153, 180, 194, and 206), and provided reference values for other select PCBs (IUPAC numbers 8, 66/95, 101/90, 105, 138/163/164, 170/190, 187/182, 209) and OCPs (γ-chlordane, *o,p'*-DDE, *trans*-nonachlor, dieldrin, *p,p'*-DDE, *o,p'*-DDE, and *p,p'*-DDD). The certified PCB values had percent differences ranging from 6-52% (mean = 26%), and the reference values ranged from 3-52% (mean = 24%). PCB-44 varied the most, not including PCB-28 which coelutes with PCB-31 by our method. OCP reference values had percent differences that ranged between -27 and 19% (mean = -10%), excluding γ-chlordane which was almost 2-fold higher by our method. Reference values for PBDE congeners were not included in the CARP-2 CRM.

Concentrations in blanks ranged from not detectable to 0.45, 0.38, 0.15 ng g^{-1} ww for PCBs, OCPs, and PBDEs, respectively. Individual compounds measurable in blanks occurred at concentrations much less than 1% of those in samples (e.g. HCB, *p,p'*-DDE, PCB-118, -153, and PBDE-47). Based on the QA/QC results, lowerbound concentrations were determined using method blank subtracted values for data handling and statistical purposes**.**

3.2.6. Stable isotope analysis

Subsamples of whole fish homogenates were freeze dried and pulverized with a ball mill grinder to a fine powder for stable isotope analysis. Samples were washed with methanol, dichloromethane and hexane in succession, and then centrifuged, discarding the supernatant. The lipid-free tissue was oven dried, ground using a mortar and pestle, and stored in desiccation vials until analysed. Approximately 0.3 mg of dried tissue was used in the simultaneous analysis of stable C and N isotopes on a Delta Plus Continuous Flow Stable Isotope Ratio Mass Spectrometer

(Thermo Finnigan/Bremen-Germany) coupled to a Carlo Erba Elemental Analyzer (CHNS-O EA1108 - Italy) at the Environmental Isotope Lab (University of Waterloo, ON, Canada). All results were expressed in conventional delta notation (δ) relative to Peedee Belemnite limestone for $\delta^{13}C$ and atmospheric nitrogen for $\delta^{15}N$. The analytical precision, assessed by the repeat analysis of approximately 15% of the samples, was 2% and 1% or less for $\delta^{15}N$ and $\delta^{13}C$, respectively.

3.2.6. Data analysis

Most biological characteristics of eels were normally distributed as determined by Shapiro-Wilk tests, and results were presented as arithmetic means ± standard deviations. The distributions of contaminant concentrations were skewed and were log-transformed to obtain log-normal distributions, confirmed with Shapiro-Wilk tests. Consequently, all concentrations were reported as geometric means (antilog of the mean log values). Differences in contaminant concentrations, lengths, weights, fat content, and ages among sites were identified by analysis of variance (ANOVA) followed by Bonferroni tests for Post Hoc comparisons of means. Pearson correlations were used to test the strength of relationships between lognormal wet weight OCP, PCB, and PBDE concentrations and biological variables (age, length, weight, and fat content); a *p*-value of <0.05 was considered statistically significant. Principal component analysis (PCA) identified chemical contaminants that differed significantly in concentration among eel samples. The PCA also helped to identify 'tracer' contaminants that were standardized and incorporated in a mean-centered PCA to assess whether individual eels could be classified by similarities in chemical profiles, rather than by sampling location. Statistical analyses were performed with Microsoft Excel 2003 (Redmond, USA) and SPSS PASW Statistics Student Version 18.0 (IBM, Somers, USA).

3.3 Results and Discussion

3.3.1. Eel sample origin before migration

On the basis of mirex concentrations, a chemical tracer related to its production at Niagara Falls, NY, and a spill in the Oswego River in the late 1950s, previous studies concluded that eels collected along the south shore of the St. Lawrence estuary near, R. Ouelle, R. Sud-Ouest, and Kamouraska, were dominated by upstream migrants from L. Ontario [28, 32]. However, they also included eels from tributaries of the St. Lawrence River, as well as from local streams entering the estuary [10, 33]. Carbon and nitrogen stable isotope and chemical tracer data were used to determine if eels collected along the south shore of the St. Lawrence estuary in the present study were upstream migrants or from local estuary tributaries.

3.3.1.1. Carbon and nitrogen stable isotopes

Carbon and nitrogen stable isotope data measured in eels collected in L. Ontario, the St. Lawrence River (Mallorytown), the St. Lawrence estuary (R. Ouelle, Kamouraska), and tributaries to the estuary (R. Sud-Ouest) were differentiated using a biplot of the $\delta^{15}N$ and $\delta^{13}C$ values (Supplementary Information (SI); Figure SI 3-1). The results suggest that all eels were freshwater inhabitants, including the eels captured in the estuary. The data also indicate that only three eels from R. Sud-Ouest were significantly different from the rest, which was confirmed by one-way ANOVA, suggesting that only these eels belong to a significantly different food web. The origins of the remaining eels could not be discriminated by these methods ($p > 0.05$), suggesting a common freshwater habitat in the St. Lawrence River watershed.

3.3.1.2. Chemical tracers

Because the carbon and nitrogen stable isotope data failed to differentiate the majority of the samples, the variations among sites in chemical contaminant concentrations in individual eels were compared by PCA. Data for OCPs and PCBs as well as polychlorinated naphthalenes,

polychlorinated dibenzo-*p*-dioxins and dibenzofurans [30] for all 78 eels were input to a standardised PCA to identify useful chemical tracers of eel origin. The PCA (not shown) discriminated 25 single and coeluting contaminants that were related to origin, having contributions higher than one in the loadings plot, and it was validated with a subset of 60 eels captured in Canada. The list of tracer chemicals included: *trans*-nonachlor, dieldrin, *p*,*p*'-DDE, *p*,*p*'-DDD, mirex, PCB-52, -99, -110, -149, -118, -153, -138, -187, -180, -170, PBDE-47, 2,3,7,8-TCDD/F, 2,3,4,7,8-PeCDF, OCDD, PCN-52/60, -66/67, and 69. These tracer chemicals, in addition to $\delta^{15}N$ and $\delta^{13}C$ values were standardized and further analysed for the 40 eels captured in the St. Lawrence River and estuary watersheds by a mean-centered PCA (Figure 3-2). Mirex, BDE-47, CN-66/67, $\delta^{15}N$ and $\delta^{13}C$ were weighed by a factor of two, since they were observed to have the largest variance among samples. The first two principal components accounted for 86.4% of the variance in the data: PC1 (75.9%) and PC2 (10.5%). The PCA identified five clusters that corresponded to different profiles of chemical contamination. Eels from L. Ontario had uniform chemical profiles, characteristic of their site of capture as almost all were found in a single cluster (LO1) with a high proportion of mirex and BDE-47. A similar observation was made for St. Lawrence R. eels captured at Mallorytown, as all were located in a unique cluster (SLR1), with high proportions of mirex and CN-66/67, but low BDE-47. In contrast, eels from R. Sud-Ouest, R. Ouelle and Kamouraska were found in different clusters, indicating a mixture of chemical profiles. Several eels captured in Kamouraska and R. Ouelle were found in the SLR1 and LO1 clusters suggesting their origin was the upper St. Lawrence R.–L. Ontario watershed. Other eels from these sites were grouped in two different clusters (SLR2 and SLE), suggesting that they grew at sites located further from the source of mirex, and with a high proportion of BDE-47. R. Sud-Ouest eels also showed a variety of chemical profiles and were found in three different clusters (SLR2, SLE and RSO). The RSO cluster, contained only three R. Sud-Ouest

eels, and was characterized by a low proportion of mirex, and distinctive $\delta^{13}C$ and $\delta^{15}N$ values. The mean and standard error of the groups were determined (Figure 3-2b), and were statistically different ($p < 0.001$). The two eels captured in L. Ontario that overlapped with the Mallorytown eels were not reclassified, because their origin was known. The reclassified groups based on predicted origin inferred from captured location and from the PCA results were used for the remaining statistical analysis represented below (Table 3-1).

Table 3- 1. Summary of the number of eels captured and their reclassification based on carbon and nitrogen stable isotopes and chemical tracers.

Geographic origin	Inferred from site of capture	Inferred from statistical grouping of chemical data[1]	Total	Label
Watersheds of the St. Lawrence R. basin				
Lake Ontario	Lake Ontario (10)	R. Ouelle (7)	17	LO1
St. Lawrence River	Mallorytown (10)	R. Ouelle (3)		SLR1[2]
		Kamouraska (3)		SLR2
		R. Sud Ouest (1)	17	
St. Lawrence estuary tributaries	R. Sud-Ouest (4)	R. Ouelle (1)		RSO
		Kamouraska (1)	6	SLE
Other watersheds, low concentrations of most contaminants				
	Miramichi R. (10)			NB
	Margaree R. (10)		20	NS
Other watersheds, high concentrations of contaminants				
	Hudson R. (9)			NY
	Canal Dessel-Schoten, BE (9)		18	BE
Total			78	

[1]Statistically inferred = captured in the estuary as migrating silver eels (R. Ouelle, R. Kamouraska) or in R. Sud-Ouest with a chemical signature similar to that of fish caught in freshwater.

[2]SLR1 = Mallorytown eels, plus one eel captured at Kamouraska and R. Ouelle; SLR2 = the rest of statistically inferred St. Lawrence River eels; SLE = one eel each captured at Kamouraska, R. Ouelle, and R. Sud-Ouest.

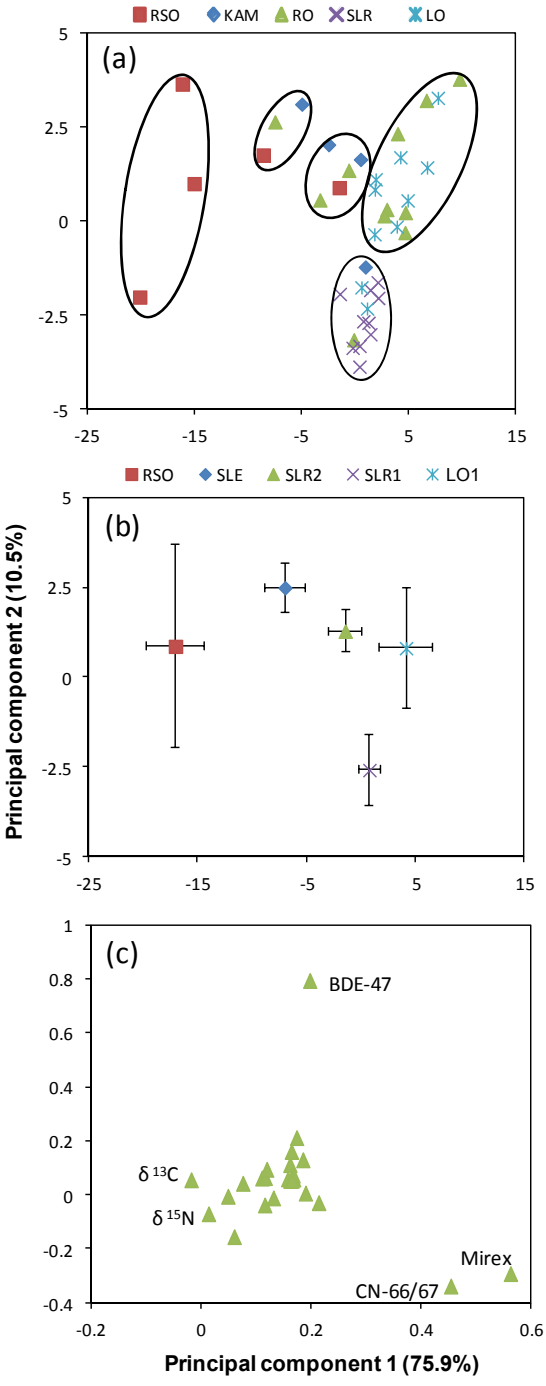

Figure 3- 2. Standardized and mean-centred Principal Component Analysis (a) scores plot on the basis of 25 tracer compounds in 40 St. Lawrence watershed and estuarine eels based on capture site, and resulted in five groupings. Mirex, BDE-47, CN-66/67, $\delta^{15}N$, and $\delta^{13}C$ values were weighed by a factor of two; (b) Mean values with standard error of the five groups based on predicted origin; (c) loadings plot. R. Ouelle = RO, Kamouraska = KAM, R. Sud-Ouest = RSO, St. Lawrence R. at Mallorytown = SLR, St. Lawrence estuary = SLE, St. Lawrence R. = SLR1/2, and L. Ontario = LO.

3.3.2. Eel biological characteristics and bioaccumulation

The length of all eels ranged from 490 to 1470 mm (mean = 840 mm) and weighed 0.215 to 3.550 kg (mean = 1.122 kg), differing significantly among sites ($p < 0.0001$). Lipid content was lowest in the Hudson R. (range 1.01-17.8%) and highest in the European eels (range 20.1-30.9%), with an overall mean of 19.5 ± 6.3%. The mean eel age was 18.7 y (range 8-39 y), and significantly different among sites ($p < 0.0001$) (Table 3-2).

All eels examined histologically were females. All eels collected in the St. Lawrence estuary, and the Margaree and Miramichi Rivers were silver migrant eels as were the majority (> 60%) of the eels collected in R. Sud-Ouest and in Belgium. Among silver eels, fat content was highest in eels from Belgium with the longest migration route, and lowest in Atlantic Canada with the shortest migration route (Table 3-2).

As described previously [34], American eels mature at a smaller size and age in Atlantic Canada than in the St. Lawrence basin, and increasing body length and fat content correlate with increasing distance from the spawning site. In contrast, all eels sampled in the Hudson R. and the majority of eels sampled in L. Ontario and the St. Lawrence R. were yellow eels. Within site, lipid content increased with body length in yellow eels [35], and it was lower in smaller-size Hudson R. yellow eels compared to larger-size yellow eels from L. Ontario or the upper St. Lawrence R. However, no significant correlations were observed between any of the biological factors and chemical contaminant concentrations. Additionally, contaminant concentrations did not differ between large yellow and silver eels captured at the same location, and in some cases the exposure time for yellow eels was longer than for silver eels (e.g. St. Lawrence R. eels were significantly older than Miramichi R. eels). The biological factors were less important for predicting contaminant loadings than origin. For example, the St. Lawrence estuary biological

outlier had similar biological characteristics to the L. Ontario and St. Lawrence R. eels (Table 3-2), yet most contaminant concentrations in this eel were more than 5-fold less.

Table 3- 2. Biological data for eels organized according to predicted origin before migration. Means sharing the same superscript letter (a - e) are not significantly different ($p > 0.05$).

Location	N	Year	Life Stage	Length (cm)	Weight (g)	Fat (%)	Age
Margaree R., (NS)	10	2007	Silver	$67 \pm 5.4^{1, d, e}$	$567 \pm 151^{b, c}$	$18 \pm 2.0^{a, b}$	$16 \pm 4.8^{c, d}$
Miramichi R., (NB)	10	2007	Silver	$74 \pm 4.1^{c, e}$	$775 \pm 227^{b, c}$	17 ± 1.8^{b}	11 ± 1.2^{c}
R. Sud-Ouest (RSO), QC	3	2008	Mixed[2] Silver	$70.2 \pm 0.2^{b, c, e}$ (108)[3]	$555 \pm 56^{a, b, c}$ (3081)	$12 \pm 4.3^{a, b}$ (25.1)	$16 \pm 4.2^{c, d}$ (12)
St. Lawrence estuary tributaries (SLE)	3	2008	Mixed[2] Silver	$86 \pm 1.4^{a, b, c}$ (109.5)	$1232 \pm 228^{a, b}$ (2690)	$18 \pm 3.9^{a, b, d}$ (19.9)	$13 \pm 2.8^{a, c}$ (28)
St. Lawrence River (SLR2)	5	2008	Silver	$98 \pm 10^{a, b}$	$1974 \pm 676^{a, e}$	$20 \pm 5.0^{a, b, d}$	$23 \pm 2.1^{a, d, e}$
St. Lawrence River (SLR1)	12	2008	Mixed[4] Yellow Yellow	$101 \pm 6.4^{a, b}$ (67.5) (88)	2115 ± 598^{a} (518) (901)	$25 \pm 3.4^{a, d}$ (7.67) (23.4)	$27 \pm 7.5^{a, b}$ (13) (13)
Lake Ontario (LO1), ON	17	2008	Mixed[2]	108 ± 12^{a}	2532 ± 736^{a}	$23 \pm 2.5^{a, d}$	$21 \pm 3.2^{a, d, e}$
Hudson R., (NY)	9	2009	Yellow	58 ± 5.7^{d}	375 ± 135^{c}	7.4 ± 5.5^{c}	$17 \pm 3.8^{c, e}$
Canal Dessel-Schoten, (BE)	9	2009	Mixed[5]	78 ± 5.1^{c}	$902 \pm 187^{b, c, e}$	25 ± 3.1^{d}	$16 \pm 2.7^{c, e}$

[1]Standard deviation. [2]Equal number of yellow and silver eels. [3](Eels with significantly different biological characteristics). [4]Four yellow, four intermediate, and two silver eels; [5]One yellow, two intermediate, and six silver eels. Location names in brackets are based on classification statistics, see Table 3-1 and Figure 3-2.

3.3.3. Differentiating eels by their chemical profiles

A standardized and mean-centered PCA using all of the OCP, PCB, and PBDE contaminants data for all of the eels analysed in the present study was used to determine if the chemical contaminant profiles varied among sites, and if so, what compounds were responsible for the differences (Figure 3-3). The two most significant principal components accounted for less than

half of the variance in the data: PC1 (23.7%) and PC2 (12.8%). Yet, the scores plot of PC1 versus PC2 showed significant clustering of eels by capture site/origin (Figure 3-3a), and the average including standard error of the groups, illustrated that contaminant profiles were location dependent (Figure 3-3b). The L. Ontario, St. Lawrence River and Estuary, and R. Sud-Ouest eels were grouped based on the results of a PCA mentioned previously, so their differentiation was not independent. However, the L. Ontario and St. Lawrence R. (1) eels were not separated by this unweighted PCA. To a lesser extent, the loadings plot of PC1 versus PC2 indicated that the European and Hudson R. eels were distinguished based on higher proportions of highly chlorinated PCBs. Eels in Atlantic Canada had higher proportions of less chlorinated PCBs and PBDEs, which resulted in their separation from St. Lawrence watershed eels (Figure 3-3c). Atlantic Canada eels were dissimilar largely because of high proportional concentrations of *p,p'*-DDE in Miramichi R. eels. This method of distinguishing eels by their chemical contaminant profile supports the fingerprinting results for European eels [36-38].

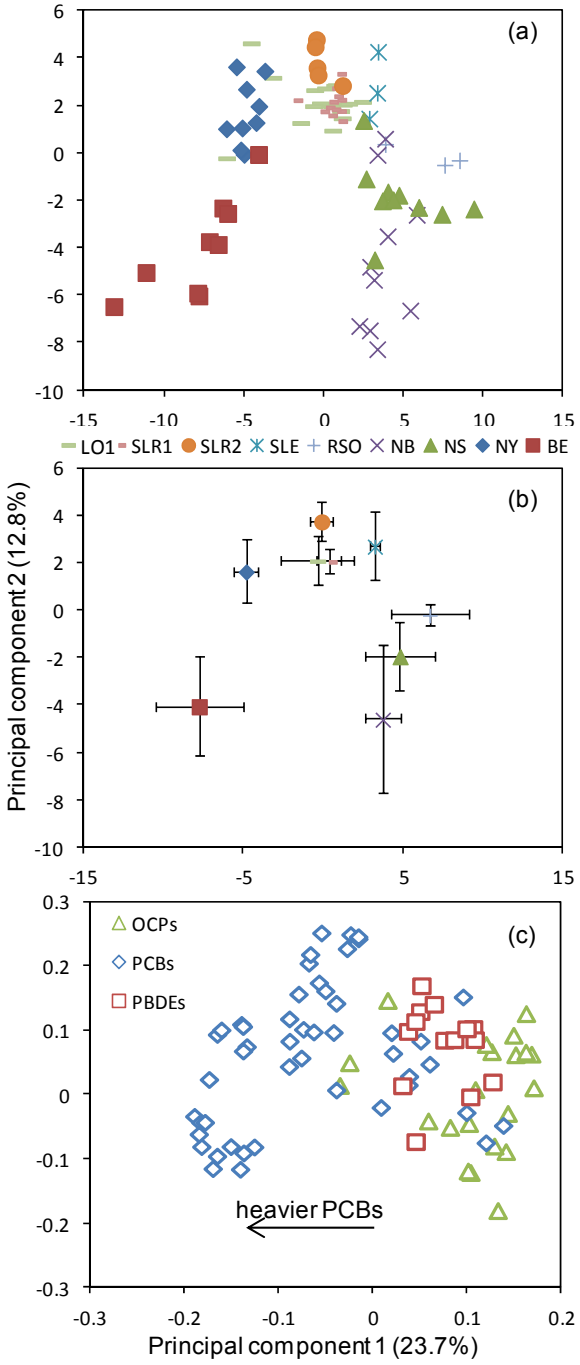

Figure 3- 3. Standardized and mean-centred Principal Component Analysis (a) scores plot on the basis of all OCPs, PCBs, and PBDEs measured in all 78 eels; (b) scores plot illustrating average values with standard error; (c) loadings plot. The L. Ontario and St. Lawrence River (1) eels were the only statistically similar samples ($p < 0.05$).

3.3.4. Polychlorinated biphenyl concentration trends

The number of PCB congeners detected, their relative abundance, and their congener profiles were origin dependent (Table SI 3-1). In eels from L. Ontario and the St. Lawrence River, 38 of 44 congeners were detected at concentrations > 0.1 ng g^{-1} ww. Twenty-one PCB congeners were detected in the R. Sud-Ouest eels, and 29 in the Margaree and Miramichi R. eels. In contrast, 41 were found in Hudson R. eels and 40 in the European eels. The sum of PCB concentrations followed the same trend as detection frequency. For eels captured in Canadian waters, highest concentrations were measured in L. Ontario, and concentrations decreased from west to east along the St. Lawrence River and Estuary, then increased slightly in Atlantic Canada (Figure 3-4). The mean sum of PCB concentrations in L. Ontario was 384 ng g^{-1} ww, 2-fold higher than in St. Lawrence R. eels, and 5-fold higher than in St. Lawrence Estuary eels; the lowest concentrations were measured in eels from R. Sud-Ouest (10.9 ng g^{-1} ww) ($p < 0.001$). In contrast, the mean sum of PCBs in eels from the Hudson R. and Europe were 1762 and 8700 ng g^{-1} ww, respectively. These trends reflected local sources. For example, there are 11 active areas of concern around the L. Ontario and St. Lawrence R. basin, and one delisted site (Oswego R., Mirex), which are largely responsible for historic inputs of POPs to this watershed [39]. There are a number of superfund sites along the Hudson R. near the sampling site in this study, which included PCB production facilities [40]. The area around the Canal Dessel-Schoten, BE sampling site is very industrialized and contaminated historically [41].

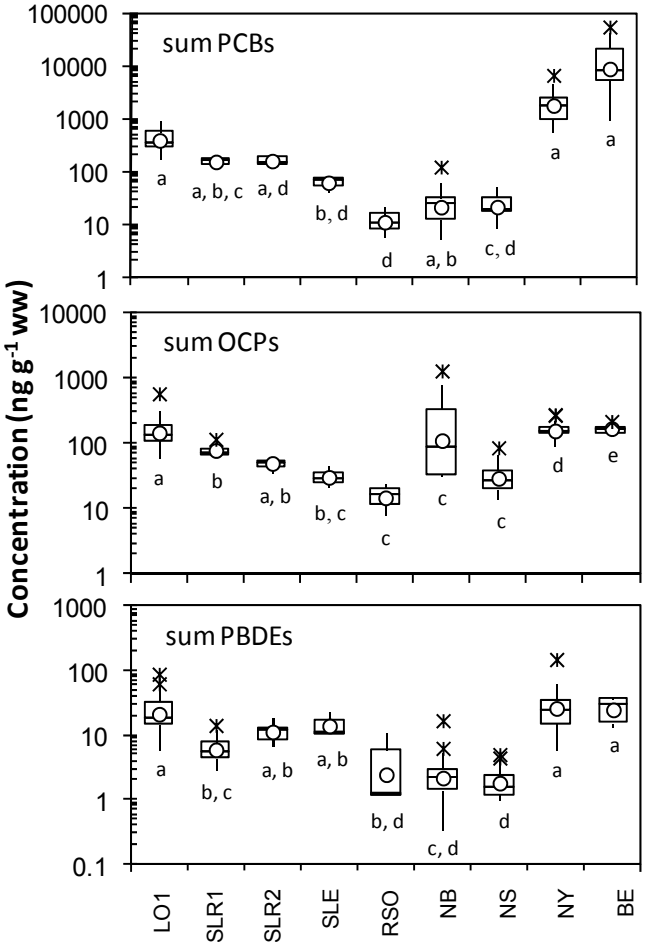

Figure 3- 4. Box-and-whisker plots (minimum, first quartile, median, ◦ geometric mean, second quartile, maximum, and ⁎ outlier) of total PCBs, OCPs, and PBDEs for eels collected between 2007-2009 from nine locations in eastern Canada, New York (USA), and Belgium. Different letters indicate a significant difference among sites ($p < 0.05$). LO = Lake Ontario; SLR1/2 = St. Lawrence River; SLE = St. Lawrence Estuary; RSO = Rivière Sud-Ouest; NB = Miramichi River; NS = Margaree River; NY = Hudson River; BE = Canal Dessel-Schoten.

The PCB congener profiles of eels captured in Canada were dominated by five congeners that averaged 52.0% of the total PCB load (Figure SI 3-2). In L. Ontario, PCB-153 > PCB-138 > PCB-180 > PCB-118 > PCB-187 were the dominant congeners. In the St. Lawrence River and Estuary and in Atlantic Canada, PCB-118 was greater than PCB-180, with the rest of the concentration order remaining the same. In the Hudson R., PCB-138 > PCB-153 > PCB-52 > PCB-180 > PCB-149 were the most abundant. In contrast, the concentration order was PCB-153 > PCB-149 > PCB-138 > PCB-151 > PCB-180 in the European eels.

To estimate the contribution of Aroclor mixtures A1260, A1254, A1248, and A1242 to the total PCB contamination in eels, the weighed mean % in eels was compared to the technical

mixtures from Frame [64] (Figure SI 3-2). In Canada, mean PCB congener profiles in eels indicate that PCB contamination was attributed mainly to A1260 (66%), A1254 (27%), and A1248 (7%). The pollution profile depended on origin with eels from R. Sud-Ouest having the highest proportion similar to A1260. Miramichi R. eels showed the lowest similarity to A1260 and the highest to A1254. In the Hudson R., PCB concentrations were more varied between A1260, A1254, A1248, and A1242, while in the European eels, A1260 predominated with A1254 making up the remainer. PCB patterns in fish from Luxembourg were predicted to be 85/15 A1260 and A1254 [42], with Belpaire et al. [43] reporting similar results in Belgium eels. The contributions from different Aroclors were estimates only because the relative contribution of individual PCB congeners differs between the technical mixtures and fish due to dissimilar rates of metabolism and degradation [42, 44].

On average, the European eels had a larger portion of the higher chlorinated PCBs compared to American eels from Canada, reflecting local use patterns. A higher proportion of lighter less viscous Aroclor mixtures are used in eastern Canada than in Europe because of lower temperatures. The difference could also result from differences in the complexity and species included in the food web.

3.3.5. Organochlorine pesticides concentration trends

Most of the OCPs measured in this study have been banned from production and use in the western world for > 30 years because of their persistence, bioaccumulation potential, and toxicity. The concentration profiles reflect this ban as in many cases, the metabolite concentrations were higher in concentration than the precursor (e.g. *p,p'*-DDE > *p,p'*-DDT; heptachlor epoxide > heptachlor; TCPM > TCPMe). In Canada, most OCP concentrations were highest in L. Ontario eels, and decreased 2-fold in the St. Lawrence R., 4-fold in the St. Lawrence estuary, and 10-fold

in R. Sud-Ouest eels ($p < 0.01$), before increasing slightly in Atlantic Canada. The exceptions were the concentrations of DDTs, which were the highest in Miramichi R. eels (Figure SI 3-4).

The most abundant OCP in all eels was *p,p'*-DDE. Relatively high concentrations of *p,p'*-DDE in eels from the Miramichi R. contributed > 85% to the sum of OCPs (Figure SI 3-2). These elevated concentrations are most likely due to the aerial application of *p,p'*-DDT at 0.55 kg/ha in the 1950s and early 1960s to control spruce bud-worm for forest protection [45, 46]. Mean total DDT concentrations ranged from 9.44 to 96.0 ng g^{-1} ww in Canada, was 125.9 ng g^{-1} ww in the Hudson R., and 127.0 ng g^{-1} ww in European eels. Ratios of DDE/DDT <1 indicate whether DDT is still being used, because DDE is only present at trace amounts in the DDT formulation and is the main degradation product of DDT [47]. Mean ratios ranged from 6.5 to 41.8 in Canada, and were 37.9 and 65.5 in the Hudson R. and Canal Dessel-Schoten, respectively, consistent with historic and widespread use of DDT.

Mean mirex concentrations were highest in L. Ontario and St. Lawrence River eels, corresponding to past production and discharge in the Lake Ontario basin, and were almost negligible in all other locations. Dieldrin, a replacement for DDT, was measured at higher concentrations than DDT at all locations except R. Sud-Ouest, and *trans*-nonachlor was more abundant than *cis*-nonachlor. Mean concentrations of HCB were significantly higher in the European eels (13.4 ng g^{-1} ww) than in American eels. The sum of TCPM and TCPMe was 3 to 100-fold higher in Hudson R. eels (mean = 1.05 ng g^{-1} ww) than in American eels captured in Canada (Table SI 3-2).

The sum of OCPs varied greatly within and among eels classified by origin (Figure 3-3). The sum of OCPs showed a concentration gradient downstream from L. Ontario to R. Sud-Ouest, decreasing about 10-fold ($p < 0.001$). L. Ontario, Hudson R., and European eels had statistically similar sum OCP concentrations, with means around 150 ng g^{-1} ww. Miramichi R. eels had the

highest mean sum of OCPs, but high standard error minimized the statistical significance. These trends correspond with the degree of anthropogenic impact within each watershed, i.e. the amount of agricultural, urban, and industrial development. For example, the L. Ontario watershed has a much greater intensity of development (> 10 million people) than the watershed of R. Sud-Ouest (< 10,000 people) [48], and the Miramichi R. watershed received high inputs of DDT historically [45, 49].

3.3.6. Polybrominated diphenyl ether concentration trends

PBDE concentrations in eels varied considerably with origin, but showed a different spatial trend across Canada than PCBs or OCPs (Figure 3-3). The sum of PBDEs was highest in L. Ontario (mean = 26.7 ng g^{-1} ww), and statistically similar to Hudson R. and European eels (Table SI 3-3). Concentrations in St. Lawrence R. eels were about 4-fold less than L. Ontario, but increased by almost double in the St. Lawrence Estuary eels. The sum of PBDEs in R. Sud-Ouest and Atlantic Canada eels were about 9-fold less than L. Ontario ($p < 0.001$).

Of the 14 PBDEs quantified in this study, six congeners made up almost 97% of the total PBDE concentration in all eel samples: BDE-47 (64.7%), BDE-100 (17.2%), BDE-49 (5.7%), BDE-154 (5.5%), BDE-153 (2.4%), and BDE-99 (1.3%) (Figure SI 3-5). These congeners are characteristic of historic use of Penta and Octa-BDE technical mixtures and have been measured previously in American and European eels [11, 50]. The absence of the highly brominated congeners including deca-BDE, is likely a sign of weak biological transfer, as well as a trademark of biotransformation or metabolism as BDE-209 can be metabolised in fish to form BDE-154 [31, 51]. Thus, the high portion of BDE-154 in L. Ontario and upper St. Lawrence River eels could have been the result of higher proportional exposure to BDE-209 in these areas. Alternatively, among the Canadian sites, there appeared to be a west to east enrichment of the lighter PBDEs, although not statistically significant, while there was a significant depletion of the higher PBDEs

(i.e. BDE-154, -153) between L. Ontario and Margaree R. eels. This may have been induced by 'longitudinal refraction' or the preferential atmospheric transport of more volatile compounds, as predicted by their Henry's Law constants [52, 53]. Longitudinal refraction of PCBs was also observed in this study.

3.3.7. Comparison with other studies and consumption guidelines

There are many studies reporting PCB, OCP, and to a lesser extent, PBDE concentrations in European eels, enough to merit a review [54], and some monitoring programs span several decades [8]. In contrast, few data are published for American eels. In Canada, two studies detailed concentrations of OCPs and PCBs in a large number of eels captured along the St. Lawrence estuary, determined to be mostly L. Ontario and St. Lawrence River migrants [10, 33]. Concentrations of sum DDTs were more than 10-fold higher historically, and mirex has also decreased over time. Concentrations of PCBs were about 10-fold higher in 1982 and about 5-fold higher in 1990 than the L. Ontario and St. Lawrence River eels in the present study (Figure 3-5). PCB concentrations in the Hudson R. decreased slightly, although not significantly compared to eels captured in 2000 [55], but were still elevated compared to eels from the Hudson R. tributaries and Delaware R. [56].

Law et al. [57] reported the mean sum of 10 PBDEs in muscle of American eel captured near Kamouraska in 1999 as 101 ng g^{-1}ww, significantly higher than in the present study (Figure 3-5). Ashley et al. [11] measured 28 PBDEs in yellow eels from the Delaware River and its tributaries in 1998 and reported total PBDEs similar to this study. However, Xia et al. [58] caught American eels along the Hudson River in 1999, 2000, and 2003 and PBDE concentrations (sum of PBDE-47, -100, -99, -154/153) in 46 eels were 10-fold higher than in the present study; the median composition of BDE-47 was 69% of the total, followed by BDE-100 (22%), BDE-154/153 (about 5%), and then BDE-99 (about 3%), similar to this study.

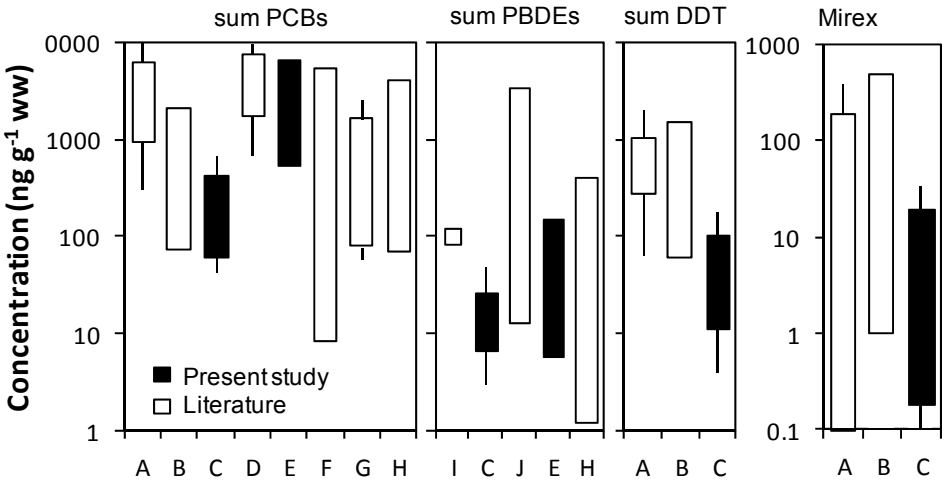

Figure 3- 5. Comparison of concentration ranges for sum PCBs, PBDE, DDT, and mirex with standard error, when available, for the present study and literature values. A = [33] – St. Lawrence Estuary 1982; B = [10] – St. Lawrence Estuary 1990-1992; C = St. Lawrence watershed – present study; D = [55] – Hudson R. 1994-1995; E = Hudson R – present study; F = [56] – Hudson R. tributaries 2004-2005; G = [55] – Delaware R. and tributaries 1994-1995; H = [11] – Delaware R. 1998; H; I = [57] – Kamouraska 1999; J = [58] – Hudson R. 1999-2003.

The Canadian guidelines for human consumption for total PCBs, DDTs, and individual OCPs in fish are 2000, 5000, and 100 ng g^{-1} ww, respectively [59]. None of the eels collected in Canada in the present study exceeded these guidelines. However, 44% of Hudson River (4 of 9) and 89% of European eels (8 of 9) exceeded these guidelines for PCBs.

The Canadian tissue residue guideline for the protection of wildlife consumers of aquatic biota is 14.0 ng g^{-1} ww for total DDT [60]. Only three eels from the Margaree R., one from the St. Lawrence estuary, and two from R. Sud-Ouest, had concentrations below the guideline. The remaining eels (72 of 78) exceeded the guideline based on concentrations of DDT metabolites.

The International Joint Commission set objectives of <LOD, 0.1 and 1.0 ng g^{-1} ww, respectively, for mirex, total PCBs, and total DDT for the protection of birds and animals that

consume fish. All eels originating from L. Ontario and the upper St. Lawrence R., as well as two eels from the Hudson R., had mirex concentrations greater than 1.0 ng g^{-1} ww; concentrations in the remaining eels were less than 1.0. All of the eels from L. Ontario and the St. Lawrence R. exceeded the objective for PCBs, but the rest of the eels captured in Canada were below the objective for the Great Lakes, except for one Miramichi R. eel. All except one of the eels in the present study had total DDT concentrations less than 1.0 ng g^{-1} ww (NB = 1.29 ng g^{-1} ww). It is worth noting that for these compounds, there are no guidelines to protect the health of fish in Canada. The Canadian environmental quality guidelines for PBDEs are 120, 88, 1, and 420 ng g^{-1} ww for the tri, tetra, penta, and hexa-BDE homolog groups, respectively [61]. The guidelines are indicators of environmental quality for assessing the ecological significance of measured concentrations and act as a way for risk managers to measure success; they do not have direct implications for the health of fish or human consumers. In L. Ontario and the St. Lawrence River and Estuary, 80% of eels (32 of 40) exceeded the penta-BDE homolog guideline, in addition to two eels from the Miramichi R. All of the eels from the Hudson R. and Europe exceeded the penta-BDE guideline. No exceedances were recorded for the other homolog groups in all samples.

Guidelines for human consumption and for the protection of top piscivorous predators differ because: (1) piscivorous predators eat much more fish than humans do, and (2) they eat whole fish and not just muscle. Guidelines for the health of fish and for human consumption should also differ because fish guidelines are based on concentrations in target organs other than muscle. Until guidelines are developed for concentrations of these compounds in eels, it is difficult to assess the risk they may pose to eel health.

3.4 Summary

The most bioaccumulated POPs were 4-6 chlorine and bromine substituted PCBs and PBDEs, respectively, as well as the metabolites of DDT, heptachlor, and TCPM. Differences in concentrations of contaminants in eels among locations primarily indicated point source pollution with some non-point sources due to atmospheric transport from agricultural, forestry, industrial, and urban activities (OCPs). Pollution fingerprinting using PCA has proven to be a useful tool for discriminating eel origin as the proportional composition of PCBs, OCPs, and PBDEs in eels was, for the most part, related to local sources based on the composition of the original technical products.

The current concentrations of PCBs, OCPs, and PBDEs in eels are generally below or within the lower range of preceding studies, consistent with a continuing decline of these POPs in biota following regulations on their use and manufacture [8, 62, 63]. Concentrations of PCBs and OCPs in eels captured in Canada were below published guidelines to protect human health, indicating that they no longer pose a significant risk to human consumers. In contrast, total PCB, DDT, and mirex concentrations were high enough to pose a risk to piscivorous predators in historically contaminated locations. The penta-BDE homolog guideline was exceeded in L. Ontario and the upper St. Lawrence R. eels, although the risk to eels and their consumers is unknown. Future studies that develop guidelines for the health of eels are needed.

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3.7 Supplementary Information

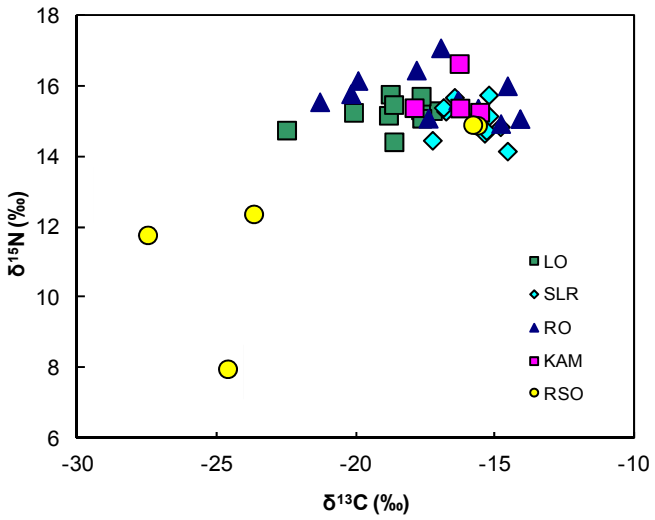

Figure SI 3- 1. Biplot comparing $\delta^{13}C$ and $\delta^{15}N$ isotopes for eels collected from L. Ontario (LO), Mallorytown (SLR), R. Ouelle (RO), Kamouraska (KAM), and R. Sud-Ouest (RSO). The three RSO eels that are distinct from the others are from the R. Sud-Ouest tributary; whereas, the other two eels captured at R. Sud-Ouest are upstream migrants.

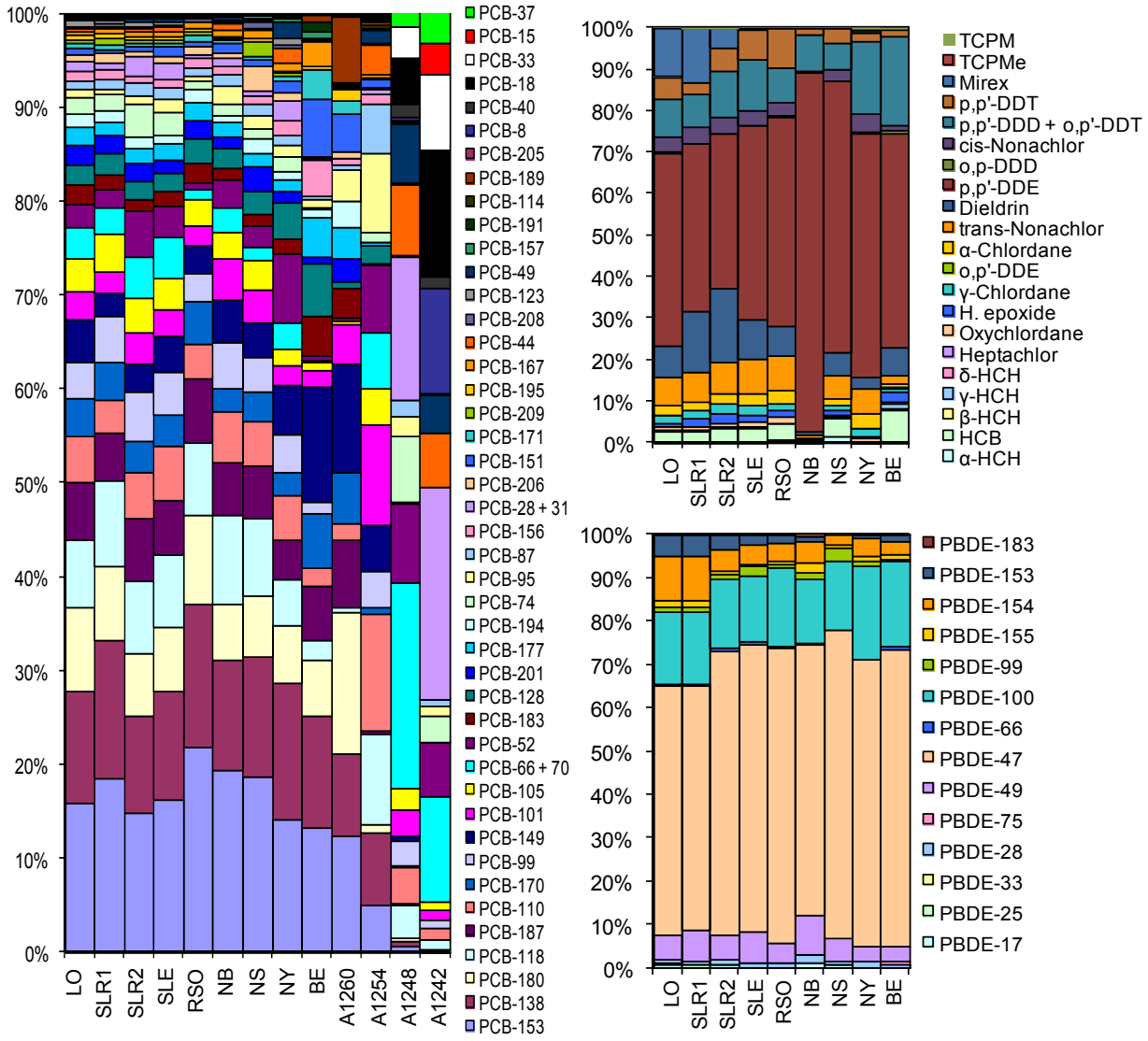

Figure SI 3- 2. Weighed % mean weight wet concentration profiles of PCBs, OCPs, and PBDEs in 78 eel samples originating from nine locations in eastern Canada, New York (USA), and Flanders (Belgium) captured in 2007-2009. Aroclor mixture profiles are from [64]. LO = Lake Ontario; SLR1/2 = St. Lawrence River; SLE = St. Lawrence Estuary; RSO = Rivière Sud-Ouest; NB = Miramichi River; NS = Margaree River; NY = Hudson River; BE = Canal Dessel-Schoten.

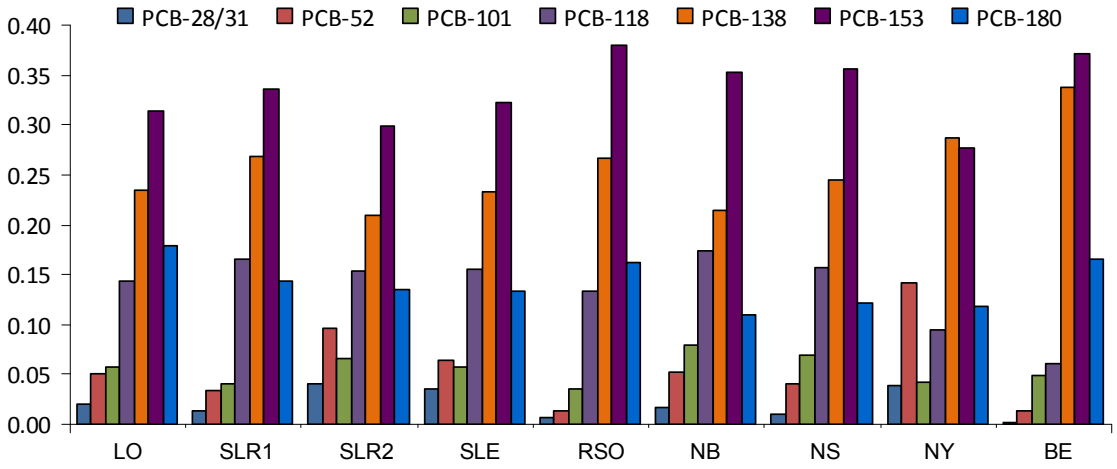

Figure SI 3- 3. Weighed mean % of the seven marker PCB congener profiles in 78 eels from eastern North America and Belgium. LO = Lake Ontario; SLR1/2 = St. Lawrence River; SLE = St. Lawrence Estuary; RSO = Rivière Sud-Ouest; NB = Miramichi River; NS = Margaree River; NY = Hudson River; BE = Canal Dessel-Schoten.

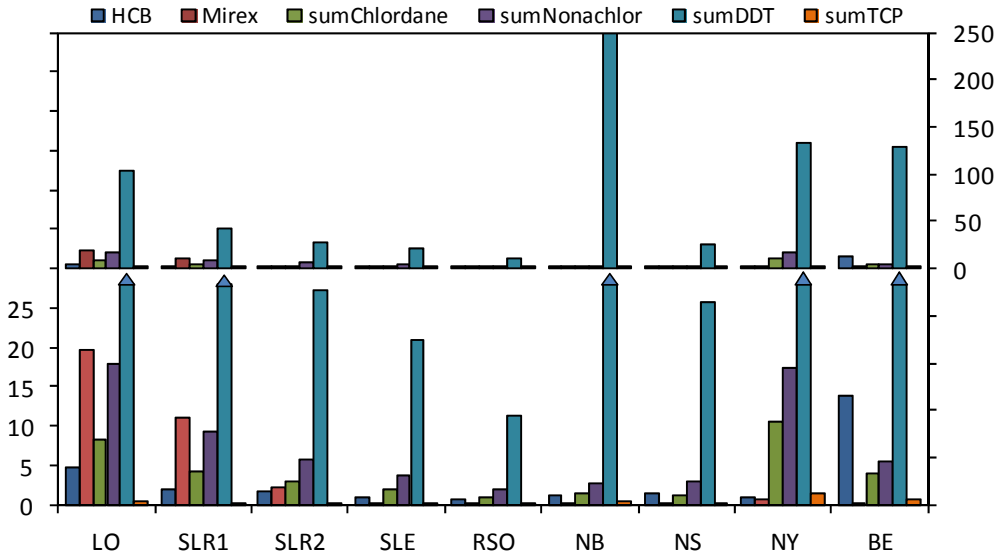

Figure SI 3- 4. Panel chart of the predominant OCP profiles (ng g^{-1} ww) in 78 eels from eastern Canada, New York (USA), and Belgium. LO = Lake Ontario; SLR1/2 = St. Lawrence River; SLE = St. Lawrence Estuary; RSO = Rivière Sud-Ouest; NB = Miramichi River; NS = Margaree River; NY = Hudson River; BE = Canal Dessel-Schoten.

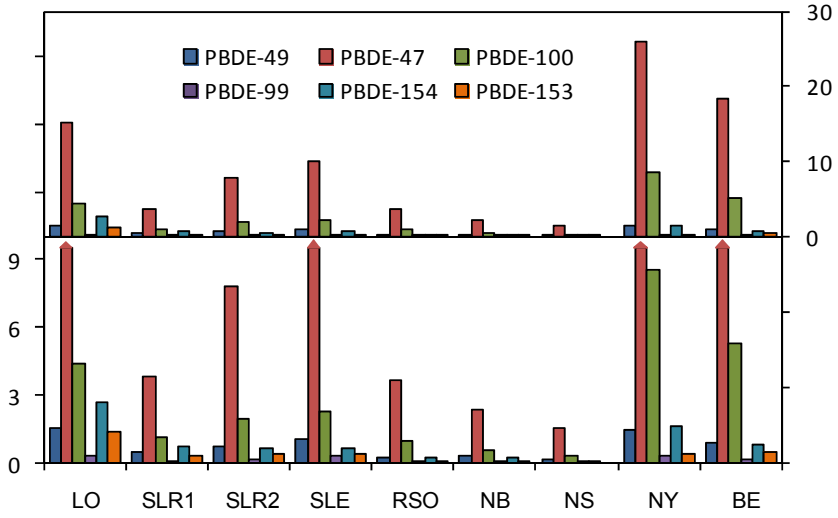

Figure SI 3- 5. Panel chart of the predominant PBDE congener profiles (ng g^{-1} ww) in 78 eels from eastern North America and Belgium. LO = Lake Ontario; SLR1/2 = St. Lawrence River; SLE = St. Lawrence Estuary; RSO = Rivière Sud-Ouest; NB = Miramichi River; NS = Margaree River; NY = Hudson River; BE = Canal Dessel-Schoten.

Table SI 3- 1. Average ± S.D. of concentrations of PCBs (ng g^{-1} ww) in eels collected in eastern Canada, New York (USA), and Belgium.

	LO	**SLR1**	**SLR2**	**SLE**	**RSO**	**NB**	**NS**	**NY**	**BE**
PCB-8	<0.01[a]	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.05	<0.02
PCB-18	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.254±0.403	0.146±0.089
PCB-15	<0.02	<0.01	<0.03	<0.02	<0.01	<0.01	<0.01	0.138±0.113	<0.05
PCB-28 + 31	4.39±5.61	1.21±0.19	3.17±1.81	1.1±0.74	0.053±0.052	0.284±0.417	0.126±0.09	46.7±46.8	7.23±3.09
PCB-33	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.03	<0.03
PCB-52	10.9±11.5	3.2±0.77	7.63±3.43	2.04±1.29	0.098±0.033	0.863±1.001	0.529±0.367	170±198	74.7±41
PCB-49	0.692±0.665	0.215±0.083	0.605±0.421	0.206±0.187	<0.01	0.111±0.2	<0.05	40.7±56.9	21.4±15.7
PCB-44	1.25±1.15	0.352±0.131	0.78±0.474	0.323±0.306	<0.02	0.184±0.305	0.067±0.033	37.9±61.8	15.1±11
PCB-37	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
PCB-40	0.051±0.092	<0.02	<0.05	<0.02	<0.01	<0.02	<0.01	7.35±11.55	2.1±1.29
PCB-74	7.37±7.35	3.01±1.07	5.43±2.67	1.55±0.99	0.101±0.025	0.386±0.525	0.265±0.152	40.5±55.5	15.6±6
PCB-66 + 70	14±12.8	4.86±1.16	7.01±2.76	2.74±1.68	0.114±0.132	0.838±1.428	0.376±0.248	65.4±94.4	31.1±14
PCB-95	3.64±2.94	0.976±0.295	1.53±0.95	0.842±0.52	0.085±0.068	0.58±0.854	0.348±0.283	27.5±33.8	150±132
PCB-101	12.3±8.1	3.74±1.32	5.2±3.27	1.81±1.05	0.249±0.19	1.31±1.92	0.893±1	49.6±45.9	275±176
PCB-99	16.5±9.4	8.39±3.08	8.18±2.29	2.79±1.19	0.379±0.102	1.46±1.76	0.939±0.71	93.5±70.4	192±196
PCB-87	4.03±2.56	1.59±0.65	2.14±0.74	0.817±0.504	0.103±0.044	0.35±0.467	0.29±0.229	24.3±33.1	56.6±34.6
PCB-110	21.4±20.9	5.98±3.3	7.77±2.63	3.56±1.91	0.474±0.14	1.65±2.04	1.17±0.78	111±118	304±319
PCB-151	2.97±2.38	0.759±0.592	0.945±0.504	0.667±0.492	0.063±0.039	0.308±0.444	0.164±0.119	27.1±39.2	954±927
PCB-149	19.5±21.6	4.22±3.09	4.95±2.09	2.47±1.2	0.379±0.159	1.41±1.98	0.86±0.522	121±133	1924±1565
PCB-123	3.16±5.95	0.841±1.455	0.747±0.779	0.304±0.338	<0.05	0.05±0.06	0.044±0.033	17.5±31.9	19±19.1
PCB-118	31±18.7	15.4±6.3	12.2±2.5	4.9±2.04	0.957±0.423	2.88±2.86	2.02±1.46	113±97	341±373
PCB-153	68±32.7	31.5±10.2	23.5±6	10.2±1.8	2.71±1.96	5.86±6.11	4.57±2.79	331±186	2068±1888
PCB-105	15.3±12.7	6.9±2.49	5.91±0.69	2.06±0.83	0.359±0.217	0.829±0.833	0.732±0.569	44±44.6	129±147

	LO	SLR1	SLR2	SLE	RSO	NB	NS	NY	BE
PCB-187	25.8±19.3	8.6±2.99	10.7±3.2	3.69±1.36	0.855±0.8	1.66±1.77	1.36±1	99.5±73.1	904±1297
PCB-183	8.74±6.82	2.57±0.99	2.19±0.76	1.01±0.2	0.271±0.246	0.418±0.48	0.302±0.221	36.8±28.5	668±1079
PCB-128	9.53±7.35	3.99±1.48	2.98±0.62	1.2±0.39	0.31±0.226	0.608±0.639	0.596±0.39	90.5±85.3	861±891
PCB-177	8.19±7.27	2.31±1.02	2.64±0.73	1.09±0.38	0.229±0.264	0.461±0.506	0.351±0.256	28.7±23.7	683±1111
PCB-171	2.39±1.95	0.758±0.244	0.635±0.225	0.294±0.066	0.091±0.106	0.128±0.144	0.085±0.057	12.8±11	495±826
PCB-156	4.48±3.45	2±1.32	1.31±0.2	0.539±0.2	0.122±0.077	0.277±0.223	0.246±0.199	37.5±37.2	611±751
PCB-180	38.8±33.1	13.5±4.2	10.7±3.5	4.2±1.07	1.16±1.02	1.83±1.42	1.55±0.97	141±109	925±1233
PCB-191	0.484±0.464	0.136±0.04	0.105±0.04	0.055±0.03	<0.02	<0.02	<0.02	1.84±1.56	159±327
PCB-170	17.3±13.1	7.01±2.09	5.5±2.08	2.16±0.46	0.549±0.511	0.777±0.642	0.758±0.434	59.3±45.6	911±1372
PCB-201	8.66±6.73	3.3±1.14	2.93±1.04	0.908±0.268	0.249±0.184	0.381±0.34	0.643±0.476	30.1±17.6	108±147
PCB-208	0.965±0.241	0.539±0.225	0.273±0.133	0.111±0.067	<0.05	0.052±0.064	0.177±0.133	7.86±4.2	3.57±6.55
PCB-195	2±1.86	0.748±0.256	0.705±0.249	0.252±0.085	<0.05	0.065±0.056	0.109±0.085	5.41±3.69	75.7±120.4
PCB-194	6.26±5.67	2.14±0.66	2.03±0.68	0.615±0.18	0.181±0.107	0.211±0.166	0.367±0.267	18.1±12.1	125±168
PCB-205	0.281±0.205	0.107±0.038	0.102±0.037	<0.05	<0.01	<0.01	<0.02	0.725±0.508	14.9±29.8
PCB-206	2.71±1.04	1.59±0.66	0.809±0.371	0.36±0.165	0.113±0.036	0.148±0.164	0.644±0.517	22.5±12.6	32.9±66.5
PCB-209	1.79±0.65	1.25±0.5	0.351±0.212	0.156±0.061	<0.05	0.041±0.07	0.357±0.275	8.63±4.49	1.52±1.8
PCB-114	0.518±0.357	0.224±0.102	0.235±0.11	0.091±0.064	<0.01	<0.03	<0.03	2.53±2.9	6.32±7.3
PCB-167	1.81±1	0.898±0.437	0.683±0.157	0.299±0.076	0.077±0.055	0.221±0.189	0.2±0.118	18.8±18.2	408±555
PCB-157	0.687±0.383	0.331±0.183	0.252±0.078	0.089±0.045	<0.03	0.063±0.059	0.054±0.043	7.51±6.94	92±125.9
PCB-189	0.412±0.306	0.175±0.061	0.15±0.042	0.044±0.013	<0.02	<0.03	<0.05	2.12±1.75	140±323
sum7PCBs	216±113	93.7±37.2	79±18.5	31.6±8.7	7.14±4.89	16.6±17.2	12.8±8	240±219	5567±5170
sumPCBs	429±253	171±67	160±37	63±20.5	12.5±7.9	30.4±33.2	24.6±14.4	2345±1979	15681±16926

[a] values < 3 x concentration in blank.

Table SI 3- 2. Average ± S.D. of concentrations of OCPs (ng g^{-1} ww) in eels collected in eastern Canada, New York (USA), and Belgium.

	LO	**SLR1**	**SLR2**	**SLE**	**RSO**	**NB**	**NS**	**NY**	**BE**
α-HCH	0.287±0.069	0.285±0.064	0.224±0.095	0.221±0.059	0.142±0.148	0.642±0.17	0.537±0.222	<0.05	0.132±0.049
HCB	4.63±7.81	2±0.38	1.63±0.69	0.962±0.254	0.64±0.342	1.11±0.63	1.54±0.45	0.852±0.695	13.9±4.6
β-HCH	0.201±0.047	0.225±0.039	0.119±0.045	0.066±0.014	<0.02	0.08±0.037	<0.05	<0.02	0.502±0.083
γ-HCH	0.126±0.039	0.138±0.035	0.084±0.045	0.051±0.034	<0.05	0.105±0.021	0.08±0.043	0.08±0.082	1.65±0.43
δ-HCH	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.05
Heptachlor	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02	<0.01	<0.01
Oxychlordane	1.33±1.12	0.768±0.338	0.495±0.297	0.353±0.147	0.275±0.097	0.238±0.196	0.207±0.208	1.12±0.72	1.1±0.38
H. epoxide	1.85±0.84	1.65±0.33	1.15±0.82	0.555±0.22	0.207±0.02	0.27±0.119	0.384±0.085	0.589±0.46	4.25±1.47
γ-Chlordane	2.95±2.35	1.59±0.48	1.12±0.42	0.699±0.245	0.295±0.174	0.401±0.539	0.324±0.119	3.72±3.64	1.34±0.78
o,p'-DDE	<0.05	<0.02	<0.01	<0.01	<0.01	0.06±0.102	<0.01	<0.05	0.084±0.043
α-Chlordane	3.9±3.7	1.86±0.64	1.24±0.46	0.879±0.281	0.487±0.282	0.839±1.221	0.589±0.28	5.62±5.53	1.55±0.74
trans-Nonachlor	11.5±9.8	5.9±2.41	3.75±0.82	2.65±0.84	1.39±0.93	2.01±2.17	1.93±1.15	10.2±7.5	3.3±1.46
Dieldrin	12.7±7.1	12.3±2.9	9.21±4.44	3.16±0.98	1.15±0.17	1.43±0.85	1.87±1.03	4.81±3.4	11.6±2.1
p,p'-DDE	78.3±67.2	33.9±15.2	18.7±6.4	14.9±9.1	8.31±4.82	223±384	22.5±18.1	99.9±30.9	88.8±20.5
o,p-DDD	0.26±0.241	0.062±0.018	0.09±0.061	0.083±0.027	0.036±0.044	0.368±0.37	0.038±0.09	0.465±0.57	0.988±0.452
cis-Nonachlor	6.4±5.13	3.38±1.27	2.03±0.31	1.15±0.27	0.502±0.277	0.77±0.81	0.995±0.596	7.15±6	2.09±0.91
p,p'-DDD + *o,p'*-DDT	15±10.3	6.45±2.55	5.5±1.59	3.86±1.52	1.42±1.4	22.5±29.6	2.11±3.44	29.9±16.4	37.3±16.3
p,p'-DDT	9.15±6.69	2.38±1.32	2.9±0.88	2.23±0.79	1.52±1.3	3.82±3.49	1.17±0.48	3.5±2.51	2.43±1.85
Mirex	19.6±14.7	11±4.8	2.31±0.81	0.18±0.093	0.037±0.062	0.058±0.032	0.064±0.053	0.771±0.542	0.237±0.296
TCPMe	<0.05	<0.03	<0.03	<0.01	<0.01	<0.04	<0.01	0.819±0.685	0.162±0.101
TCPM	0.38±0.227	0.28±0.088	0.187±0.052	0.066±0.051	<0.01	0.428±0.372	0.056±0.1	0.528±0.429	0.606±0.428
sumChlordane	8.18±7.1	4.22±1.41	2.85±1.1	1.93±0.54	1.06±0.51	1.48±1.93	1.12±0.49	10.5±9.7	3.99±1.74
sumNonachlor	17.9±14.9	9.28±3.65	5.78±1.08	3.8±1.1	1.89±1.2	2.78±2.97	2.92±1.73	17.3±13.5	5.39±2.31
sumDDT	103±82	42.8±18.2	27.2±6.6	21±10.8	11.3±7.4	250±398	25.8±21.6	134±46	130±28

Table SI 3- 3. Average ± S.D. of concentrations of PBDEs (ng g^{-1} ww) in eels collected in eastern Canada, New York (USA), and Belgium.

	LO	**SLR1**	**SLR2**	**SLE**	**RSO**	**NB**	**NS**	**NY**	**BE**
PBDE-17	0.216±0.408	0.031±0.054	0.08±0.099	0.065±0.073	<0.02	0.035±0.057	<0.02	0.132±0.086	0.05±0.024
PBDE-25	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
PBDE-33	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.037±0.057	<0.01
PBDE-28	0.248±0.32	0.055±0.051	0.116±0.072	0.103±0.062	0.033±0.04	0.076±0.17	<0.02	0.321±0.507	0.142±0.054
PBDE-75	0.049±0.065	<0.01	<0.02	<0.01	<0.01	<0.01	<0.01	<0.02	0.146±0.033
PBDE-49	1.5±1.17	0.483±0.242	0.682±0.428	1.05±0.53	0.237±0.196	0.342±0.435	0.117±0.145	1.46±2.04	0.914±0.266
PBDE-47	15.3±14.3	3.82±1.98	7.85±2.87	10±5.6	3.64±3.88	2.35±3.14	1.52±1.04	26.1±33.5	18.4±8.6
PBDE-66	0.135±0.097	0.036±0.028	0.06±0.07	0.09±0.102	<0.02	<0.02	<0.01	0.048±0.072	0.148±0.144
PBDE-100	4.38±3.46	1.13±0.76	1.93±0.77	2.29±0.99	0.973±0.617	0.547±0.615	0.344±0.211	8.51±5.81	5.27±2.19
PBDE-99	0.294±0.259	0.057±0.037	0.13±0.107	0.313±0.275	0.047±0.026	0.062±0.061	0.067±0.034	0.345±0.586	0.154±0.112
PBDE-155	0.473±0.288	0.105±0.066	0.059±0.038	0.104±0.076	0.025±0.021	0.078±0.159	<0.02	0.523±0.266	0.305±0.149
PBDE-154	2.68±1.65	0.703±0.567	0.624±0.361	0.669±0.314	0.242±0.227	0.192±0.266	0.052±0.074	1.61±1.87	0.795±0.267
PBDE-153	1.35±0.81	0.342±0.221	0.406±0.18	0.359±0.272	0.096±0.062	0.041±0.059	<0.01	0.366±0.375	0.448±0.284
PBDE-183	<0.02	<0.01	<0.01	<0.01	<0.01	<0.03	<0.01	<0.01	<0.02
sumPBDEs	26.7±21.4	6.78±3.85	12±4.5	15.1±7.8	4.33±5.48	3.75±4.88	2.14±1.45	39.4±44.4	26.8±10.4

[a] values < 3 x concentration in blank. LO = Lake Ontario; SLR1/2 = St. Lawrence River; SLE = St. Lawrence Estuary; RSO = Rivière du Sud-Ouest; NB = Miramichi River; NS = Margaree River; NY = Hudson River; BE = Canal Dessel-Schoten.

Chapter 4
Spatial trends of dioxin-like compounds in Atlantic anguillid eels

Abstract

Atlantic *anguilld* eel stocks have experienced unsustainable declines, yet to be explained. The decline of lake trout (*Salvelinus namaycush*) in Lake Ontario has been linked to aryl-hydrocarbon receptor agonists such as polychlorinated dibenzo-p-dioxins/dibenzofurans (PCDD/Fs), dioxin-like polychlorinated biphenyls (dl-PCBs), and polychlorinated naphthalenes (PCNs), and the question remains whether eels are affected similarly by these compounds. Concentrations of PCDD/Fs, dl-PCBs, and PCNs were determined in eels collected at seven locations in eastern Canada including L. Ontario, one location in New York, USA, and one location in Flanders, Belgium. Concentrations varied greatly among origins and there was no correlation among classes of compounds, indicating dissimilar historic loadings to local areas. The risk to eel reproduction was evaluated with 2,3,7,8-TCDD toxic equivalents, and increased by 10-fold from the least to most contaminated site. The risk to eel recruitment from dioxin-like compounds in American eel using available guidelines is low. The development of a more comprehensive model for eel recruitment risk assessment due to dioxin-like compounds, using eel-specific guidelines, is recommended. Toxic equivalents were 5-fold higher when based on mammalian toxic equivalency factors compared to fish values. About half of the eels captured in L. Ontario exceeded the Canadian guideline for fish consumption (20 pg TEQ g^{-1} ww), but there were no other exceedances in Canada. The current risk to eel consumers in Canada is low overall.

4.1. Introduction

During the mid-1900s, there was an influx of chlorinated persistent organic pollutants (POPs) in most industrialized countries. This was highlighted by the production of polychlorinated naphthalenes (PCNs) and polychlorinated biphenyls (PCBs), and the discovery of polychlorinated dibenzo-*p*-dioxins and dibenzofurans (PCDD/Fs) as unintentional byproducts of industrial processes [1, 2]. PCBs, PCNs, and PCDD/Fs are classes of compounds that have similar physical-chemical properties, and some have been shown to bioaccumulate and cause toxicity in fish and wildlife [3-6]. Of particular interest are the coplanar structures that have chlorine substituted in both the *meta* and *para* positions, analogous to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD). Twelve dioxin-like PCBs (dl-PCBs), 17 PCDD/Fs, and six PCNs meet these criteria and they act additively via a common aryl-hydrocarbon receptor (AhR) mediated mechanism [7-12]. The effects of these AhR agonists are well documented and include a combination of toxic responses such as embryotoxicity, hepatotoxicity, immunotoxicity, teratogenicity, and carcinogenicity [7].

Cook et al. [13] demonstrated the significance of these compounds for fish. They showed that recruitment failure of lake trout (*Salvelinus namaycush*) from Lake Ontario in the mid-1960s could be explained by historic loadings of dioxin-like chemicals to L. Ontario. Cook's model predicted a period from about 1940 to 1980 when there was 100% mortality of lake trout embryos. Based on induction of cytochrome enzymes, American eel (*Anguilla rostrata*) may be as sensitive to TCDD as lake trout [14, 15]. As such we hypothesize that eels, like lake trout, accumulate progressively higher concentrations of POPs from their diet as they grow to sexual maturity. We would predict, therefore, that the maternal transfer of these POPs to eggs should cause embryo-toxicity and impaired recruitment analogous to the impacts on lake trout. There may be added stress on American eel embryos compared to lake trout embryos because eels (1) are longer lived, meaning they have more time to accumulate contaminants, (2) are semelparous,

so they do not depurate contaminants during successive reproductive cycles, and (3) concentrate hydrophobic contaminants during their spawning migration as they consume a large portion of their stored fat for energy [15-17].

There is significantly more information available in the literature for European eel than American eel. Where possible, information not readily available for American eel will be inferred from European eel studies due to their physiological and ecological similarities, illustrated by their overlapping spawning sites and the occurrence of hybrids [18, 19].

In this study, the geographic variation in concentration of dioxin-like compounds in American eel was determined across eastern Canada. Concentration data were used to calculate toxic equivalents (TEQs) in adult eel, and the TEQs were compared to published thresholds for embryo-toxicity to other species, and to fish consumption guidelines.

4.2. Experimental details

4.2.1. Sampling

Whole fish homogenates were prepared from 60 eels captured throughout eastern Canada in 2007-2008 (10 each from L. Ontario, ON; Thousand Islands or Mallorytown, ON; Miramichi R., NB; Margaree R., NS; 11 eels from St. Lawrence estuary near R. Ouelle, QC; 4 from R. Sud-Ouest, QC; 5 from Kamouraka, QC). Attempts were made to acquire large yellow-silver females only. Eels were collected in the Fall at the onset of their spawning migration. In addition, nine eels each from the United States (*A. rostrata*) and Belgium (*A. anguilla*) were collected in 2009 and used as positive controls (Table 4-1). Further details of the sample collection including a map of sampling locations, biological characteristics, and age determination were described elsewhere [20].

Table 4- 1. Summary of length, weight, age, condition factor, and residency for eels collected from nine locations used to examine spatial differences in contaminants. Residency refers to eels that were present in, and associated with, the upper St. Lawrence River and Lake Ontario, as determined from transitional otolith growth characteristics.

System and location	N	Total length (mm) Avg ± 95 CI	Total weight (g) Avg ± 95 CI	Age (yr) Avg ± 95CI	Min	Max	Condition factor Fulton's K	Percent showing residency
Lake Ontario, Outlet Basin	10	1,062 ± 104	2,229 ± 558	20.0 ± 2.1	16	25	0.182 ± 0.023	100
Upper St. Law. R., 1,000 Islands	10	974 ± 88	1,837 ± 514	26.1 ± 5.8	13	39	0.187 ± 0.021	100
St. Law. Estuary, Rivière-Ouelle	11	1,108 ± 42	2,854 ± 236	21.1 ± 3.3	16	33	0.211 ± 0.016	36.4
St. Law. E., Kamouraska	4	923 ± 163	1,794 ± 1,151	17 .8 ± 8.6	11	23	0.219 ± 0.036	
Rivière Sud-Ouest, QC	5	860 ± 202	1,366 ± 1,301	16.8 ± 6.6	12	25	0.183 ± 0.046	
Miramichi River, NB	12	735 ± 24	779 ± 133	11.2 ± 0.9	9	13	0.192 ± 0.075	
Margaree River, NS	10	667 ± 39	567 ± 108	16.3 ± 3.4	8	22	0.179 ± 0.017	
Hudson River, United States	9	585 ± 44	375 ± 104	16.6 ± 2.9	12	24	0.179 ± 0.017	
Dessel-Schoten Canal, Belgium	9	829 ± 39	942 ± 148	15.7 ± 2.1	11	20	0.167 ± 0.030	

4.2.2. Chemical analysis

Individual eel tissue extracts were prepared for chemical analysis from approximately 20 g of homogenate. About 5 g of tissue was used to determine 17 PCDD/F, 21 PCN, and 12 dl-PCB (four non- and eight mono-ortho) concentrations. Samples were spiked with a solution of 15 [$^{13}C_{12}$]-PCDD/F, eight [$^{13}C_{10}$]-PCN, and 12 [$^{13}C_{12}$]-dl-PCB surrogates. The PCDD/F and dl-PCB standards were purchased from Wellington Laboratories (Guelph, ON), and the PCN standards were acquired from Cambridge Isotope Laboratories (Andover, MA). Lipids were removed by gel permeation chromatography with Biobeads SX-3, and a 2-layered packed 5% deactivated silica-alumina column. The dl-PCBs, PCNs, and PCDD/Fs were separated on a Cosmosil 5PYE column by high performance liquid chromatography. One fraction contained the dl-PCBs and the mono-tetra PCNs, and another contained the penta-octa PCNs and the PCDD/Fs. The fractions were reduced in volume and spiked with additional [$^{13}C_{12}$]-PCDD and [$^{13}C_{12}$]-PCB instrument performance standards.

Gas chromatography- high resolution mass spectrometry (GC-HRMS) analyses was carried out on a Micromass AutoSpec mass spectrometer (Micromass, Manchester, UK), operated in electron ionization (EI) and selected ion-monitoring (SIM) modes, connected to a Hewlett-Packard 6890 GC (Hewlett Packard, Palo Alta, CF, USA) equipped with a CTC A200S autosampler (Leap Technologies, Chapel Hill, NC, USA). Chromatographic separation was achieved using a Restek Dioxin-2, 60 m x 0.25 mm x 0.25 µm column (Bellefonte, PA) under the following conditions: He carrier gas: 1.5 mL/min; source temp: 280 °C; front Inlet temp: 280 °C; transfer line temp: 280 °C; splitless injection: 1.5 min at 30 mL/min. The GC-HRMS was tuned with perfluorokerosene as a reference compound (10,000 resolution at 5% peak height definition) over the mass range of the PCDD/F and non-ortho PCB congeners (no-PCBs). PCNs were separated on a Restek Rtx-5, 60 m, 0.25 mm x 0.25 µm capillary column.

The remaining dl-PCBs concentrations, eight mono-ortho PCBs (mo-PCB), were determined separately, and have been detailed elsewhere [20]. Briefly, chromatographic

separation was by a ThermoQuest Trace GC on a DB-5, 60 m, 0.25 mm x 0.25 μm column (J&W Scientific, Palo Alto, CA). The GC was coupled to a Thermo Finnigan PolarisQ ion trap MS (Waltham, MA), operated in EI and MS/MS modes. The limit of detection for the mo-PCBs was one to two orders-of-magnitude less sensitive (tens of pg g^{-1} ww) than for the PCNs, no-PCBs, and PCDD/Fs, but the mo-PCBs were several orders-of-magnitude higher in concentration.

4.2.3. QA/QC

Samples were analysed by site with one procedural blank, one spiked lab blank, one in-house reference material (lake trout), and one certified reference material (CARP-2, National Research Council of Canada (NRC), Ottawa, ON). Replicate samples had an average percent coefficient of variation of 9, 5, and 10 for PCDD/Fs, dl-PCBs, and PCNs, respectively. CARP-2 reference values were compared to this study with a paired t-test using mean values from eight replicates for the nine PCDD/F congeners reported. Two congeners, 1,2,3,7,8-PeCDD and 1,2,3,6,7,8-HxCDD, had slightly but statistically lower concentrations than the reference values determined with a Student's *t*-test ($t = 2.27$ and 2.87, respectively), while the remaining PCDD/F congeners were statistically similar to the reported values. The only other dioxin-like compound reported by the NRC in the certified reference material was PCB-118, and our results were in agreement with the reference value ($t = 1.25$). Blank samples had concentrations below the limit of quantitation (LOQ) for all 17 PCDD/F congeners > 97% of the time, with the remaining having concentrations <0.44 pg g^{-1} ww (OCDD). Concentrations of the no-PCBs in blanks were below the LOQ, except for two instances for CB-77 (5.19 and 6.25 pg g^{-1} ww); PCNs ranged from non-detectable to 5.30 pg g^{-1} ww. The results were blank subtracted for PCNs only. The mean percent recoveries of spiked blanks were 101% (range = 82-126%) and 108% (range = 88-124%) for PCDD/Fs and dl-PCBs, respectively; native PCNs were not added to the spiked lab blanks. For statistical purposes, non-detectable concentrations were assigned a value of half the LOQ.

4.2.4. Toxic equivalents

Toxic equivalents (TEQs) were calculated using the World Health Organization Toxicity Equivalency Factor (WHO-TEF) method for fish and mammals [7, 8]. The TEQs calculated using fish-specific TEFs were denoted as $FISH_{1998}$, and mammalian TEQs were notated as WHO_{1998} and WHO_{2005} using mammalian TEFs from 1998 and the reevaluated TEFs from 2005, respectively. The PCN TEFs were available primarily for mammals, and only for a limited number of congeners (penta-octa). Their values were obtained from a variety of sources [9-12].

4.2.5. Residency of Lake Ontario-upper St. Lawrence River eels

Studies have shown that eels captured in the St. Lawrence estuary are predominately migrants from upstream origins, with some eels from estuarine tributaries [21, 22]. Residency for the eels captured in L. Ontario-upper St. Lawrence R. (Thousand Islands or Mallorytown) was confirmed by otolith growth characteristics (Table 4-1). Additionally, four eels captured at R. Ouelle showed L. Ontario residency which supports the above statement. To examine accurately spatial differences in chemical contamination it was necessary to classify eels captured in the St. Lawrence estuary (R. Ouelle, Kamouraska, R. Sud-Ouest) by their origin rather than their capture location. These eels were reclassified previously based on results from the principal component analysis (PCA) of their stable isotope and chemical contaminant concentrations [20]. The results of the reclassification are presented in Supplementary Information. For statistical purposes, eels were grouped by sampling location, R. Sud-Ouest (RSO), Miramichi R. (NB), Margaree R. (NS), Hudson R. (NY), and Canal-Dessel-Schoten (BE), or by predicted origin, L.Ontario (LO1), St. Lawrence R. (SLR1 and SLR2), and St. Lawrence estuary (SLE).

4.2.6 Statistical analysis

For the nine different groups of eels, the concentration data followed skewed probability distributions. The contaminant concentrations were log-transformed to obtain normally distributed data, confirmed with Shapiro-Wilk tests. Toxic equivalency factors were determined on a logarithmic scale, and geometric means (mean) were used to examine individual congener

and TEQ concentrations. Analysis of variance (ANOVA) of the log-transformed data tested significance of spatial trends in eel samples, and geometric means were compared with Bonferroni post-hoc tests. Principal component analysis was used to assess the differences, if any, in dioxin-like compound concentrations and congener patterns among samples, and the significance of the groupings was validated using ANOVA. Statistical analyses were performed using Microsoft Excel 2003 (Microsoft Corp., Redmond, USA) and SPSS PASW Statistics Student Version 18.0 (IBM, Somers, USA)**.**

4.3. Results

4.3.1. Concentration trends

Concentrations of dl-PCBs, PCNs, and PCDD/Fs were determined in eels caught in the Lake Ontario/St. Lawrence Watershed, Atlantic Canada, the Hudson R., NY, and Canal Dessel-Schoten, Belgium. The dl-PCBs had a high detection frequency; typically, only four of the eight mo-PCB congeners (PCB-118, 105, 156, and 167) were measured in eel tissues from the most eastern Canadian origins, R. Sud-Ouest (RSO), Miramichi R. (NB), and Margaree R. (NS), and all eight congeners were present in every sample from L. Ontario (LO1), the St. Lawrence River (SLR1 and SLR2), and the St. Lawrence Estuary (SLE). The no-PCBs 77, 81, 126, and 169 were detected in 93-100% of the samples. The most frequently detected PCDD/F congeners were 2,3,7,8-TCDF, present in every sample, and 2,3,4,7,8-PeCDF, present in all but two samples. Conversely, 1,2,3,4,7,8,9-HpCDF and OCDF were measured in 7% and 27% of the eels, respectively, and have been reported to occur less frequently in biota because of their low solubility [2]. The most commonly detected PCNs were CN-52/60 and -66/67, which were quantified in every sample.

In general, the mean sum of PCDD/F concentrations decreased from west to east in eels starting in the L. Ontario- upper St. Lawrence River to the estuary; e.g. L. Ontario (7.85 pg g^{-1} ww) to R. Sud-Ouest (3.01 pg g^{-1} ww), then increased slightly but insignificantly in Atlantic

Canada (Table 4-2). Average concentrations for individual congeners are summarized in the Supplementary Information (Table SI 4-1).

Table 4- 2. Geometric mean concentrations of PCDD/Fs, PCNs (pg g^{-1} ww), and dl-PCBs (ng g^{-1} ww) in Atlantic eels; fish and mammalian TEQs for PCDD/Fs, PCNs, and dl-PCBs (pg g^{-1} ww). Different superscript letters indicate a significant difference ($p < 0.05$) among sites.

	LO	SLR1	SLR2	SLE	RSO	NB	NS	NY	BE
ΣPCDD/F	$7.85^{a, c}$	$7.22^{a, c}$	$4.86^{a, d}$	$3.7^{a, d}$	3.01^{d}	$4.7^{a, d}$	$5.29^{a, b, d}$	10.9^{c}	$9.64^{b, c}$
Σdl-PCB	50.2^{a}	$22.9^{a, b}$	$21.5^{a, b}$	$7.85^{b, c}$	1.57^{c}	3.24^{c}	2.98^{c}	163^{d}	642^{e}
ΣPCN	321^{a}	247^{a}	$70.1^{d, e}$	$28.2^{c, d, e}$	4.17^{f}	$11.8^{c, f}$	$20.4^{b, c}$	$51.8^{b, e}$	272^{a}
$FISH_{1998}$									
ΣPCDD/F-TEQ	$2.96^{a, b}$	$2.18^{a, d}$	$1.22^{c, d}$	0.77^{c}	0.61^{c}	0.84^{c}	0.70^{c}	$2.33^{a, d}$	$2.9^{a, b}$
Σdl-PCB-TEQ	$0.86^{a, d}$	$0.42^{a, b}$	$0.34^{a, b, d}$	$0.14^{b, c}$	0.04^{c}	0.07^{c}	0.08^{c}	1.16^{d}	4.80^{e}
Total TEQs	3.87^{a}	$2.62^{a, b}$	$1.60^{b, c}$	$0.91^{b, c}$	0.65^{c}	0.92^{c}	0.79^{c}	$3.70^{a, d}$	8.24^{d}
WHO_{1998}									
ΣPCDD/F-TEQ[1]	$2.94^{a, b}$	$2.19^{a, d}$	$1.23^{c, d}$	0.78^{c}	0.62^{c}	0.84^{c}	0.70^{c}	$2.37^{a, d}$	$2.92^{a, b}$
Σdl-PCB-TEQ[1]	$19.0^{a, d}$	$9.16^{a, b}$	$7.31^{a, b}$	$2.98^{b, c}$	0.77^{c}	1.34^{c}	1.45^{c}	31.4^{d}	165^{e}
ΣPCN-TEQ[3]	$0.76^{a, c}$	$0.65^{a, c}$	$0.16^{a, d}$	$0.02^{b, d}$	$<0.01^{e}$	0.01^{b}	0.02^{b}	0.07^{d}	0.44^{a}
Total TEQs	$23.1^{a, d}$	$12.1^{a, b}$	$8.90^{a, b}$	$3.79^{b, c}$	1.41^{c}	2.27^{c}	2.24^{c}	34.7^{d}	170^{e}
WHO_{2005}									
ΣPCDD/F-TEQ[2]	$2.59^{a, b}$	$1.94^{a, d}$	$1.09^{c, d}$	0.70^{c}	0.57^{c}	0.78^{c}	0.62^{c}	$2.13^{a, d}$	$2.32^{a, b}$
Σdl-PCB-TEQ[2]	$13.8^{a, b}$	$6.90^{a, b}$	$5.20^{a, b}$	$2.21^{b, c}$	0.65^{c}	1.09^{c}	1.22^{c}	12.1^{a}	54.4^{d}
Total TEQs	17.4^{a}	$9.56^{a, b}$	$6.65^{a, b}$	$2.93^{b, c}$	1.25^{c}	1.95^{c}	1.92^{c}	14.8^{a}	57.7^{d}

LO = Lake Ontario (N = 17); SLR1 and SLR2 = St. Lawrence River (N = 12 and 5); SLE = St. Lawrence Estuary (N = 3); RSO = Rivière Sud-Ouest (N = 3); NB = Miramichi River (N = 10); NS = Margaree River (N = 10); NY = Hudson River (N = 9); BE = Canal Dessel-Schoten (N = 9). [1]van den Berg et al. [7]; [2]van den Berg et al. [8]; [3]Fernandes et al. [30].

The total PCDD/F concentrations in the American eel samples from the Hudson R. (NY) and the European eels from Belgium (BE) were similar to those of L. Ontario eels. PCDD/F congener profiles of eels from L. Ontario and the St. Lawrence R. were dominated by 2,3,4,7,8-PeDF and 2,3,7,8-TCDD, whereas, eastern Canadian and Hudson R. eels had high proportions of

OCDD. In the European eels, 2,3,4,7,8-PeCDF represented more than 30% of the total PCDD/F concentration. The eels captured in the Miramichi R. had a significantly higher proportion of 2,3,7,8-TCDF at 12 ± 4.9% than eels from all other locations (5.0 ± 2.9%; ANOVA $p < 0.001$).

The mean sum of dl-PCB concentrations in eels caught in Canadian waters decreased more than 30-fold from L. Ontario to R. Sud-Ouest, and then increased slightly but not significantly in Atlantic Canada. By comparison, the Hudson R. and European eels had much higher concentrations with values ranging from 48.4 to 644 ng g^{-1} ww (mean = 163 ng g^{-1} ww) and 88.8 to 4976 ng g^{-1} ww (mean = 642 ng g^{-1} ww), respectively. The dl-PCB patterns in eels captured in Canada were dominated by CB-118 and to a lesser extent CB-105 (Figure SI 4-2). These two congeners contributed over 80% on average to the total dl-PCBs for these eels. Comparatively, CB-118 and -105 contributed about 70% for the Hudson R. eels. For the European eels, CB-118 and -105 were responsible for less than 40%, with 60% from the heavier PCBs in the following order: CB-156 > 167 > 189 > 157.

In Canadian waters, the mean sum of PCN concentrations in eels were highest in L. Ontario (321 pg g^{-1} ww) and lowest in R. Sud-Ouest (4.17 pg g^{-1} ww), demonstrating a similar pattern to the other dioxin-like compounds in this study. Concentrations in Hudson R. eels were intermediate (mean = 51.8 pg g^{-1} ww), and total concentrations in European eels (272 pg g^{-1} ww) were similar to L. Ontario. The PCN congener composition for L.Ontario to R. Sud-Ouest was dominated by CN-66/67 followed by CN-52/50, totaling about 60% and 30% of the total PCNs, respectively. The Atlantic Canada eels had a concentration order: CN-52/60 > CN-66/67 > CN-61 > CN-69; whereas, for the Hudson R. eels: CN-52/60 > CN-69 > CN-66/67 > CN-71/72 > CN-61, and European eels: CN-52/60 > > CN-66/67 > CN-69 > CN-61.

The PCA in Figure 4-1 indicates three principal components that account for 70% of the variance in the data, PC1 (44.3%), PC2 (13.6%) and PC3 (12.1%). PC1 appears to differentiate primarily on dl-PCB profiles, PC2 by PCDD/F profiles, and PC3 by mo-PCBs only. The PCA result suggests that the eels may be separated into three distinct groups based on their

contamination profiles. The scores plot of PC1 versus PC3 illustrates the three groups: Group 1 (LO1, SLR1, SLR2, and SLE), Group 2 (RSO, NS, and NB), and Group 3 (NY and BE). The corresponding loadings plot shows the distinction in mo-PCB composition among groups, with Group 1 and Group 3 having higher proportional concentrations of the lighter and heavier mo-PCBs, respectively. Group 1 was also separated due to higher proportional loadings of most PCN congeners and PCB-126. Group 2 had the highest percent concentration of PCDD/Fs to total dioxin-like compounds**.**

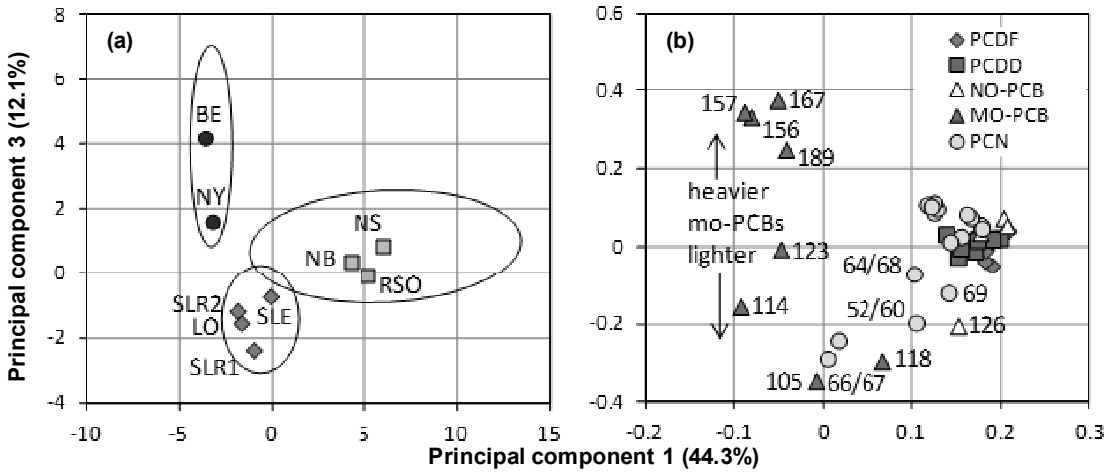

Figure 4- 1. (a) Scores and (b) loadings plots from the principal component analysis separating eel origins by differing congener pattern. BE = Canal Dessel-Schoten; NY = Hudson River; LO1 = Lake Ontario; SLR1/2 = St. Lawrence River; SLE = St. Lawrence Estuary; RSO = Rivière Sud-Ouest; NB = Miramichi River; NS = Margaree River; PCDF = polychlorinated dibenzofuran; PCDD = polychlorinated dibenzo-*p*-dioxin; NO-PCB = non-ortho polychlorinated biphenyl; MO-PCB = mono-ortho PCB; PCN = polychlorinated naphthalene.

4.3.2. Toxic equivalents

A summary of the TEQ results are shown in Table 4-2. PCNs were included in the mammalian calculations, but not in the fish-specific calculations because a sufficient number of relative potency values or TEFs for fish were not available in the literature. However, relative potencies were readily available only for CN-52/60, -53, -*66/67*, -64/68, -69, -71/72, -63, -*73*, -

74, -*75* (*italicized congeners are meta-para-substituted similar to TCDD*). These congeners accounted for > 90% of the sum of PCNs at most locations, except for Miramichi (81%) and Margaree R. (68%) eels. Box and whisker plots of $FISH_{1998}$ and WHO_{1998} TEQs are shown in Figure 4-2. Eels collected from the L. Ontario and St. Lawrence R. had similar values to the Hudson R. eels with geometric means around 3 pg $FISH_{1998}$ TEQ g^{-1} ww; Atlantic Canada eels had the lowest TEQs (mean < 1 pg $FISH_{1998}$TEQ g^{-1} ww), and the European eels had the highest concentrations (mean = 8.24 pg $FISH_{1998}$ TEQ g^{-1} ww). The sum of PCDD/F + dlPCB-TEQs ranged between 0.36 and 16.1 pg $FISH_{1998}$ TEQ g^{-1} ww for American eels, compared to 2.46 – 30.2 pg $FISH_{1998}$ TEQ g^{-1} ww for the European eels. For mammalian consumption, TEQs ranged from 0.83 to 51.1 pg WHO_{2005}TEQ g^{-1} ww for eels captured in Canadian waters, 5.21 to 54.2 pg WHO_{2005} TEQ g^{-1} ww for the Hudson R. eels, and 15.0 to 234 pg WHO_{2005}TEQ g^{-1} ww for the European eels.

In the American eels, 2,3,7,8-TCDD, 1,2,3,7,8-PeCDD, 2,3,4,7,8-PeCDF, CB-126, and -118 contributed the most to $FISH_{1998}$-TEQs, totaling between 77 and 90% of the total TEQs; whereas, CB-156,-118,-126, -167, and 2,3,4,7,8-PeCDF contributed the majority to the European eels (73%), with 2,3,7,8-TCDD contributing less than 3% to the total TEQs. For the WHO_{1998} and WHO_{2005}-TEQs, PCBs had a more significant contribution, e.g. CB-156, -118, -126, -105, and -157 contributed 93% and 85% to the total TEQs, respectively. The mean percent contribution of PCDD/F-TEQs increased from west to east for the Canadian sites, and correspondingly the percent of dl-PCB-TEQs decreased (Figure SI 4-3). The European eels had the highest proportion of dl-PCB-TEQs followed by Hudson R. > L. Ontario.

PCDD/F-TEQs represented 76 to 91% and 12 to 35% of the total TEQs for $FISH_{1998}$ and WHO_{1998}, respectively, for the Canadian sites; compared to 62% and 28%, and 6% and 1%, respectively, for the Hudson R. and European eels. The mean percent contribution of dl-PCB-TEQs to the total TEQs decreased, but not significantly, between WHO_{1998} and WHO_{2005} values

for all sites, and the dl-PCB profile varied greatly. The percentage of mo-PCB-TEQs decreased by almost one half when using the re-evaluated TEFs from 2005 instead of the 1998 values.

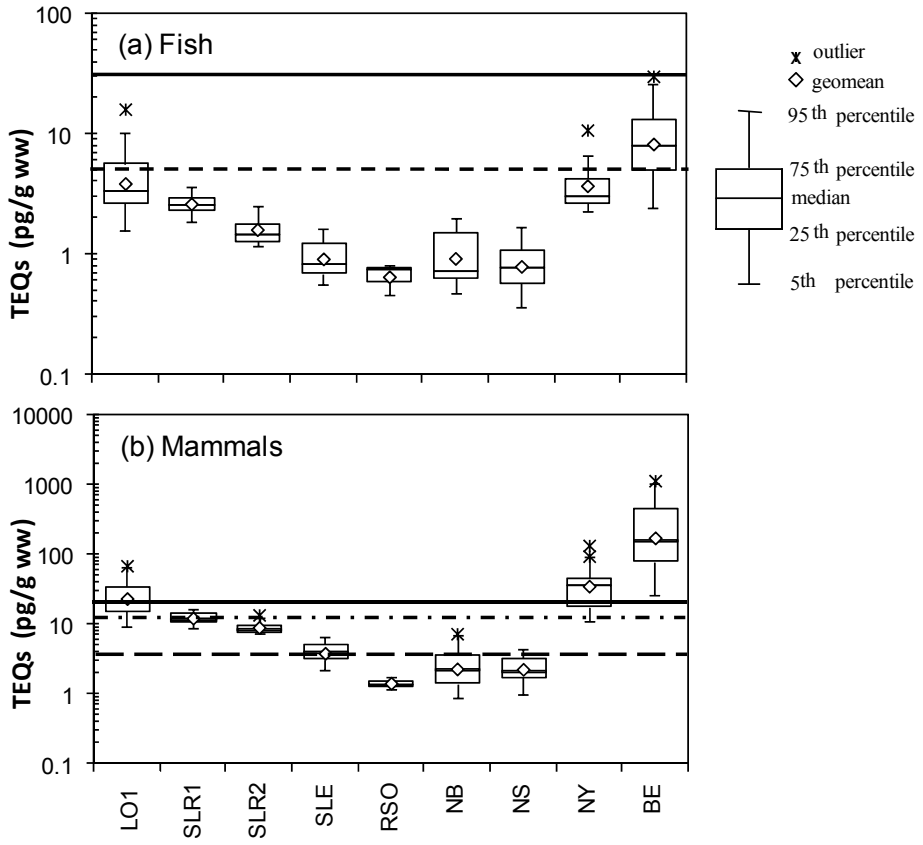

Figure 4- 2. (a) Box and whisker plots of total dl-PCB and PCDD/F TEQ concentrations (pg $FISH_{1998}$ TEQ g^{-1} ww), calculated using $FISH_{1998}$ TEFs, in eel tissue from nine sampling sites. As a guideline used for Lake trout, (- - -) no observable adverse effects level = 5 pg $FISH_{1998}$ TEQ g^{-1} ww egg, (—) mortality threshold = 30 pg $FISH_{1998}$ TEQ g^{-1} ww egg [13].

(b) Box and whisker plots of total dl-PCB, PCN, and PCDD/F-TEQ concentrations (pg TEQ g^{-1} ww), calculated using WHO_{1998} TEFs and PCN TEFs described in text, in eel tissue from nine sampling sites. For comparison, (— —) disrupting effects level = < 4 pg WHO_{1998} TEQ g^{-1} ww [15], (· — ·) European Commission guideline for human consumption of eels = 12 pg WHO_{1998} TEQ g^{-1} ww [43], (—) Canadian guideline for human consumption of fish is equivalent to 20 pg WHO_{1998} TEQ g^{-1} ww.

4.4. Discussion

4.4.1. Concentration patterns related to historic loadings

Chemical contamination in eels, in addition to biological factors such as diet and age, is often a reflection of their local environment [23, 24], and the congener profile for eels in this study supports this finding. Eels migrating from L. Ontario and the St. Lawrence R. had the highest concentration of total PCDD/Fs + dl-PCBs + PCNs in Canadian waters (Table 4-2 and Figure SI 4-1). Concentrations were related to the amount of industrialization and/or urbanization, and are consistent with known historic loadings; sources to L. Ontario included the Love-Canal dumpsite in Niagara Falls, NY, chlor-alkali facilities, and by-products from Agent Orange, PCB (Aroclors) and PCN (Halowaxes) production [3-5]. The R. Sud-Ouest, QC eels had congener patterns primarily reflective of atmospheric inputs. The Atlantic Canada eel congener profiles were also dominated primarily by atmospheric inputs, generally showing an enrichment of the lighter, less chlorinated compounds with respect to L. Ontario, consistent with a phenomenon known as longitudinal refraction [20]. However, they also showed local source signatures which account for these sites having higher PCDD/F concentrations than R. Sud-Ouest. For example, the Miramichi R. eels had the highest concentration of 2,3,7,8-TCDF in this study, which is linked to the use of elemental chlorine in pulp and paper production [1]. Until recently, there was a bleached-kraft paper mill near the mouth of the Miramichi R. where the eels were collected. The size of the eels indicate that they were upstream residents caught during their migration, which may account for their lower concentrations compared to other fish species collected downstream of the mill [25]. High OCDD concentrations in the Atlantic coast eels may be related to sources of pentachlorophenol and combustion [26]. Pentachlorophenol was used primarily in the timber processing industry [27], and may explain the high concentrations of OCDD in the Miramichi and Margaree Rivers; however, in the Hudson R., disposal of coal ash into the river from about 1960 to the mid-1970s was the most likely cause [26].

PCB contamination in the Hudson R. eels was most likely related to historic inputs from General Electric capacitor plants located on the upper Hudson R. [26]. The differing PCB congener patterns between the Canadian, Hudson R., and European samples was related to differences in Aroclor technical mixtures in response to climate (latitude). Eels captured in Canada had a larger proportion of lower molecular weight Aroclor 1254 compared to higher molecular weight Aroclor 1260 use in New York and Belgium. For a detailed discussion of PCBs in these eels see [20].

PCN sources were primarily from PCN technical mixtures (e.g. Halowaxes), thermal sources, and by-products of PCB production (e.g. Aroclors) [4]. However, bioaccumulation of PCN congeners by vertebrates is governed by the amount of chlorine substitution and position, and K_{ow}. The PCN congeners with adjacent chlorines and without adjacent unsubstituted carbons are more likely to bioaccumulate than congeners without adjacent chlorines, and higher chlorinated congeners have a higher K_{ow} [28]. Congeners like CN-73,-74,-75, and to a lesser extent CN-71/72, may have a K_{ow} that is too high to be bioaccumulative, and they may also be more susceptible to biotransformation and degradation [28]. Eels exhibited a high proportion of CN-66/67, -52/60, -61 and -69, and a low proportion of the remaining congeners in this study. Our results were similar to the study by Helm et al. [28] on other fish species in L. Ontario, as well as to reports on fish from Sweden [29] and the UK [30].

4.4.2. Toxic equivalency profiles

In this study, the PCDD/F-TEQs were equivalent using $FISH_{1998}$ and WHO_{1998} TEFs, but the WHO_{2005} concentrations were about 6% less on average. The difference between the WHO_{1998} and WHO_{2005} TEQs occurred because half order-of-magnitude increments on a log scale were used in the re-evaluated TEFs. Also, the $FISH_{1998}$ TEFs for mo-PCBs were all assigned a value <0.000005 and the TEFs for no-PCBs ranged from 0.005 to 0.00005, whereas the WHO_{1998} TEFs ranged from 0.0001 to 0.00001 for mo-PCBs, and from 0.1 to 0.0001 for no-PCBs. This decrease

in mo-PCB TEFs by more than one order-of-magnitude, except for PCB-167, reduced the mo-PCB contribution to the total TEQs by one-half, and significantly lowered the total TEQs (Table 4-2). The less sensitive LOD for the mo-PCBs had no effect on the TEQ calculations.

4.4.3. Concentrations in European and American eel

Table 4-3 shows a summary of other studies that have measured dioxin-like compounds in eels. Limited dioxin-like compound data are available for American eel. Only two studies have reported dioxin-like compounds in eels from Canada previously, both occurring before the WHO-TEFs were established, and with incomplete datasets. To make these data comparable to this study, TEQs were estimated for these data using a combination of the congener composition information reported by Bhavsar et al. [31, 32] and profiles in this study, before applying the TEF approach to develop TEQs. The TEQs for American eels in the present study were lower than these historic data, which is consistent with other studies that showed decreasing concentrations of chlorinated POPs since the late 1960s [13, 33].

Comparatively, there are many more dioxin-like compound data available in the literature for European eel; however, most studies consider human consumption only and report WHO_{1998} data. These data share similar concentration ranges with the American eel data. Most comparably, 38 pooled eel samples from Belgium, which included two pools of small yellow eels collected in 2003 from the same canal as the present study [34] revealed concentrations of PCDD/Fs ranging from 0.2 to 9.8 pg WHO_{1998} TEQ g^{-1} ww (mean = 1.2), and total WHO_{1998} TEQs from 1.1 to 141.9 pg g^{-1} ww (mean = 17.4). Eels from Canal Dessel-Schoten (N=10) had values of 0.57 and 1.68 pg WHO_{1998} TEQ g^{-1} ww for PCDD/Fs, and 9.43 and 38.0 pg g^{-1} ww total WHO_{1998} TEQs. These concentrations fall within the lower range of our Belgian eel data. However, by analysing individual eels instead of pooled samples, we recorded three instances where the total dl-PCB-TEQs were > 400 pg WHO_{1998} TEQ g^{-1} ww (individual data not shown), which would have been lost otherwise. As eels grow toward maturity increasing in weight and fat content, they may also

have a corresponding increase in their body burden of dioxin-like compounds (Table SI 4-2). The Belgian eels in our study were much larger-silvering eels and had a much higher fat content than the yellow eels analysed by Geeraerts et al. [34], which may explain the greater concentrations in the present study.

Table 4- 3. Fish and mammalian TEQs calculated using World Health Organization 1998 TEFs for European and American eels in this and other studies.

Location	N	Year	$FISH_{1998}$-TEQs (pg g^{-1} ww)	WHO_{1998}-TEQs (pg g^{-1} ww)	Reference
Canada	60	2007/08	0.36-16.1	0.89-67.9	This study
USA	9	2009	2.3-10.8	10.8-133	This study
Belgium	9	2009	2.46-30.2	25.2-1120	This study
Canada	6	1980	17.9-107*	100-583*	[38]
Canada	100	1990	8.25-45.7*	41.1-228*	[22]
Baltic Sea	28‡	2005/06		1.35-16.8	[39]
Ireland	76	2005/07	0.21-5.0	0.48-4.93	[27]
Norway	4	2000/01		6.4-27.7	[40]
Netherlands	39	2001		0.2-52	[42]
Belgium	38‡	2003		1.1-141.9	[34]

*Retrospective estimate of full set of WHO dioxin-like compounds determined from composition profiles in Bhavsar et al. [31, 32] and TEFs from van den Berg et al. [7]. ‡Pooled samples.

4.4.4. Sources of uncertainty and potential effects on eel recruitment and fish consumption

The TEQ approach to risk assessment has several underlying assumptions that result in uncertainty, including that all species show similar relative potencies, and that all fish TEFs can be borrowed from lake trout data. However, to date it remains the most widely applied method for dioxin-like compound risk assessment.

To assess the effects of dioxin-like compounds on eel recruitment, ideally one would determine the concentration of TEQs in the eggs of spawning eels, and compare this

concentration to a mortality threshold. If the concentration in the eggs was greater than the mortality threshold, recruitment failure would likely occur. A population level effect could be determined by assessing the percentage of eel eggs exceeding the threshold. Unfortunately, concentrations of dioxin-like contaminants in wild eel eggs at spawn have never been measured. Eels metabolize a higher portion of their fat during migration and use a larger portion of their body fat to produce eggs than iteroparous fish [34-36] and are estimated to require >13.5% body fat to successfully migrate and spawn [37]. Hodson et al. [22] revealed concentrations of dioxin-like compounds in tissues of migrating silvering eels to be highest in gonads with gonad/maternal concentration ratios averaging about 1.4 for PCBs. Calculating egg TEQs using this multiplication factor, mean concentrations were 3.55, 20.0, and 14.3 pg TEQ g^{-1} ww for $FISH_{1998}$, WHO_{1998}, and WHO_{2005}, respectively.

Reliable mortality thresholds for dioxin-like compounds have not been established for eels. Although, Palstra et al. [15] correlated the concentrations of dioxin-like compounds with impaired development and survival of European eel embryos and estimated a threshold below 4 pg WHO_{1998} TEQ g^{-1} ww gonad. However, there is uncertainty associated with this value because TEQs in eggs were determined by DR-CALUX assays based on rat liver cells, and all embryos appeared to have died with 35-42 h, even when TEQs equaled zero. The $FISH_{1998}$ egg TEQs can be evaluated against early life stage mortality guidelines for lake trout [13]. The sublethal effects guideline of 5 pg TEQ g^{-1} ww egg was exceeded by 17% of the samples, but none exceeded the mortality threshold of 30 pg TEQ g^{-1} ww egg, indicating a lower risk of eel recruitment failure. The method of mortality evaluation could have a dramatic impact on the outcome of recruitment risk assessment. Therefore, it is essential for future research to focus on the development of relative potencies for eels and resultant eel specific dioxin-like compound mortality thresholds.

The European guideline for consumption of eels is 12 pg total TEQs g^{-1} ww [43], and the Canadian guideline is equivalent to 20 pg total TEQs g^{-1} ww [44]. Using WHO_{2005}-TEFs compared to WHO_{1998}-TEFs resulted in fewer exceedances of the guidelines (Table 4-4). For

example, in Canada when considering WHO_{1998}-TEQs against the Canadian guideline, 10 of 17 eels exceeded the guideline in L. Ontario compared to 7 with the WHO_{1998} values. Also, due to the stricter threshold, there were almost twice as many exceedances when considering the European Commission guideline as opposed to the Canadian guideline.

Table 4- 4. Summary of the number (percent) of guideline exceedances by eel origin with reference to the Canadian and European consumption guidelines for fish.

Location	**N**	**EC guideline (12 pg TEQ g^{-1}ww)**		**Canadian guideline (20 pg TEQ g^{-1}ww)**	
		WHO_{1998}	WHO_{2005}	WHO_{1998}	WHO_{2005}
BE	9	9 (100)	9 (100)	9 (100)	8 (89)
NY	9	8 (89)	6 (67)	6 (67)	2 (22)
LO	17	14 (82)	13 (76)	10 (59)	7 (41)
SLR1	12	5 (42)	2 (17)	0	0

BE = Canal Dessel-Schoten; NY = Hudson River; LO = Lake Ontario; SLR1/2 = St. Lawrence River; SLE = St. Lawrence Estuary; RSO = Rivière Sud-Ouest; NB = Miramichi River; NS = Margaree River. Note: SLR2 (N=5), SLE (N=3), RSO (N=3), NB (N=10), and NS (N=10) had no guideline exceedances.

4.5. Conclusion

Dioxin-like compound contamination was greatest in eels collected from waters surrounded by highly industrialized and urbanized areas such as L. Ontario, Hudson R., NY, and Canal Dessel-Schoten, BE, and lowest in remote areas (e.g. R. Sud-Ouest). The congener profile varied geographically, supporting the concept of pollution fingerprinting. For example, eels in L. Ontario-St. Lawrence River had a higher proportion of 2,3,7,8-TCDD and a lower proportion of OCDD compared to eels from the east coast of North America. The European eels had a higher proportion of 2,3,4,7,8-PeCDF and higher chlorinated PCBs and PCNs compared to American eel. Also, a majority of the TEQs for fish and mammalians was contributed by three compounds, CB-126, -118, and 2,3,7,8-TCDD for American eels; in the European eels, CB-156, -126, and -

118 predominated. Overall, the risk to eel recruitment from dioxin-like compounds in American eel using available guidelines is low. These data may be used in the future to assess the effect of dioxin-like compounds on eel recruitment when embryo-toxicity data become available for eels. The development of a more comprehensive model for eel recruitment risk assessment due to dioxin-like compounds, using eel-specific guidelines, is recommended. The risk to consumers of eels is moderate-low, except in highly industrialized and urbanized areas where concentrations were above consumption guidelines (i.e. L. Ontario, Hudson R., and Canal Dessel-Schoten).

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4.8. Supplementary Information

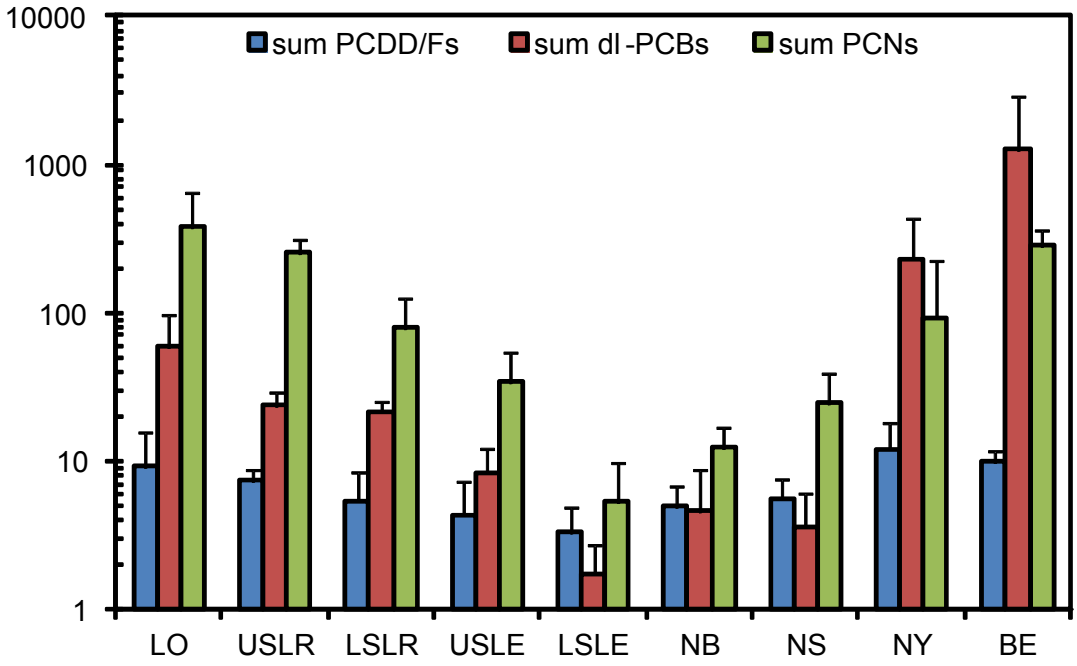


Figure SI 4- 1. Bar graph showing origin dependent average concentrations with standard error of PCDD/Fs, PCNs (pg g^{-1}ww), and dl-PCBs (ng g^{-1}ww). LO = Lake Ontario (N = 17); SLR1 and SLR2 = St. Lawrence River (N = 12 and 5); SLE = St. Lawrence Estuary (N = 3); RSO = Rivière Sud-Ouest (N = 3); NB = Miramichi River (N = 10); NS = Margaree River (N = 10); NY = Hudson River (N = 9); BE = Canal Dessel-Schoten (N = 9).

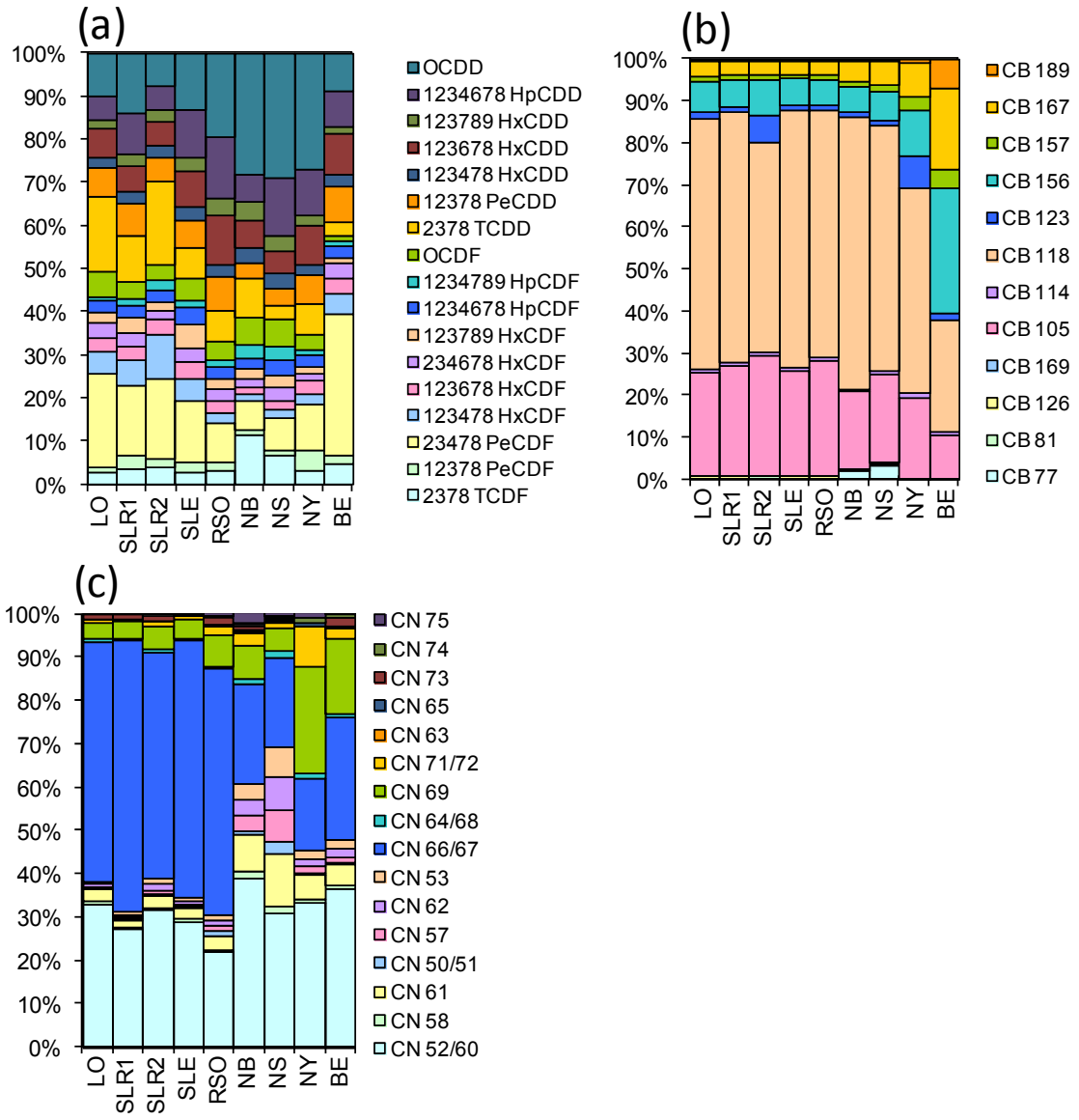


Figure SI 4- 2. Congener pattern of (a) PCDD/Fs, (b) dl-PCBs, and (c) PCNs in eel tissue, expressed as mean % of the wet weight concentration. LO = Lake Ontario (N = 17); SLR1 and SLR2 = St. Lawrence River (N = 12 and 5); SLE = St. Lawrence Estuary (N = 3); RSO = Rivière Sud-Ouest (N = 3); NB = Miramichi River (N = 10); NS = Margaree River (N = 10); NY = Hudson River (N = 9); BE = Canal Dessel-Schoten (N = 9).

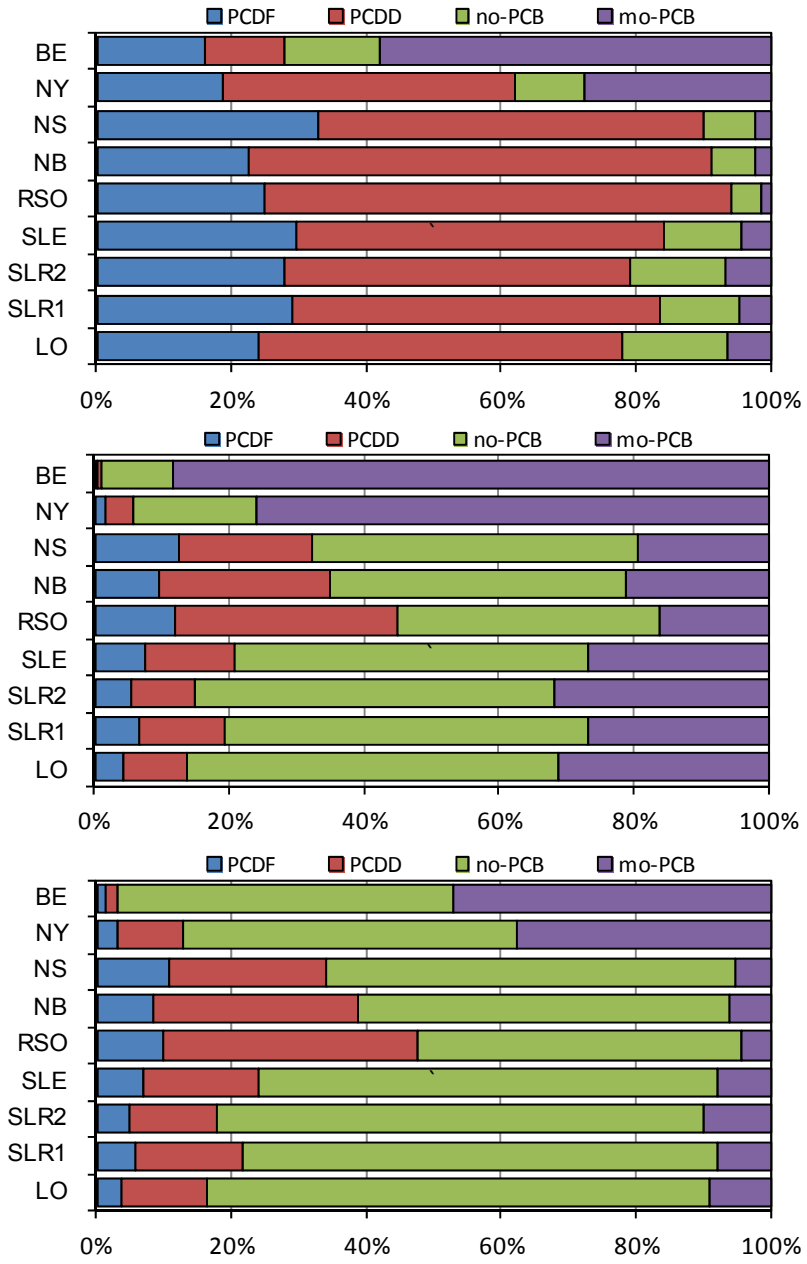


Figure SI 4- 3. The TEQ congener profile for (a) $FISH_{1988}$, (b) WHO_{1998}, and (c) WHO_{2005}, excluding PCNs. LO = Lake Ontario (N = 17); SLR1 and SLR2 = St. Lawrence River (N = 12 and 5); SLE = St. Lawrence Estuary (N = 3); RSO = Rivière Sud-Ouest (N = 3); NB = Miramichi River (N = 10); NS = Margaree River (N = 10); NY = Hudson River (N = 9); BE = Canal Dessel-Schoten (N = 9).

Table SI 4- 1. Average ± standard deviation concentrations of PCDD/Fs, non-ortho PCBs, and PCNs (pg/g ww) in Atlantic eels.

	LO	**SLR1**	**SLR2**	**SLE**	**RSO**	**NB**	**NS**	**NY**	**BE**
PCDD/Fs:									
2,3,7,8-TCDF	0.25±0.14	0.29±0.1	0.23±0.13	0.25±0.27	0.13±0.08	0.56±0.23	0.36±0.12	0.4±0.22	0.47±0.29
1,2,3,7,8-PeCDF	0.13±0.11	0.19±0.09	0.17±0.27	0.16±0.2	0.07±0.04	0.06±0.04	0.08±0.07	0.55±0.36	0.2±0.13
2,3,4,7,8-PeCDF	1.83±0.92	1.35±0.46	0.73±0.27	0.41±0.2	0.24±0.11	0.33±0.2	0.41±0.2	1.27±0.85	3.21±1.36
1,2,3,4,7,8-HxCDF	0.78±1.77	0.45±0.17	0.24±0.19	0.17±0.23	0.1±0.05	0.08±0.05	0.11±0.08	0.33±0.26	0.46±0.1
1,2,3,6,7,8-HxCDF	0.33±0.39	0.2±0.08	0.2±0.17	0.15±0.21	0.09±0.05	0.06±0.02	0.1±0.08	0.37±0.24	0.34±0.09
2,3,4,6,7,8-HxCDF	0.24±0.19	0.21±0.05	0.2±0.24	0.17±0.19	0.09±0.06	0.1±0.08	0.18±0.11	0.19±0.15	0.36±0.09
1,2,3,7,8,9-HxCDF	0.21±0.17	0.24±0.05	0.2±0.22	0.21±0.27	0.08±0.04	0.12±0.1	0.16±0.1	0.17±0.1	0.12±0.06
1,2,3,4,6,7,8-HpCDF	0.25±0.33	0.18±0.09	0.21±0.16	0.17±0.19	0.07±0.07	0.12±0.09	0.19±0.16	0.35±0.18	0.24±0.09
1,2,3,4,7,8,9-HpCDF	0.14±0.26	0.14±0.01	0.1±0.05		0.15±0.12	0.05±0.01	0.14±0	0.19±0.23	0.13±0.1
OCDF	0.38±0.23	0.25±0.06	0.29±0.3	0.41±0.32	0.12±0.03	0.32±0	0.35±0.17	0.46±0.25	0.1±0.06
2,3,7,8-TCDD	1.76±2.25	0.94±0.25	0.38±0.19	0.24±0.04	0.17±0.16	0.46±0.38	0.17±0.24	0.84±0.52	0.32±0.2
1,2,3,7,8-PeCDD	0.54±0.31	0.53±0.22	0.38±0.31	0.25±0.23	0.24±0.1	0.17±0.11	0.23±0.14	0.81±0.44	0.81±0.23
1,2,3,4,7,8-HxCDD	0.24±0.14	0.19±0.09	0.18±0.15	0.12±0.1	0.11±0.11	0.17±0.01	0.19±0.1	0.32±0.3	0.27±0.14
1,2,3,6,7,8-HxCDD	0.53±0.34	0.51±0.09	0.43±0.19	0.26±0.18	0.37±0.43	0.31±0.16	0.29±0.18	1.08±0.83	0.92±0.33
1,2,3,7,8,9-HxCDD	0.21±0.16	0.21±0.12	0.21±0.2	0.17±0.16	0.14±0.12	0.22±0.13	0.2±0.11	0.32±0.23	0.18±0.06
1,2,3,4,6,7,8-HpCDD	0.48±0.36	0.71±0.27	0.48±0.35	0.43±0.31	0.62±0.47	0.3±0.24	0.75±0.39	1.29±0.78	0.8±0.22
OCDD	0.71±0.55	1.06±0.44	0.78±0.32	0.62±0.17	0.65±0.41	1.4±1.83	1.62±0.58	3.26±4.35	0.87±0.25
Sum PCDD/F	**9.01±6.72**	**7.66±1.43**	**5.41±3.1**	**4.34±3.02**	**3.31±1.66**	**4.94±1.74**	**5.58±1.94**	**12.1±6.2**	**9.82±2.05**

Table SI 4-1. continued

	LO	SLR1	SLR2	SLE	RSO	NB	NS	NY	BE
Non-ortho PCBs:									
3,3',4,4'-TCB 77	58.8±51.6	32.9±36.3	43.8±54.6	48.1±47.3	5.45±5.76	73.4±13.2	104±21	25±19.3	31.4±17.1
	LO	**SLR1**	**SLR2**	**SLE**	**RSO**	**NB**	**NS**	**NY**	**BE**
3,4,4',5-TCB 81	5.04±6.26	3.4±2.3	3.99±3.06	9.17±11.48	2.78±1.93	3.54±0.91	5.14±1.87	4.77±3.97	3.2±1.58
3,3',4,4',5-PeCB 126	124±86	90.8±86.8	47.3±20.3	21.5±13.4	5.31±0.37	11.8±10.8	11.3±5.9	83.9±77.7	304±181
3,3',4,4',5,5'-HxCB 169	16±19.6	7.34±2.86	3.5±1.67	1.57±1.04	2.38±1.81	2.96±1.95	3.56±1.31	25.1±19.7	330±457
Sum non-ortho PCB	**204±139**	**134±119**	**98.6±71.7**	**80.4±54.3**	**15.9±8**	**91.7±15.8**	**124±22**	**139±111**	**669±572**
Sum dl-PCB (ng g^{-1} ww)	**56.7±37.7**	**27.1±12.0**	**21.7±3.34**	**8.44±3.56**	**1.74±1.02**	**4.56±4.22**	**3.59±2.41**	**231±204**	**1266±1580**
PCNs:									
1,2,3,5,7-PeCN 52/ 1,2,4,6,7-PeCN 60	119±105	74.9±31.4	22.1±15.7	14.3±7.9	2±2.45	4.76±2.68	7.59±5.31	30.9±46.9	102±54
1,2,4,5,7-PeCN 58	2.32±2.69	1.17±0.52	0.53±0.49	0.55±0.4	0.11±0.12	0.24±0.09	0.42±0.28	0.84±1.5	2.62±1.74
1,2,4,6,8- PeCN 61	9.36±9.25	5.21±2.55	2.45±2.17	3.57±3.04	0.66±0.57	1.03±0.43	2.98±3.04	5.09±8.74	13.9±6.6
1,2,3,4,6-PeCN 50/ 1,2,3,5,6-PeCN51	0.74±0.55	0.75±0.3	0.49±0.39	0.5±0.59	0.16±0.07	<0.2	0.67±0.55	0.48±0.63	1.03±0.75
1,2,4,5,6-PeCN 57	1.68±1.22	1.43±0.99	1.07±1.09	1.2±1.4	0.23±0.15	0.46±0.44	1.81±1.71	1.36±1.04	3.29±1.38
1,2,4,7,8-PeCN 62	2.99±2.13	2.16±1.56	1.66±1.84	1.84±2.15	0.29±0.18	0.43±0.46	1.95±2.31	1.69±1.21	5.13±2.11
1,2,3,5,8-PeCN 53	2.73±2.35	1.86±1.15	1.35±1.08	1.22±1.18	0.18±0.11	0.42±0.16	1.72±1.81	2.04±1.92	6.37±2.1
1,2,3,4,6,7-HxCN 66/ 1,2,3,5,6,7-HxCN 67	201±124	167±49	43.8±28.2	5.34±4.04	0.49±0.41	2.84±1.79	5.07±3.6	15.3±15.9	79.5±18.2
1,2,3,4,5,7-HxCN 64/ 1,2,3,5,6,8-HxCN 68	2.87±3.67	1.12±0.49	0.32±0.25	0.34±0.28	<0.1	0.17±0.05	0.35±0.33	1.18±1.84	1.93±1.2
1,2,3,5,7,8-HxCN 69	16.4±15.5	11.1±4.4	4±1.78	3.1±1.94	0.49±0.59	0.94±0.37	1.31±0.71	22.7±33.8	48.9±20.3
1,2,4,5,6,8-HxCN 71/ 1,2,4,5,7,8-HxCN 72	3.77±5.48	1.3±0.75	0.65±0.44	1.34±0.85	0.35±0.4	0.37±0.1	0.37±0.28	8.81±17.95	6.65±3.23

Table SI 4-1. continued

	LO	SLR1	SLR2	SLE	RSO	NB	NS	NY	BE
1,2,3,4,5,6-HxCN 63	0.18±0.22	0.13±0.06	<0.1	<0.1	<0.1	<0.1	<0.1	<0.2	0.37±0.29
1,2,3,4,5,8-HxCN 65	0.21±0.44	0.13±0.1	<0.1	0.2±0.14	<0.1	<0.1	<0.1	0.48±1.14	0.95±0.73
1,2,3,4,5,6,7-HpCN 73	4.53±4.07	2.88±0.88	0.5±0.5	<0.2	<0.1	<0.2	<0.2	<0.2	5.98±3.48
1,2,3,4,5,6,8-HpCN 74	0.65±1.12	0.11±0.06	<0.1	<0.1	<0.1	<0.1	<0.2	1.11±2.14	2.56±1.36
OCN 75	0.46±1.49	0.21±0.11	0.13±0.1	<0.1	<0.1	0.3±0.06	0.17±0.09	0.88±0.21	0.36±0.4
Sum PCN	**368±264**	**272±83**	**79.2±44.5**	**33.9±20.1**	**5.26±4.38**	**12.3±4.3**	**24.7±14.7**	**93.2±134**	**282±78**

LO = Lake Ontario (N = 17); SLR1 and SLR2 = St. Lawrence River (N = 12 and 5); SLE = St. Lawrence Estuary (N = 3); RSO = Rivière Sud-Ouest (N = 3); NB = Miramichi River (N = 10); NS = Margaree River (N = 10); NY = Hudson River (N = 9); BE = Canal Dessel-Schoten (N = 9). Note that as concentration increased, so did the standard deviation, highlighting the necessity of log-transformed and geometric mean data.

Table SI 4- 2. Comparison of eels collected from Canal Dessel-Schoten in 2003 and 2009 with respect to the number of eels (N), average length (LM), average weight (WM), fat content (%), absolute PCDD/F and dl-PCB concentrations (pg g^{-1} ww), and the body burden (ng g^{-1} ww).

Site	N	LM (cm)	WM (g)	Fat %	ΣPCDD/Fs	Σdl-PCBs	Body burden ΣPCDD/F + dl-PCBs
KDS1*	10	48.1	181.9	10.6	6.5	125000	22700
KDS2*	10	40.4	126	2.3	1.8	23400	2950
BE¤	8	83.1	924	25.5	9.8	802000	741000

*KDS = Canal Dessel-Schoten eel data from Geeraerts et al. (2011). ¤Arithmetic mean data for the Belgian eels from Canal Dessel-Schoten in this study, excluding the outlier shown in Figures. 4-3 and 4-4.

Table SI 4- 3. Pearson correlation matrix of seven variables; dl-PCB-TEQs, PCDD/F-TEQs, PCN-TEQs, age, fat content, length, and weight.

		PCDD/F-TEQs	dl-PCB-TEQs	PCN-TEQs	Length	Weight	Fat	Age
Correlation	PCDD/F-TEQs	1.000						
	dl-PCB-TEQs	.220	1.000					
	PCN-TEQs	.600	.127	1.000				
	Length	.405	-.015	.676	1.000			
	Weight	.411	-.067	.645	.952	1.000		
	Fat	.299	.163	.523	.522	.446	1.000	
	Age	.105	.000	.406	.591	.574	.332	1.000

The only significant correlation was between length and weight (correlation factor = 0.952). This suggests that loadings of PCNs, dl-PCBs, and PCDD/Fs to eels originate from different sources, irrespective of their size, fat content, and age.

Chapter 5

Historical concentrations (1988-2008) of emerging halogenated flame retardants in American eel captured in Eastern Lake Ontario (Canada)

Abstract

Select halogenated flame retardants such as Mirex, hexabromobiphenyl, and polybrominated diphenylethers have been banned or withdrawn from the marketplace in recent years. Replacements are being produced by industry, but little is known about their occurrence in the environment. Recent studies of Lake Ontario sediment and biota have discovered Dechlorane Plus and other similar substances, in addition to emerging brominated flame retardants. However, their occurrence in fish such as American eel (*Anguilla rostrata*), a benthic predator, is unexplored. Populations of American eel in Lake Ontario are sustained only by stocking. Although emerging halogenated flame retardants are not the likely cause of their demise, they may be one of the factors retarding their recovery. Concentrations of nine chlorinated flame retardants and seven emerging brominated flame retardants were determined in American eel from 1988 to 2008 by gas chromatography - high resolution mass spectrometry. Concentrations of chlorinated flame retardants have all decreased between 1988 and 2008 in eels, whereas, most of the emerging brominated flame retardants have remained constant or increased significantly. Concentrations of the measured replacement brominated flame retardants are expected to continue to increase, and hence we recommend ongoing monitoring.

5.1 Introduction

Halogenated flame retardants (HFRs) are used in a variety of consumer products such as textiles, plastics, and electronics to increase their fire resistance, and several are large volume production chemicals [1-3]. First generation HFRs like Mirex, also known as Dechlorane, and 2,2',4,4',5,5'-hexabromobiphenyl (BB-153), a main component in FireMaster, were banned in the 1970s because of their persistence, bioaccumulation potential, and toxicity. Similarly, the use and production of polybrominated diphenyl ether (PBDE) technical mixtures, namely PentaBDE and OctaBDE, have been banned recently or restricted worldwide [4]. Nevertheless, flame retardency regulations sustain the demand for flame retardants, so industry continues to manufacture replacements. For example, Dechlorane Plus, Dechlorane 602, 603, and 604 were designed as replacements for Mirex, and PBDE technical mixtures are being replaced by a variety of BFRs. Little is known about the production and usage of many new and emerging HFRs, and even less information is available regarding their environmental fate.

American eels (*Anguilla rostrata*) are a benthic predatory fish that reach sizes > 1m in length and 3 kg in weight, before returning to the Sargasso sea to spawn and die [5]. Prior to the 1990s, American eel thrived in Lake Ontario. Their population collapse caused the closure of the commercial yellow eel fishery in Lake Ontario in 2004, and registry as an endangered species under the Species at Risk in Ontario List in 2008 [5, 6]. Chemical contaminants may have contributed to their demise, and the present study is an extension of previous studies focused on legacy contaminants [7-9]. The main objective was to measure several novel chlorinated and brominated flame retardants in archived and freshly-caught American eel to determine historical concentrations between 1988 and 2008 in Lake Ontario, as well as characteristics related to bioaccumulation. For comparative purposes, Mirex, BB-153, and 2,2',4,4'-tetrabromodiphenyl ether (BDE-47) were also considered.

5.2 Experimental Details

Large, sexually maturing female American eels, about the same age (≈ 20 years), were captured by electrofishing from Lake Ontario in 1988, 1998, and 2008 (10 each) (Figure 2-1; Table 5-1). Sampling details were summarized in Byer et al. [9] (Chapter 2). All fish were gutted and stored frozen until analysis. Whole fish homogenates were prepared for each fish, and extracted for chemical analysis according to the following: about 5 g of wet weight (ww) equivalent of tissue was first dried with anhydrous sodium sulfate, spiked with [^{13}C]-PCB-170 (Wellington Laboratories, Guelph, ON), and eluted with dichloromethane. Lipid content was determined gravimetrically. Extracts were further spiked with [^{13}C]-PBDE-47, -99, -153, and -209 surrogate standards [10]. Bulk-lipids were removed by gel permeation chromatography (SX-3 biobeads) with a mixture of 1:1 dichloromethane:hexane (v:v). Final clean-up consisted of a two-layered packed, 5% deactivated silica-alumina column, eluted with hexane and 1:1 dichloromethane :hexane. The cleaned-up fractions were reduced in volume and spiked with [^{13}C]-PCB-111 and -189 internal standards.

Table 5- 1. Biological data for eels captured in Lake Ontario in 1988, 1998, and 2008.

Year	No.	Length (cm)	Weight (g)	Age	Lipid %
1988	10	95 ± 7	2075 ± 418	22 ± 3	30.7 ± 4.0
1998	10	87 ± 5	1582 ± 440	20 ± 3	28.9 ± 3.8
2008	10	106 ± 15	2211 ± 780	20 ± 3	23.2 ± 2.7

Concentrations of chlorinated and brominated flame retardants were determined with gas chromatographic-high resolution magnetic sector mass spectrometry (GC-HRMS). A CTC A200S autosampler (Leap Technologies, Chapel Hill, NC, USA) was connected to a Hewlett-Packard 6890 GC (Hewlett Packard, Palo Alta, CA,USA). The GC column was a DB-5HT 15 m x 0.25 mm x 0.1 μm film (J&W Scientific, USA). Mass spectrometric analysis was carried out

using a Micromass AutoSpec MS (Micromass, Manchester, UK), operated in electron-ionisation mode with selected-ion-monitoring, tuned to 10,000 resolution at 5% peak height.

The chlorinated flame retardants (CFRs) monitored include, Dechlorane Plus (*syn*-DP and *anti*-DP), dechlorinated Dechlorane Plus (aCl10DP and aCl11DP), 1,5-Dechlorane Plus mono-adduct (DPMA), Dechloranes 602, 603, 604 (Dec-602, Dec-603, Dec-604), as well as Chlordene Plus (CP), and Mirex. The brominated flame retardants were allyl 2,4,6-tribromophenyl ether (ATE), BB-153, 2-bromoallyl-2,4,6-tribromophenyl ether (BATE), bis(2-ethylhexyl)tetrabromophthalate (TBPH), 1,2-bis(2,4,6-tribromophenoxy)ethane (BTBPE), 2,3-dibromopropyl-2,4,6-tribromophenylether (DPTE), 1,2-bis(pentabromophenyl)ethane (DBDPE), hexabromobenzene (HBB), (1R,2R,5R,6R,9S,10S)-5,6-dibromo-1,10,11,12,13,13-hexachlorotricyclo[8,2,1,0(2,9)]-tridec-11-ene (HCDBCO), 4,5,6,7-tetrabromo-1,1,3-trimethyl-3-(2,3,4,5-tetrabromophenyl)-indane (OBIND), pentabromoethylbenzene (PBEB), pentabromotoluene (PBT), and 2,3,5,6-tetrabromo-p-xylene (p-TBX). Chemical structures are represented in Table 5-2.

Quantitation was based on [^{13}C]-PBDE surrogate standards and individual relative response factors, using a four point calibration curve. Peaks were identified based on retention time (± 0.2 s) and ion ratios (± 15% theoretical). Method blanks were performed with each set of 10 fish samples. For the emerging CFRs, blank values ranged from non-detectable to 2.8 pg/g ww. Blank values for the BFRs ranged from non-detectable to 16 pg/g ww. All reported data were blank subtracted and lipid normalised. For statistical purposes, concentrations below the limit of detection (LOD) were given values of half the limit of quantification. Concentrations of BATE, DBDPE, DPTE, HCDBCO, and pTBX did not exceed their LOD in any of the eel samples.

Table 5- 2. Halogenated flame retardant chemicals.

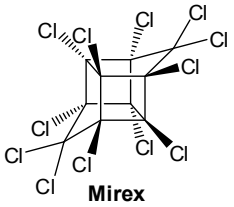
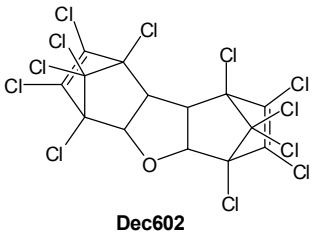
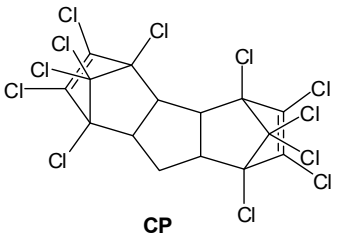
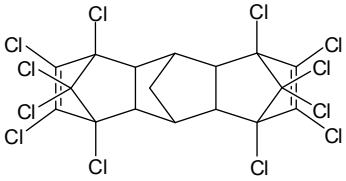
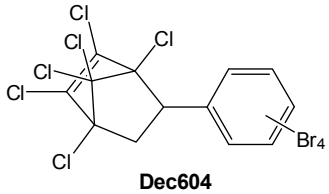
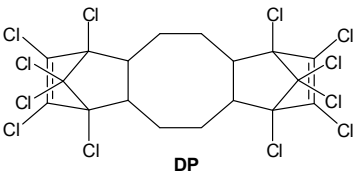
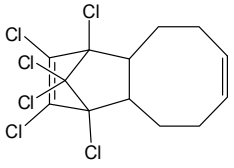
Compound Name	Formula	MW (g/mol)	Structure
Mirex (Dechlorane)	$C_{10}Cl_{12}$	545.54	 Mirex
Dechlorane 602	$C_{14}H_4Cl_{12}O$	613.62	 Dec602
Chlordene Plus	$C_{15}H_6Cl_{12}$	611.64	 CP
Dechlorane 603	$C_{17}H_8Cl_{12}$	637.68	 Dec603
Dechlorane 604	$C_{13}H_4Br_4Cl_6$	692.50	 Dec604
Dechlorane Plus	$C_{18}H_{12}Cl_{12}$	653.72	 DP
1,5-Dechlorane Plus mono-adduct	$C_{13}H_{12}Cl_6$	380.95	 1,5- DPMA

Table 5- 2. Halogenated flame retardant chemicals (continued).

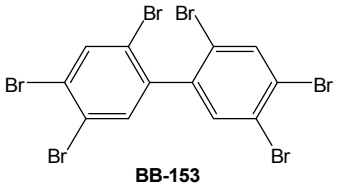
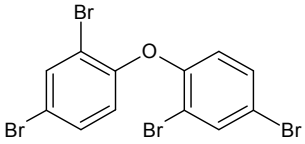
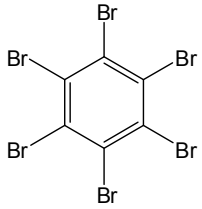
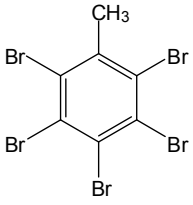
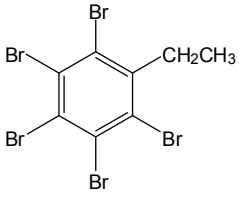
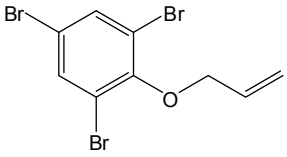
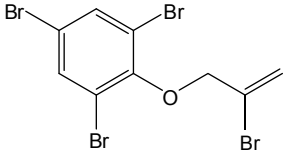
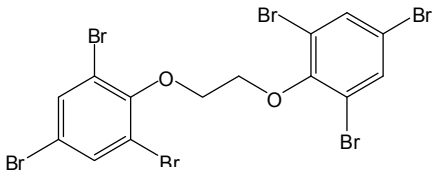
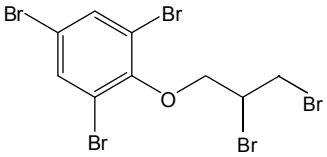
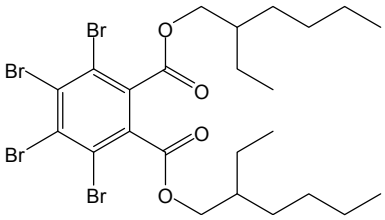
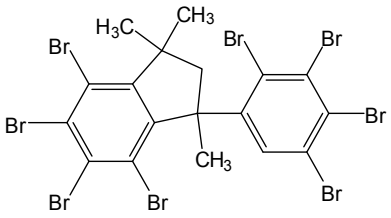
Compound Name	Formula	MW (g/mol)	Structure
2,2',4,4',5,5'-Hexabromobiphenyl	$C_{12}H_4Br_6$	627.58	 BB-153
2,2',4,4'-Tetrabromodiphenyl ether	$C1_2H_6Br_4O$	485.79	 BDE-47
Hexabromobenzene	C_6Br_6	551.49	 HBB
Pentabromotoluene	$C_7H_3Br_5$	486.62	 PBT
Pentabromoethyl-benzene	$C_8H_5Br_5$	500.65	 PBEB
allyl 2,4,6-tribromophenyl ether	$C_9H_7Br_3O$	370.87	 ATE
2-bromoallyl-2,4,6-tribromophenyl ether	$C_9H_6Br_4O$	449.76	 BATE

Table 5- 2. Halogenated flame retardant chemicals (continued).

Compound Name	Formula	MW (g/mol)	Structure
1,2-bis(2,4,6-tribromo-phenoxy) ethane	$C_{14}H_8Br_6O_2$	687.64	 BTBPE
2,3-dibromopropyl-2,4,6-tribromophenyl ether	$C_9H_7Br_5O$	530.67	 DPTE
bis(2-ethylhexyl)-tetrabromophthalate	$C_{24}H_{34}Br_4O_4$	643.72	 BEHTBP
4,5,6,7-tetrabromo-1,1,3-trimethyl-3-(2,3,4,5-tetrabromophenyl)-indane	$C_{18}H_{12}Br_8$	867.53	 OBIND

Concentration differences among years were evaluated for significance with ANOVA, both for unadjusted and trophic position $\delta^{15}N$ adjusted concentrations in Microsoft EXCEL using the DATA ANALYSIS tool pack. The $\delta^{15}N$ data were reported in Chapter 2 [9]. The adjusted concentrations were determined by linear regression of $\delta^{15}N$ values against the natural logarithm of measured concentrations as described previously [11]. The residuals of the regression analysis represent the adjustments for trophic position, and when added to the grand mean, can be back transformed to obtain the final $\delta^{15}N$ adjusted concentrations. Concentration differences between unadjusted and $\delta^{15}N$ adjusted data were assessed for significance using paired Student's *t*-tests at a 95% confidence interval.

5.3 Results and Discussion

5.3.1 Chlorinated flame retardants

Dechlorane Plus was measured in Lake Ontario eels because of a known source from Oxychem in Niagara Falls, NY, and previous reports in Lake Ontario sediment and biota [12-17]. The exposure dynamics of eels are different than other fish studied previously, such as lake trout (*Salvelinus namaycush*). They inhabit a benthic rather than a pelagic habitat, residing mainly in sediments of near-shore areas versus open water, and they are omnivores, feeding at a variety of trophic levels. Biological factors enhancing bioaccumulation include longevity, high fat content, and semelparous reproduction.

Concentrations of emerging CFRs in eels were unexpectedly low, especially when compared to Mirex (Table 5-3). Dechlorane 602 was by far the most bioaccumulative, having a maximum concentration of 1895 ± 987 pg/g lipid weight (lw) in 1988, more than two orders-of-magnitude higher in concentration than the next most abundant CFRs, *anti*-DP (55.2 ± 41.6 pg/g lw) and *syn*-DP (51.3 ± 37.4 pg/g lw). Although CP and Dec602 are very similar structurally (cyclopentadiene as opposed to furan), CP was detected at concentrations <1.0% of Dec602. This may be reflective of production, since Dec602 is produced commercially, whereas CP is a byproduct in chlordane and chlordene.

Concentrations of total DP in Lake Ontario fish collected between 2000 and 2003 ranged from 15 ± 4 pg/g lw in smelt to 1554 ± 500 pg/g lw in sculpin, and its distribution among species indicated that DP does not biomagnify in the Lake Ontario food-web [17]. Data from the present study for eel were added to this food-web study for comparative purposes, and support the existing pattern showing no biomagnification (Figure 5-1). Shen et al. [15] reported concentrations of Mirex, Dec602, 603, 604, and total DP in Lake Ontario whitefish (*Prosopium cylindraceum*) and lake trout (Table 5-4). A similar rank order of concentration was observed in eels where mirex >> Dec602 > DP > Dec603 > Dec604. It was somewhat unexpected for eels to have lower concentrations of CFRs than lake trout and whitefish, because eels reside in Lake

Ontario up to four times longer. However, eels occupy a lower trophic position than lake trout in Lake Ontario (Figure 5-1), and diet and biotransformation capacity may be more relevant factors than age or fat content.

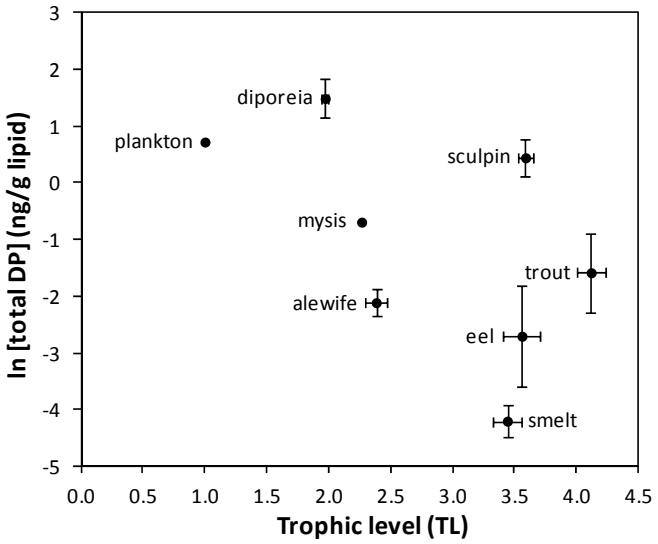


Figure 5- 1. Dechlorane Plus does not biomagnify in the Lake Ontario food web. Figure adapted from Tomy et al. [16]. There appears to be a relationship between total DP concentration and trophic level, however, linear regression analysis proved the correlation to be insignificant (r^2 = 0.31; p = 0.15). Trophic levels were estimated using $\delta^{15}N$ values reported in Tomy et al. [16] for all organisms except eel, which were from Byer et al. [9], according to eq 1. An average of 1998 and 2008 $\delta^{15}N$ values was used for eels (N = 20), since samples for the Tomy et al. study were collected between 2000 and 2003. Mysis and Plankton (N = 1); Diporeia (N = 3); Sculpin, Alewife, Smelt, and Trout (N = 4). Error bars represent standard deviation.

$$TL = [(\delta^{15}N_{pred} - \delta^{15}N_{base})/ \Delta\delta^{15}N] + TL_{base} \quad \{1\}$$

where $\delta^{15}N_{pred}$ is the $\delta^{15}N$ signature of the predator (eel), $\delta^{15}N_{base}$ is the $\delta^{15}N$ signature of the representative baseline for the predator, TL_{base} is the trophic level of that baseline, and $\Delta\delta^{15}N$ represents the trophic fractionation of $\delta^{15}N$, estimated at 3.4‰ [24, 30]. Plankton was used as the baseline, so TL_{base} was equal to 1.

Table 5- 3. Mean ± standard deviation concentrations of chlorinated flame retardants (pg/g lw) in American eel (1988-2008) both unadjusted and $\delta^{15}N$ adjusted (N =10/yr).

	Unadjusted			$\delta^{15}N$ Adjusted		
Year	**1988**	**1998**	**2008**	**1988**	**1998**	**2008**
Mirex[+]	345000±129000[a]	306000±109000[a]	78400±56100[b]	317000±200000[a]	247000±148000[a,b]	120000±68000[b]
DPMA	32.7±52.6	21.6±37.6	0.37±0.57	21.7±26.4	11.8±26	0.7±0.67
CP	20.0±29.3	1.93±4.86	6.28±9.27	24.2±35.1	1.45±3.03	4.38±6.55
Dec602	1900±987[a]	991±514[b]	882±515[b]	1800±1000[a]	884±446[b]	1030±612[b]
Dec603	18±7.41	12.2±5.14	12.4±5.08	17.6±7.82	11.6±4.84	13.2±5.37
Dec604	6.41±11.1[a]	16.1±14.9[b]	1.67±4.26[a]	10.5±27	7.00±6.51	4.51±11.4
aCl10DP	11.5±20.9	0.23±0.1	4.43±9	11.1±19.1	0.3±0.16	3.51±7.01
aCl11DP	25.1±32.2	7.09±3.66	8.01±9.73	24.2±31.6	6.36±3.62	9.57±11.8
sDP	51.3±37.4	33.8±16	28.3±16.9	48.2±35.7	29.4±13.8	34.1±20.7
aDP	55.2±41.6	34.4±20	38.6±31.2	52.3±39.2	30.9±18.4	44.1±35.7
f_{anti}	0.52±0.02	0.50±0.04	0.56±0.08	0.52±0.03	0.50±0.04	0.56±0.08

[+]Data from [8, 9]. Means sharing the same superscript letter (a or b) are not significantly different (ANOVA, $p > 0.05$).

Table 5- 4. Concentration range (pg/g lw) of chlorinated flame retardants in Lake Ontario fish.

	Mirex	Dec 602	Dec 603	Dec 604	Total DP
Lake trout[†]	190000-390000	7400-32000	33-95	63-400	220-390
Whitefish[†]	200000-540000	15000-34000	73-550	230-1300	640-2600
Lake trout[*]	83000-4300000	8200-180000	30-40	-	180-1900
American eel[‡]	78400-345000	882-1900	12-18	2-16	67-107

[†]Shen et al. [15]. [*]Shen et al. [13] [‡]Present study mean range among years.

Changes in the concentration of CFRs in Lake Ontario eels between 1988 and 2008 are shown in Figure 5-2. Concentrations of Dec602, Dec603, and total DP decreased between 1988 and 1998, but only Dec602 decreased significantly ($p < 0.01$). DPMA declined dramatically between 1998 and 2008, but the change was not statistically significant due to high standard deviations. Dechlorane 604 decreased from 1998 to 2008, and CP did not increase or decrease consistently among years ($p>0.1$). For comparison, Mirex decreased slightly between 1988 and 1998, and significantly to 2008 ($p < 0.05$).

The concentration of *anti*-DP as a proportion of the total DP (f_{anti}), ranges between 0.64 and 0.8 in technical products [13]. In eels, f_{anti} ranged from 0.50 ± 0.04 to 0.56 ± 0.08 between 1998 and 2008, increasing over this time period ($p <0.03$). This trend has also been observed in other fish from Lake Ontario, indicating that *anti*-DP is less bioaccumulative than *syn*-DP [13]. However, this may be food web dependent as studies in China show *syn*-DP to be less bioaccumulative [18, 19].

A temporal study of lake trout in Lake Ontario between 1979 and 2004 [13], highlighted concentration ranges for mirex, total DP, CP, Dec602, and Dec603 (Table 5-4); however, the total DP concentrations reported in these fish were up to 10-fold different then first reported by Ismail

et al. [12]. The discrepancy may be related to differing analytical methodologies. Both used GC-MS, but the ionization and resolution of the MS analysers differed. Ismail et al. [12] used electron-capture negative ionisation with selected-ion-monitoring of *m/z* 651/653 by low resolution mass selective dxetector (MSD); whereas, Shen et al. [13] operated in electron ionisation mode and monitored the two most abundant fragment clusters of DP *m/z* 271.8102/273.8072. This may explain the stronger agreement between results from the present study and the latter. Shen et al. [13] reported that concentrations of most CFRs peaked in the mid-1980s and declined to the late-1990s, before leveling off in recent years below 1970s concentrations. Likewise in eels, concentrations of most CFRs were highest in 1988, and did not change significantly between 1998 and 2008.

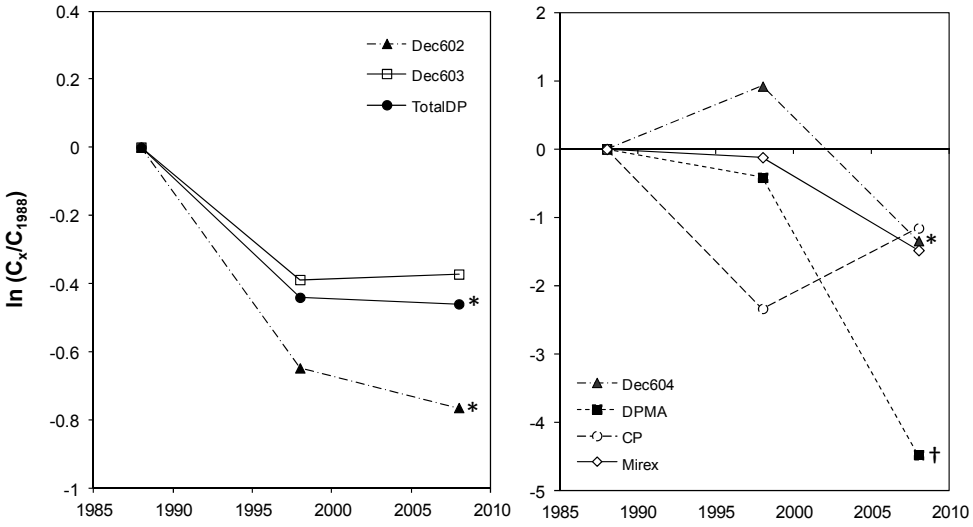


Figure 5- 2. Concentration differences among years of total Dechlorane Plus (*syn*-DP + *anti*-DP), Dechloranes 602, 603, 604 (Dec-602, Dec-603, Dec-604), 1,5-Dechlorane Plus mono-adduct (DPMA), Chlordene Plus (CP), and Mirex in American eel captured in Lake Ontario (N =10/yr). Values are presented normalized to the natural logarithm of the 1988 mean concentration. Statistically significant differences ($p<0.05$) between 1988 and 2008 are denoted with an asterisk (*). †Not significant because of high standard deviation.

5.3.2 Brominated flame retardants

Concentrations of legacy BFRs were the most abundant in eels, averaging about 100 and 1000-fold higher for BB-153 and BDE-47, respectively, than the most abundant emerging BFRs. Of the emerging BFRs, ATE was the most concentrated (mean = 226 ± 223 pg/g lw in 2008). The structure of ATE is similar to DPTE and BATE (Table 5-2), and typically, DPTE and BATE occur at higher concentrations in the environment. In biota, ATE has been attributed to the biotransformation of DPTE and BATE [20]. If this is the case, eels are extremely effective at converting DPTE and BATE to ATE, since both BATE and DPTE were not detectable. Biotransformation of other BFRs occurs in eels; Law et al. [21] showed concentration ratios of BDE-99/100 of around two for most organisms, while the ratio was significantly less in eels (0.1). Alternatively, DPTE and BATE may be transformed by other organisms in the food web prior to eels.

The next most significant emerging BFR was BTBPE, which had a mean concentration near 80 pg/g lw between 1988 and 2008. Concentrations of TBPH were similar to BTBPE in 1988, but were about 3-fold less in 1998 and 2008. OBIND was detectable in very low quantities (< 5 pg/g lw), until 2008 (mean = 26 pg/g lw). The other BFRs, HBB, PBT, and PBEB, were detectable at low concentrations (< 10 pg/g lw) (Table 5-5).

Temporally, HBB and PBT concentrations declined, but not significantly and not to the same extent as BDE-47 and BB-153, which declined from 1988 to 2008 ($p < 0.001$). In contrast, TBPH decreased from 1988 to 1998 ($p < 0.04$), but was statistically similar between 1988 and 2008, as was PBEB and BTBPE. Two BFRs, ATE and OBIND were the only emerging flame retardants to increase sharply from 1998 to 2008 ($p < 0.002$ and 0.02, respectively) (Figure 5-3). Overall, this meant that the concentration order changed over time. In 1988 and 1998, the order was BDE-47 >> BB-153 > BTBPE > TBPH > ATE > HBB > OBIND > PBEB > PBT. In 2008,

the legacy BFRs were still the most abundant, but the order of the other BFRs changed dramatically: ATE > BTBPE > OBIND > TBPH > PBEB > HBB > PBT. This suggests a significant change in the production and use of these BFRs between 1998 and 2008, particularly for ATE (or DPTE and BATE if ATE occurs in eels due to biotransformation), although limited production information is available to support this hypothesis. Alternatively, there could have been a change in the ecosystem structure as evident by the significant change in $\delta^{15}N$ (Figure 5-4) and fat content (Table 5-1) between 1998 and 2008.

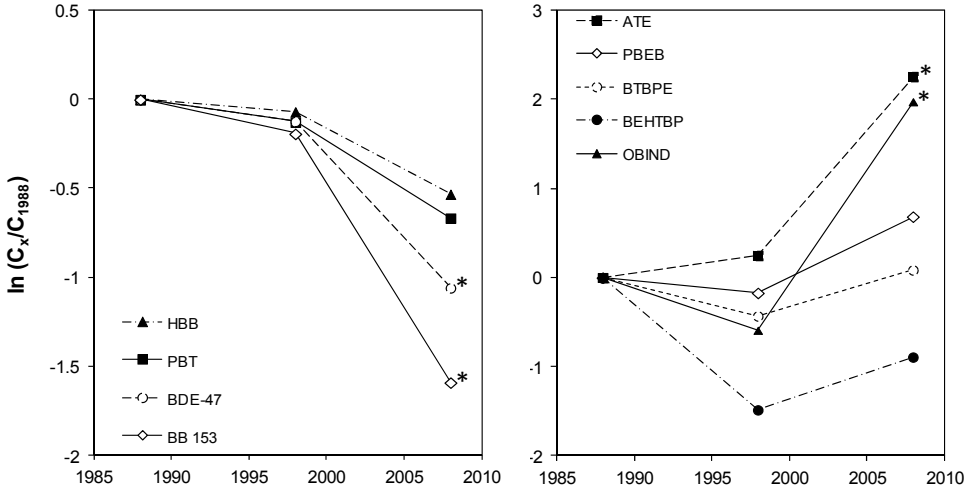


Figure 5- 3. Concentration differences among years of hexabromobenzene (HBB), pentabromotoluene (PBT), 2,2',4,4'-tetrabromodiphenyl ether (BDE-47), 2,2',4,4',5,5'-hexabromobiphenyl (BB-153), bis(2-ethylhexyl)tetrabromophthalate (TBPH), 1,2-bis(2,4,6-tribromophenoxy)ethane (BTBPE), allyl 2,4,6-tribromophenyl ether (ATE), pentabromoethylbenzene (PBEB), and 4,5,6,7-tetrabromo-1,1,3-trimethyl-3-(2,3,4,5-tetrabromophenyl)-indane (OBIND) in American eel captured in Lake Ontario (N =10/yr). Values are presented normalized to the natural logarithm of the 1988 mean concentration. Statistically significant differences ($p<0.05$) between 1988 and 2008 are denoted with an asterisk (*).

Table 5- 5. Mean ± standard deviation concentrations of brominated flame retardants (pg/g lw) in American eel (1988-2008) both unadjusted and $\delta^{15}N$ adjusted (N =10/yr).

	Unadjusted			$\delta^{15}N$ Adjusted		
Year	**1988**	**1998**	**2008**	**1988**	**1998**	**2008**
BDE-47[+]	314000±61000[a]	278000±111000[a]	109000±91000[b]	275000±94000[a]	212000±106000[a,b]	165000±111000[b]
BB-153	7160±2390[a]	5910±2000[a]	1460±769[b]	6090±2780[a]	4990±3990[a,b]	2370±1050 [b]
ATE	23.6±7.87[a]	30.3±28.3[a]	226±223[b]	33±20.6[a]	60.8±66.1[a,b]	126±106[b]
PBT	1.77±1.8	1.56±1.47	0.91±1.09	1.78±1.8	1.59±1.48	0.85±1
PBEB	3.17±3.71	2.67±2.74	6.29±9.87	3.69±4.59	4.1±4.82	3.77±4.77
HBB	6.33±5.71	5.91±8.37	3.72±4.06	6.94±6.4	6.34±8.57	2.91±3.24
TBPH	62.7±62.7[a]	14.2±19.4[b]	25.7±26.3[a,b]	65±65.3[a]	14.8±19.9[b]	24.1±24.7[b]
BTBPE	80.9±86.4	52.6±48.4	88.2±71.4	83.9±82.7	64.1±58	64±52
OBIND	3.63±3.1[a]	2.01±2.26[a]	26.2±35[b]	4.89±4.45	3.54±3.55	13.1±15.5

[+]Data from [8, 9]. Means sharing the same superscript letter (a or b) are not significantly different (ANOVA, $p > 0.05$).

A study by Ismail et al. [12] on the same lake trout from Lake Ontario as Shen et al. [13], reported that concentrations of BTBPE ranged from 620 to 2600 pg/g lw between 1979 and 2004, up to 20-fold higher than in eels. Concentrations peaked in the mid-1990s, before leveling off recently in lake trout, whereas there was no overall trend in eels. Concentrations of PBEB ranged from 17000 to 320000 pg/g lw, up to five orders of magnitude higher in concentration than in eels. Zhu et al. [22] measured concentrations of BB-153 from 1980 to 2000 in lake trout from Lake Ontario and reported values between 4620 and 92400 pg/g lw, peaking in the early 1980s and then steadily declining overtime. For the years that were comparable between the Zhu et al. and the present study, concentration ranges were slightly but significantly lower in eels.

5.3.3 Possible influence of trophic position changes on temporal trends

As predators consume their prey, ^{15}N is transferred up the food chain. In general, there is an enrichment of about +3.0‰ per trophic transfer [23]. The $\delta^{15}N$ value in eels has changed by about half this value (Figure 5-4), indicating that they have not changed an entire trophic level, but the source of nitrogen in their diet has changed significantly. Trophic position can be determined by measuring the degree of ^{15}N enrichment in an organism with respect to the base organism of the food chain [24]. Hebert and Weseloh [11] have shown that adjusting for changes in trophic position can lessen rates of chemical decline in biota. Ismail et al. [12] indicated that changes to the Lake Ontario food web have had an impact on the trophic position of lake trout, and consequently on the interpretation of chemical temporal trends. For example, if an organism changes to a lower trophic position, their contaminant exposure through diet could increase if the contaminant does not biomagnify but is bioavailable, and vica versa. The point being that a change in contaminant concentration does not necessarily reflect a change in environmental loadings. To examine if the shift in trophic position of eels affects the interpretation of our temporal data, $\delta^{15}N$ adjust concentrations were calculated [11] using the $\delta^{15}N$ measurements from

the same eels [9]. Figure 5-4 shows examples where adjusting for $\delta^{15}N$ reduced the concentration differences among years in eels, e.g. Dec604, BDE-47, and OBIND no longer had statistically different concentrations among years using the $\delta^{15}N$ adjusted data. The only instance where unadjusted and $\delta^{15}N$ adjusted concentrations were statistically different for an individual contaminant was for BB-153 in 2008. Accounting for trophic position, concentrations of only five HFRs, Mirex, Dec602, BB-153, ATE, and TBPH changed significantly between 1988 and 2008 in eels. Complete listings of the unadjusted and $\delta^{15}N$ adjusted concentrations are shown in Tables 5-3 and 5-5.

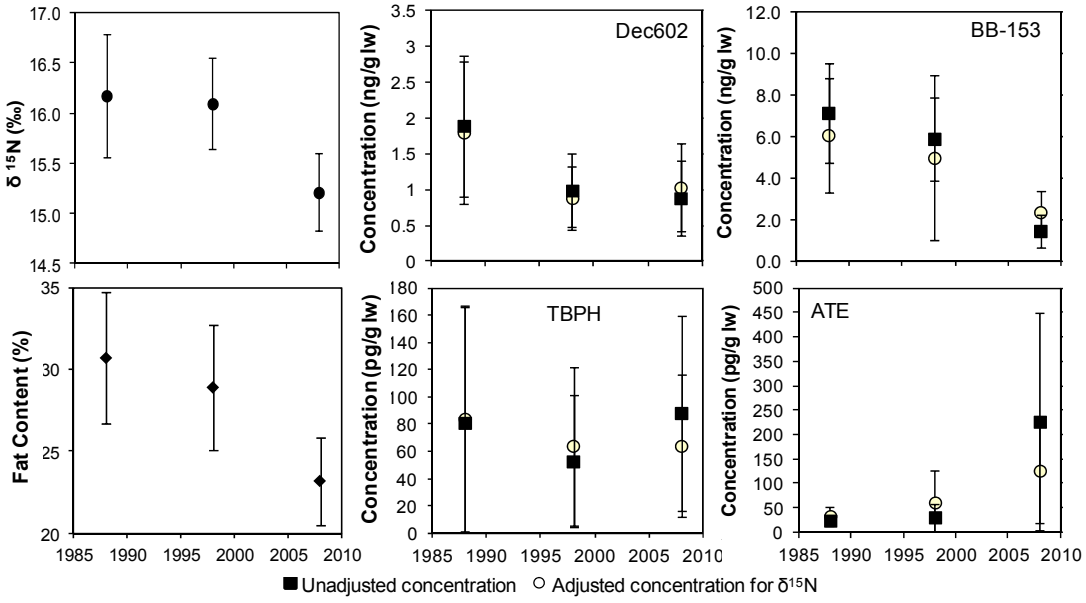


Figure 5- 4. Differences among years of $\delta^{15}N$ and percent fat content, in addition to actual, unadjusted and $\delta^{15}N$ adjusted concentrations of Dechlorane 602, allyl 2,4,6-tribromophenyl ether (ATE), 2,2',4,4',5,5'-hexabromobiphenyl (BB-153), and bis(2-ethylhexyl)tetrabromophthalate (TBPH) in American eel collected in Lake Ontario (N =10/yr). Data are shown as mean with standard deviation.

5.3.4 Bioaccumulation of halogenated flame retardants.

Eels have been used in the past as biomonitoring tools for pollution fingerprinting, and are an indicator of the health of an aquatic ecosystem [25, 26]. In Canada, a chemical with an octanol-water partition coefficient (log K_{ow}) ≥ 5, and a bioconcentration factor (log BCF) or a bioaccumulation factor (log BAF) ≥ 3.7 is considered to be bioaccumulative [27]. Using the Arnot-Gobas model, chemicals with a log K_{ow} between 3.7 and 8.2 are potentially bioaccumulative under the Stockholm Convention criteria [27]. Table 5-6 provides a summary of a variety of factors related to bioaccumulation in fish, obtained using the U.S. Environmental Protection Agency's EPI Suite [28]. The only CFRs that met the Arnot-Gobas model log K_{ow} criteria were Mirex (6.89), DPMA (7.16), and Dec602 (8.05). Correspondingly, Mirex and Dec602 were by far the most abundant CFRs in eels; DPMA was not nearly as abundant, most likely because it was not produced intentionally for commercial use, but instead was a byproduct of DP production [2, 16]. Only CFRs that had a predicted log BAF and log BCF > 3.7 appeared to bioaccumulate.

Similar to DP, TBPH and OBIND are super-hydrophobic chemicals (log K_{ow} > 11; WS < 10^{-6} mg/L), and should not bioaccumulate, as inferred by their concentrations in eels. Most of the other emerging BFRs have log K_{ow}s in the 4 to 9 range, which is important for bioaccumulation potential. However, only two of the emerging BFRs meet any other criteria: BTBPE has a log BAF > 3.7, and ATE exceeded all of the bioaccumulation criteria (K_{ow}, BCF, and BAF).

Based on the estimated values in Table 5-6, and the concentrations determined in eels, Dec602 and ATE were the only two emerging flame retardant chemicals measured in this study that represented a genuine cause for concern. In addition to their potential to bioaccumulate, they both have the potential to undergo long-range atmospheric transport. Dec602 has a small Henry's law constant (2.66 x 10^{-9} atm-m^3/mol), but has been measured in remote regions because it is transported in the atmospheric bound to the particulate phase [29].

Table 5- 6. Chemical properties related to the bioaccumulation of chlorinated and brominated flame retardants predicted (experimental) using EPI Suite [28].

Name	log K_{ow}	WS (mg/L)	Henry's Law (atm-m^3/mol)	log BCF		log BAF‡
Mirex	7.01 (6.89)	4.76E-4 (0.085)	1.3E-6 (8.1E-4)	4.21† (4.31)	3.76‡	6.19
DPMA	7.16	3.1E-3	1.17E-3	4.05	3.80	6.7
CP	9.8	5.79E-7	3.18E-6	2.75	2.11	5.81
Dec602	8.05	1.75E-5	2.66E-9	3.61	3.48	7.04
Dec603	11.2	2.45E-8	2.47E-6	2.06	0.79	4.43
Dec604	10.56	3.75E-8	1.12E-6	2.38	1.26	4.91
syn-DP	11.27	1.7E-8 (4.4E-8)	7.44E-6	2.03	0.73	4.36
anti-DP	11.27	1.7E-8 (4.4E-8)	7.44E-6	2.03	0.73	4.36
BDE-47	6.77	1.46E-3	2.97E-6	4.13	3.70	5.83
BB-153	9.10	1.80E-6	1.65E-6	3.68	2.47	6.05
ATE	5.59	7.79E-2	2.65E-5	3.36	3.89	4.89
PBT	6.99	9.35E-4	5.97E-5	4.28 (2.43)	2.01	2.10
PBEB	7.48	2.90E-4	7.92E-5	3.89 (2.52)	1.78	2.08
HBB	7.33 (6.07)	2.2E-3 (1.6E-4)	2.15E-5	3.67 (3.00)	2.95	3.10
TBPH	11.95	1.98E-9	2.98E-7	1.10	-0.041	0.38
BTBPE	9.15	6.55E-7 (0.2)	7.32E-9	3.07	2.69	6.37
OBIND	13.03	1.96E-11	4.60E-7	1.17	-0.021	2.45

†Regression-based estimate, ‡Arnot-Gobas upper trophic model estimate, and Henry's law constant estimated using Bond method options in EPI Suite [28]. WS = water solubility.

ATE has a predicted Henry's law constant of 2.65 x 10^{-5} atm-m^3/mol, which makes it susceptible to long-range transport; it is also a large production volume chemical in Europe, and it has been identified as a potential Arctic contaminant [20]. These characteristics satisfy two of the three requirements under the Stockholm Convention to include a chemical on the globally-

restricted chemicals list (*persistence*, *bioaccumulation*, and toxicity). Limited information is available regarding the toxicity of ATE and Dec602.

The other compound very close to meeting the bioaccumulation criteria was BTBPE, especially when considering that the values in Table 5-6 were calculated, and not experimentally derived. The predicted log K_{ow} for BTBPE was similar to BB-153, and it had the highest log BAF of any of the BFRs. It may also be susceptible to long-range transport like Dec602, although, concentrations of BTBPE were low by comparison, and did not vary significantly among years.

5.4 Conclusion

Concentrations of emerging HFRs were much less than those of legacy contaminants in eels, and were not of concern for the most part. Most of the HFRs measured in this study had decreased or had not changed in concentration between 1988 and 2008, with the exception of ATE and OBIND, which increased significantly. Adjusting concentrations for trophic position minimized the rates of change for all compounds studied, but was statistically significant for only a few HFRs. The only emerging HFRs to have characteristics contributing to bioaccumulation were BTBPE, ATE, and Dec602, with the latter two compounds being the most abundant emerging HFRs in 2008; a food web study of these compounds is an important research need, and would be mandatory to support the bioaccumulation and amplification of these compounds. Toxicity studies are also needed to assess if these chemicals should be added to the globally restricted chemicals list, because they both meet the persistence and bioaccumulation criteria.

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Chapter 6

Qualitative analysis of halogenated organic contaminants in American eel by gas chromatography/time-of-flight mass spectrometry

Abstract

Target compound analysis with scanning mass spectrometers such as quadrupole or magnetic sector instruments is used extensively in the environmental field because of the selectivity, sensitivity, and robustness of these detectors. Yet, target compound analysis selectively ignores the majority of compounds present in a sample, especially in complex matrices like fish. In this study, time-of-flight mass spectrometry was used to screen for halogenated compounds in American eels (*Anguilla rostrata*) that had been analysed previously using targeted methods for a variety of halogenated persistent organic pollutants. Individual and then pooled eel samples were analysed using electron ionization and electron capture negative ionization modes with mass range (45-800 Da). Eels were differentiated by their chemical profile using principal component analysis, and were grouped corresponding to their capture location, all with a single instrument injection per sample. The selectivity of electron capture negative ionization was used to screen pooled eel samples for bromine containing compounds by utilizing the Br^- ion m/z 79 and 81. A total of 51 brominated compounds were detected in the samples, including nine that were not polybrominated diphenyl ethers; these were identified using authentic standards, library searching, and/or chemical formula prediction based on accurate mass measurements. The majority of the 'new' brominated compounds identified were bromophenols, bromoanisoles, and bromobenzenes. These classes of compounds are synthesized for use in flame retardant production either as intermediates or as final products. However, their occurrence in eels was most likely the result of metabolism or break-down products of high production volume flame retardants like polybrominated diphenyl ethers and bromophenoxy compounds.

6.1 Introduction

American eel (*Anguilla rostrata*) are a catadromous, semelparous species that spawn in the Sargasso Sea. Offspring migrate up the Atlantic coast of North America and into continental waters where they often reside in less than pristine environments. In Canada, eels are native to rivers and lakes of Ontario, Quebec, and all of the Atlantic Provinces. American eel populations have undergone severe declines over the past three decades, and their status has been recently designated as threatened by the Committee on the Status of Endangered Wildlife in Canada [1]. Eels are a primarily benthic, long lived, and fatty fish, and as such can accumulate lipophilic persistent organic pollutants (POPs). As a result, they are an ideal species to investigate local sources of pollution [2-6].

We have reported concentrations for a variety of targeted halogenated POPs in eels captured at seven locations in eastern Canada previously [5, 6], in an attempt to determine if POPs reduced spawner quality in eels migrating from this region. We found that concentrations were generally less than established guidelines for most legacy compounds, except for in highly urbanized and industrialized regions. Furthermore, concentrations of most chlorinated POPs have decreased significantly in the environment since their peak in the 1960-70s [7-9], and similarly, so have concentrations of polybrominated biphenyls and diphenyl ethers in recent years (Chapter 2; [10, 11]). This trend is most likely due to stricter environmental regulation, e.g., the Stockholm Convention on POPs [12]. But if concentrations of legacy POPs are declining in eels and, thus far, eel populations are not recovering, then either legacy POPs accumulation is not related to declining eel populations or perhaps other non-targeted compounds are to blame. In the present study, we investigate the latter question.

Target analysis is excellent for providing sensitive and selective results for known compounds. Instruments that use chromatographic separation coupled to mass spectrometry (MS), such as gas chromatography-quadrupole MS (GC-MS) or high-resolution magnetic sector MS (GC-HRMS) and liquid chromatography-triple quadrupole MS (LC-MS/MS), are popular choices for target analysis of environmental samples. However, by design, target analysis filters out all of the other ions not corresponding to the target analytes, making it obviously inappropriate for new compound identification. Time-of-flight mass spectrometers (ToF-MS) have gained popularity over scanning instruments for screening applications (non-target and post-target analysis) because full mass range spectra are acquired, and high acquisition rates can be achieved (up to 1,000 Hz) with minimal mass bias [13, 14]. This provides a number of advantages including the possibility of deconvolving chromatographic interferences using modern software, which enhances the ability to isolate and identify a greater number of compounds.

In the present study, two main research questions were addressed: Can we achieve a similar spatial resolution using qualitative comprehensive analysis by GC-ToF-MS to what we reported previously using target analysis? Are there any other halogenated organic contaminants of consequence in eels? The first question was investigated using principal component analysis of the acquired eel samples to differentiate sample location based on chemical profile. The second question was more involved, and was answered via non-target analysis using peak identification and mass spectral characterization, and post-target analysis by accurate mass searching for lipophilic brominated compounds known to be in commerce.

Experimental

Sample collection

A total of 60 eels were collected from seven locations in eastern Canada from the Bay of Quinte, Lake Ontario to the Margaree River, Nova Scotia in 2007 and 2008. Sampling specifics were detailed elsewhere [5]. Whole fish homogenates were prepared according to the standard laboratory practices outlined by Kiriluk et al. [15], less 10% of the muscle for other analysis, the liver, and sagittal otoliths. Homogenates were stored at -80°C in Environment Canada's National Aquatic Biological Specimen Bank and Database until chemical extraction.

Table 6- 1. Summary of American eel sample collection in eastern Canada.

Capture location	**Number**	**Coordinates**	
Margaree River, NS	10	46°25.20'N	61°05.27'E
Miramichi River, NB	10	47°02.44'N	65°27.04'E
Rivière du Sud-Ouest, QC	4	48°20.40'N	68°46.27'E
St. Lawrence estuary, Kamouraska, QC	5	47°34.00'N	69°51.58'E
St. Lawrence estuary, Rivière Ouelle, QC	11	47°25.48'N	70°01.11'E
St. Lawrence River, Thousand Island, ON	10	44°26.98'N	75°49.24'E
Prince Edward Bay, Lake Ontario, ON	10	43°57.01'N	76°58.01'E

Extraction and fractionation procedure

About 20 g of whole fish homogenate for each fish was dried chemically with anhydrous sodium sulfate, spiked with $^{13}C_{12}$-2,2',3,3',4,4',5-heptachlorobiphenyl (CB-170) and 2,3,7,8-[$^{37}Cl_4$]-tetrachlorodibenzo-p-dioxin, and extracted with dichloromethane. About 2 g wet weight equivalent (ww eq) was used for gravimetric lipid determination, 5 g ww eq was used for legacy contaminant analysis [5], another 5 g ww eq was used for dioxin-like compounds analysis [6],

leaving about 8 g ww eq for other analyses. For the screening of emerging halogenated hydrophobic contaminants, about 40% (3.2 g ww eq) of the back-up fraction underwent gel permeation chromatography for the removal of bulk lipids. No other clean-up was done on these fractions before they were concentrated to a 100µL final volume. Individual samples were considered first then pooled by capture location for instrumental analysis.

Instrumental analysis

Sample extracts were introduced into a GC/ToF-MS using a CTC Combi Pal autosampler through a Gerstel CIS-4 PTV injection port (Linthicum, MD). Gas chromatographic separation was performed using an Agilent 7890 GC equipped with a 30m Restek Rxi-XLB column, 0.25mm i.d., 0.25µm film thickness (Bellefonte, PA). Oven temperature program was: initial temperature 100^{o}C, held for 2 min, raised to 325^{o}C at 10^{o}C/min and held for 19.5 min with a transfer line temperature of 280°C. The accurate mass GC/ToF (Waters GCT Premier) was operated in both electron capture-negative ion (ECNI) and electron ionization (EI) modes with an electron energy of 70eV. For ECNI, a rhodium filament was used with a source temperature of 150°C, and for EI, a tungsten filament was selected with a source temperature of 200°C for library searching. Mass spectra were acquired between m/z = 45 and 800 Da, with a resolution > 7000 at FWHM using continuum and dynamic range enhancement (centroid). The acquisition rate was 0.30 s per scan with an interscan delay time of 0.05 s, which enabled about 7-10 data points per chromatographic peak to be acquired.

Data processing

Mass spectral data were accurate mass corrected using perfluorotributylamine (PFTBA) ions as references. The processing software used was MassLynx 4.1, which included ChromaLynx and MarkerLynx. The ChromaLynx software was used to identify unique peaks in the acquired data based on their m/z and retention time, and where possible the NIST AMDIS_32 software was used for deconvolution. MarkerLynx facilitated the principal component analysis of data based on unique retention time and exact mass features called markers, which were used to differentiate samples. Markers were defined over the mass range m/z = 45-800 Da and chromatographic run time from 7 to 45 min, with a minimum peak height more than 10% of the base peak. Markers that were identified in the loadings plot to have a significant degree of variance from the origin, and thus, contributed to the differentiation among samples, were subsequently subjected to compound identification.

Method for compound identification

The retention time and exact mass information for the differentiated markers from MarkerLynx were used to populate a list of potential compounds for identification. Identities of these compounds were first compared against a list of previously targeted contaminants. Accurate mass data of the remaining unknown compounds were used to calculate possibly elemental compositions, which were reduced using isotope pattern, fragmentation pattern, and double bond equivalence. The <strip> scripting function in MassLynx helped to identify halogenated clusters in the chromatograms. EI spectra were searched against the NIST 08 library. Additionally, due to the fact that the ECNI spectrum of brominated compounds contain a predominate signal at m/z = 79 and 81, corresponding to $[Br]^-$, all of the brominated compounds were recognized in the chromatogram. Tentatively identified compounds were compared to authentic standards when possible (Figure 6-1).

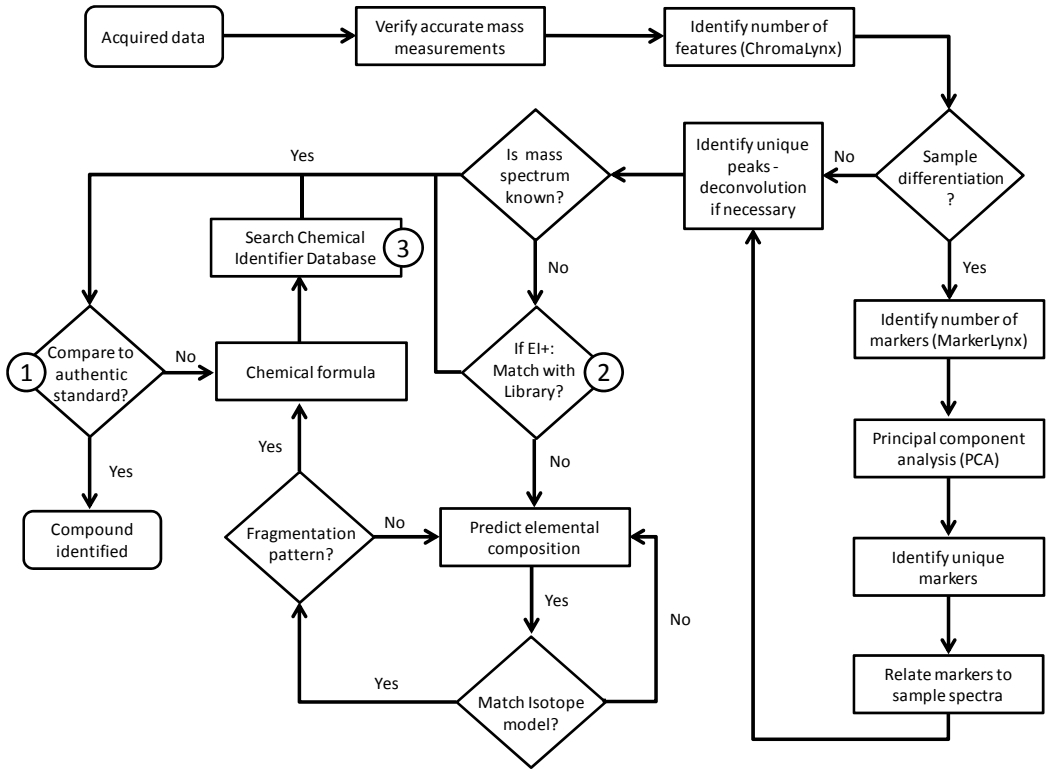


Figure 6- 1. Flowchart diagram of the compound identification methodology. The circled numbers 1-3 indicate the level of confidence for the identification, with 1 being most confident and 3 least confident.

6.2 Results and discussion

Mass spectra were collected for each eel sample in EI^+ and ECNI mode, accurate mass corrected using PFTBA. The EI^+ features identified were library searched using NIST 08. The majority of the features were naturally occurring organic molecules in animals like sterols, proteins, and fatty acids. Background chemical noise, which included many siloxane compounds, was about 8-fold higher for the EI^+ data than ECNI, limiting its utility for screening (Figure 6-2). ECNI is a much more selective and sensitive technique than EI, especially for halogenated compounds. Therefore, ECNI data were relied upon to identify halogenated compounds in the eel samples.

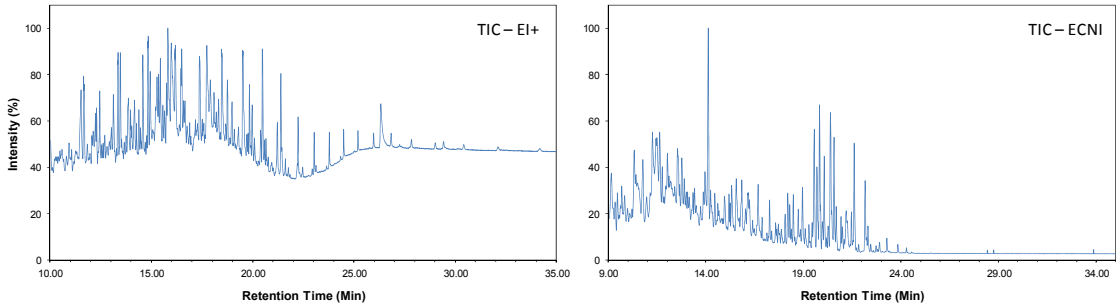


Figure 6- 2. Summed total ion chromatogram for pooled eel samples collected in EI^+ and ECNI. ChromaLynx Identify found 1218 features in EI^+ mode and 855 features in ECNI mode.

Mass spectral data for individual eel samples were collected in ECNI mode and input into MarkerLynx to investigate if eels may be differentiated based on their chemical profile from a single injection per sample. Figure 6-3 shows the principal component analysis results using normalized, mean-centred, and Pareto-scaled data for PC 1 vs PC 2 and PC 1 vs PC 3. These principal components were the most significant, accounting for more than 41% of the variance in the data.

The data show that the chemical profile of eels varied depending on capture location. The scores plots of PC 1 vs PC 2 indicate six differentiated locations with Lake Ontario and Rivière du Sud-Ouest being statistically similar. The scores plots of PC 1 vs PC 3 also indicate six differentiated capture locations with Rivière du Sud-Ouest and Margaree River being statistically similar. When considering PC 1 to 3 in 3-Dimensional space (not shown) all of the locations were significantly different from each other. Sixty-two features in the loadings plots had significance values $p > 0.05$. Most of the features were identified as pesticides, polychlorinated biphenyls, and other known POPs. The data suggest that legacy compounds still dominate the contaminants profile of eels from eastern Canada. This method was successful at differentiating eel samples by their chemical profile, although, it did not help to identify any unknown compounds.

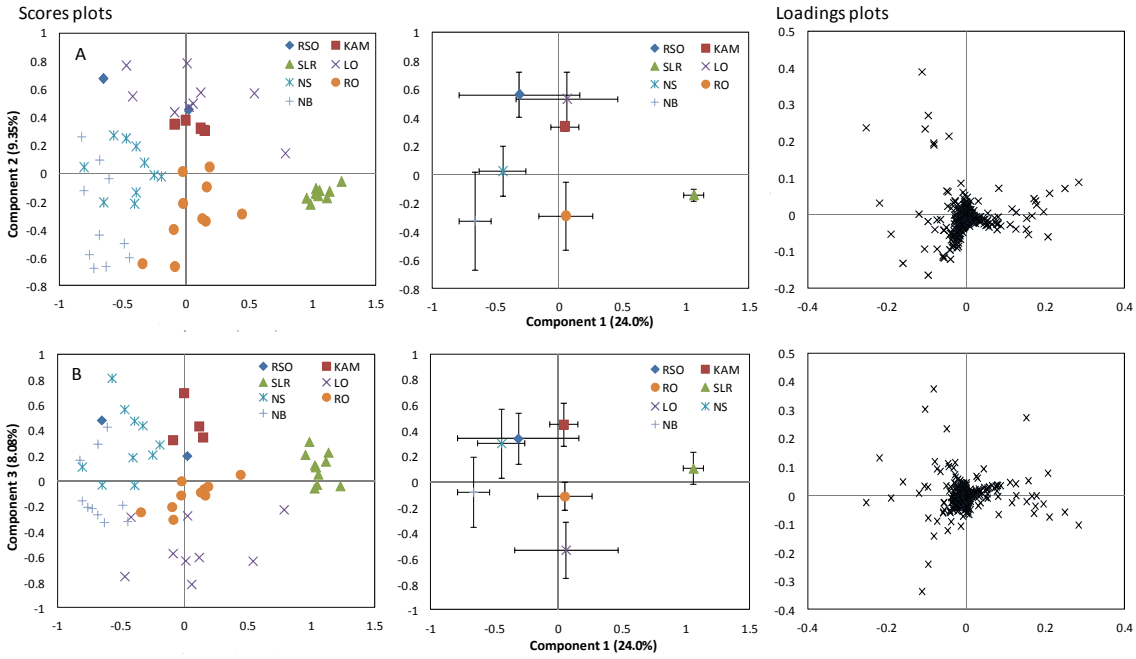


Figure 6- 3. Normalised, mean-centred principal component analysis from MarkerLynx using Pareto-scaled ECNI data; 1141 markers were identified with more than 500 ion counts. (A) PC 1 vs PC 2; (B) PC 1 vs PC 3.

Time-of-flight MS of complex samples like eels generates a large amount of data that requires extensive data processing. The lack of direct deconvolution in MassLynx made it extremely difficult to identify unique peaks in chromatograms. ChromaLynx and MarkerLynx are tools designed to help with data processing, but scripting and chemical intelligence are also useful. As a method of data filtering, we decided to use the predominant signal at m/z=79 and 81 corresponding to Br^- in the ECNI spectrum to identify all of the brominated compounds in the chromatograms. Initially, we investigated the differences in organobromine compounds among all of the sampling sites using PCA analysis of pooled eel samples. All peaks containing m/z 79 and 81 were integrated and submitted to MarkerLynx for PCA analysis (Figure 6-4). A total of 50 markers were identified with 19 markers having a $p > 0.05$. Visual inspection of the significant

markers revealed that there was one co-elution, yielding a total of 20 unique features in the chromatograms. Retention times of the significant bromine containing compounds with their predicted chemical formula and proposed identification are shown in Figure 6-5.

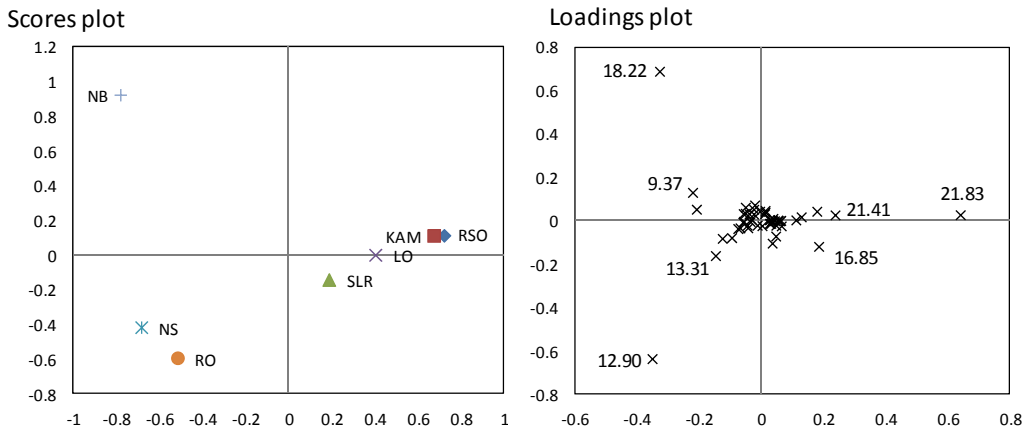
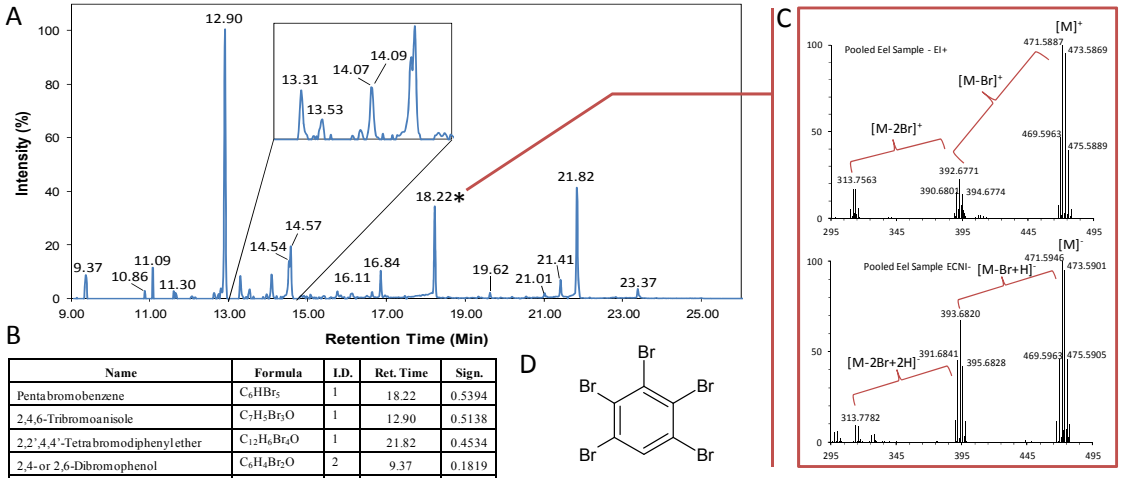


Figure 6- 4. Normalised, mean-centred principal component analysis from MarkerLynx using Pareto-scaled ECNI data of pooled eel samples.

The data used to generate Figure 6-4 are presented in Table 6-2. The loadings indicate how the chemical profiles for the brominated compounds defer by sampling location. Interestingly, the two most significant peaks (R.T. = 18.22 and 12.9 min), both with p-values > 0.5, were not measured previously in these eel samples by targeted methods. The chromatographic peak at 18.22 min, identified as pentabromobenzene (PBBz), was present in eels from the Miramichi River, NB, at significantly higher levels than all other locations. PBBz is a suspected metabolite or break-down product of many fully brominated aryl rings, e.g., PBBz is the primary metabolite of hexabromobenzene in rats [16]. The peak at 12.90 min corresponded to 2,4,6-tribromoanisole, which is produced by O-methylation of 2,4,6-tribromophenol (TBP). The production and use of TBP is primarily as a fungicide, as a wood preservative, and as a reactive

intermediate in flame retardant production like tetrabromobisphenol-A. TBP and other bromophenols may also occur as break-down products of PBDEs [17].



Name	Formula	I.D.	Ret. Time	Sign.
Pentabromobenzene	C_6HBr_5	1	18.22	0.5394
2,4,6-Tribromoanisole	$C_7H_5Br_3O$	1	12.90	0.5138
2,2',4,4'-Tetrabromodiphenylether	$C_{12}H_6Br_4O$	1	21.82	0.4534
2,4-or 2,6-Dibromophenol	$C_6H_4Br_2O$	2	9.37	0.1819
2,2',4,5'-Tetrabromodiphenylether	$C_{12}H_6Br_4O$	1	21.41	0.1689
		4	16.84	0.155
2,4,6-Tribromophenol	$C_6H_3Br_3O$	2	13.31	0.1544
2,4-or 2,6-Dibromoanisole	$C_7H_6Br_2O$	2	11.09	0.1526
2,2',4,4',6-Pentabromodiphenylether	$C_{12}H_5Br_5O$	1	23.37	0.13
		4	11.30	0.1061
2,2',4-Tribromodiphenylether	$C_{12}H_7Br_3O$	1	19.62	0.0917
		4	10.86	0.0874
Tetrabromodiphenylether	$C_{12}H_6Br_4O$	3	21.01	0.0785
2,4,6-Tribromoaniline	$C_6H_4NBr_3$	3	14.09	0.0772
2,4,6-Tribromotoluene	$C_7H_5Br_3$	3	14.07	
		4	16.11	0.0598
Allyl 2,4,6-Tribromophenylether	$C_9H_7Br_3O$	1	14.54	0.0593
	$C_9H_8Br_2O$	3‡	14.57	0.0572
		4	13.53	0.0541
		4	16.98	0.0535

Pentabromobenzene EI+

Mass	Calc. Mass	PPM	mDa	Formula
469.5963	469.5975	-2.6	-1.2	C6HBr5
471.5887	471.5954	-14.2	-6.7	
473.5869	473.5934	-13.7	-6.5	
475.5889	475.5914	-5.3	-2.5	
390.6801	390.6791	2.6	1.0	C6HBr4
392.6771	392.6771	0.0	0.0	
394.6774	394.6750	6.1	2.4	
311.7585	311.7608	-7.4	-2.3	C6HBr3
313.7563	313.7588	-8.0	-2.5	

Pentabromobenzene ECNI-

Mass	Calc. Mass	PPM	mDa	Formula
469.5963	469.5975	-2.6	-1.2	C6HBr5
471.5946	471.5954	-1.7	-0.8	
473.5901	473.5934	-7.0	-3.3	
475.5905	475.5914	-1.9	-0.9	
391.6841	391.6870	-7.4	-2.9	C6H2Br4
393.6820	393.6849	-7.4	-2.9	
395.6828	395.6829	-2.5	-0.1	
313.7782	313.7764	5.7	1.8	C6H3Br3
315.7730	315.7744	-3.2	-1.0	

Figure 6- 5. (A) Mathematically combined extracted ion chromatogram in ECNI mode (78.92±0.05 Da) of all seven pooled eel samples. (B) Brominated compounds with significant contribution ($p > 0.05$) to PCA discrimination in Figure 6-4. (C) Mass spectrum of pentabromobenzene in EI^+ and ECNI modes. (D) Structure of pentabromobenzene and mass accuracy measurements resulting from accurate mass correct mass spectral data. Identification followed the method outlined in Figure 6-1. Formula with an I.D. = 1 were confirmed with authentic standards; I.D. = 2 were predicted based on accurate mass measurements and similarity matching using NIST 08 library searching; I.D. = 3 were assigned a chemical formula based on exact mass measurements, but the mass spectrum was not in the NIST library; I.D. = 4 were identified as bromine containing, but no other chemical information was discernible.

The list of identified brominated compounds in Figure 6-5B may be classified into four groups: bromobenzenes, bromophenols, bromoanisoles, and diphenyl ethers. Polybrominated diphenyl ethers are well studied and have been reported in eels in the present study elsewhere [5]. Bromophenols occur naturally in marine environments but are not known to be biosynthesized in fresh waters [17], so their occurrence in eels captured in freshwater is most likely the result of anthropogenic activity. The bromobenzenes and bromophenoxys identified are also synthetic compounds. Interestingly, all of the non-PBDE brominated compounds identified in this study, except for 2,4,6-tribromoaniline, are potential metabolites or break-down products of replacement BFRs in commerce or BFRs that have been identified as potential replacements. Many of these BFRs are shown in Chapter 1 (Table 1-1); e.g., bromophenols can be formed from bromophenoxy compounds like allyl 2,4,6-tribromophenylether, 2-bromoallyl 2,4,6-tribromophenyl ether, and 1,2-bis(2,4,6-tribromophenoxy)ethane. Furthermore, 1,2-bis(2,4,6-tribromophenoxy)ethane has been suggested to replace octa-BDE [18]; Firemaster 550 and BZ-54 contain 2-ethylhexyl-2,3,4,5-tetrabromobenzoate and bis(2-ethyl-1-hexyl)tetrabromophthalate and have been suggested to replace penta-BDE [19]; and decabromodiphenyl ethane is expected to replace dec-BDE [20]. The occurrence of these potential break-down products in eels is perhaps an indication that many of the suggested replacements are already prevalent in the environment. The break-down products occurred at much higher intensities than the above precursors in eels, which further supports our previous findings that eels are effective at metabolizing brominated compounds [5] like other species of fish [21-23].

Table 6- 2. Normalised peak height for the 20 significant markers using m/z 78.92±0.05 Da.

	Ret. Time	LO	SLR	RO	KAM	RSO	NS	NB
Pentabromobenzene	18.22	0	42	0	18	0	0	4264
2,4,6-Tribromoanisole	12.90	2751	3242	6329	2170	2396	4900	2306
2,2',4,4'-TetraBDE	21.82	2762	1848	811	3893	4600	125	0
Dibromophenol	9.37	0	0	129	0	0	523	656
2,2',4,5'-TetraBDE	21.41	469	372	0	586	421	0	0
Unknown	16.84	477	756	361	784	358	224	3
2,4,6-Tribromophenol	13.31	195	282	280	97	0	723	159
Dibromoanisole	11.09	136	120	468	150	130	459	609
2,2',4,4',6-PentaBDE	23.37	219	0	0	307	433	0	0
Unknown	11.30	0	0	0	0	0	600	82
2,2',4-TriBDE	19.62	188	83	0	220	99	0	0
Unknown	10.86	0	0	0	0	0	392	31
TetraBDE	21.01	166	133	0	112	95	0	0
2,4,6-Tribromoaniline	14.09*	435	477	291	213	351	386	199
2,4,6-Tribromotoluene	14.07*	435	477	291	213	351	386	199
Unknown	16.11	108	177	120	88	30	0	0
ATE	14.54	699	735	940	672	462	539	750
Unknown	14.57	0	0	0	0	0	24	56
Unknown	13.53	88	66	101	97	65	203	111
Unknown	16.98	35	39	0	0	0	0	63

* Coeluting features. BDE = bromodiphenyl ether

6.3 Summary

The first objective of this study was to differentiate the eel samples based on their chemical profile with a single injection as opposed to the multiple injections required for targeted analysis. Principal component analysis of individual eel samples was able to differentiate eels using their chemical profile, which corresponded to their capture location. The chemical profile in eels was significantly different for all capture locations when considering the first three principal components.

The second objective was to screen for and identify halogenated contaminants using non-targeted analysis. A total of 51 brominated compounds were detected in these eel samples, eight of which were identified and were previously unknown to occur in these samples. A number of brominated compounds (38 of 51), as inferred by their m/z 79/81 characteristic, were unidentifiable under the method detailed herein. Thus, these samples can be examined further focusing on these unidentified compounds, in addition to all of the other non-halogenated compounds that were largely disregarded in this study.

It is unknown at this time if the non-PBDE brominated compounds discovered in this study exhibit toxic effects in eels. However, this study may be used as a proof-of-concept for the discovery of analytes by non-targeted methods in complex environmental samples like fish. It may also be used to highlight potentially significant contaminants for toxicological testing.

6.4 Acknowledgments

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Chapter 7
Summary and areas of further research

7.1 Conclusions

American eels that spend their continental phase in Canada are more than 95% female, and represent a significant contribution to the fecund American eel population. This thesis demonstrated that American eels accumulate significant concentrations of halogenated persistent organic pollutants (POPs). However, there remains a question as to whether these measured concentrations are sufficient to cause a decrease in spawner quality and inhibit recruitment.

7.1.1 Summary of hypotheses tested

The four null hypotheses for this work were that (1) there are no temporal trends in concentrations of POP in eels or dioxin-like toxicity; (2) there are no differences geographical in concentrations of POPs in adult eels; (3) concentrations of POPs in adult eel do not exceed published thresholds for toxicity to eels or surrogate species; (4) there are no other compounds of concern that accumulate in eels. All four null hypotheses were rejected based on the results presented in this thesis. It was demonstrated that there was a temporal trend in concentration of POPs and dioxin-like toxicity in eels from Lake Ontario, Canada, and that these concentrations did exceed toxicity thresholds historically for surrogate species (European eel and lake trout). Concentrations of POPs varied significantly depending on geographic origin. There were also a number of new compounds measured in American eels that were previously undiscovered.

7.1.2 Legacy persistent organic pollutants

The legacy POPs temporal study (Chapter 2) showed that concentrations of all classes of POPs measured, PCDD/Fs, PCBs, OCPs, and PBDEs, have decreased in eels from historic values, most likely a result of environmental regulations. Dioxin and dioxin-like toxicity was also

measured to have decreased correspondingly. Concentrations of chlorinated POPs in eels exceeded toxicity thresholds for surrogate species historically, so chlorinated POPs may have had an effect on spawner quality in the past. Still, the extent to which eels were affected remains undetermined, as well as the correlation, if any, to recruitment decline.

Spatially, concentrations of legacy POPs in eels were dependent on where eels spent their continental growth phase (Chapters 3 and 4). Eels that resided in highly urbanized and industrialized regions tended to have higher concentrations of contaminants than eels that inhabited less developed regions. For eels migrating from Canada and chemically characterized in the present thesis, the rank order of contamination was L. Ontario > St. Lawrence River > St. Lawrence estuary > Miramichi River, NB > Margaree River, NS > St. Lawrence estuary tributaries.

7.1.3 Emerging persistent organic pollutants

Regulations and environmental improvements have reduced concentrations of organochlorine chemicals in the environment, yet there is little-to-no evidence of eel stock recovery. Geeraerts and Belpaire [1] have recently reviewed the potential for other legacy and emerging contaminants to contribute to the overall decline in European eels. The emergence of these ‘new’ environmental contaminants such as organobrominated and organofluorinated compounds may be linked to the lack of recovery of eels in recent years, since their concentrations have increased since the early 1980s [2-7]. Chapters 5 and 6 highlighted a number of halogenated flame retardants that are emerging in American eels, particularly brominated flame retardants like tribromophenoxy compounds. For many of the emerging brominated environmental contaminants, limited toxicological information is available. Consequently, the effect of these compounds on eel spawning quality is unknown.

7.2 Recommendations for future research

Limitations due to a lack of knowledge about the early life stages of the eel (spawning has yet to be observed in the open ocean), and other factors such as sex determination and genetic variability complicate risk assessment and evaluating the effects of POPs on spawning quality. These knowledge gaps need to be better understood to reduce the number of variables possibly affecting eel recruitment decline.

There is a lack of embryo-toxicity data for eels. Currently embryo-toxicity thresholds must be related to other fish species, and eels may have a different dose response relationship for dioxins and dioxin-like compounds. There is also insufficient detail regarding species-specific toxic equivalency factors for eels. Toxicological similarities between American eels and the surrogate species used (lake trout) have only recently been suggested, and are by no means certain [8]. Because discrepancies could lead to inaccurate assumptions about eel embryo-toxicity, further study in this area is recommended. In the future, when eel-specific thresholds are established, this thesis may be used as a resource for determining the past risk of a number of POPs to eels.

Dioxins and dioxin-like compounds can cause toxic effects at low concentrations (sub ppb), so analytical methodologies must continue to improve. Concurrently, risk assessment approaches will have to be adaptable, since dioxins occur in the environment as mixtures. It may be prudent to perform risk assessment studies using actual environmental mixtures, chemically characterized to reduce confounding factors.

In the future, sediment cores could be analysed from the eel collection areas and related to temporal and spatial concentrations in eels. The determination of BMFs and BSAFs along with eel-specific toxicity testing would provide a more holistic understanding of contaminant accumulation in eels and potential implications on recruitment.

The continued emergence of new chemicals in environmental matrices creates difficulties for risk assessors trying to pinpoint or establish cause-effect relationships. Additive models and mixture approaches for risk assessment need to be developed concurrently since chemical manufacturers continue to market new chemical formulations.

One positive aspect of this thesis is the observed decrease of legacy POP concentrations in American eel from Lake Ontario. For this reason, and assuming that no new loadings of these legacy persistent bioaccumulative compounds occur, the threat of these compounds could be eliminated. Perhaps one day we will be able to predict the recovery of recruitment of American eel.

7.3 References

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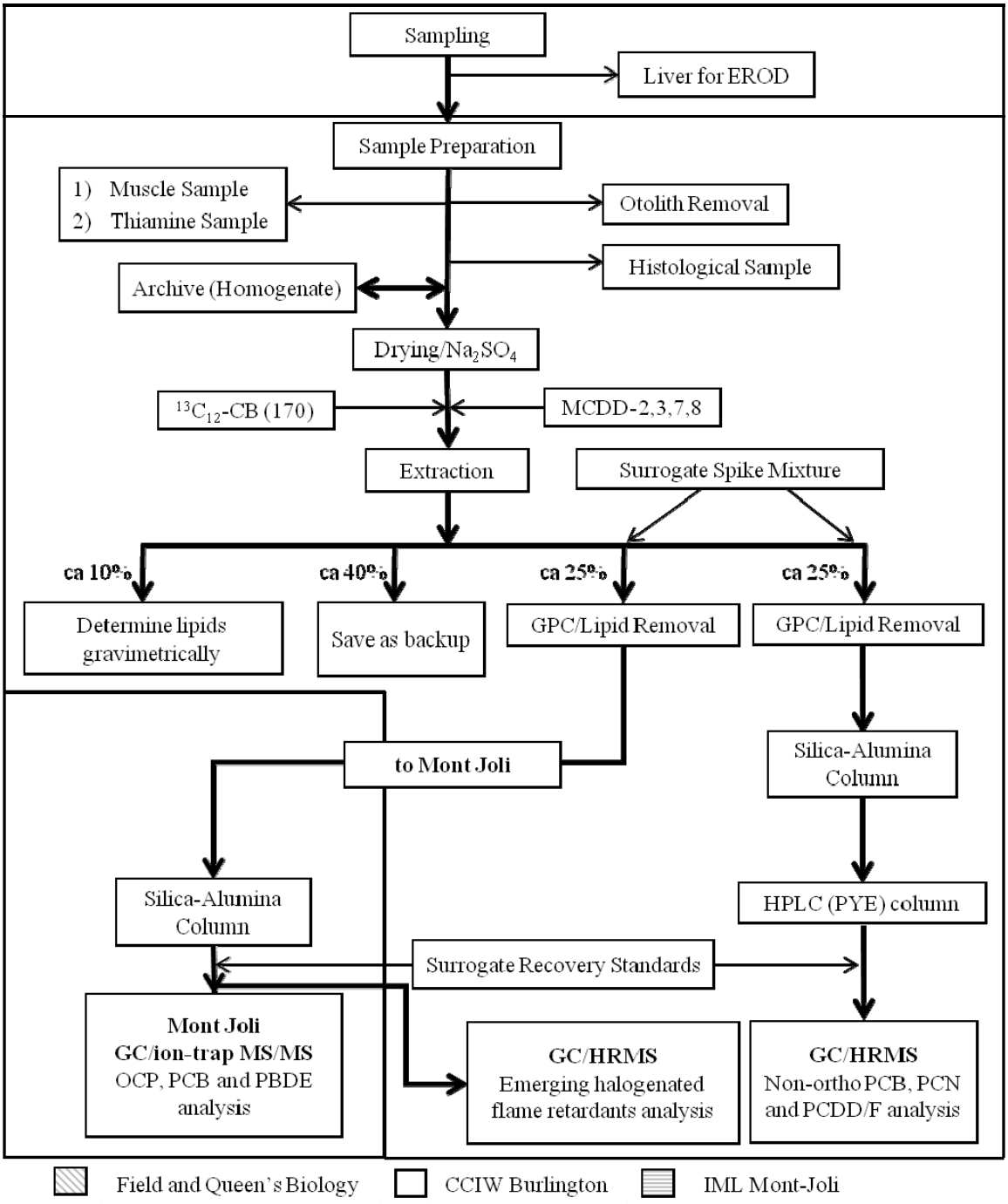
Appendix A
Sample preparation methods for the determination of organohalogenated persistent organic pollutants in eels

A1. Overview

Sample preparation methods for the chemical characterization of eels from sampling to extracts ready for instrumental analysis at Environment Canada in Burlington, Ontario, and the Department of Fisheries and Oceans in Mont Joli, Quebec, are described. A number of classes of persistent organic pollutants were targeted including organochlorine pesticides (OCPs), polybrominated diphenylethers (PBDEs), polychlorinated biphenyls (PCBs), polychlorinated naphthalenes (PCNs), and polychlorinated dibenzo-*p*-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs). Samples were whole eels that were sacrificed then stored frozen in contaminant-free plastic bags until homogenization and subsequent utilization. All whole fish homogenated samples were extracted by grinding with sodium sulphate, packing into glass columns, and eluting with dichloromethane (DCM), prior to fractionation for lipid determination. Bulk lipids were removed using automated gel permeation chromatography (GPC). Additional clean-up was performed using a 2-layered alumina-silica column. Extracts for dioxin-like compounds analysis were fractioned on a 5-pyrenyl silica (PYE) high performance liquid chromatography (HPLC) column. Instrumental analysis was carried out by gas chromatography (GC)-ion trap (IT)-mass spectrometry (MS) or GC- high resolution magnetic-sector MS; however, details are not discussed here because they were mentioned in the experimental sections of Chapters 2-5.

A2. Experimental Workflow



A3. Sampling of Live Eels

When live eels are received in the laboratory, they are transferred immediately to fresh, flowing water in covered tanks suitable for aquaculture purposes in an animal care facility certified by the Canadian Council on Animal Care. Most eels are shipped in a small amount of water in bags inflated with air or oxygen, and chilled with ice. A low water temperature (<10°C) is recommended to reduce stress and oxygen demand. All tanks are aerated, checked daily, and dead eels removed as soon as discovered. A once-daily check of eels is recommended as more frequent checking may cause stress. All lids had a device to lock them securely and all drains had a fine-mesh screen over the outlet to prevent escape.

It is best practice to sample eels as soon as is practical after receipt. Sampling requires that the eels be anesthetised before being killed, preferably by immersion in an accepted fish anaesthetic (Ethyl 3-aminobenzoate methanesulfonic acid (tricaine methane sulfonate or MS-222); or clove oil (mixture of eugenol, isoeugenol and methyl eugenol; please note: govdocs.aquake.org/cgi/reprint/2003/813/8130070.pdf). Eels are extremely difficult to subdue, so patience and high concentrations of anaesthetics are required!

1. Before sampling, prepare a sample sheet (or preferably a note book) that shows the date the eels were received, the date they were sampled, the personnel doing the sampling, and columns and rows that show the unique eel number that accompanies all samples, the total weight and length of the eel, the apparent sex (will be confirmed histologically), the liver weight, any obvious lesions or deformities, and any additional remarks. For each eel the date of transfer of samples to another scientist (e.g. to Burlington for homogenization) should also be recorded.

2. Anaesthetize the eels one at a time, preferably in a closed, dark container to reduce stress. Do not start anaesthesia unless you are certain you will be prepared to sample each one as soon as it is ready.
3. When the eel is limp, weigh it to the nearest gram in a pre-tared clean basin.
4. Lay the eel on clean foil or fresh absorbent paper towel, preferably in a tray, and measure the total length (tip of nose to tip of tail) to the nearest cm.
5. Cut the spine just behind the opercula using a clean sharp knife or scissors – this may require considerable force, and there will be considerable blood loss.
6. Open the eel's peritoneal cavity from the anus to the base of the jaw with either a fresh scalpel or freshly cleaned scissors.
7. Identify the liver (near the head, brown-red coloured, large egg-shaped and about 2-5% of body mass), and lift up the distal end to find the gall bladder
8. Using a clean hemostat, clamp off the neck of the gall bladder so that no bile can escape, and tease or cut the gall bladder away from the liver. Put the gall bladder whole in a cryovial, or use a syringe to sub-sample bile for storage in a cryovial. Cryovials should be labeled with a unique fish number (below) and frozen at -20°C or lower.
9. Remove the liver and weigh in a pre-tared plastic weigh boat.
10. Using clean scissors, chop the liver into small 5 mm x 5 mm cubes, mix, and fill a 1.0 or 2.0 mL labelled cryovial with liver pieces. Freeze immediately in liquid Nitrogen or on dry ice.
11. Lift the remaining carcass of the eel on to solvent-rinsed foil, wrap carefully using tape if needed on the outside to keep the foil in place, and place the foil-wrapped eel into a plastic bag for freezing.

12. Each bag should be labelled on the inside with a paper or plastic tag that contains the fish number, date sampled, and the name of the person and agency who did the sampling (or 'owns' the fish). Where paper tags are used, place the tag inside a small plastic bag to avoid the tag going to mush.
13. Label the outside of the bag with a black magic marker that resists freezing.
14. Freeze all eels at -20°C and group all eels from one origin in one larger plastic bag.
15. Between eels, clean all dissecting tools to remove any traces of blood or tissue, and dry using paper towel. Trays used for weighing and dissecting should also be cleaned and re-lined with absorbent paper, and fresh foil, weigh boat, and cryovials prepared for the next samples.
16. Enter all data in an Excel spread sheet and ensure that more than one person/computer has a copy. Keep the paper copy in an appropriate file.

A4. Preparation of Homogenates

A4.1 Samples

Samples used in this study are sexually-maturing female silver eels that have stopped feeding at the onset of their spawning migration from contaminated and reference sites to the Sargasso Sea. Live eels are to be collected preferentially, which can be euthanized with clove oil before their livers are removed and immediately frozen with liquid nitrogen and stored until determination of EROD activity. Samples received already frozen have their livers removed as part of the sample preparation procedure for consistency. Whole carcasses are stored frozen at -80°C in individually sealed aluminium foil packets, logged and tracked through Environment Canada's (EC) fish contaminants lab information database until sample preparation.

Note of caution: All procedures should be done quickly and efficiently to minimize thaw times. The more frequently fish are frozen and thawed, and the longer they remain thawed, the greater is the risk of a loss of water, which biases wet weight, and an associated lost of protein, lipid and contaminants, which will bias all results.

All tools must be clean and solvent rinsed with acetone or dichloromethane and hexane.

A4.2 Sample Preparation

For each eel carcass:

1. Before homogenization samples, prepare a sample sheet that shows the date they were homogenized, the personnel doing the homogenizing, and columns and rows that show the unique eel number that accompanies all samples, the total weight and length of the eel, the apparent sex (will be confirmed histologically), the weight in grams to three decimals samples of the histologically sample, the muscle sample, the thiamine sample, and the liver (if there), any obvious lesions or deformities, and any additional remarks. For each eel the number of jars of homogenate collected and their size should also be recorded.
2. Measure and record length and weight verifying against data sheet for the frozen eel.
3. Remove a plug of muscle for thiamine analysis (refer to "Thiamine Muscle Sample").
4. Thaw carcass at 4°C (2-3 hrs).
5. Scrape down the exterior of the eel using the aluminium foil packaging, removing any excess foreign material.
6. Note any obvious physical anomalies.
7. Verify the liver has been removed; if not, remove, weigh, and store the liver in a labelled glass jar.

8. Identify/verify sex.
9. Remove gonads and measure length and weight. Dissect a 0.5 cm thick section from the left lobe and place in a tissue cassette for histology, recording weigh (refer to "Gonad Sample"). The gonads not used for sex determination are to be homogenized (Step 12).
10. Identify stomach contents where discernible and look for signs of parasites (*Anguillicolla crassus*) in the swim bladder.
11. Remove a steak of the eel muscle behind the anus (refer to "Muscle Sample Dissection") weighing dissected portion before freezing the portion (as necessary).
12. Dissect out sagittal otoliths and store in numbered vials (refer to "Otolith Removal").
13. Homogenize whole eel four times through a Hobart meat processor, and once using a Polytron® benchtop homogenizer.
14. Fill up to seven solvent rinsed jars (50 g and 100 g) with all available homogenate, label and store at -80°C. All homogenate should be retained for the temporal, Lake Ontario archived samples.
15. All jars of homogenate of each eel were contributed to EC's National Biological Tissue Archive at -80°C.
16. Clean and rinse equipment between eels and solvent-rinse equipment (acetone-hexane) between locations.

A4.3 Homogenate Sample Labelling

Sample jars are to be labelled with the following information:

- Fish Number (i.e. NB-1)
- Peter Hodson and Mehran Alaee's names
- Collection date
- Species

- # of #
- EC five digit archive number (i.e. 52630)

Labels are to be placed on the top and sides of each jar covered with tape suitable for -80°C. The caps of each jar should be shrink-sealed.

A4.4 Thiamine Muscle Sample

Materials:

- 1 sharp filleting knife
- Whirl-pak®
- Aluminium foil

Note of caution: Eels should be/remain frozen (at least -20°C) for thiamine sample removal as thiamine can break down and/or be lost in the drip fluid post-mortem.

Remove a sample (10-20 g) just posterior to the head and anterior of the dorsal fin, above the lateral line as follows:

1. Make two vertical incisions about 3 cm apart across the dorsal side, down to the lateral line.
2. Cut through the fish along the lateral line, just above the backbone, connecting the two vertical incisions.
3. Remove and weigh the plug/muscle sample, and store immediately at -20°C in a labelled Whirl-pak® bag.
4. The sample should be approximately 2% of the whole wet weight of the fish.

A4.5 Gonad Sample

Materials:

- 1 sharp scalpel
- Tissue/histological cassette
- 2-L plastic container of 10% phosphate buffered formalin

Method summary:

1. Open the abdomen of the eel and localize the gonad (frilled whitish ribbons slightly granular for females and deeply lobe organs, smooth for males)
2. Weigh and measure the gonad.
3. Cut a transversal section (0.5 cm thick maximum) with a sharp scalpel blade in the middle of the left gonad lobe.
4. Put the gonad section directly in a histological cassette and label the cassette with a HB pencil with the ID number of the eel.
5. If there are any obvious abnormalities take a picture and an addition histological slice.
6. Place the labelled cassette(s) in 10% phosphate buffered formalin (20 volumes of formalin per volume of gonad tissue).

Several cassettes can be put in a 2-L plastic jar filled with formalin. Respect the 20 volume of fixative per volume of tissue in the jar.

10 % phosphate buffered formalin:

- 200 mL 40 % formalin (Read MSDS for safe handling instructions)
- 1.80 L distilled water
- 8 g $NaH_2PO_4 \bullet H_2O$
- 12 g Na_2HPO_4

A4.6 Muscle Sample

Materials:

- 2 scalpels
- 1 pair of tweezers
- 1 pair of forceps
- 1 pair of large tweezers
- Whirl-pak® plastic bag

Method Summary:

1. With a sterilized scalpel, dissect a section starting from behind the anus, making sure not to remove more than 10% of the eel's length as well as weight (5 to 8cm for an eel that measures 1 to 1.2 m). Ideally the sample should be approximately 8% of the whole wet weight of the fish.
2. Weigh the dissected sample to be sure it does not exceed the allowed 10% of the eel's weight.
3. Wrap the sample in contaminant-free aluminium foil and place it in a Whirl-pak® with the proper fish label on the bag.
4. Store the labelled sample at -20°C.

The remaining steps were performed in Mont-Joli after the section of muscle has been thawed.

5. Holding the dissected sample with the large tweezers, make a lengthwise incision on one side.
6. Remove the skin from the dissected sample using the forceps; catching one corner of the skin, pull the skin away from the sample while cutting it from the flesh with the help of a scalpel.

7. Once the skin has been successfully removed, thinly slice off each end of the dissected sample.
8. Carefully remove sections of the eel's tissue, avoiding rupturing the aorta and the pink underlying tissue.
9. Repeat for the other side.

4.7 Otolith Removal

Materials:

- 1 sharp filleting knife
- 1 pair of forceps
- 1 pair of sharp scissors
- labelled vial

Method Summary:

1. Cut off the head of the eel.
2. With a pair of sharp scissors cut along the length of the head, horizontally through the mouth, towards the posterior of the eel.
3. Repeat for the other side.
4. Cut down the center line (bone) of the head. Use a sharp filleting knife for larger eels (> 600 g) or scissors for smaller eels (< 600 g).
5. Split the head into two sections exposing the canals containing the otoliths.
6. Using forceps, extract the white calcified structures (one from either side of the center line).
7. Remove all tissue from the otoliths and place them in a labelled vial.
8. Allow the otoliths to sufficiently dry for 24 - 72 hrs, before capping and storing for aging.

A4.8 Silica Gel Control Blank Samples

These control samples are designed to provide QA/QC information and assess contamination during the dissection and homogenization of fish samples. The silica gel is designed to represent real tissue and should be treated similarly to real fish samples, subjecting the silica to the tools and environment used for fish samples.

During dissection the silica gel should be poured onto aluminium foil and cut and sliced like what is done with a real sample. The gel should be exposed to air for a similar amount of time needed to dissect an eel. Afterwards, the silica gel can be poured back into the glass jar and shipped along with the rest of the samples for homogenization. Similarly for homogenization, the silica gel should be processed like an eel carcass and homogenized into two 50 g jars.

Note: silica gel should be processed with every 10 eels (one per site for spatial study) and stored along with the other samples in each batch.

Materials:

- 1 kg of silica
- 19 jars 250 mL
- Hexane
- DCM
- MilliQ water

Method Summary:

1. Extract the MilliQ water with hexane
2. Wash the silica gel with hexane and DCM
3. Mix the silica and water 1:1 by weight (50% deactivated silica)
4. Divide the silica in 19 jars each containing approximately 100 g.

To extract MilliQ water with hexane:

- In a separating funnel (2 L) rinsed with hexane beforehand, add 1.2 L of MilliQ water
- Extract the water with 350 mL of hexane by shaking the bulb and degassing the solution until no more gas is released
- Recover the water (lower phase)
- Repeat two more times
- Bottle

To wash silica with hexane and DCM:

- Wash the silica three times with hexane and three times with DCM
- Allow it to evaporate in a fume hood for three days covered with aluminium foil
- Condition the silica for 12 hrs in a drying oven beginning at 120°C, raising the temperature 30°C every hour

In total, 980 g of washed silica and 980 mL of extracted water was mixed and well homogenized and distributed into 19 jars (Table A1).

Table A1: Silica Control Sample Jars

Jar No.	1	2	3	4	5	6	7	8	9	10
Mass (g)	100.0	100.1	100.1	100.0	100.3	100.0	100.0	100.0	100.0	100.7
Jar No.	11	12	13	14	15	16	17	18	19	
Mass (g)	100.7	100.2	100.1	100.2	99.8	101.0	101.0	99.4	101.0	

A5. Sample Preparation

A5.1 General Information

Samples removed from the archive are tracked through Environment Canada's database. Changes in sample custody should be recorded both by the person removing the sample from the archive

and the analyst of the sample. Sample batches should be ranked and processed such that the least-contaminated (reference sites) are analyzed first and the more contaminated sites are analyzed last, where possible. In addition, individual sample batches should be processed in a similar fashion with the procedural blank run first followed by the silica gel control, homogenate samples, and reference material(s).

Each step of the procedure from homogenization to sample analysis should be document first with hard copy and second electronically. This information is essential for inter-laboratory communication, as well as for reporting on data.

Total number of samples for chemical analysis:

- 20 temporal samples
- 78 spatial samples

} Homogenate

- 10 chemical determinations from toxicological extracts
- 10 sub samples of muscle tissue from R. Ouelle

Each homogenate sample batch for chemical analysis consists of 14 samples:

- 1 Silica Gel Blank
- 1 Spiked Silica Gel Sample
- 1 Reference Material (RM)
- 1 Certified Reference Material CRM (NRC)
- 10 Homogenate samples (one site for spatial trends)

The RM was prepared at EC from extra lake trout homogenate generated by the Fish Contaminants Laboratory. Left-over fish was homogenized five times through a Hobart meat processor and divided into about 60 x 20 g jars. Ten of the jars were used to evaluate/validate the

RM and establish acceptable chemical ranges; the remaining 50 were divided between EC in Burlington and DFO in Mont-Joli.

RM jars were labelled with the following information, top and side:

- RM-1 to 60
- Peter Hodson and Mehran Alaee
- Date: March 7, 2008
- L. Trout composite

A5.2 Moisture Determination (Optional)

For each homogenate sample:

1. Weigh and record the mass of two aluminium weighing boats to at least 3 decimal places.
2. Accurately weigh 3 g of sample into each of the pre-weighed boats.
3. Dry the samples at 70°C in a vented oven at least overnight.
4. Cool the sample in a desiccator and record the weight.
5. Calculate the percent moisture using the following equation:

$$\frac{(\text{wet sample weight})-(\text{dry sample weight})}{\text{wet sample weight}} \times 100\%$$

A5.3 Sample Extraction

Materials:

- 1 jar of homogenate
- 1 Aluminium weighing boat and analytical scale capable of at least 3 decimal places
- Anhydrous sodium sulphate: baked at 450°C at least overnight and cooled to room temperature in a desiccating chamber
- 1 Porcelain mortar, 750 mL capacity and pestle

- 1 Large chromatography column (22 mm I.D. x 500 mm length), carbon trap, and reservoir
- Silanized glass wool
- 1 Large long stem funnel
- 350 mL DCM (distilled in glass)
- 1 of each 500 mL, 250 mL, and 100 mL round bottom flask
- Rotary evaporator

For each homogenate sample:

1. Remove from storage and thaw whole fish homogenate (50 g jar) mixing thoroughly to recombine any separated lipid with the tissue, remembering to document jar information.
2. Transfer a 20 g portion of the homogenate, weighed in a solvent-rinsed aluminium boat, quantitatively into a large (750 mL) mortar with 200 g (2 x 100) of anhydrous Na_2SO_4.
3. Grind the sample mixture manually until it is a free-flowing mixture (dry).
4. Transfer the mixture into a large chromatography column (22 mm I.D. x 500 mm length) plugged with silanized glass wool, quantitatively with rinses of DCM.
5. Spike the column with 40 ul of surrogate internal standard $^{13}C_{12}$ -CB(170) and 2,3,7,8-($^{37}Cl_4$)-TCDD.
6. Elute the balance of the DCM from an extraction reservoir such that 350 mL of solvent is passed through the column at a rate of 5-10 mL/min, and collect the eluted solvent in a pre-weighed 500 mL round bottom flask.
7. Add 200 µL of toluene (or isooctane) keeper.
8. Split the extract into three fractions: 1) 10% by weight used to determine lipids gravimetrically; 2) 40% by weight saved as back-up; 3) 50% by weight for chemical analysis.

9. Sub-divide the fraction for chemical analysis in half and spike each portion, 25% for coplanar PCBs and PCDD/Fs and 25% for PBDEs and PCBs, with their respective ^{13}C surrogate mixture.
10. Reduce each fraction in volume to a few mLs by rotary evaporation. Quantitatively transfer with rinses of 1:1 DCM:hexane the back-up fraction into a 40 mL amber sealed vial.

A5.4 Lipid Determination

Materials:

- 10% by weight fraction: reduced to 1 mL
- Aluminium weighing boat
- Desiccating chamber with anhydrous calcium sulphate

Method Summary:

1. Using the 10% by weight fraction from the "Sample Extraction" method (Step 8), quantitatively transfer the extract into a pre-weighed aluminium weighing boat with rinses of 1:1 DCM:hexane.
2. Dry the weighing boat and solvent in a 40°C vented oven overnight.
3. Cool the sample completely in a desiccator over anhydrous calcium sulphate, and record the weight of the lipid sample minus the weighing boat.
4. Calculate percent lipid using the following equations

$$\frac{\text{Weight lipid}}{\text{Weight Sample}} \times 100\%$$

where *Weight Sample* equals 10% of the original sample weight before extraction.

A6. Sample Clean-up

A6.1 Gel Permeation Chromatography

Materials:

- AccuPrep GPC (J2 Scientific) LP direct inject
- 1:1 DCM:hexane
- Disposable glass centrifuge tube
- Disposable glass test tube with cap and septum
- 500 mL or 1L collection flask
- Rotary evaporator

Method Summary:

1. For the PBDE and bulk PCB extract, dilute it with 1:1 DCM:hexane to 5 mL in preparation for gel permeation chromatography (GPC).
2. For the dioxin-like compound extract, dissolve the extract in 1:1 DCM:hexane so that lipid levels are about 0.5 g of lipid per 5 mL of solvent. Use the percent lipid value determined previously to calculate the amount of solvent necessary.
3. Turn on the GPC computer, autosampler (wait for it to initialize), degasser, the autoinjector system, and load (double click) the AuccuPrep program (Accup 307F). Left-click “Use autoinjector.”
4. Left-click the “config” button to check probe depth, method and tray selection. For pcbs select method “ocpcb”, 24 flask tray, and set autosampler probe depth to 123 mm. For dioxins select “dioxin” method, 6 flask tray, and set probe depth to 126 mm. If the tray is changed the AccuPrep program will need to be restarted.

5. Click “Put column in line”. Prime the pump by clicking on “Prime”. Using the pump component in AccuPrep, left-click “enable” button so that pump goes on-line. Pump should start pumping. Column should condition for approx. 1 hour prior to running first sample.
6. Quantitatively transfer extract to a disposable glass tube and cap with disposable caps and septum. Place in autosampler tray and label each tube.
7. Place collection flasks in appropriate spots in tray to match autosampler. Do NOT cover with foil or anything else as the probe does not penetrate well and is easily broken.
8. Ensure solvent reservoir is full (400 mL of 1:1 DCM:hexane per loop is required). Top it up if the run is to be overnight. Ensure waste bottle is empty enough to accommodate overnight flow to waste. About 200 mL from each loop goes to waste. Programming on AccuPrep includes shutdown for pump so that it will stop flowing after last sample collection, and column will go off-line.
9. In AccuPrep, prepare the tray by selecting sequence and entering the information as required. Samples should be given numbers and method chosen. If it is to be for dioxins, once the sequence is saved, view sequence must be selected and more information entered here. The number of injections required per tube, which corresponds to the number of loops and grams of lipid found, will need to be added to each position for the dioxin sequence.
10. After the run, remove flasks from tray and stopper with a glass stopper. Discard tubes in auto-sampler. Turn off autosampler and degasser. Computer will indicate whether run ran successfully. Pump should not be flowing. Exit out of all screens and out of AccuPrep program. It is not necessary to completely shut down computer.
11. Add 500 µL of isooctane keeper.

12. Gently rotary evaporate sample to 1 mL (for PCB and PBDEs) or 0.5 mL (for dioxin-like compounds).
13. Quantitatively transfer sample into a GC vial with rinses of 1:1 DCM:hexane up to 1.5 mL (for PCB and PBDEs) or 0.7 mL (for dioxin-like compounds).

Note: At this point, the sample extracts for OCP, PCB and PBDE analyses are ready to be shipped to Mont Joli for final clean-up and GC-MS analysis.

A6.2 Silica-Alumina Column

This method is used to purify biological tissue extracts obtained after GPC clean-up. This column has the capacity to separate organochlorinated pesticides, PCBs, and PBDEs of most aliphatic hydrocarbons, as well as residual lipids following GPC. This method also functions as a clean-up step for PCDD/Fs and PCNs prior to fractionation on a 5-PYE HPLC column.

Materials:

- 1 Glass column (10 mm I.D. x 240 mm length) with a Teflon cap and a 200 ml reservoir
- Stand equipped with a support for column
- Washed glass wool
- 2 small tweezers
- 1 glass stirring rod (approx. 9 x 400 mm)
- 3 Pasteur pipettes (7") having an end with internal diameter ≈ 2 mm
- 2 Pasteur pipettes (9")
- 2 graduated cylinders (25.0 ml)
- 1 graduated cylinder (100.0 ml)
- 3 beakers (50 ml)
- 1 beaker (100 ml)

- 1 Zymark evaporation glass tube (200 ml)
- Aluminum foil
- Washed and activated (5% H_2O): neutral silica, 70-230 mesh, neutral alumina, 70-230 mesh
- Washed anhydrous sodium sulfate
- Hexane and solution of 1:1 DCM:hexane (pesticide residue analysis grade)

A6.3 Preparation of Reagents

Anhydrous sodium sulfate

1. Add approx. 350 g of sodium sulfate to a 1500 ml beaker.
2. Fill the beaker to 1400 ml with DCM.
3. Using a clean glass stirring rod, stir for several minutes before elutriating and removing the remaining solvent.
4. Repeat the washing step two more times.
5. Repeat steps 2 to 4, using hexane in place of DCM.
6. Afterward, cover the beaker with hexane rinsed aluminum foil.
7. Air-dry the sodium sulfate under the fume hood, stirring on occasion with a clean glass stirring rod.
8. When dried, put in a drying oven at 30°C, increasing the temperature by 30°C even hour.
9. Leave the temperature at 130°C overnight.
10. Cool to room temperature in a desiccator and store the sodium sulfate in one or more hermetic containers, noting the date and number.

Glass wool

1. In a clean 1 L beaker, add enough glass wool so as to occupy a volume of 300 ml.
2. Wash the glass wool with three portions of DCM (approx. 600 ml/portion).

3. For each wash, stir well with a clean glass stirring rod, before elutriating and removing the remaining solvent.
4. Repeat steps 2 and 3 using hexane.
5. Air-dry under the fume hood, and condition in a drying oven at 30°C, increasing the temperature by 30°C even hour, leaving the temperature at 130°C overnight.
6. Store glass wool in a cupboard in a jar rinsed with DCM and hexane beforehand, covered with aluminum foil.

Water extracted with hexane

1. In a 2 L separating funnel rinsed with hexane beforehand, add 1.2 L of MilliQ water.
2. Extract water with 350 ml of hexane: shake the bulb then degas. Repeat until there is no more gas.
3. Recover water (lower phase).
4. Repeat two more times.
5. Bottle.

Deactivated silica gel (5%)

1. The day before assembling the columns, roughly place the necessary quantity of silica in a pre-weighed Erlenmeyer flask with its stopper. Do not label. Remove the stopper and place a piece of hexane rinsed aluminum foil on the container. Place the container in a furnace at 200°C overnight.
2. After removing the Erlenmeyer from the furnace, replace the stopper. It should, however, be done with care so that the stopper does not become wedged while cooling.
3. Weigh the container and its stopper in order to determine the mass of the silica, and use the value to calculate the amount of extracted water with hexane to add for 5% w/w.
4. On the balance, always using a Pasteur pipette, add the quantity of water necessary.

5. Immediately close the Erlenmeyer flask with its stopper and vigorously stir until the powder is homogeneous.
6. Using Teflon tape, tape around the joint sealing the containing, and store it in a dessicator.

Deactivated alumina (5%)

Follow the same procedure as for neutral silica gel.

Method Summary:

1. Attach the column to a support, and stopper the column with a Teflon cap.
2. Using a clean pair of tweezers, insert the washed glass wool into the column and compact it using a clean glass rod. Rinse the column three times with DCM and hexane, making sure that there are no air bubbles between the glass wool and the stopcock.
3. Close the stopcock and fill the column approximately ¾ full with hexane.
4. In a clean 50 ml beaker, accurately weigh 5.0 g of 5% activated silica, and promptly add enough hexane to cover the silica in the beaker.
5. Transfer the silica into the column filled with hexane (open the stopcock) using a clean Pasteur pipette having an end internal diameter of ≈ 2mm (if the end has a diameter less than this value, shorten it to resize the end).

Note: From this point forward, the column must never go dry. i.e. there must always be solvent inside the column.

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6. Tap along the wall of the column in order to pack the silica well and to obtain a level surface (one can give oneself a benchmark by making a small pencil mark on the column in order to if the silica is well compacted).

7. Repeat steps 4 to 6, this time using 10.0 g of 5% activated alumina.
8. Accurately weigh 1.25 g of anhydrous sodium sulfate, cover with hexane, and repeat steps 5 and 6.
9. Cover the column with aluminum foil to avoid the deposition of impurities.

Note: It should be noted that "real" elution will be carried out in two parts: 15.0 ml of hexane (waste), followed by, at least, 70.0 ml of 1:1 DCM:hexane. Thus, one ensures oneself to recover the maximum amount of compounds.

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10. Close the stopcock when the hexane reaches the sodium sulphate. Measure 15.0 ml of hexane using a 25.0 ml graduated cylinder. Cover it with a piece of hexane rinsed aluminum foil.
11. Place an Erlenmeyer flask as waste under the column, open the stopcock and load the column with the extract. It is very important that this extract is a small volume (less than 0.7 ml) so that cleaning is effective. Use a clean 9" Pasteur pipette to transfer the sample.
12. Rinse the tube having contained the extract with 3 portions of ≈2 ml of hexane, and load each rinse on the column. Always wait until the solvent meniscus reaches the sulfate before loading. The hexane used for the rinsing comes from the 15.0 ml measured previously. Two 9" Pasteur pipettes are thus necessary: one for transferring the sample and the rinses onto the column and another for transferring hexane from the beaker to the Zymark tube for the rinsing and for the column. Therefore, it is very important that the later pipette is only in contact with hexane and especially not with the sample, because it is re-used with the subsequent samples (to fulfill the same function).

13. After 15 ml of hexane is collected (when the meniscus reaches the surface), close the stopcock, remove the collected fraction and discard it as waste, and place a clean Zymark tube under the column.
14. Completely elute 70.0 ml (1:1 DCM:hexane).
15. Add 200 µl of isooctane (keeper) to the collected eluate.
16. Put the Zymark tube in the TurboVap and evaporate to ≈ 0.7 ml using to the following conditions: bath temperature of 21ºC with a pressure of 3 psi.

Note: For the PCDD/F fraction, Zymark tubes and TurboVap evaporator can be replaced with Kuderna-Danish (K-D) concentrator tubes and a centrifugal evaporator, or nitrogen evaporation.

A6.4 Pyrenyl-Silica HPLC Column

This procedure describes the fractionation of extracts containing PCDDs, PCDFs, and non-ortho PCBs from planar and bulk PCBs, following silica-alumina column clean-up, in preparation for instrumental analysis.

Materials:

- Hexane (HPLC grade)
- Toluene (HPLC grade)
- Agilent 1200 Series quaternary LC pump and vacuum degasser, manual injector equipped with a 500 µL loop, and a 2/10 switching valve
- 1 beaker 100 mL
- Cosmosil 5-PYE HPLC column [2-(1-prenyl)ethyldimethylsilylated silica gel] 250 x 4.6 mm
- 2 K-D concentrator tubes and centrifugal evaporator
- 500 µL syringe

- 2 mL syringe
- Amber glass GC vial and insert

Note: Use needles with 0.028-inch outer diameter (22 gauge) × 2-inch long needle, without electro-taper, and with 90º point style (square tip).

Method Summary:

1. Further evaporate the extract down to ≈ 50 µL in a centrifugal evaporator under the following conditions: Rotational frequency of 3000 rpm, pressure of 1.3 kPa, and temperature of 43°C.
2. Turn on the HPLC computer, LC pump, and vacuum degasser. The degasser light should turn green when it is ready. Load the ChemStation software.
3. Purge the system with isopropanol, toluene and hexane (at least 30 mL).
4. Condition the HPLC system by running hexane at a flow rate of 2 mL/min for 10 minutes. Afterward, reduce to flow rate to 1 mL/min.
5. Set up a binary system of hexane-toluene using the ChemStation software beginning with hexane for the first 15 minutes, followed by toluene for 35 minutes, and hexane for 10 minutes (column regeneration) all at a flow rate of 1 mL/min.
6. With the manual injector in the "Load" position, rinse the injection port with three 1.5 mL portions of hexane using a 2 mL syringe. The hexane should pass through the injection loop and out the vent port into a 100 mL beaker. This may be done while the system is being conditioned.
7. Fill the 500 µL syringe with 15 µL of hexane, 10 µL of air, whole sample, three 50 µL tube rinses of hexane, 10 µL of air, and 15 µL of hexane.

8. Load the sample into the injection loop with the injection valve in the "Load" position. Leaving the syringe in the port, turn the valve to the "Inject" position. The binary system of hexane-toluene should automatically start running after injection.
9. Fraction I (0 to 7.5 min) and Fraction II (7.5 to 9.5 min) may be collected in disposal test tubes, labelled, capped, and stored (-20°C) as backup. Collect Fraction III (9.5 to 16.5 min) in a K-D concentrator tube and Fraction IV (16.5 to 50 min) in a 100mL round bottom flask.
10. While the column is being regenerated with hexane (after 50 minutes), the injection valve may be returned to the "Load" position, and the injection port/loop rinsed with hexane according to Step 6.
11. The 500 µL syringe should also be rinsed with three 500 µL portions of hexane, ensuring that the exterior of the needle is rinsed as well.
12. Reduce Fraction III down to 50 µL in a centrifugal evaporator under the same conditions as in Step 1. Rotary evaporate Fraction IV down to 1mL, transfer it with rinses to a K-D tube, and reduce to 50 µL by centrifugal evaporation.
13. Quantitatively transfer both fractions into amber glass GC vials fitted with a glass insert.
14. Add the instrument recovery spike to the vials (Fraction III: 50 µL of 5-fold diluted WP-ISS; Fraction IV: 10 µL of EPA-1613ISS). Evaporate the samples just to dryness under nitrogen, and make up the "dioxin-like" PCB and PCDD/F with 100 µL and 20 µL of nonane, respectively. Cap and seal the vial until instrumental analysis.

Note: Weigh the vials and record the information if they are to be stored for a prolonged period or mark the solvent levels on the vials.

List of Publications

Articles published or accepted in refereed journals

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Byer, J.D., Pacepavicius, G., Lebeuf, M., Brown, R.S., Hodson, P.V., and Alaee, M., (2013) Post-target determination of brominated flame retardants and related compounds in American eels captured in eastern Canada. Proceedings of the Sixth International Symposium on Brominated Flame Retardants.

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