ENDOCRINE DISRUPTING CHEMICALS IN WATER, SEDIMENTS AND FISH OBSERVED IN URBAN TRIBUTARIES OF THE FRESHWATER TIDAL POTOMAC RIVER: OCCURRENCE, BIOACCUMULATION AND TISSUE DISTRIBUTION

by

Golala Arya

A Dissertation
Submitted to the
Graduate Faculty
of
George Mason University
in Partial Fulfillment of
The Requirements for the Degree
of
Doctor of Philosophy
Chemistry and Biochemistry

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Date: 30 July 2015	Summer Semester 2015 George Mason University Fairfax, VA

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A Dissertation submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy at George Mason University

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DEDICATION

This work is dedicated to my loving husband Heewa, without whose patience and support it would not exist, and our wonderful daughter Cheyenne, who inspired me to never abandon my goals. To my wonderful and supportive parents, Arif and Parvin, whose sacrifices made my progress possible. To my loving sister, Shatao, and brothers Goran and Soran who never stopped believing in me. Last but not least, to my committee chair Dr. Gregory Foster whose relentless support, patience and guidance were essential to my success.

ACKNOWLEDGEMENTS

Although this document bears my name alone, a number of people contributed to the project, including but not limited to ones named below. For those whose names are not listed, please know that you are appreciated as well.

Special gratitude goes to:

My committee chair, Dr. Gregory D. Foster and committee members, Dr. Kim de Mutsert, Dr. John Schreifels and Dr. Barney Bishop, who were always available to provide advice and encouragement.

My research partners, Sara Tadayon, who turned fish into data and provided moral support, as well as James Sadighian, who turned water into data points and was always the "handy man" of the laboratory. Also special thanks are owed to Adam Moody and David Nadrchal in familiarizing me with the environmental chemistry laboratory as well as instrument training.

Jennifer Jones and Ron Bacon, our intelligent and capable undergraduate students whose work was paramount to this research.

Field work crew from Environmental Science and Policy department, with supervision of Dr. Chris Jones and Dr. Joris van der Ham, including C. J. Schlick, Amanda Sills, and Alex Graziano.

Dr. Kristy Lewis, whose support, whether editorial or moral was essential.

Dr. Tom Huff, whose unwavering support of Team Foster is well recognized and appreciated by all of us.

And last, but certainly not least, the wonderful staff of various departments of George Mason University, who at some point or another provided advice and support. Mery Tucker and Fabiola Suarez from Chemistry and Biochemistry; Roslyn Cress, Susan Cheselka and Lisa Bair from Environmental Science and Policy; Melissa Hayes of College of Science Academic Affairs, and Sally Evans with the University Dissertation and Thesis Services.

This project was funded by Alexandria Renew Enterprises in collaboration with Potomac Environmental Research and Education Center (PEREC), which provided two years of graduate research assistantship, critical to completion of my graduate research.

I am grateful to the Office of the Provost at George Mason University for providing Dissertation Completion Grant.

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LIST OF ABBREVIATIONS

2,3,7,8-Tetrachlorodibenzo-para-dioxin ..... TCDD
Acetonitrile ..... ACN
Agency for Toxic Substances and Disease Registry ..... ATSDR
American Chemical Society ..... ACS
Aryl hydrocarbon receptor ..... AHR
Below detection limit ..... <DL
Dextromethorphan ..... DXM
Dichloromethane ..... DCM
Dioxin-like polychlorinated biphenyls ..... dl-PCBs
Dispersed solid phase extraction ..... dSPE
Double distilled water ..... DDW
Dry weight ..... dwt
Electron impact ..... EI
Endocrine Disrupting Chemicals ..... EDCs
Environmental Protection Agency ..... EPA
Ethyl Acetate ..... EtOAc
Gas chromatographer mass spectrometer ..... GCMS
Large volume injection LVI
Lethal concentration 50 ..... LC_{50}
Lethal dosage 50 ..... LD_{50}
Limits of detection ..... LOD
Methanol ..... MeOH
Method detection limit ..... MDL
Microwave assisted extraction ..... MAE
Microwave assisted saponification ..... MAS
Minimum effect threshold ..... MET
Multi-mode inlet ..... MMI
N-Methyl-N-(trimethylsilyl) trifluoroacetamide ..... MSTFA
Organic carbon ..... OC
Over the counter ..... OTC
Persistent Organic Pollutants ..... POPs
Polychlorinated biphenyls ..... PCBs
Polycyclic aromatic hydrocarbons ..... PAHs
Potomac Environmental Research and Education Center ..... PEREC
Programmable temperature vaporizing ..... PTV

Selected ion monitoring ..... SIM
Solid phase extraction ..... SPE
The International Agency for Research on Cancer ..... IARC
The International Programme on chemical Safety ..... IPCS
Threshold effect concentration ..... TEC
Toluene ..... TOL
Total polychlorinated biphenyls ..... tPCB
Total suspended matter ..... TSM
Toxic equivalency factor ..... TEF
Triclosan ..... TCS
Dextromethorphan ..... DXM
Wastewater treatment plant ..... WWTP
Wet weight ..... wwt
Bioaccumulation factor ..... BAF

ABSTRACT

ENDOCRINE DISRUPTING CHEMICALS IN WATER, SEDIMENTS AND FISH OBSERVED IN URBAN TRIBUTARIES OF THE FRESHWATER TIDAL POTOMAC RIVER: OCCURRENCE, BIOACCUMULATION AND TISSUE DISTRIBUTION

Golala Arya, PhD

George Mason University, 2015

Dissertation Director: Dr. Gregory D. Foster

The goal of the present study was to assess the ecological impacts of legacy and contemporary endocrine disrupting chemicals (EDCs) in the upper tidal Potomac River associated with WWTP discharge. Legacy EDCs includes polychlorinated biphenyls (PCBs) and the present study quantified total-PCBs and dioxin like PCBs (dl-PCBs), which are the more toxic PCB congeners, in bed sediments and biota from Hunting Creek and Gunston cove to evaluate the significance of dl-PCBs in the upper Potomac River.

Dl-PCBs were consistently detected in sediments and fish from both Gunston Cove and Hunting Creek. PCB 118 and 123 had the highest concentration (0.56 ± 0.03 ng/g dwt) followed by PCB105 (0.28 ± 0.01 ng/g dwt) in Gunston Cove sediment while PCB105 and 118 dominated the Hunting Creek sediment with concentrations of 1.66 ± 0.71 and 1.31 ± 0.57 (ng/g dwt) respectively. The highest

fish dl-PCB congener detection belonged to PCB118 and 123 (62.9 ± 25.3 ng/g lipid) followed by PCB105 (29.5 ± 9.83 ng/g lipid), reflecting the sediments profile observed in the Gunston Cove. PCB118 (31.3 ± 27.8 ng/g lipid) was the dominant dl-PCB, followed by PCB105 (13.6 ± 6.70 ng/g lipid) in Hunting Creek fish, reflecting the Hunting Creek sediment profile as well. Statistical analysis indicated geospatial differences among the sites as well as differences among fish species.

This study also assessed the ecological impacts of contemporary endocrine disruptor chemicals (EDCs) in Hunting Creek, Accotink Creek and Pohick Creek associated with WWTP discharge. Triclosan and dextromethorphan were the two analytes detected in water, sediments and fish samples. Triclosan (TCS) is a broad-spectrum antibacterial mostly used in soap and dextromethorphan (DXM) is an antitussive agent readily available in over-the-counter (OTC) drugs. Tissue distribution analysis for DXM and TCS in fish revealed selective bioaccumulation of the two analytes in skin, stomach and gonads, without any correlation to the lipid content of the perspective tissues.

Mean concentrations of triclosan observed in Hunting Creek water, sediments and whole fish (banded killifish and white perch) were 15.5 ± 3.71 ng/L, 72.5 ± 9.41 ng/g dwt, 72.2 ± 4.56, and 81.8 ± 5.84 ng/g wwt, respectively. Mean observed dextromethorphan concentrations in Hunting Creek water, sediments and whole fish (banded killifish and white perch) were 74.9 ± 11.8 ng/L, 103 ± 84.2 ng/g dwt, 470 ± 7.06, and 304 ± 43.3 ng/g wwt, respectively.

Mean concentration of dextromethorphan in Accotink Creek whole fish, alewife and gizzard shad, and sediments were 45.3 ± 9.12 ng/g wwt, 101 ± 11.1 ng/g wwt and 91.1 ± 3.52 ng/d dwt, respectively. Triclosan was not detected in Accotink sediments and mean TCS concentration in alewife and gizzard shad whole fish homogenate were 76.1 ± 9.13 ng/g wwt and 45.5 ± 7.04 ng/g wwt, respectively.

Mean concentration of dextromethorphan in Pohick Creek whole fish alewife and gizzard shad and sediments were 49.1 ± 9.68 ng/g wwt, 134 ± 11.8 ng/g wwt, and 233 ± 48.9 ng/g dwt, respectively. Triclosan was not detected in Pohick Creek sediments and whole fish homogenate for alewife and gizzard shad were 47.9 ± 6.89 ng/g wwt and 51.7 ± 5.32 ng/g wwt, respectively.

The greatest DXM concentration was observed in alewife stomach (2000 ± 156 ng/g wwt) and lowest in alewife muscle (74.3.5 ± 5.34 ng/g wwt). The greatest TCS concentration was observed in gizzard shad testes (274 ± 60.5 ng/g wwt) and lowest concentration was observed in alewife muscle (192 ± 87.4 ng/g wwt).

Triclosan and dextromethorphan gonad concentrations ranged from 102 to 274 ng/g wwt (for TCS) and 265 to 1,273 ng/g wwt (for DXM) suggesting parental transfer to offspring is possible, warranting further research and analysis.

The consistent detection of TCS and DXM across water, sediment and fish species warrants further research considering the recent well documented increase in use of TCS and reported risks to the aquatic environment and fish. DXM is one of the top five over-the-counter (OTC) drugs being abused in many countries, and would manifest psychiatric symptoms induced by chronic abuse.

OVERVIEW

This study determined the concentrations, bioaccumulation and partitioning of legacy and contemporary endocrine distrusting chemicals (EDCs) in urban tributaries of the freshwater tidal Potomac River. Legacy EDCs, such as dichloro-diphenyl-trichloroethane (DDT) and polychlorinated biphenyls (PCBs) are chemicals that are no longer used and manufactured in most countries, while contemporary EDCs, such as flame-retardants and pharmaceuticals are currently used and manufactured in most countries.

PCBs are a class of legacy persistent organic pollutants (POPs) that possess physical and chemical properties leading to high environmental persistence. PCBs are sparingly soluble in water, with water solubility decreasing with increasing chlorine substitution, hydrophobic, readily dissolving in nonpolar organic solvents, and lipophilic, accumulating in the fatty tissues of terrestrial and aquatic biota including humans.[1]

Total PCBs were analyzed in Gunston Cove and Hunting Creek bed sediments and fish for the legacy EDCs portion of this study. The PCB study was focused on dioxin-like PCB occurrence and matrix profiles since this topic is relatively new in the PCB toxicity perspective. Dioxin-like PCBs are the more toxic congener due to their structural resemblance to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD, referred

to as dioxin), which is considered the most toxic synthetic organic contaminant yet identified in environmental samples.[1]

A total of eight fish species from both embayments were processed and analyzed for tPCBs and dl-PCBs. Hunting Creek and Gunston Cove both recived treated water from local WWTPs, Alexandria Renew Enterprises and Noman Cole Jr. plant. These species included spottail shiner (*Notropis hudsonius*), mummichog (*Fundulus heteroclitus*), bluegill (*Lepomis macrochirus*), inland silverside (*Menidia beryllina*), redbreast sunfish (*Lepomis auritus*), white perch (*Morone americana*), banded killifish (*Fundulus diaphanous*) and striped bass (*Morone saxatilis*). Only three species were common among Gunston Cove and Hunting Creek fish samples; white perch, banded killifish and striped bass.

Legacy EDCs, due to their prolonged use and persistence in the environment are still of concern, even decades after their manufacturing has stopped. EDCs are emitted to the aquatic environment through industrial waste discharge, urban stormwater runoff and wastewater treatments discharge.

The two embayments receive treated water from local WWTPs. Hunting Creek is a tributary of the Potomac River, formed by the confluence of Cameron Run and Hooff Run. Alexandria Renew Enterprises WWTP is located on the east shore of the Hunting Creek. Alexandria Renew WWTP discharges 150,000 m^3 (average) of wastewater daily.[2] Gunston Cove, a Y-shaped embayment of the Potomac River, is located 20.0 km downstream from Washington DC. Gunston Cove has water area of about 5 km^2 and is formed from the confluence of Pohick Creek and Accotink Creek.

Gunston Cove incorporates recreational parks, marinas, urban housing developments, Fort Belvoir, and Noman Cole wastewater treatment plant (WWTP). The Noman Cole WWTP is located 10 km to the west of the Potomac River and discharges 17,000 m^3 (average) of wastewater daily into Pohick Creek.[3] Both treatment plants are categorized as major WWTP (>2 MGD, mega gallons daily).[4]

The use of pharmaceuticals and personal care products, a subset of contemporary EDCs, in the past few decades has increased exponentially, and the subsequent environmental pollution is increasingly recognized as a major threat to both wildlife and humans. Pharmaceuticals and PCBs have very different physical and chemical properties. Pharmaceuticals and PCBs are both hydrophobic and lipophilic compounds and tend to accumulate in fatty tissues. Pharmaceuticals relative to PCBs, have higher boiling points rendering their analysis with GCMS troublesome. GCMS analysis of pharmaceuticals requires derivitization while PCBs do not require this step.

The remaining three chapters aimed at determining the most dominant contemporary EDCs in the Hunting Creek water, bed sediments and fish; as well as bed sediments and fish from Accotink Creek and Pohick Creek. Study objectives were to determine if there is a geospatial pattern, profile the tissue distribution among fish tissue homogenate extracts, assess potential parental transfer to offspring, and also determine the relationship between water, sediment and fish showing the direction of flow or establishment of equilibrium. In all samples

(water, sediment and fish) dextromethorphan (DXM, antittusive) and triclosan (TCS, antibacterial) were the prominent analytes detected.

The consistent detection of TCS and DXM across water, sediment and fish species warrants further research considering the recent well documented increase in use of TCS and reported risks to the aquatic environment and fish. DXM as one of the top five over-the-counter (OTC) drugs being abused in many countries, and would manifest psychiatric symptoms induced by chronic abuse.

CHAPTER 1 DIOXIN-LIKE POLYCHLORINATED BIPHENYLS (DL-PCBS) PROFILES IN FISH AND SEDIMENTS FROM HUNTING CREEK AND GUNSTON COVE REGIONS OF THE TIDAL FRESHWATER POTOMAC RIVER (VIRGINIA, USA)

Introduction

Structure, Sources and Properties of PCBs

Polychlorinated biphenyls (PCBs) are synthetic organic compounds composed of the elements carbon, hydrogen and chlorine. PCBs have the base structure of biphenyl with one to five chlorine atoms substituted in each phenyl group (Figure 1.1). PCBs represent a complex mixture of 209 possible congeners (unique structural homologs), which are defined by the number of substituted chlorine atoms (1 through 10) and geometric isomer combinations. Individual PCB congeners are numbered 1 through 209 following nomenclature rules established by the Chemical Abstract Service (CAS) of the American Chemical Society.[5] PCBs are thermally stable chemicals, making them ideal in applications such as dielectric fluids in electrical capacitors and transformers, heat transfer fluids and as lubricants.[1] They have been used in minor applications as plasticizers, adhesives, inks, caulk and sealants.

Monsanto Corporation (St. Louis, MO), the primary manufacturer of PCBs in the United States, supplied PCBs under the trade name Aroclor® from 1929 to 1979.

The nomenclature of Aroclors, dedicates the first two digits to the number of the carbon atoms in the phenyl rings (12 for the biphenyl rings of PCBs) and the next two digits indicates the percentage of chlorine by mass in the mixture. Aroclor 1248 has the highest percentages of tetra-chloro, tri-chloro followed by penta-chloro. Aroclor 1254 contained penta-chloro as the highest percentage followed by hexa-, teta- and hepta-chloro congeners. Aroclor 1260 contained hexa-chloros as the highest percentage, followed by hepta-, penta- and octa-chloro congeners. Approximately 610 to 635 million kg of commercial PCBs were produced in the U.S. by Monsanto[6] and it has been estimated that 10% of this amount remains cycling through the environment or has the potential to reach the environment.[7]

PCBs persist in the environment due to their high un-reactivity and resistance to breakdown by acids, bases and heat. The more highly chlorinated congeners adsorb strongly to sediment[7] with half lives ranging from months to years in sediment as high as 25 year half-lives.[8] Contemporary emissions of PCBs to the environment primarily include spills, leaky industrial storage containers, contaminated superfund sites (e.g., military facilities) and landfills.[1]

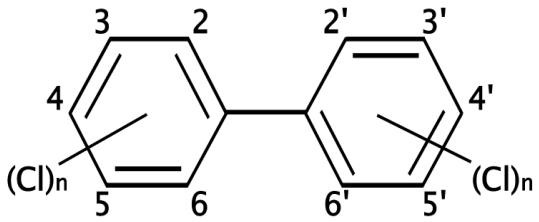


Figure 1.1. Parent structure of polychlorinated biphenyls.

PCBs are a class of legacy persistent organic pollutants (POPs) that possess physical and chemical properties leading to high environmental persistence. PCBs are sparingly soluble in water, with water solubility decreasing with increasing chlorine substitution, hydrophobic, readily dissolving in nonpolar organic solvents, and lipophilic, accumulating in the fatty tissues of terrestrial and aquatic biota including humans.[1] PCBs are generally relatively water-insoluble, with the highest solubilities among the ortho- congeners (5 mg/L for PCB1)[7], water solubility (Table 1.1) decreases as chlorination degree increases specially in ortho-vacant congeners as the para positions are filled.[7] PCBs are soluble in non-polar organic solvents and lipids[9], and the water to lipids solubility shift increases with increasing octanol-water partition coefficient (reported as Log K_{ow}).

Table 1.1. Water solubility and Log K_{ow} values for a few PCBs. Included PCB 1 to signify the different solubility values.

	PCB 1	PCB 77	PCB 105	PCB 118	PCB 156
No. chlornies	1	4	5	5	6
Position	non-ortho	mono-ortho	mono-ortho	mono-ortho	mono-ortho
Water solubility (mg/L, 25 ^{0}C)	4.83	0.175	0.0034	0.0134 20 ^{0}C	0.00533 20 ^{0}C
Log K_{ow}	4.53	6.63	6.50	7.12	7.60

Solubility and Log K_{ow} values were obtained from EPA EPI Suite.[10]

PCBs are unreactive and as a consequence have very long environmental half-lives. PCB half-lives of a few congeners in blood serum of exposed children have been reported ranging 3-4 years for PCB 138, 4.5-5.5 years for PCBs 105 and 118,

6.5-7.5 years for PCBs 156, 170 and 187, and 7-9 years for PCBs 153 and 180.[11] Seegal et al.,[12] reports half-lives of PCB congeners in former capacitor workers measured over a 28 year period and indicates greater half-lives for heavy vs. light occupational congeners (9.6 yrs vs. 17.8 yrs), also greater half-lives for women vs. men (19.8 yrs vs. 9.0 yrs). The historical record of PCBs in dated lacustrine sediment cores has shown concentrations reached maximum levels in the early 1950s and have declined since then, the most rapid decline occurred following 1979 when PCB industrial production was banned.[13] However, some areas such as Chesapeake Bay, have high enough levels to adversely affect the health of humans and wildlife and all of the Bay jurisdictions, Potomac River included, have water bodies identified as impaired for human consumption of fish.[14] PCBs are still regulated through the U.S. EPA Clean Water Act and remain listed as pollutants in the Chesapeake Bay Toxics of Concern.[15] To this day, PCBs undergo intensive environmental study as potential human toxicants. The products manufactured before the ban, which contain PCBs, are still in use, but registered with the EPA to ensure accurate response in cases of spills. Incidents involving PCB spills lead the list of accidental release of toxic organic chemicals to the environment.[16]

Toxicity of PCBs

PCBs are listed as probable human carcinogens by the Environmental Protection Agency (EPA) and the International Agency for Research on Cancer.[1] Agency for Toxic Substances and Disease Registry (ATSDR), in 2000, summarized

studies that reported lower birth weights of infants born to women who had consumed high amounts of PCB-contaminated fish. These infants had motor skill issues along with problems with short-term memory. A high accidental exposure to PCBs during fetal development or to dioxins in childhood reduces semen quality in adulthood.[16]

All 209 PCB congener are not equally toxic. The most toxic congeners are those that (i) either lack the ortho-substitution of chlorine entirely (i.e., 2, 2', 6 and 6' positions) or are mono-substituted in these positions, because these congeners have the ability to exist in planar ring conformations (i.e., both rings can lie flat in a single plane) and thus are more able to effectively intercalate into the structures of biomolecules such as DNA and proteins, and (ii) coupled with substitution of chlorine in the lateral positions (i.e., 3 3',5, 5' substituted) . Such PCB congeners are termed dioxin-like PCBs (dl-PCBs) because they resemble the structure of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) (Figure 1.2), considered the most toxic synthetic organic contaminant yet identified in environmental samples.[1] TCDD causes chloracne in humans as well as soft-tissue sarcomas, lymphomas and stomach carcinomas, and EPA has classified TCDD as a probable human carcinogen; group B2 category.[17] The International Agency for Research on Cancer (IARC) classifies TCDD as a Group 1 carcinogen to humans.[18]

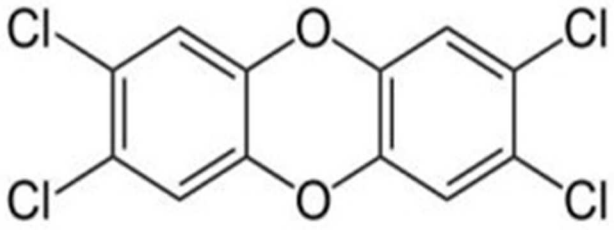


Figure 1.2. Structure of 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD)

Dioxins such as TCDD have half-lives in the human body estimated to be 7 to 11 years.[19] Twelve PCB congeners have been designated as "dioxin-like" (Table 1.1). Dioxin-like PCBs and TCDD both act through the aryl hydrocarbon hydroxylase receptor (AHR) to cause a full range of toxic responses such as changes in gene transcription.[20] AHR induces the transcription of the genes for aromatase. Aromatase catalyzes the last steps of estrogen biosynthesis from androgens. Specifically, it transforms androstenedione to estrone and testosterone to esteradiol.[21]

The dl-PCBs bind AHR and cause toxicity in fish, birds and mammals, but the mono-ortho dl-PCBs lack such effect in fish.[20] dl-PCBs are rated by their toxic equivalency factor[22] (TEF), which is based on the relative toxicity of each congener to the toxicity of TCDD in rats. TCDD has a TEF of 1.

Table 1.2. List of dl-PCBs.

IUPAC #	Homolog Group	IUPAC Name	TEF value
non-*ortho* substituted PCBs			
77	tetra-CB	3,3',4,4'-tetra-CB	0.0001
81*	tetra-CB	3,4,4',5-tetra-CB	0.0003
126*	penta-CB	3,3',4,4',5-penta-CB	0.1
169*	hexa-CB	3,3',4,4',5,5'-hexa-CB	0.03
mono-*ortho* substituted PCBs			
105	penta-CB	2,3,3',4,4'-penta-CB	0.00003
114	penta-CB	2,3,4,4',5-penta-CB	0.00003
118	penta-CB	2,3',4,4',5-penta-CB	0.00003
123	penta-CB	2,3',4,4',5'-penta-CB	0.00003
156	hexa-CB	2,3,3',4,4',5-hexa-CB	0.00003
157	hexa-CB	2,3,3',4,4',5'-hexa-CB	0.00003
167	hexa-CB	2,3',4,4',5,5'-hexa-CB	0.00003
189	hept-CB	2,3,3',4,4',5,5'-hepta-CB	0.00003

* Not included in this analysis.
TEF values obtained from World Health Organization.[22]

Environmental Occurrence of dl-PCBs

Dioxin-like PCBs occurrence in the environment is documented in numerous studies, although much less is known about the occurrence and distribution of dl-PCBs than total-PCBs. Nunes et al.,[23, 24] reported dl-PCBs detected in sediment and biota, with PCBs 118 and 105 as the dominant congeners. The total dl-PCB concentrations in sediments were 199 pg/g dwt and varied from 18.1-2,800 pg/g wwt in several fish species.

Ssebugere et al.,[25] reported dl-PCB detection frequency of 75.5% from Lake Victoria (East Africa) with average concentration of 136 pg/g dwt. An extensive study of occurrence of dl-PCBs in food items, ranging from vegetables to meats and dairies purchased from markets in Valencia region of Spain indicated PCB118

having the highest abundance among the samples and varied from 3.88 pg/g wwt (in vegetables) to 3467 pg/g wwt (in fish oil).[26] These studies all report PCB118 as the most abundance in biota as well as sediment followed by PCB105 and 156.

Study Objectives

The goal of the present study was to assess the ecological impacts of polychlorinated biphenyls in the upper tidal Potomac River associated with WWTP discharge. The primary objectives of the present study were (i) to quantify total-PCBs and dl-PCBs in bed sediments and biota from Hunting Creek and Gunston cove to evaluate the significance of dl-PCBs in the upper Potomac River, (ii) assess geospatial differences between two highly urbanized embayments, (iii) compare the fish and sediment profiles of dl-PCBs to identify differences in distributions between matrices, and (iv) determine whether sediment concentrations and profiles of dl-PCBs can be used to predict corresponding concentrations and profiles in fish species.

Materials and Methods

Sample Sites

Gunston Cove, a Y-shaped embayment (Figure 1.3) of the Potomac River, is located 20.0 km downstream from Washington DC. Gunston Cove has water area of about 5 km^2 and is formed from the confluence of Pohick and Accotink creeks.

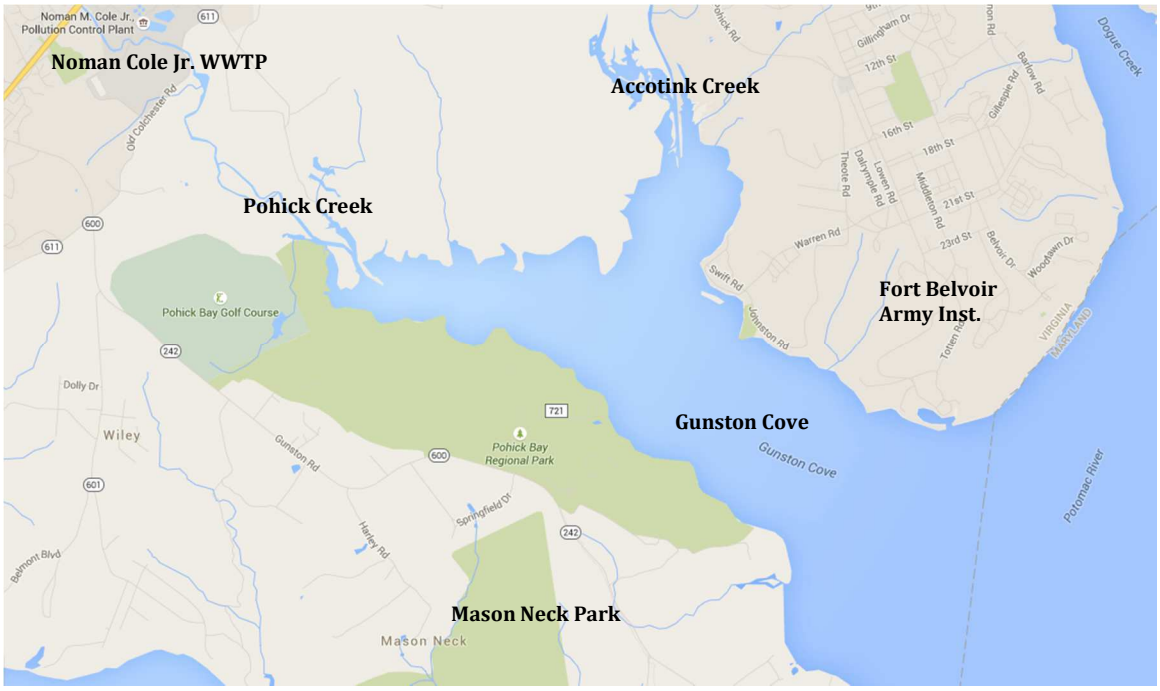


Figure 1.3. Gunston Cove location in relation to the WWTP and its boundaries.

The Cove forms the northern boundary of Mason Neck State Park and the southern boundary of Fort Belvoir (US Army base) in northern Virginia. Gunston Cove incorporates recreational parks, marinas, urban housing developments, Fort Belvoir, and the Noman Cole wastewater treatment plant (WWTP). Accotink Creek

watershed has an area of 132 square kilometers[27] and Pohick Creek watershed is smaller with an area of 95 square kilometers.[28] Half of Pohick Creek is forested and only 7.26% of the land use is commercial/industrial[29] while Accotink Creek is below 38% forested and has a higher percentage of commercial/industrial land use at 17.7% (Table 1.3).[30]

Table 1.3. Pohick creek and Accotink Creek land use.

Land use classification	Pohick Creek	Accotink Creek
	Percent	Percent
Forested	50.5	37.6
Field/Pasture	7.52	5.65
Low Intensity Residential	28.7	33.5
High Intensity Residential	0.06	0.02
Commercial/Industrial	7.26	17.7
Exposed Land	2.09	3.23
Wetlands	1.98	1.88
Open Water	1.85	0.47

Land use data obtained from Fairfax County.[30, 31]

The Noman Cole WWTP, the local treatment municipality, is located 10 km to the west of the Potomac River and discharges 17,000 m^3 (on average) of wastewater daily into Pohick Creek.[3] One of the major factors influencing pollutant emission into rivers is land use (Figures 1.4 and 1.5). Urban watersheds are known to release toxic pollutants, such as PCBs, into the aquatic environment during runoff events.[32]

As a major discharger of treated wastewater into the Gunston Cove and consequently into the Potomac River, Fairfax County has been proactive in reducing the nutrient loading into the freshwater embayment since the late 1970s. In such

effort at the request of Fairfax County an ecological study of the cove has been in effect since 1983 (Figure 1.6). The nearly 30 year record of data from Gunston Cove and the nearby Potomac River has revealed improved water quality and biological resources which validate the effectiveness of County initiatives to improve and aid in the continued management of the watershed and point source inputs.[33]

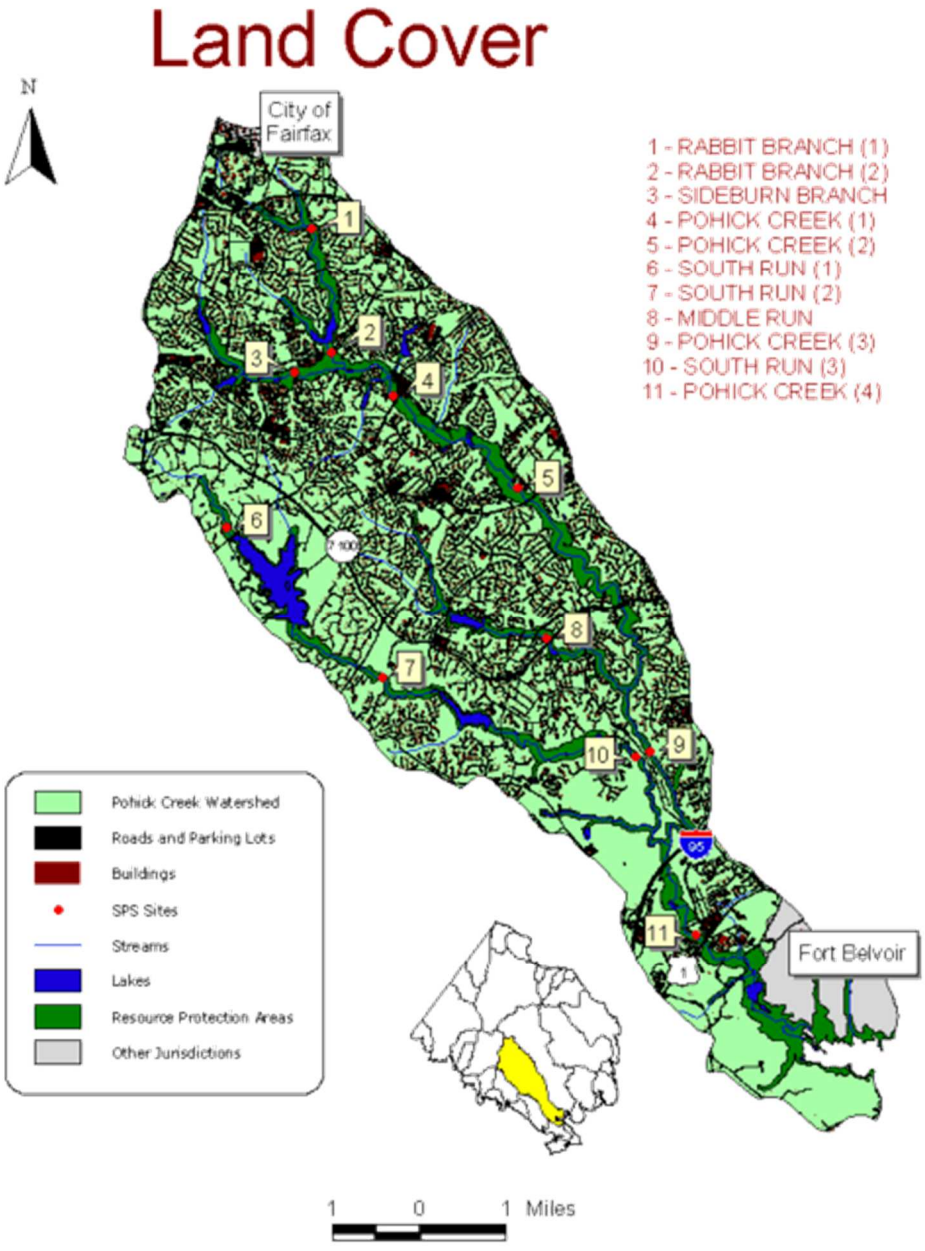


Figure 1.4. Pohick Creek land use map obtained from Fairfax County.[31]

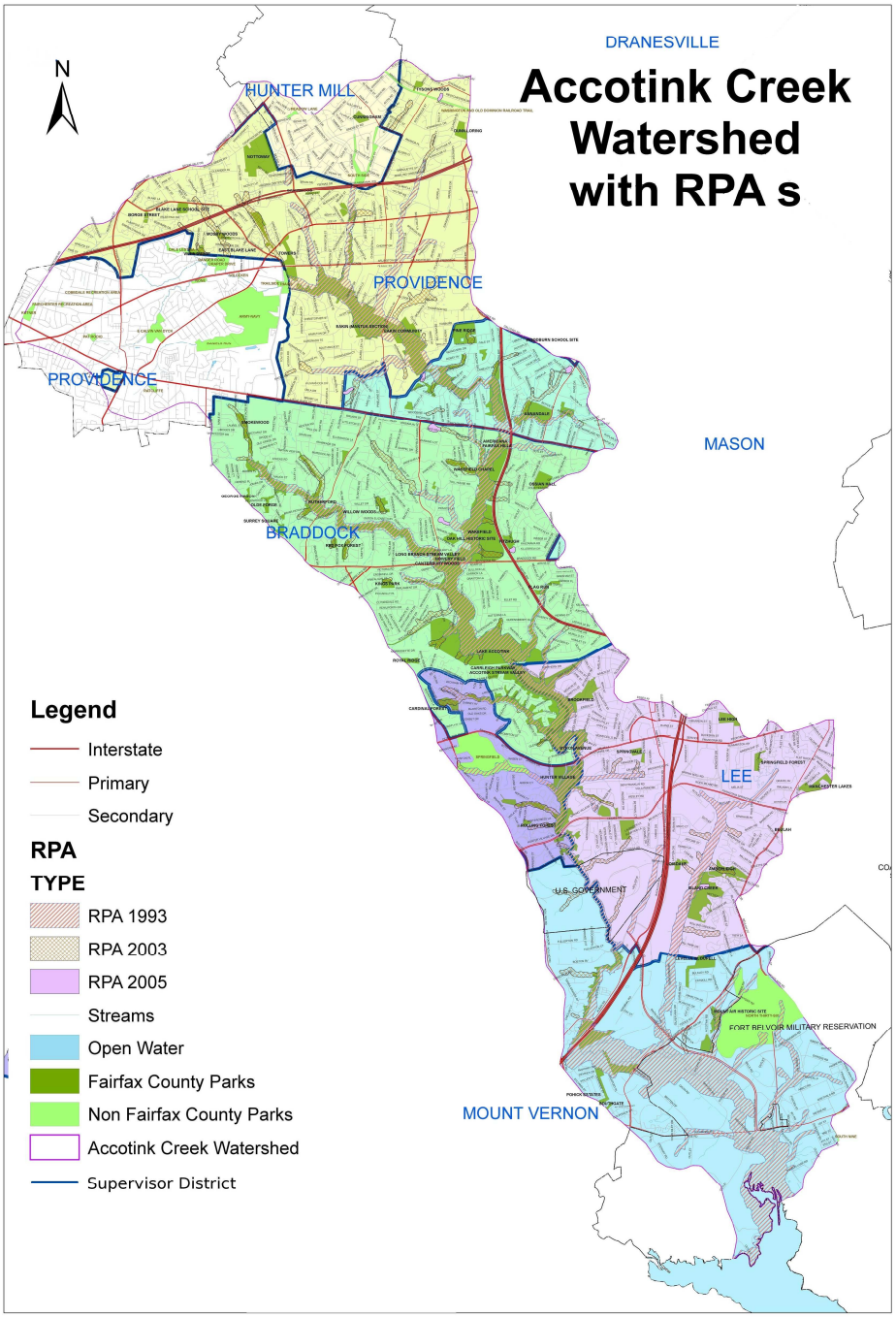


Figure 1.5. Accotink Creek land use map obtained from Fairfax County.[30]

Table 1.4. Coordinates of sampling sites for sediment and fish from Gunston Cove.

Collection Site	Abbrev.	Lat (deg min) N	Long (deg min) W
Gunston Cove 1	GC1	38 41.006	77 10.369
Gunston Cove 2	GC2	38 40.780	77 10.469
Gunston Cove 3	GC3	38 40.989	77 9.433
Gunston Cove 4	GC4	38 40.718	77 9.650
Gunston Cove 5	GC5	38 40.245	77 8.139

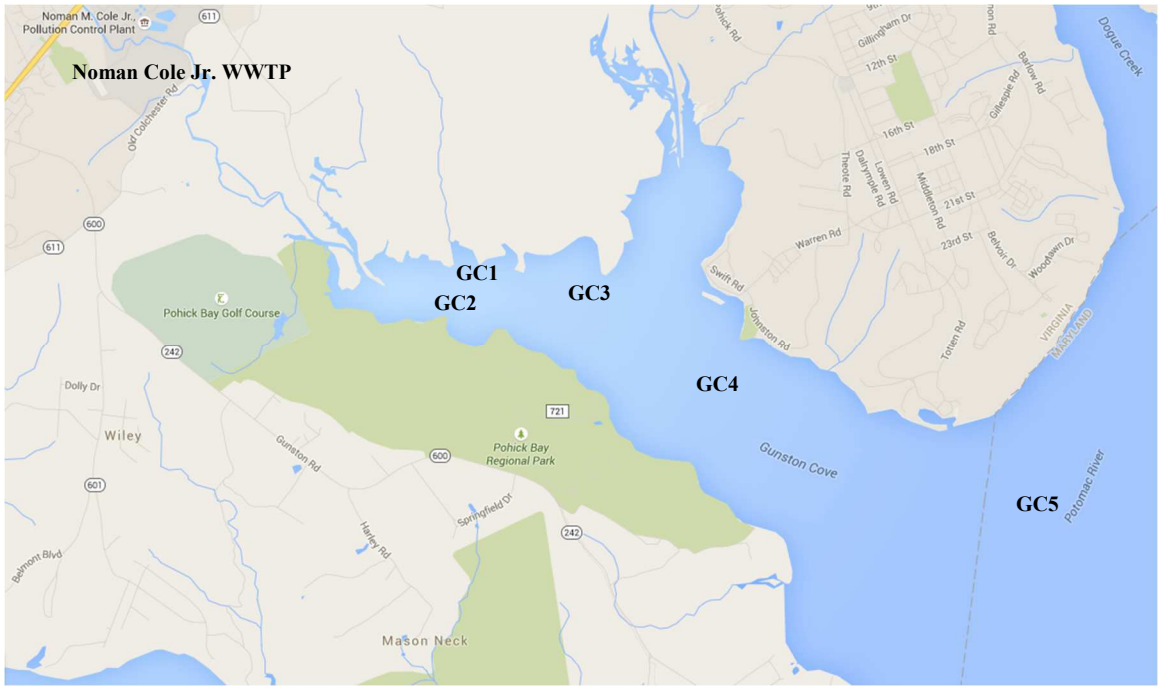


Figure 1.6. Sampling locations for sediment and fish from Gunston Cove.

Hunting Creek is a tributary of the Potomac River, formed by the confluence of Cameron Run and Hooff Run. Jones Point forms the northern boundary and the southern boundary is Dyke Marsh (Figure 1.7). Hunting Creek as a tributary in

Figure 1.7. Location of Hunting Creek relative to the WWTP and geographic boundaries.

Cameron Run watershed that is situated in a very populated area of which more than 50% consists of low to high density residential area as well as 13.6% combined commercial and industrial land usage[34] (Table 1.5).

Table 1.5. Cameron Run land use data.

Land use classification	Percent
Open space	13.7
Multi-family Common Area	3.60
Low Density Residential	16.0
Medium Density Residential	31.5
High Density Residential	5.10
Low Intensity Commercial	8.40
High Intensity Commercial	1.90
Industrial	3.30
Transportation	15.9
Open Water	0.60

Data obtained from Fairfax County.[34]

The Alexandria Renew Enterprises WWTP is located on the east shore of the Hunting Creek. Alexandria Renew discharges 150,000 m^3 (average) of wastewater daily.[2] In 2013 the Potomac Environmental Research and Education Center (PEREC) in collaboration with Alexandria Renew initiated a program to monitor water quality and biological communities in the Hunting Creek area including stations in the embayment itself and the adjacent river mainstem (Figure 1.8, Table 1.6).

Table 1.6. Coordinates of Hunting Creek sampling sites.

Collection Site	Abbrev.	Lat (deg min) N	Long (deg min) W
Hunting Creek 1	HC1	38 47.285	77 2.905
Hunting Creek 2	HC2	38 47.166	77 2.652
Hunting Creek 3	HC3	38 47.017	77 2.494
Hunting Creek 4	HC4	38 46.909	77 2.167
Hunting Creek 5	HC5	38 47.415	77 2.413
Hunting Creek 6	HC6	38 46.983	77 3.200

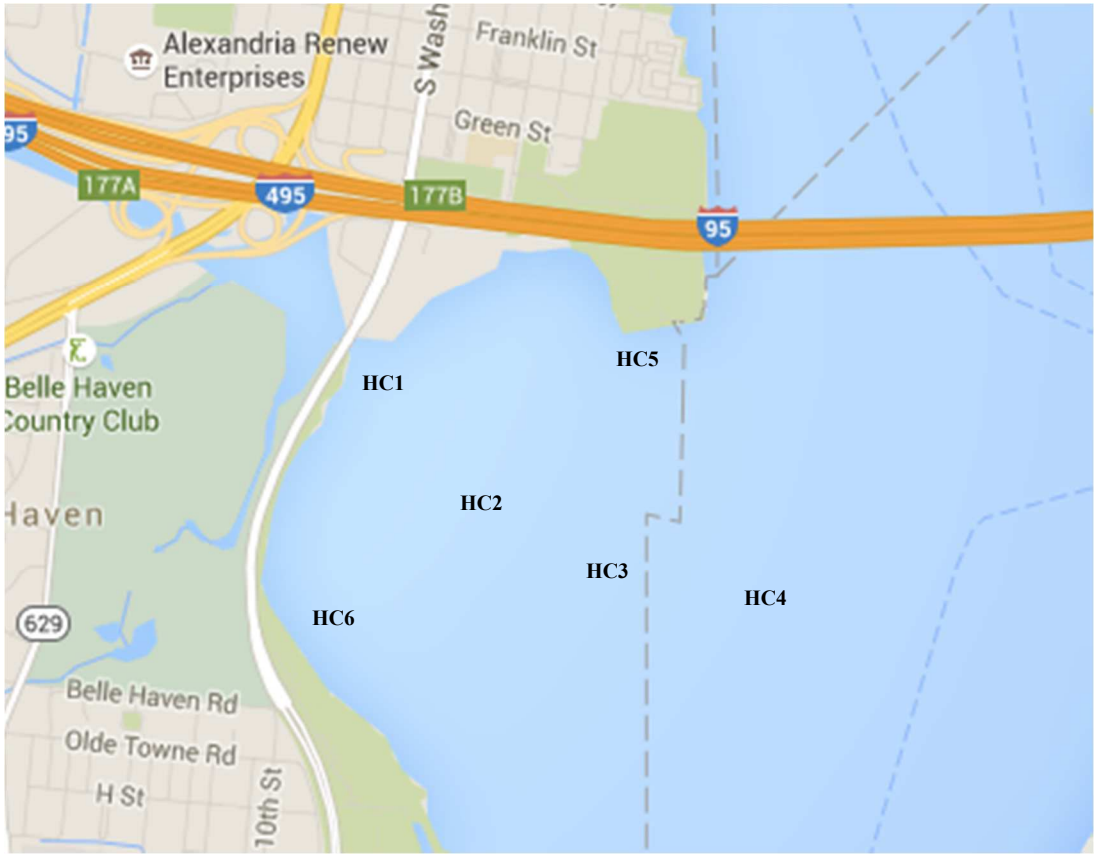


Figure 1.8. Hunting Creek sampling locations.

Field Sampling

Bed sediments from Gunston Cove, collected on May 22, 2012 (Table 1.4) and Hunting Creek, collected May-June 2013 (Table 1.6) were obtained by boat. Alluvial sediment was collected manually using a Petite Ponar grab sampler (Wildco, Saginaw, MI). Sediment obtained in the Ponar was expelled into a stainless steel tray onboard the boat, where approximately 10 g of the top surface layer was placed directly into ashed amber jars using a stainless steel spoon. The jars were sealed

with Teflon-lined lids and stored on ice for transportation. Upon return to the laboratory all samples were stored at -20°C until sample processing.

Gunston Cove fish (collected May 22, 2012) included six species, which were harvested using a 16.7 mm seine net at sites 2 and 3 (Figure 1.6), and by otter trawl net at sites 5 and 1. Fish species collected from Gunston Cove are typical to the mid-Atlantic tidal fresh water rivers.[35] Five of the six species included benthivorous intermediary trophic level species (level III) whose primary diets consist of benthic dwelling aquatic organisms (mosquito: *Culicidae*), midge fly (*Chironimidae*) larvae, oligocheate worms (*Oligocheata*), fingernail clams (*Sphaeriidae*), fish eggs and zooplankton.[35-37] These species included spottail shiner (*Notropis hudsonius*), mummichog (*Fundulus heteroclitus*), bluegill (*Lepomis macrochirus*), inland silverside (*Menidia beryllina*), and redbreast sunfish (*Lepomis auritus*). The sixth species, white perch (*Morone americana*), common in both sets of samples from Gunston Cove and Hunting Creek, is an important commercial and popular game fish in eastern North America. It is the most common fish found in Gunston Cove and Hunting Creek. White perch of the size range captured during sampling feed on a mixed diet of benthos, zooplankton and smaller fish and can be classified as trophic level III/IV.[35-37] Fish from Hunting Creek were collected (April-August 2013) using a 16.7 mm seine net at sites 5 and 6, and by otter trawl net at site 3 (Figure 1.8). Fish species collected from Hunting Creek are also typical to the mid-Atlantic tidal fresh water rivers. A total of five species, ranging from intermediary trophic level III to intermediary trophic level IV were harvested for chemical analysis. White perch,

spottail shiner and bluegill were also harvested in Hunting Creek and were similar in size of the Gunston Cove samples. The other two species, not present in the Gunston Cove samples, were banded killifish (*Fundulus diaphanous*) and striped bass (*Morone saxatilis*). Banded killifish are benthivorous and feed on small crustaceans, mollusks and worms.[35] Banded killifish are the most common species in Gunston Cove but coincidentally were not among the fish samples collected from Gunston Cove. Striped bass are predators on a variety of fish, but the size collected (2-4 cm), indicate a diet of crustaceans, and worms.[35, 37]

Table 1.7. Fish samples collected from Gunston Cove (GC) and Hunting Creek (HC)

		Species		
Date	Station	Common Name	Scientific Name	Trophic level
22-May-12	GC 2 and 3	Mummichog	*Fundulus heteroclitus*	3.6 ± 0.48
	GC 4	Spottail shiner	*Notropis hudsonius*	2.1 ± 0.09
	GC 2	Inland silverside	*Menidia beryllina*	3.2 ± 0.30
	GC 4 and 1	Bluegill	*Lepomis macrochirus*	3.2 ± 0.16
	GC 1	Redbreast sunfish	*Lepomis auritus*	3.2 ± 0.01
	GC 5	White perch	*Morone americana*	3.1 ± 0.35
10-Apr-13	HC 6	Banded killifish	*Fundulus diaphanous*	3.3 ± 0.17
	HC 6	White perch	*Morone americana*	3.1 ± 0.35
	HC 6	Bluegill	*Lepomis macrochirus*	3.2 ± 0.16
	HC 6	Spottail shiner	*Notropis hudsonius*	2.1 ± 0.09
8-May-13	HC 3	White perch	*Morone americana*	3.1 ± 0.35
	HC 3	Spottail shiner	*Notropis hudsomius*	2.1 ± 0.09
17-Jul-13	HC 6	White perch	*Morone americana*	3.1 ± 0.35
26-Jul-13	HC 5	Striped bass	*Morone saxatilis*	4.7 ± 0.25
21-Aug-13	HC 5	Striped bass	*Morone saxatilis*	4.7 ± 0.25

Trophic levels obtained from FishBase.[38]

Fish ranged in length from 5-20 cm and were tallied and separated according to species before being wrapped in ashed aluminum foil and placed on ice for transport to the laboratory.

Materials

Florisil and hydrochloric acid (ACS grade) were purchased from J.T. Baker (Philipsburg, NJ). Sodium sulfate was supplied by VWR International Inc. (Bridgeport, NJ). Solvents (hexane, isooctane, dichloromethane, methanol, ethyl acetate and toluene) were pesticide grade and purchased from Thermo Fisher Scientific (Rockville, MD). The Internal Standards, used for GCMS quantitation, consisted of 2,4,6-trichlorobiphenyl (PCB 30), and 2,2',3,4,4',5,6,6'-octachlorobiphenyl (PCB 204), supplied by AccuStandard Inc. (New Haven, CT). Surrogate Standards were also supplied by AccuStandard Inc. and included 2,2',4,5',6-pentachlorobiphenyl (PCB 103), and 2,2',3,4,4',6-hexanchlorobiphenyl (PCB 140). All the standards were in hexane. PCBs were purchased from AccuStandard Inc. as four prepared congener mixtures dissolved in isooctane. A working mixture containing the analytes was prepared at 400 pg/L for the analysis.

GCMS consumables were purchased from Agilent Technologies (Santa Clara, CA). Laboratory grade water was produced in house by a Corning Megapure quartz element immersion still. All glassware used for sample and preparation are cleaned by washing with soap, rinsing with distilled water followed by double distilled water, and ashed at 400 °C overnight. All laboratory materials are made of glass,

stainless steel or Teflon to avoid sample contamination. The Teflon materials are cleaned the same way as glass, but without ashing. All materials, both glass and Teflon, are rinsed with methanol and air dried before use.

Sample Processing

Composite homogenates for each fish species were prepared following EPA method 823B with some modifications. Preserved fish were thawed initially. Several whole fish were combined and homogenized in a pre-cleaned, according to the protocol for glassware cleaning, with the exception of ashing and rinsed copiously with methanol and hexane, die-cast 10-speed blender (Oster, Boca Raton, FL). The composite fish tissue homogenates were stored at -20 ^{0}C prior to extraction and cleanup. Aliquots of the composite fish tissue homogenate were processed for gravimetric total lipid determination according to the method of Ramalhosa et al. [39]

PCBs were extracted from fish homogenate using microwave-assisted saponification (MAS) (MARS, CEM Corp., Matthews, NC). This method (Figure 1.9) is a combination of EPA Method 3546 and Xiong et al.[40] In summary, wet tissue homogenate (~ 1 g) was thawed and ground to a fine powder after desiccating with a 3:1 (wt/wt) ratio of anhydrous sodium sulfate using mortar and pestle. The dry tissue mixture was transferred into a 100 mL GreenChem MARS extraction vessel, filled partially with ~10 mL of 1 M potassium hydroxide in methanol, and spiked with 120 ng of surrogate standards (PCB 103 and 140). The MAS of tissue was conducted in the MARS at 100 ^{0}C for 15 minutes at 600 Watts. Following MAS, the

vessels were cooled and the liquid phase was decanted into a clean, ashed 60 mL conical centrifuge tube. The MAS extractions were completed separately in triplicate and the individual extracts were combined. The combined MAS extracts were subsequently back extracted sequentially three times with 25 mL of hexanes. The back extraction mixture was vortexed for 10 seconds and then allowed to separate into two phases. The upper phase (hexane) from all three back extractions were transferred to a clean 60-mL centrifuge tube and solvent volume reduced to ~1.0 mL using an N-VAP model 112 nitrogen evaporator (Organomation Associates Inc., Berlin, MA).

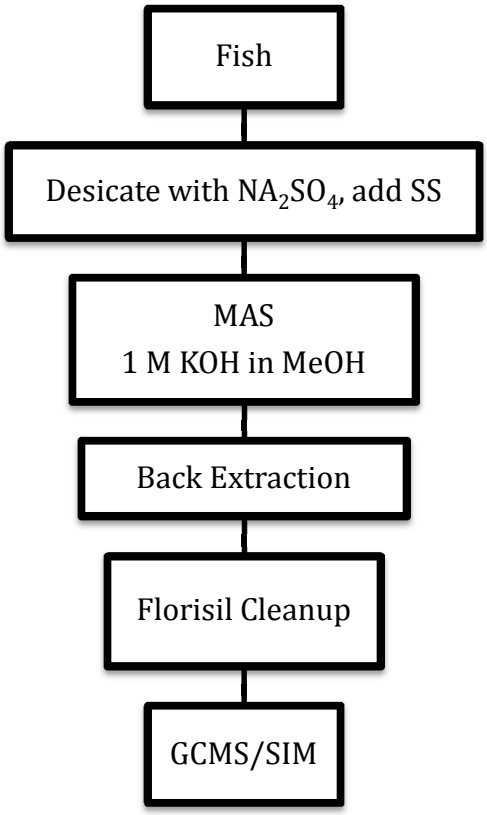


Figure 1.9. Microwave assisted saponification (MAS) method for fish.

PCBs in sediment were extracted using EPA Method 3546 modified according to Navarro et al.,[41] (Figure 1.10). Sediments were thawed and dewatered by centrifuging at 1500 rpm (International Equipment Company Model H N, Needham Heights, MA). Sediment PCB extraction was carried out using microwave assisted extraction (MAE). Briefly, 5 g of dewatered sediment along with 5 g anhydrous sodium sulfate, as desiccant, were added into a 100 mL MARS vessel along with 5.6 ng of surrogate standards (PCB 103 & 140) and mixed with a metal spatula. Extraction (100 ^{0}C, 600 watts, 15 minutes) was performed with 20 mL of 1:1 (v/v)

hexane:acetone in triplet. The sediment and fish MARS extraction programs were identical. The resulting three sequential extracts were combined into a 60-mL conical centrifuge vial and solvent exchanged with hexane and solvent volume reduced to ~1 mL using the N-VAP.

Both sediment and fish extracts were subjected to cleanup using Florisil chromatography, following EPA Method 3620C, to remove matrix substances interfering with GCMS analysis. The Florisil column was constructed by packing a stoppered glass chromatography column from bottom to top with 2 g of anhydrous sodium sulfate, 6 g of 2% (v/w) deactivated Florisil, and another 2 g of anhydrous sodium sulfate. The contents of the column were initially rinsed with 100 mL of hexane prior to loading the extracts.

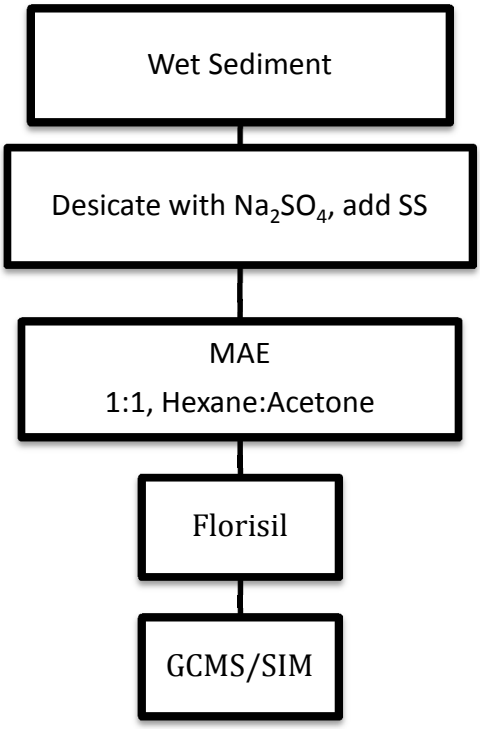


Figure 1.10. Microwave assisted extraction method for sediment

PCB analytes were eluted using 75 mL of hexane. The eluents for both bed sediment and fish tissue were solvent exchanged with toluene and solvent volume reduced to ~0.5 mL using the N-VAP, transferred to 1-mL GCMS vials and spiked with 5.6 ng of internal injection standards (PCB 30 and 204) prior to analysis.

GCMS Analysis

Quantitative analysis of PCBs was performed utilizing an Agilent (Agilent Technologies, Santa Clara, CA) 7890A gas chromatograph (GC) coupled to a 5975C mass spectrometer (MS). The GCMS was fitted with an Agilent DB-5 (5% biphenyl

95% dimethylsiloxane) 30 m x 0.25 mm (ID), with a 0.25 μm coating of bonded stationary phase, fused silica capillary column with He (Ultra High Purity Grade) as the mobile phase carrier gas. The GCMS was equipped with a multi-mode inlet (MMI), which enabled programmable temperature vaporizing injection (PTV), fitted with an Agilent inert dimpled injection liner (Agilent part number 5190-2296). To enhance detection, a 10 μL large volume injection (LVI) was employed using an Agilent model G4513A auto-injector in the PTV mode. The initial inlet temperature was 100 °C, after 0.25 minute the inlet temperature was raised by 600 °C/min to 280 °C. The initial oven temperature was 90 °C, held for 1 minute, then temperature ramped at 60 °C/min to 150 °C, followed by 5 °C/min to 300 °C, with a constant column flow of 2.5 mL/min. The temperature of transfer line, ion source and quadruple were held constant at 290 °C, 230 °C and 150 °C, respectively. The mass spectrometer was operated in selected ion monitoring (SIM) mode with the targeted ions for analytes and the corresponding congeners listed in (Table 1.8). Nine of the twelve dl-PCBs were analyzed in this study as well as total PCBs, (Table 1.2) and therefore the focus of the study will be on these nine dl-PCBs in comparison to the total-PCBs. The missing dl-PCBs are PCB 81, PCB 126 and PCB 169.

Table 1.8. ΣCB homolog classes and corresponding target ions.

Homolog class	Quant Ion	Qual Ions
ΣMonochlo	188	152, 153, 190
ΣDichloro	152[a], 222[b]	151, 152, 187, 222, 224
ΣTrichloro	186[c], 256[d]	150, 186, 221, 256, 258, 260
ΣTetrachloro	220[e], 292[f]	222, 290, 292, 294
ΣPentachloro	326	254, 256, 324, 328
ΣHexachloro	360	288, 290, 358, 362
ΣHeptachloro	394	324, 326, 396, 398
ΣOctachloro	430	358, 428, 432
ΣNonachloro	464	392, 462, 466
ΣDecachloro	498	428, 496, 500

[a] PCBs 4 & 10.
[b] PCBs 5,6,7,9,12 & 15.
[c] PCBs 17,18 & 19.
[d] PCBs 16,20,22,24,25,26,27,28,29,31,32,33,34 & 37.
[e] PCBs 40 & 46.
[f] PCBs 41,42,44,45,47,48,49,52,56,59,60,63,64,66,67,69,70,71,74 & 77.

Calibration standards were evaluated through ChemStation (version E.02.02.1431) using internal standard injections standards at 8 concentration levels, using PCB 30 and 204 as internal standards. PCBs were quantitated using the evaluated calibration standards. Co-eluting congeners' amounts were added together in the compound database since one peak would correspond to two or three PCB congeners. Total PCBs (tPCBs) were determined by summation of homologs (Table 1.9).

Table 1.9. List of PCB congeners measured in the Gunston Cove and Hunting Creek samples by GCMS

Number of Chlorines	CAS Structural PCB Number	Number of Congeners
1	1, 2, 3	3
2	4, 5, 6 7, 8, 9, 10, 12, 15	9
3	16, 17, 18, 19, 20, 24, 25, 27, 28, 29, 30[a], 31, 32, 33, 34, 37	16
4	40, 41, 42, 44, 45, 46, 47, 48, 49, 52, 56, 59, 60, 63, 64, 66, 67, 69, 70, 71, 74, (77)[c]	22
5	82, 83, 84, 85, 87, 91, 92, 93, 95, 97, 99, 101, 103[b], 104, (105), 109, 110, (114), 115, (118), 119, (123), 137	23
6	(128), 129, 131, 132, 134, 135, 136, 138, 140[b], 141, 144, 147, 149, 151, 153, (156), (157), 158, 164, (167)	21
7	170, 171, 172, 173, 174, 176, 177, 178, 179, 180, 183, 185, 187, (189), 190, 191, 193	17
8	194, 195, 196, 197, 199, 203, 204[a], 205, 206, 207, 208	8
9	206, 207, 208	3
10	209	1
	Total Number of Congeners	**123**

[a] IS = Internal Injection Standard

[b] SS = Surrogate Standard

[c] Dioxin-like PCB congeners listed in parenthesis

Quality Assurance

Analytical limits of detection (LODs) for the Gunston Cove fish and sediment samples were determined by multiplying the standard deviation of 10 replicate runs of the least concentrated calibration standard (0.80 pg/L) by the Student t-test for the 95% confidence level (± 0.02, range of 0.74-0.76). To determine the method detection limits (MDLs) in ng/g units for both fish tissue and bed sediment samples the calculated values (0.03-0.86 ng) were then divided by the perspective sample mass (fish 1 g, bed sediment 5 g). The individual LODs are presented in Appendix A. Analytical LODs for the Hunting Creek fish and sediment samples were determined same as the Gunston Cove samples according the above mentioned protocol and presented in Appendix A.

Method performance was assessed from recoveries of individual analytes spiked into the matrices. Recoveries evaluated extraction, cleanup efficiency and analyte recovery through varying spike levels in fish and sediments. The Gunston Cove and Hunting Creek recovery percentages in both fish tissue (MAS method) and bed sediment (MAE method) are presented in Appendix A. Gunston Cove surrogate recoveries of PCB 103 and PCB 140 ranged from 88% ± 8% and 94% ± 7% respectively in fish tissue and from 95% ± 2% and 72% ± 5% respectively, in bed sediment. The blank concentrations from Gunston Cove ranged from below detection limit (<DL) to 6.11 ng/g dwt (dry weight refers to the corrected sediment weight once the water content has been subtracted from the wet weight of the sample) for total PCBs (tPCBs) in fish tissue and <DL to 21.9 ng/g dwt for tPCBs in

bed sediment, which were consequently subtracted from the native sample concentrations in each sample.

Hunting Creek surrogate standard recoveries of PCB 103 and PCB 140 ranged from 81% ± 5% and from 84% ± 4% respectively in sediment and 83% ± 9% and 83% ± 10% respectively, in fish tissue. Blanks for sediments showed below detection limits (0.01 ng/g dwt for the individual congeners) except for congeners 153, 180, 128, 118, 189 and 196, which were subtracted from the sample concentrations. Sample chromatograms from fish, sediment and calibration standard are presented in figures 1.11, 1.12 and 1.13 respectively.

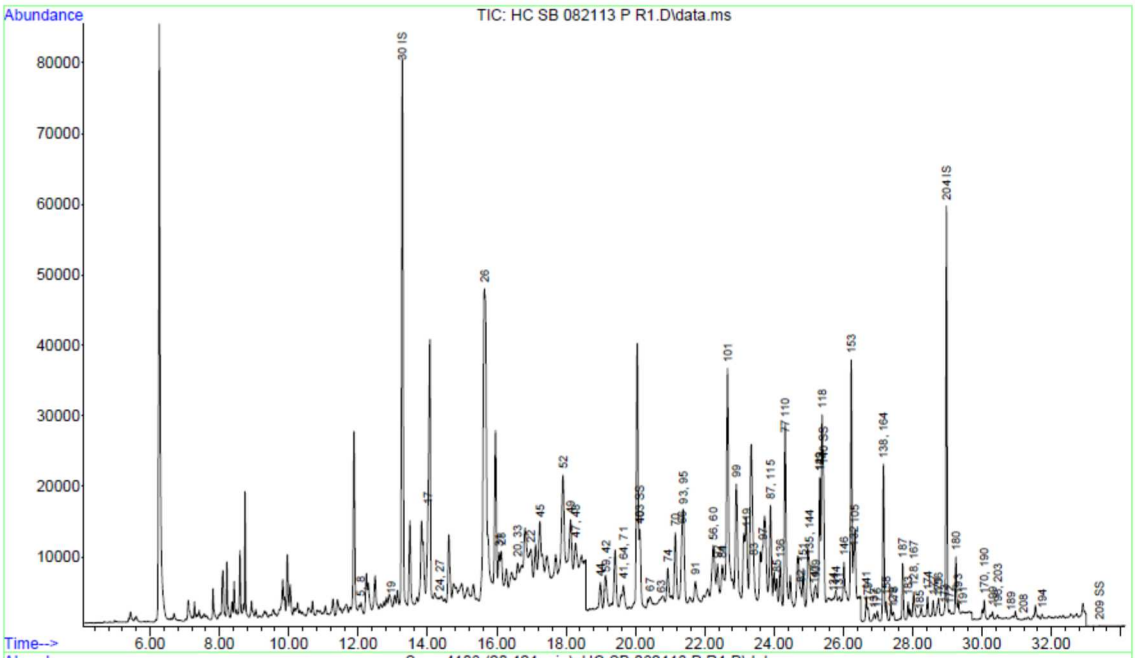


Figure 1.11. Fish sample chromatogram. Striped bass from Hunting Creek.

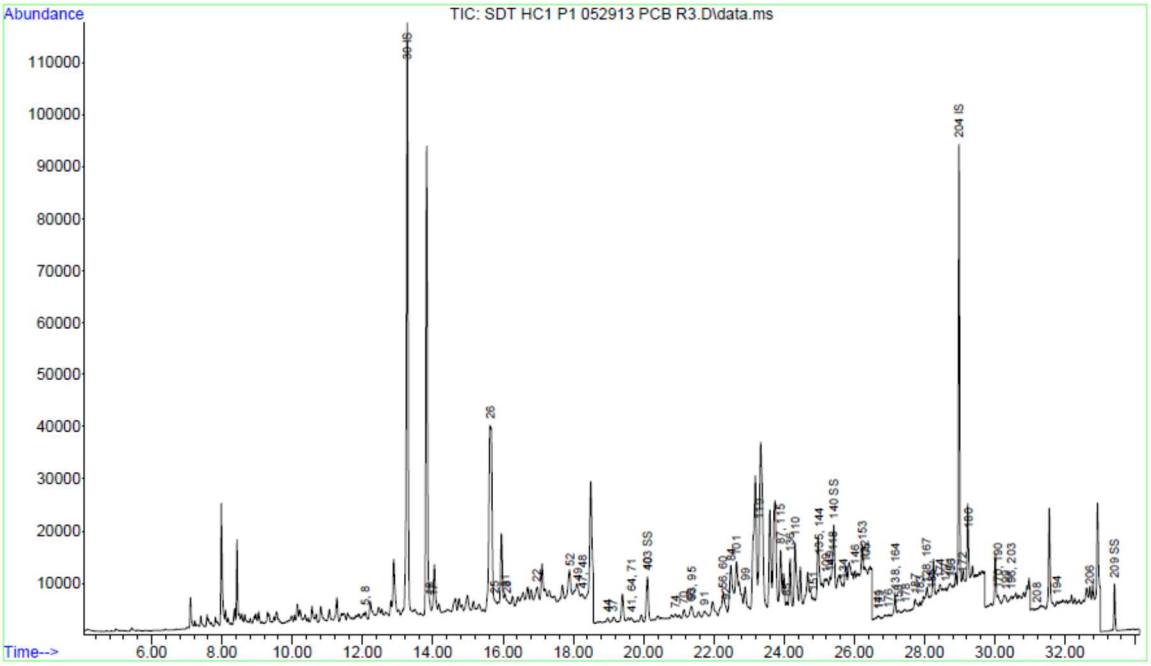


Figure 1.12. Sediment sample chromatogram. Sediment from HC1, Hunting Creek.

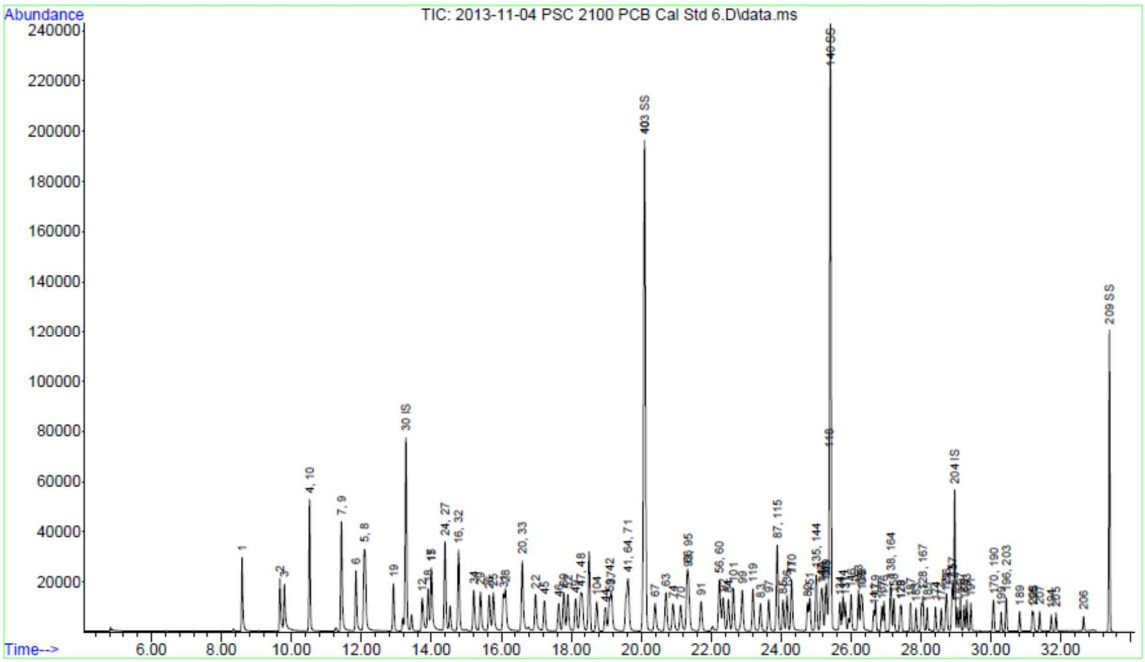


Figure 1.13. Calibration standard chromatogram.

Ancillary Measurements

Ancillary measurements were conducted on bed sediment to determine grain-size[42], moisture content[42] and organic carbon composition. Organic carbon content of the sediment was determined using a soil CN Analyzer (Flash 2000, ThermoScientific, Waltham, MA). Approximately 1 g of sediment was oven dried and ground to a fine powder using mortar and pestle. The ground sample was transferred to a silver combustion cup and treated with 1 M HCl to degas carbon dioxide. Degassed sediment was re-dried and analyzed for organic carbon content using aspartic acid as the reference standard. All sediment samples were analyzed in triplicate.

The average lipid content of homogenized fish was determined by MAS and gravimetric analysis of the residue upon complete evaporation. Evaporation of the tissue extracts in a pre-weighted vial was performed to determine the amount of lipid present in 1 g of tissue sample.

The lipid content of each fish species will be used to lipid normalize the concentrations. Lipid normalization will be obtained by dividing the detected amount of PCB congener (ng) by the amount of lipid present in the processed fish tissue (g).

The organic carbon (OC) content of sediments will be used to organic carbon normalize the concentrations. OC normalization will be obtained by dividing the detected amount of PCB congener (ng) by the amount of organic carbon present in the processed sediment (g).

Fugacity Estimates and Equilibrium Assessment

To determine the equilibrium distribution of dl-PCBs between fish and sediment fugacity theory was used. Fugacity (F) is defined as the escaping tendency of a chemical in a phase, or its vapor pressure, and is calculated in pressure units (atm) for sediments and fish using phase-specific Henry's law constants (atm-kg/mol) in conjunction with mean individual dl-PCB congener concentrations (kg/mol) measured by GCMS (Equation 1.1).[43] Mean concentrations of individual dl-PCB congeners in sediments and fish were converted from ng/g to mol/kg by using the molar mass of the PCB congeners.

$$F_{ij} = K_{H\,i,j} \times C_{i,j} \qquad \text{Equation 1.1}$$

$F_{i,j}$ = Fugacity of i^{th} chemical in j^{th} phase (atm), phase equivalent vapor pressure
$K_{H\,i,j}$ = Henry's Law Constant of i^{th} chemical in j^{th} phase (atm.kg/ml)
$C_{i,j}$ = Concentration (mol/kg) of i^{th} chemical in j^{th} phase measured by GCMS

Phase specific Henry's law constants (atm.kg/mol) for sediment and fish were estimated using equation 1.2, adapted from Schwarzenbach et al.[43].

$$K_{H\,i,j} = \frac{K_{H_i}}{K_{d_{i,j}}} = \frac{K_{H\,i}}{(\alpha_j \beta_j) K_{ow\,i}} \qquad \text{Equation 1.2}$$

$K_{H\,i,j}$ = Henry's Law Constant of i^{th} chemical in j^{th} phase (atm.kg/ml)

$K_{d\,i,j}$ = Distribution constant of i^{th} chemical in j^{th} phase (L/kg)
α_j = Constant for j^{th} phase (fish=1, sediments=0.41)
β_j = Phase constant (fish=f_L=lipid fraction, sediment=f_{oc}= organic carbon fraction)
$K_{ow,i}$ = Water octanol partition coefficient (K_{ow}) of i^{th} chemical
$K_{H,i}$ = Henry's law constant of i^{th} chemical (atm.kg/mol)

Individual K_H and K_{ow} values were determined either from EPA EPI suite[10] or obtained from MacKay et al.,[44] and presented in Appendix A. MacKay et al. offered several values for air-water K_H and K_{ow}, which were either calculated using Quantitative Structure Property Relationship (QSPR) or determined experimentally. The experimentally determined values were significantly different and were not considered for this analysis. Mean value of the QSPR calculated constants were utilized in all of the fugacity calculations.

To determine equilibrium or direction of flow, fish fugacity was divided by sediment fugacity of the corresponding sampling location. A fugacity ratio ($F_{fish}/F_{sediment}$) of 1 is accepted as equilibrium partitioning. In this study a ratio between 0.5 to 1.5 was considered equilibrium. A >1.5 ratio indicates fish to sediment flow of analytes and a <0.5 ratio indicates sediments to fish flow.

Results

Ancillary Data

A summary of ancillary measurements for Hunting Creek and Gunston Cove sediments is presented in Table 1.10. The %sand content of Hunting Creek sediments varied from 56.3%(± 2.52%) at HC1, to 8.33% (± 0.58%) at HC3. The %sand content of Gunston Cove sediments varied from 12.0% (±3.31%) at GC4 to 15.4% (±0.71%) at GC1.

Sediment %moisture contents varied from 56.7% (±1.21%) at GC4 to 58.1% (±1.20%) at GC1 for Gunston Cove sediments. Sediment %moisture contents varied from 42.0% (±1.00%) at HC1 to 54.7% (±0.58%) at HC3 for Hunting Creek sediments.

Table 1.10. Ancillary data for Hunting Creek and Gunston Cove sediment samples.

Date	Station	% Moisture	% Sand	%Silt/Clay[a]	% OC
14-May-13	HC 2	45.2 ± 8.30	14.7 ± 1.15	83.0 ± 1.06	3.90 ± 0.01
22-May-12	GC 4	56.7 ± 1.21	12.0 ± 3.31	88.0 ± 3.29	2.81 ± 0.11
	GC 1	58.1 ± 1.20	15.4 ± 0.71	84.6 ± 0.70	3.52 ± 0.10
29-May-13	HC 1	42.0 ± 1.00	56.3 ± 2.52	50.0 ± 2.69	2.58 ± 0.01
12-Jun-13	HC 3	54.7 ± 0.58	8.33 ± 0.58	92.0 ± 0.11	3.65 ± 0.01

OC: organic carbon

[a] %Silt/Clay was tabulated by subtracting %sand from 100.

Percent organic carbon (%OC) was below 4% in sediment at all sites in Hunting Creek and Gunston Cove. Gunston Cove and Hunting Creek had similar %OC in sediments and ranged from 2.58% ± 0.01% to 3.90% ± 0.01%.

Gunston Cove station GC1 displayed higher % sand content compared to GC4, as seen in Table 1.10. Typically higher % sand corresponds to higher water content and lower organic carbon content.

The % total lipid varied among species with the lowest lipid content observed in bluegill from site HC6 (1.29% ± 0.11%) and highest in spottail shiner (7.52% ± 0.33%) from HC3 (Table 1.11). Fish species collected from Gunston Cove displayed a narrower % lipid ranging from 2% to 6%, while Hunting Creek species had a wider range, from 2% to 8%. Ashley et al.,[45] reported lipid content for several fish species collected from Delaware, including white perch (whole fish content) ranging from 4.3% to 10.3%. Henderson and Tocher reported lipid content for whole fish bluegill of 5.7% ± 2.2%.[46]

Table 1.11. Total lipid percent for fish species harvested from Gunston Cove and Hunting Creek.

	Species		
Station	Common Name	Scientific Name	% Total Lipids
GC 2 and 3	Mummichog	*Fundulus heteroclitus*	3.33 ± 0.11
GC 4	Spottail shiner	*Notropis hudsonius*	4.72 ± 0.32
GC 2	Inland silverside	*Menidia beryllina*	2.14 ± 0.29
GC 4 and 1	Bluegill	*Lepomis macrochirus*	4.71 ± 0.34
GC 1	Redbreast sunfish	*Lepomis auritus*	3.71 ± 0.11
GC 5	White perch	*Morone americana*	6.13 ± 0.20
HC 6	Banded killifish	*Fundulus diaphanous*	2.70 ± 0.17
HC 6	White perch	*Morone americana*	2.31 ± 0.26
HC 6	Bluegill	*Lepomis macrochirus*	1.29 ± 0.11
HC 6	Spottail shiner	*Notropis hudsonius*	1.80 ± 0.22
HC 3	White perch	*Morone americana*	4.45 ± 0.31
HC 3	Spottail shiner	*Notropis hudsomius*	7.52 ± 0.33
HC 6	White perch	*Morone americana*	3.49 ± 0.32
HC 5	Striped bass	*Morone saxatilis*	2.15 ± 0.16
HC 5	Striped bass	*Morone saxatilis*	1.70 ± 0.25

PCBs in Sediments

The mean total-PCB (tPCB) concentration detected in Gunston Cove sediments was 39.0 ± 0.5 ng/g dwt, while the mean tPCB concentration found in Hunting Creek sediment was 35.5 ± 1.1 ng/g dwt. The mean total PCBs sediment concentrations at GC4 and GC1 were 42.3 ± 0.51 and 35.8 ± 0.56 ng/g dwt, respectively. The mean total PCBs sediment concentration at HC1, HC2 and HC3 were 0.49 ± 0.16, 0.20 ± 0.0.4 and 1.84 ± 0.1.06 ng/g dwt, respectively. There were

no significant differences between mean tPCB concentrations (ng/g dwt) of Hunting Creek and Gunston Cove sediments (one-way ANOVA, $p>0.05$) (Figure 1.14).

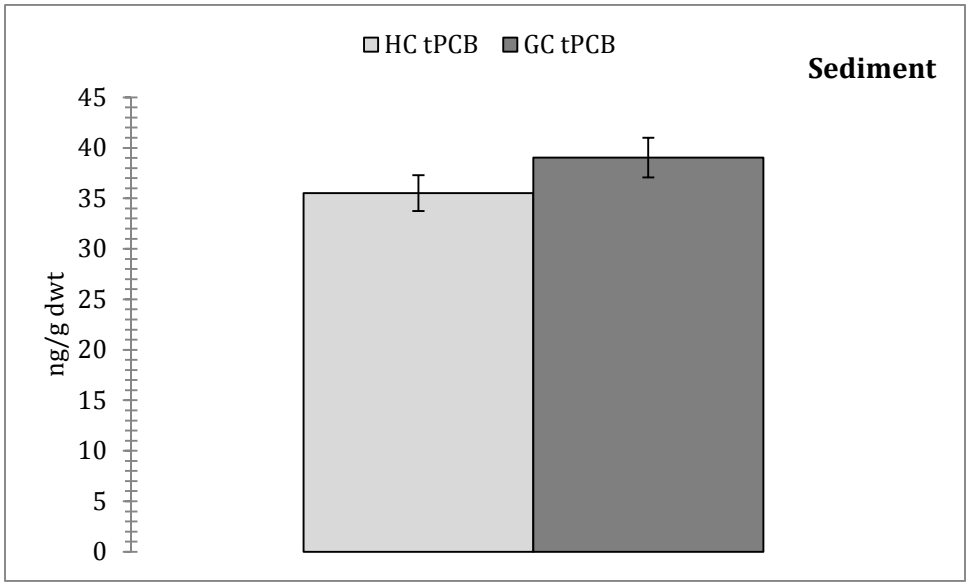


Figure 1.14. Sediment mean total-PCB concentrations (ng/g dwt) found in Hunting Creek and Gunston Cove

The sediment PCB homolog profiles (Figure 1.15) of both embayments indicated penta-, hexa- and hepta-chloros were the most prominent congener class of PCBs. Homolog concentrations are determined by summing individual PCB congener concentrations (ng/g dwt) of each homolog class. For instance, total PCB concentrations (ng/g dwt) of monochlorinated biphenyl congeners are added to yield the concentration (ng/g dwt) of mono-chloro homologs. The highest homolog concentrations in Hunting Creek was observed in penta-chloro PCBs while Gunston Cove sediment was dominated by hexa- and hepta-chloro PCBs. Homolog profiles

are used to assess if a specific homolog class is derived from an Aroclor mixture. Aroclors 1242, 1254 and 1260 dominated the market and were most used. Aroclor 1260, by definition is 60% chlorine by weight and is compromised of the heavier penta-, hexa-, hepta- and octa-chlorinated biphenyls (Table 1.12). Comparing

Table 1.12. Percent homolog abundance in Aroclor 1242, 1254 and 1260.

% Homolog Abundance	Aroclor 1242	Aroclor 1254	Aroclor 1260
Dichloro	14.7	0.0	0.0
Trichloro	46.0	1.8	0.0
Tetrachloro	30.6	17.1	0.0
Pentachloro	8.7	49.3	9.2
Hexachloro	0.0	27.8	46.9
Heptachloro	0.0	3.9	36.9
Octachloro	0.0	0.0	6.3
Nonachloro	0.0	0.0	0.7
Decachloro	0.0	0.0	0.0

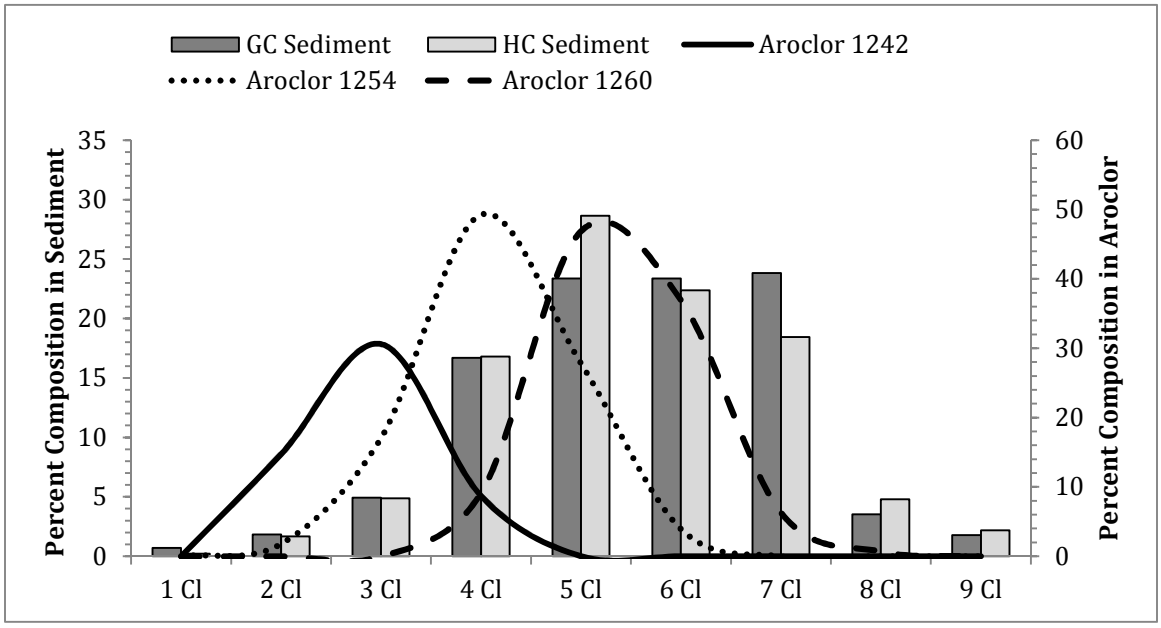


Figure 1.15. Percent homolog composition in sediments of Hunting Creek (HC) and Gunston Cove (GC), compared to percent composition in Aroclors.

percent homolog composition in Hunting Creek and Gunston Cove sediments with percent composition in Aroclors (Figure 1.15) indicates Aroclor 1260 and Aroclor 1254 as the possible sources, and displaying higher percentage of penta-, hexa- and hepta-chlorinated biphenyls points to higher abundance of Aroclor 1260.

The dioxin-like PCB (dl-PCBs) mean individual concentrations from Hunting Creek and Gunston Cove showed significant differences (one-way ANOVA, $p<0.05$) (Figure 1.16). PCB 114 was not detected in Hunting Creek sediments and the most prominent congeners in Hunting Creek sediments were PCB 105, 118 and 167. The

most prominent dl-PCB congeners from Gunston Cove sediments were PCBs 118 and 123, followed by PCB 105 and 114.

Interestingly, mean tPCB concentrations (ng/g dwt) (Figure 1.14) and percent homolog distribution patterns (Figure 1.15) were similar among sediments in the two embayments, while the individual mean individual dl-PCB concentrations (ng/g dwt) differed significantly (Figure 1.16).

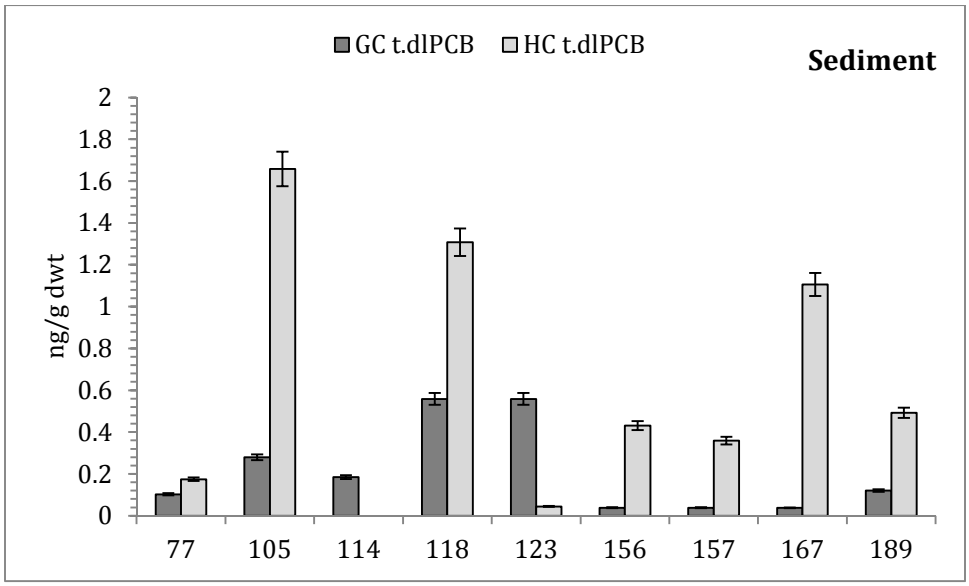


Figure 1.16. Total dl-PCB mean concentrations (ng/g wwt) in sediment from Hunting Creek and Gunston Cove.

The sediment individual dl-PCB concentrations from Hunting Creek and Gunston Cove varied from below detection to 1.66 ± 0.35 (ng/g dwt). Mean individual dl-PCB concentrations (ng/g dwt) in sediments from Hunting Creek and Gunston Cove were significantly different between the two embayments as well as

between individual sampling sites from both Hunting Creek and Gunston Cove (one-way ANOVA, $p<0.05$).

Total PCB and total dl-PCB congener concentrations were normalized to organic carbon contents, and the highest normalized concentration in Gunston Cove sediments was observed for PCBs 118 and 123 (18.0 ± 3.95 ng/g OC) and highest concentration in Hunting Creek sediments was observed for PCB 105 (54.9 ± 1.39 ng/g OC). Hunting Creek sediments had higher overall concentrations (ng/g dwt) of dl-PCBs (Figure 1.16), as well as highest average percent of total dl-PCBs relative to tPCBs, 16% ± 0.01% compared to 10% ± 0.01% for Gunston Cove.

To compare inter and intra- sample variability replicates of sediments were collected, processed and analyzed. Statistical analysis indicated no significant differences between the replicates (one-way ANOVA, $p>0.05$). The standard deviation for tPCB concentration (ng/g dwt) replicates (n=3) from HC1 varied from 0.00 to 0.06, in contrast standard deviation for tPCB concentrations (ng/g dwt) in sediments from Hunting Creek (n=12) ranged from 0.00 to 3.59.

PCBs in Fish

The greatest mean tPCB concentration was observed in Gunston Cove fish, which was 228 ± 8.64 ng/g wwt, averaged for all fish collected at this embayment. The greatest mean tPCB concentration for Hunting Creek fish was 78.1 ± 1.89 ng/g wwt, averaged for all fish collected at this embayment (Figure 1.17).

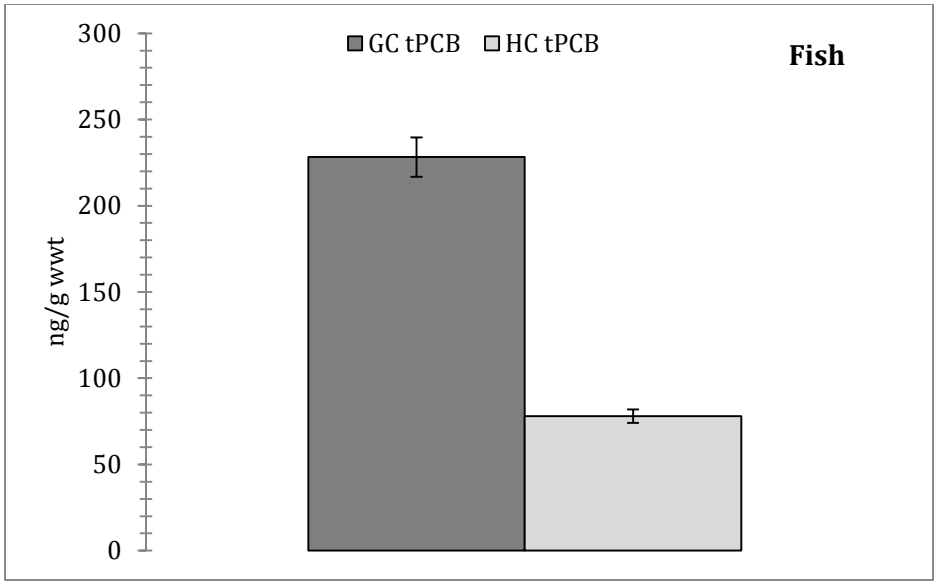


Figure 1.17. Fish mean tPCB concentration (ng/g wwt) for Hunting Creek and Gunston Cove.

Statistical analysis of mean total PCB concentrations (ng/g wwt) in individual fish species indicated significant differences between fish species collected from Hunting Creek and fish species collected from Gunston Cove (one-way ANOVA, $p<0.05$).

The highest lipid normalized mean tPCB concentration was observed in inland silverside (10.2 ± 0.24 ug/g lipid) from Gunston Cove, and the lowest lipid normalized mean tPCB concentration was observed in striped bass (0.31 ± 0.01 ug/g lipid) from Hunting Creek.

The average percent homolog distributions across all fish species in both Gunston Cove and Hunting Creek resembled one another with the exception of the higher concentrations of PCBs observed in Gunston Cove (Figure 1.18).

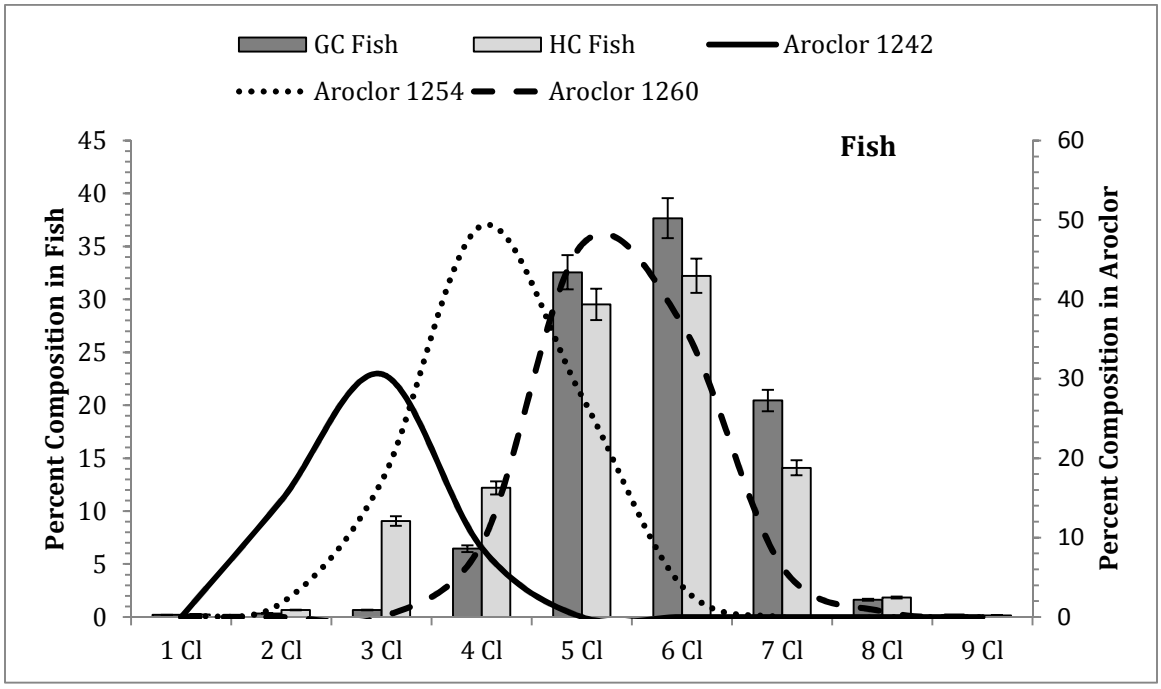


Figure 1.18. Percent homolog composition in fish of Hunting Creek (HC) and Gunston Cove (GC), compared to percent composition in Aroclor.

Comparing percent homolog composition in fish and sediments of both embayments showed two interesting facts; both fish and sediments showed higher abundance of penta-, hexa- and hepta-chlorinated biphenyls, but Hunting Creek sediments had higher percent homolog composition, which was not reflected in Hunting Creek fish percent homolog composition. Gunston Cove fish had higher percent homolog composition.

To assess Aroclors as a possible source for PCBs, homolog percent composition in Aroclors was compared to homolog percent composition in fish (Figure 1.18) and consistent with the sediments results indicated Aroclor 1260 as

the possible source with penta-, hexa- and hepta-chlorinated biphenyls at the highest percentages.

All nine of the analyzed dl-PCBs were detected in Hunting Creek and Gunston Cove fish. The highest dl-PCB concentration in Hunting Creek fish belonged to PCB 118, followed by PCBs 157, 105 and 167. The highest dl-PCB concentration (ng/g dwt) in Gunston Cove fish was observed in PCBs 157 and 156, followed by PCB 105 and 114 (Figure 1.20). The mean total dl-PCB (t.dl-PCB) congener concentration in fish species of both embayments were significantly different (one-way ANOVA, $p<0.05$), and did not resemble each other, neither were they reflective of the t.dl-PCB concentration profiles of the perspective sediments. For instance, PCB 114 was not detected in Hunting Creek sediment, but was detected in Hunting Creek fish (Figures 1.16 and 1.19).

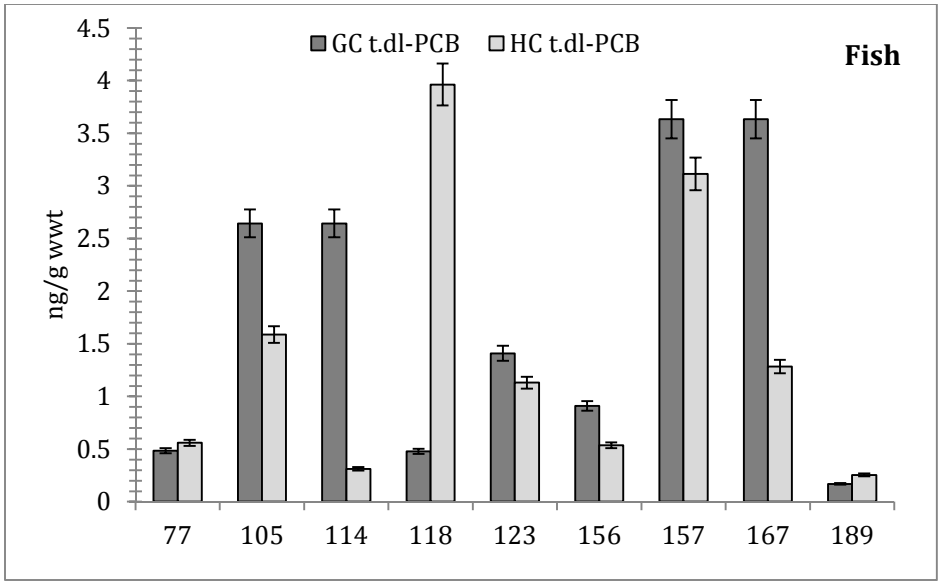


Figure 1.19. Total dl-PCB mean concentrations (ng/g wwt) in all fish from Hunting Creek and Gunston Cove

Mean lipid normalized tPCB concentrations for all fish were found to be 5.35 ± 0.21 µg/g lipid in Gunston Cove, in contrast to 0.68 ± 0.01 µg/g lipid for all fish in Hunting Creek (Table 1.13). Gunston Cove showed twice the concentration of t.dl-PCBs (0.20 ug/g lipid) relative to Hunting Creek (0.10 ug/g lipid), while Gunston Cove showed only 4% relative abundance of dl-PCBs in comparison to 14% found in Hunting Creek.

The highest lipid normalized mean concentration of dl-PCB congeners was observed for PCB 118 in bluegill (81.5 ± 2.38 ng/g lipid) from Hunting Creek and spottail shiner (108 ± 11.8 ng/g lipid) from Gunston Cove. The lowest lipid normalized mean concentration belonged to PCB 156 across all fish species and both embayments, and was observed in striped bass (1.95 ± 0.27 ng/g lipid) from Hunting Creek, and redbreast sunfish (0.99 ± 1.98 ng/g lipid) from Gunston Cove.

Table 1.13. Lipid normalized PCB concentrations for Gunston Cove and Hunting Creek fish.

	tPCB (µg/g lipid)	t.dl-PCB (µg/g lipid)	Avg. %dl-PCB per tPCB
		Gunston Cove	
Total fish data	5.35 ± 0.21	0.20 ± 0.01	4.13% ± 0.01%
Bluegill	2.63 ± 0.06	0.13 ± 0.01	5.12%
Mummichog	3.91 ± 0.20	0.16 ± 0.02	3.98%
White perch	5.51 ± 0.24	0.25 ± 0.01	4.05%
Spottail shiner	6.26 ± 0.29	0.30 ± 0.02	4.23%
Inland silverside	10.2 ± 0.24	0.20 ± 0.01	1.98%
Redbreast sunfish	3.61 ± 0.21	0.16 ± 0.02	3.91%
		Hunting Creek	
Total fish data	0.68 ± 0.01	0.10 ± 0.01	14.4% ± 0.05%
Banded killifish	0.92 ± 0.01	0.14 ± 0.01	14.9%
Bluegill	1.03 ± 0.03	0.14 ± 0.01	13.1%
Spottail shiner	0.51 ± 0.01	0.07 ±0.00	13.9%
White perch	0.61 ± 0.02	0.13 ± 0.02	20.8%
Striped bass	0.31 ± 0.01	0.03 ± 0.00	9.32%

tPCB: total PCB concentration
t.dl-PCB: total dioxin-like PCB concentration
dl-PCB per tPCB: total dl-PCBs divided by tPCBs

To assess sample variability replicates of fish were processed and analyzed. Statistical analysis indicated no significant differences between the replicates (one-way ANOVA, $p>0.05$). The variability between samples is the actual field variability

because the standard deviation between replicates (0.00 to 0.33) is much lower than field samples (0.00 to 7.01).

Distribution and Fugacity of dl-PCBs in Sediments and Fish

Individual fugacities were estimated for the dl-PCB congeners in both fish and sediments to assess equilibrium. Fugacity ratios of fish-sediments were determined and indicated fish to sediment flow (>1.5 ratio) for congeners numbers 77, 105, 114, 118, 123, 167 and 189 in spottail shiner and inland silverside from Gunston Cove (Figure 1.20). Congener numbers 156 and 157 were not detected in spottail shiner and inland silverside from Gunston Cove.

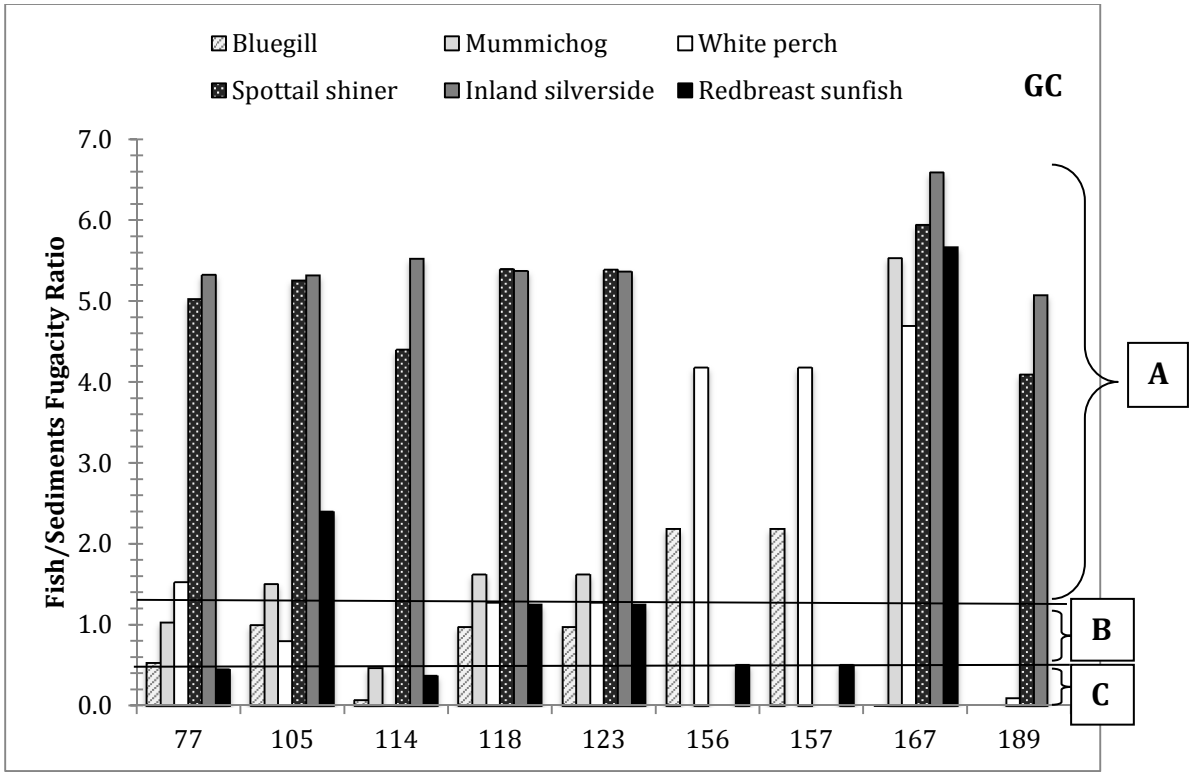


Figure 1.20. Fugacity ratio (fish-sediment) of Gunston Cove samples. A ratio of 0.5-1.5 indicates equilibrium, while a <0.5 is sediment to fish flux and >1.5 is fish to sediment flux. **A**: fish → sediments flow, **B**: equilibrium, **C**: sediments → fish flow.

PCBs 114 and 167 in bluegill and white perch, and PCB 189 in white perch from Gunston Cove indicated sediment to fish flow (<0.5 ratio). Congener numbers 77, 105 and 123 indicated equilibrium between fish and sediments (0.5-1.5 ratio) in bluegill, Mummichog, white perch and redbreast sunfish, and from Gunston Cove. PCBs 156 and 157 fugacity ratios could be determined for only three of the species; bluegill, white perch and redbreast sunfish. PCBs 156 and 157 indicated equilibrium (0.5-1.5 ratio) in redbreast sunfish and had sediments to fish flux in bluegill and white perch from Gunston Cove.

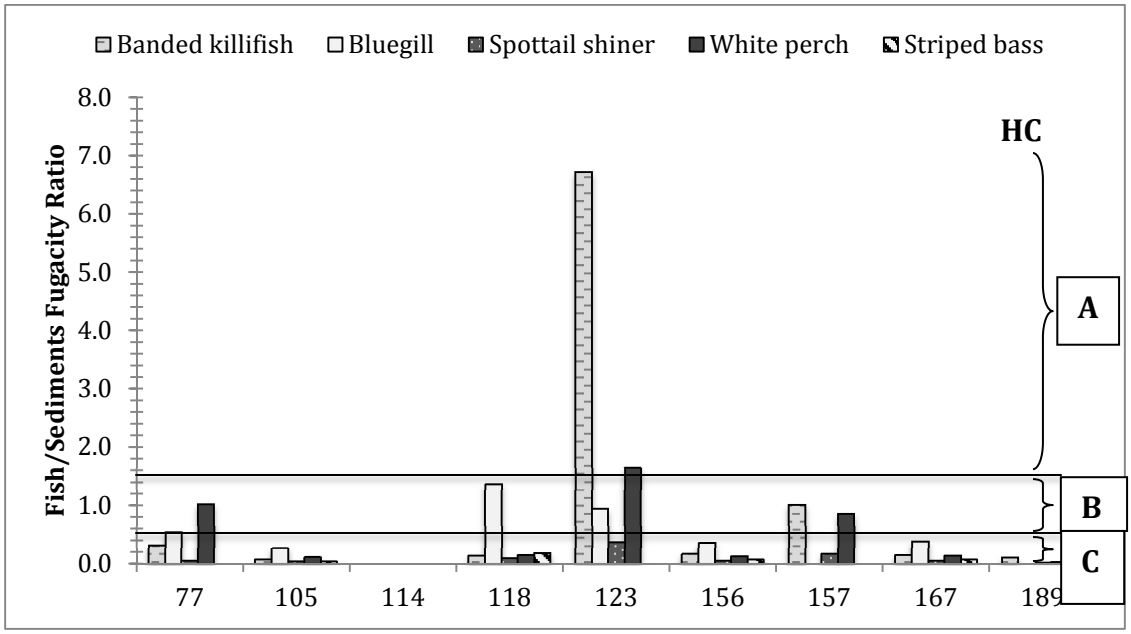


Figure 1.21. Fugacity ratios of Hunting Creek samples. A ratio of 0.5-1.5 indicates equilibrium, while a <0.5 is sediment to fish flux and >1.5 is fish to sediment flux. **A**: fish → sediments flow, **B**: equilibrium, **C**: sediments → fish flow.

In contrast to Gunston Cove, Hunting Creek fugacity ratios displayed sediments to fish flow only in PCB 123 for banded killifish and white perch (Figure 1.21). Only a few congeners were at equilibrium (0.5-1.5 ratio); PCB 77 in white perch, PCBs 118 and 123 in bluegill, PCB 157 in banded killifish and white perch.

The majority had sediment to fish flux; PCBs 105, 156, 167 and 189 indicated sediment to fish flow in all five species of fish (banded killifish, bluegill, spottail shiner, white perch and striped bass) collected from Hunting Creek. PCB 118 indicated sediments to fish flow in four species (banded killifish, spottail shiner, white perch and striped bass) and showed equilibrium in bluegill.

Discussion

The mean tPCB concentrations (ng/g dwt) from Hunting Creek and Gunston Cove were 35.5 ± 1.07 and 39.0 ± 0.53 respectively, which is well below 1829 (ng/g dwt) observed in Quantico Marine Base which includes an old land fill[47], and well below the 205 ± 244 (ng/g dwt) value reported by Shen et al.,[48] in a long term monitoring study of the Baltimore Harbor. The National Oceanic and Atmospheric Administration (NOAA) completed a bay-wide survey of Chesapeake Bay from 1998-2001[49], and the tPCB sediment concentrations ranged from below detection limit to 122 ng/g dwt.

The homolog distribution profiles of fish and sediments from Hunting Creek and Gunston Cove resembled each other and indicated penta-, hexa- and hepta-chlorinated biphenyls as the prominent homolog class. Homolog profiles were comparable to the profile reported from other tributaries of the bay, indicating penta-, hexa- and hepta-chlorinated biphenyls as showing the highest concentrations.[50-53]

The most likely source of PCBs in fish and sediments in both embayments is most likely Aroclor 1260 (Figure 1.22). Aroclor 1260 is comprised of penta-, hexa- and hepta-chloro biphenyls, homolog profiles of sediments and fish (GC and HC) both indicate penta-, hexa- and hepta-chlorinated biphenyls as the homolog class with highest concentrations (ng/g dwt) and highest percentages of abundance.

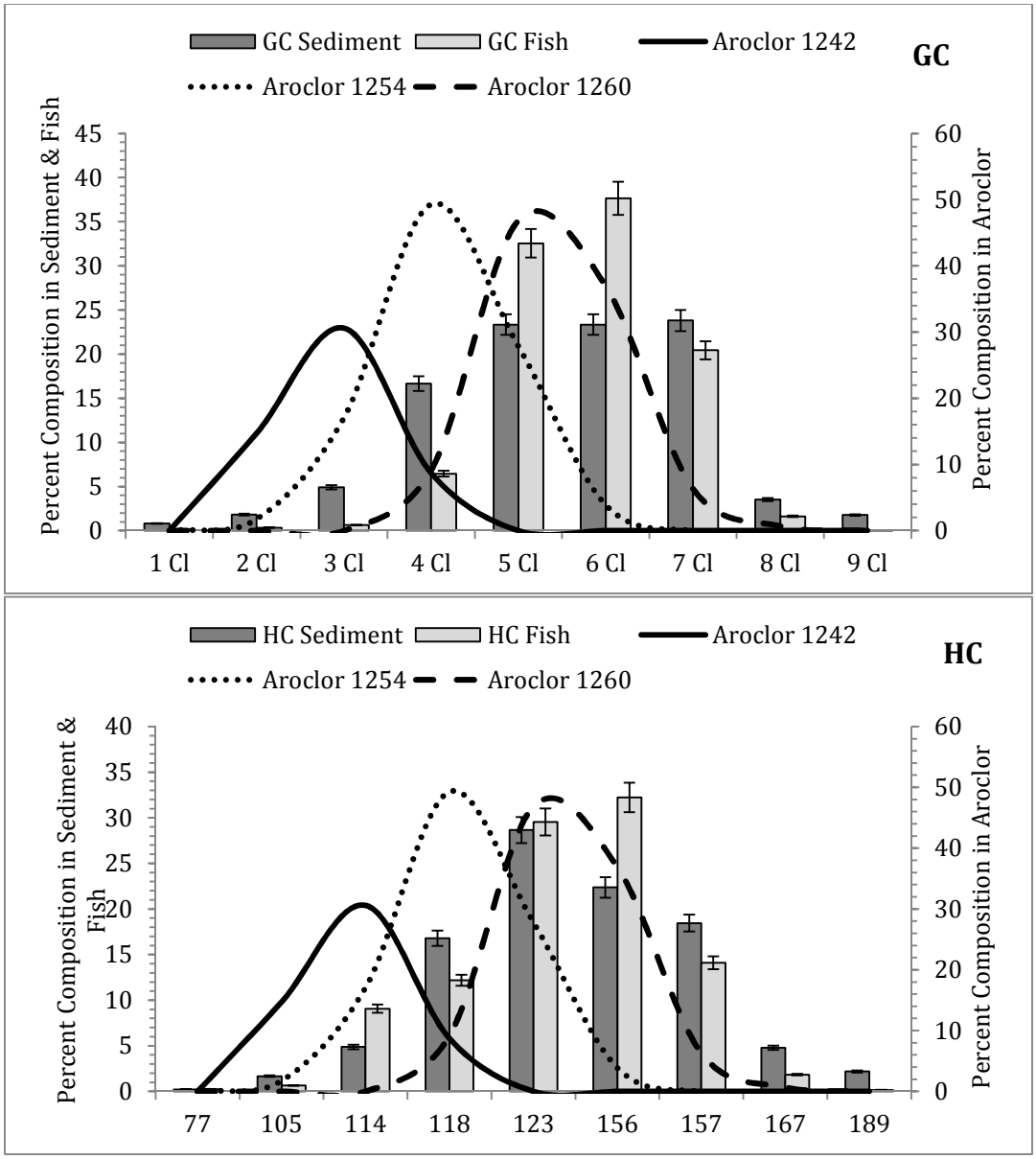


Figure 1.22. Percent homolog distribution pattern comparison in Gunston Cove fish and sediments (GC) and Hunting Creek fish and sediments (HC).

Hexa-chloros had the highest mean concentration in fish from Gunston Cove and Hunting Creek, 0.28 ± 0.02 µg/g OC and 0.25 ± 0.06 µg/g OC, respectively,

consistent with literature indicating maximum bioaccumulation observed for hexachlorobiphenyls, not hepta- or octa-chloros[54, 55] and penta-chlorobiphenyls with maximum accumulation in sediment[56] consistent with data observed in Hunting Creek and Gunston Cove, showing 320 ± 10.0 ng/g OC and 8,700 ± 99.0 ng/g OC respectively.

The highest total dl-PCB concentration in sediments was 1.66 ± 0.35 ng/g dwt well within the numerous reported values of 0.085-1.99 ng/g dwt.[23, 25, 26, 57, 58] The most prominent dl-PCB congeners were PCBs 105, 118, 156 and 157, consistent with studies indicating that PCBs 105, 118, 156 and 157 are among the most detected and highest concentrations within the dl-PCB congeners.[23, 26, 57, 58]

Total PCB mean concentrations in fish and sediments, as well as dl-PCB profiles in fish and sediments of Hunting Creek and Gunston Cove were determined and revealed several interesting facts. Foremost, mean individual dl-PCB congener concentrations in Hunting Creek sediments (ng/g OC) was higher than mean individual dl-PCB congener concentrations in Hunting Creek fish (ng/g lipid) with the exception of PCBs 123 and 157 indicating higher concentration in fish rather than sediments (Figure 1.23 A). In contrast mean individual dl-PCB congener concentrations in fish (ng/g lipid) from Gunston Cove were higher than mean individual dl-PCB concentrations in sediments (ng/g OC) by a factor of 2 to 6 (Figure 1.23 B).

Mean tPCB concentrations in sediments were very similar in both Hunting Creek (35.5 ± 1.07 ng/g dwt) and Gunston Cove (39.0 ± 0.53 ng/g dwt) and were not

significantly different (one-way ANOVA, $p>0.05$), in contrast mean tPCB concentrations observed in Gunston Cove fish (228 ± 8.64 ng/g wwt) was larger than mean tPCB concentration observed in Hunting Creek fish (78.1 ± 1.89 ng/g wwt) by a factor of 3 (Figure 1.23 C). Harris et al.,[52] evaluated tPCB accumulation in several fish species, including white perch, bluegill and striped bass and with tPCB concentrations ranging from 69 ± 24 ng/g wwt to 398 ± 67 ng/g wwt.

The overall higher concentrations of PCBs in Gunston Cove fish could be a result of several factors, including feeding habits and trophic guild interactions among the GC fish species. Only three species of fish were common among the collected fish from both embayments; white perch, spottail shiner and bluegill.

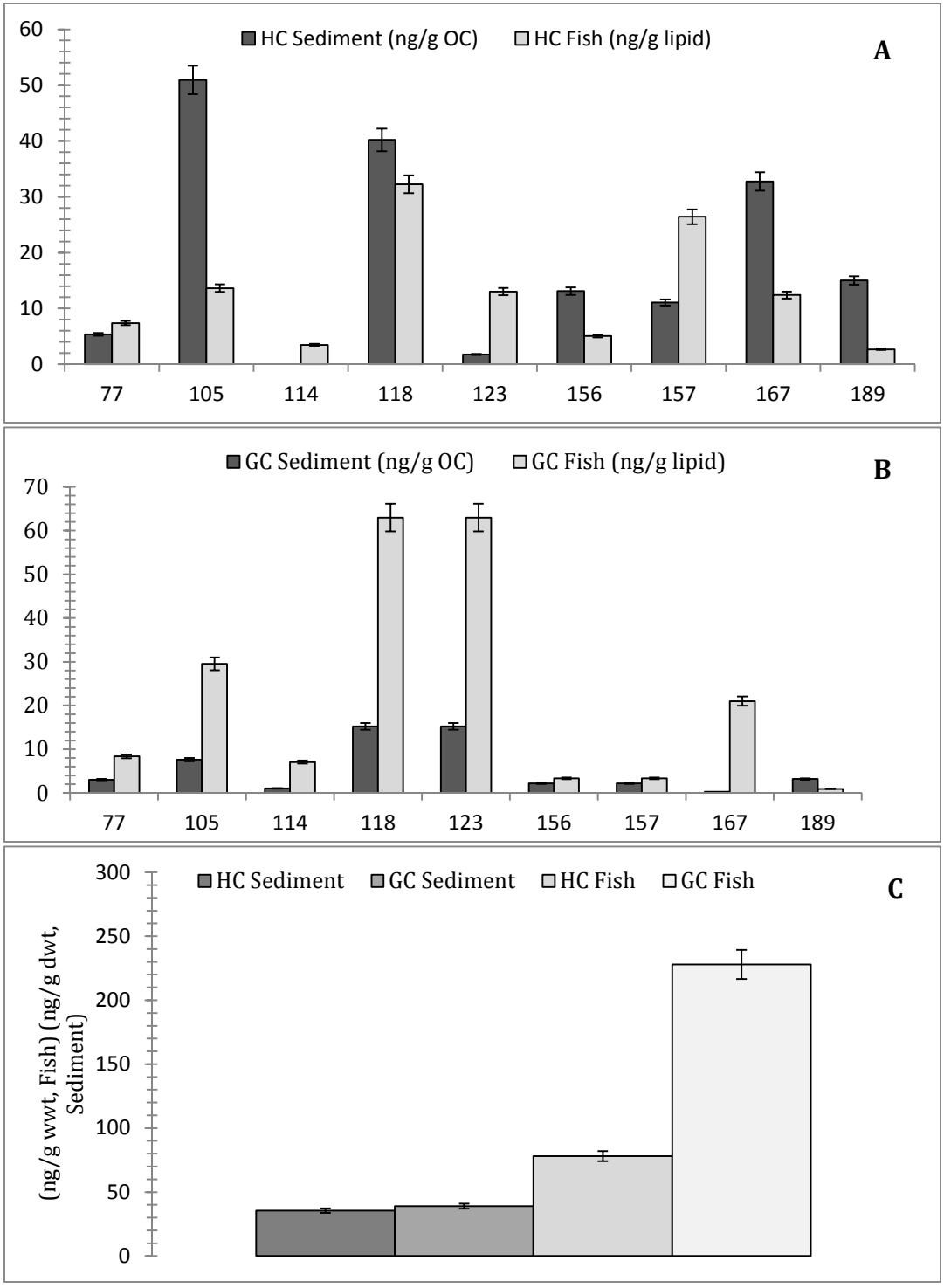


Figure 1.23. Mean dl-PCB concentrations in Hunting Creek (HC) sediments and fish (graph **A**), Gunston Cove (GC) sediments and fish (graph **B**) and mean tPCB concentration in Hunting Creek and Gunston Cove sediments and fish (graph **C**).

Fugacity calculations for Hunting Creek sediments and fish (Figure 1.21) did not show a consistent pattern of direction of flux, but the dl-PCB concentrations were consistently higher in sediments. The natural tendency of the chemicals is to reach equilibrium between the organism and environmental media,[59] and net passive chemical flow occurs from high fugacity medium to low fugacity medium.

Fugacity values can be utilized as a tool to profile distribution patterns of contaminants across ecosystem and biota. Fugacity is analogous to partial pressure of an ideal gas and directly related to concentration.[60] Fugacity ratios of analytes in different media can be calculated and the magnitude of the ratios will determine equilibrium or direction of flow.

Total PCB sediment concentrations and dl-PCB abundance profiles can be a useful tool to determine corresponding profiles in fish, but would not be a good indicator for the corresponding concentrations in fish due to trophic guild interactions and different metabolic rates of fish species.

Replicate analysis of fish and sediments from Hunting Creek and Gunston Cove showed no significant differences among replicates consistent with no variability observed between collection sites within each embayment (one-way ANOVA, $p>0.05$). To analyze the toxic effect the observed sediment concentration would have on the aquatic biota two threshold criteria are utilized (Figure 1.24). Consensus based threshold minimum effect (TEC) is used to establish toxic effects in benthic organisms and is 58.9 ng/g dwt.[61] Minimum effect threshold (MET) is the concentration that is considered to be clean to highly polluted but showing little

toxicity to the majority of sediment dwelling organism and is 200 ng/g dwt.[61] The mean concentrations in sediments of both embayments are well below the TEC and MET criteria.

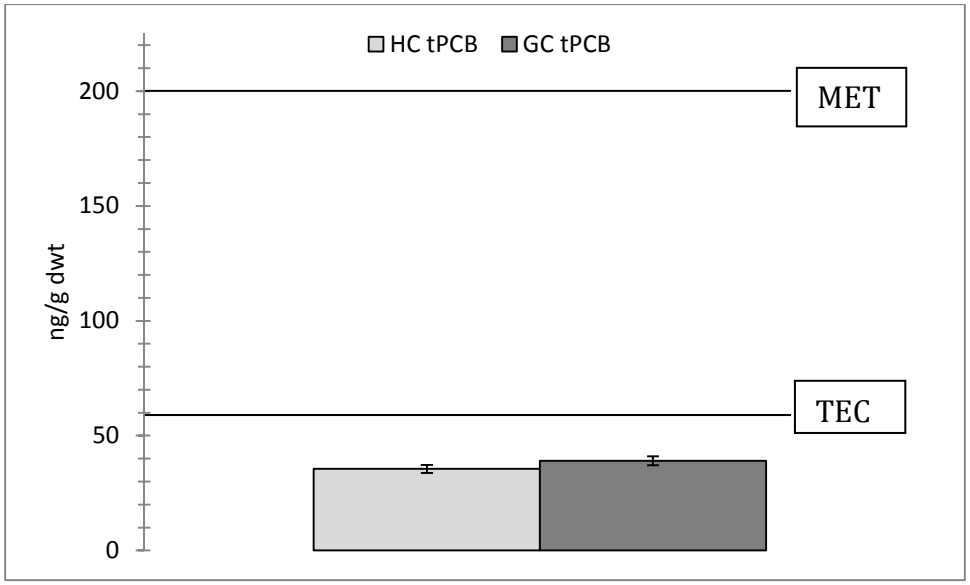


Figure 1.24. Mean tPCB concentrations of sediments from Gunston Cove and Hunting Creek relative to toxicity thresholds.

The commonwealth of Virginia, District of Columbia and State of New York have identified all of the Chesapeake Bay jurisdictions with impaired water bodies and general fish consumption advisories.[14]

Laboratory experiments have determined various LD_{50} (lethal dose resulting in 50% mortality) values of TCDD (dioxin) in hamster and guinea pig at 5 mg/kg and 0.0006 mg/kg respectively[62] and PCB LD_{50} of 1-11 g/kg for rodents. The PCB LC_{50} (lethal concentration resulting in 50% mortality) at 96 hour exposure, for bluegill is

reported at 2.74 mg/L.[63] Congener specific LD_{50} values are limited to a few congeners, however several studies report commercial mixture oral LD_{50} values of 0.4-11 g/Kg in rats.[64] Several studies have indicated adverse health effects on aquatic organisms due to contaminants in water.[65-67]

Different lipid normalized concentrations (Figure 1.25) observed in fish can be attributed to several factors, such as species specific metabolic rates, as well as congener specific accumulation patterns.[7] Bioaccumulation and elimination of PCBs in aquatic organisms are both species and congener specific.[54, 68-70]

Bluegill from Hunting Creek showed the highest concentration of PCB 118 (81.5 ± 2.38 ng/g lipid), and spottail shiner from Gunston Cove had the highest concentration of PCBs 118 and 123 (108 ± 11.8 ng/g lipid; Figure 1.25).

Overall higher mean dl-PCB concentrations (ng/g lipid) were observed in fish from Gunston Cove, even among the common species of both embayments; white perch, bluegill and spottail shiner. This raises an interesting issue regarding feeding habits and trophic guild interactions in Gunston Cove, especially considering that fish from Hunting Creek and Gunston Cove are exposed to the same concentrations of tPCBs in sediments.

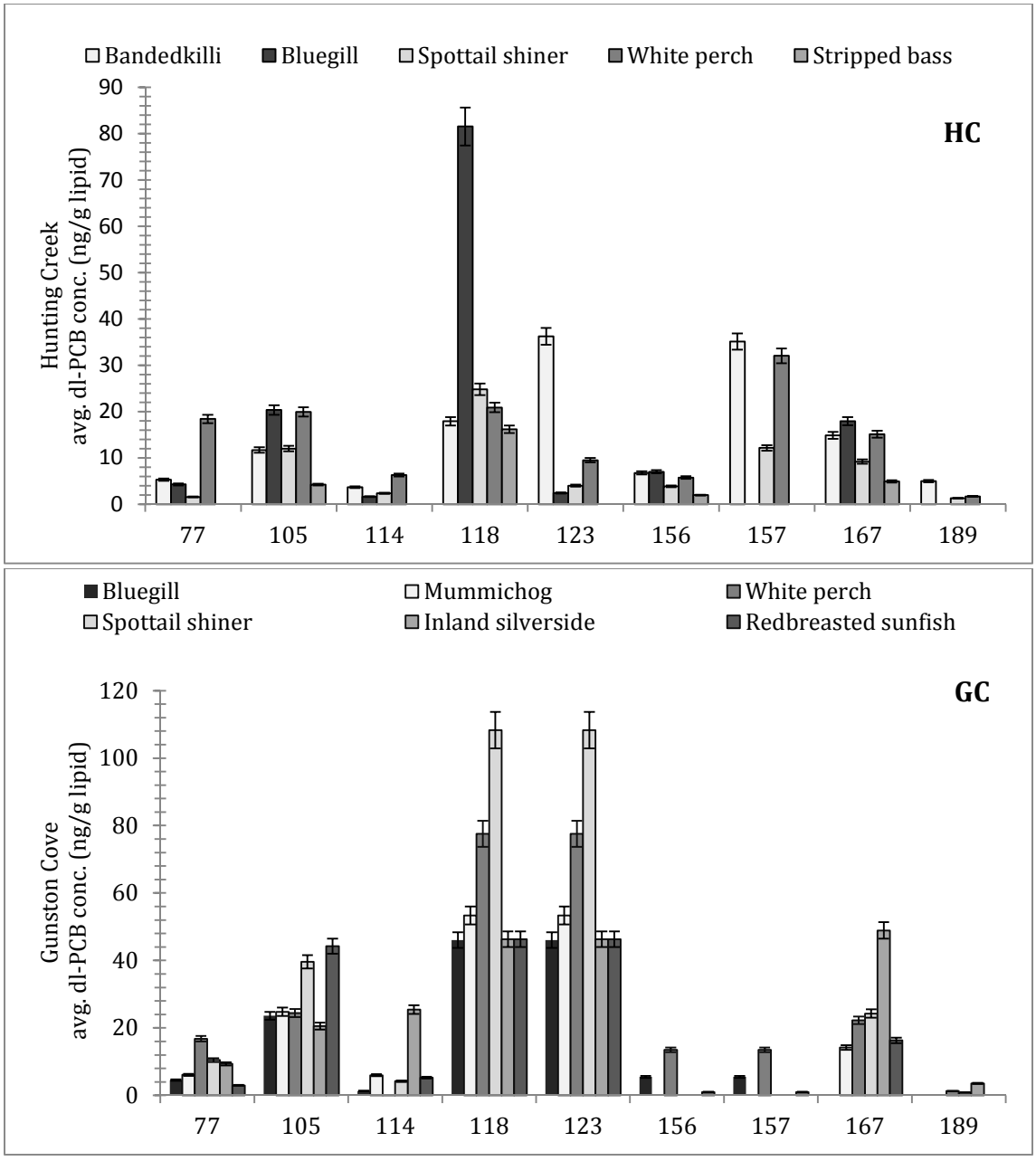


Figure 1.25. Mean total dl-PCB concentration in fish species analyzed from Hunting Creek (**HC**) and Gunston Cove (**GC**).

Conclusion

Gunston Cove and Hunting Creek both receive treated water from two local WWTPs. Significant testing indicated no differences among mean tPCB concentrations in sediments from Hunting Creek and Gunston Cove indicating no geospatial pattern, thus the WWTPs would not be considered the source of contaminants in the tributaries since PCB contamination is ubiquitous throughout the studied water bodies.

Analyzing Gunston Cove and Hunting Creek sediments and fish for PCB contamination revealed three significant facts. First, fish PCB profiles can be related to the observed PCB profiles in sediments. Organic carbon and lipid normalized total dl-PCB concentrations in both embayments demonstrated a direct relationship between sediment profiles and those observed in fish from the corresponding sites.

Second, the same congeners were detected in both types of samples, enabling predictability of what can be expected in fish.

Third, this study also demonstrated how effective utilizing fugacity of analytes in organisms and medium is to predict direction of flow. The natural tendency of a chemical is to reach equilibrium and the net passive flow of chemicals is from high fugacity medium to low fugacity medium, so if analyte fugacity is higher in sediment, the direction of flow is sediments to the organism.

Mean tPCB sediment concentrations in both Hunting Creek and Gunston Cove were very similar and not significantly different, in contrast the mean tPCBs and

mean total dl-PCBs in fish samples from Hunting Creek and Gunston Cove were significantly different. Gunston Cove fish showed higher concentrations of tPCBs and total dl-PCBs in fish species possibly due to the feeding habits and different trophic guild interactions. Fugacity ratios and direction of flux, fish to sediments, for Gunston Cove PCBs also demonstrated fish species are enriched in PCBs, while Hunting Creek fish exposed to the same concentrations of tPCBs showed significantly lower tPCB and total dl-PCB concentrations in fish and only PCB 123 in banded killifish showed fish to sediments flux.

CHAPTER 2 CONTEMPORARY ENDOCRINE DISTRUPTING CHEMICALS IN WATER AND SEDIMENTS FROM HUNTING CREEK (VA).

Introduction

Pollutants of concern to the fisheries in the Chesapeake Bay watershed include endocrine disrupting chemicals (EDCs). The International Programme on Chemical Safety (IPCS) defines endocrine disruptors as "exogenous substance or mixture that alters function(s) of the endocrine system and consequently causes adverse health effects in an intact organism, or its progeny, or (sub) populations." EDCs include legacy pollutants such as polycyclic aromatic hydrocarbons (PAHs), pesticides, polychlorinated biphenyls (PCBs) and emerging pollutants, such as pharmaceuticals, plasticizers, fragrances, flame retardants, cleaning agents and other household chemicals. Legacy EDCs, such as DDT and PCBs are chemicals that are no longer used and manufactured in most countries, while contemporary EDCs, such as flame retardants are currently used and manufactured in most countries. EDCs are emitted to the aquatic environment through industrial waste discharge, urban stormwater runoff and wastewater treatments discharge.

Increased number of reproductive abnormalities, such as presence of

feminine features in male fish, have been observed and well documented in Potomac River fish, most likely stemming from increased levels of EDCs in the environment.[71,66] Jiang et al.,[72] reviewed occurrence, fate and transport of contemporary EDCs in 14 countries (UK, Germany, France, Spain, Finland, Belgium, Italy, Greece, Switzerland, the Netherlands, Canada, USA, China and Japan), and indicated ubiquitous presence of contemporary EDCs in fish, water and sediments.[73-106]

When released into natural waters these chemicals accumulate in organic matter and fine-grained sediments or suspended sediment particles. Because storm runoff and wastewater discharge represent a sizable fraction of the annual surface water flow in urban regions, these sources are often sufficient for allowing the in-stream accumulation of EDCs. Thus, the entire aquatic community may be exposed throughout entire life cycles and across generations to mixtures of biologically-active chemicals in urban areas.[107] To better understand the implications of EDCs in the Potomac River ecosystem, further ecological baseline investigations are needed since little is known regarding the fate, effects and distribution, of endocrine disrupting pollutants in the aquatic environment.

Study Objectives

The goal of the present study was to assess the ecological impacts of contemporary endocrine disrupting chemicals in Hunting Creek associated with

WWTP discharge. The primary objectives of the present study were (i) to quantify EDCs in water and bed sediment to evaluate the significance of endocrine disruptors in the upper Potomac River, (ii) assess geospatial differences between collection sites within the creek, (iii) compare the water and sediment profiles of EDCs to identify differences in distribution between matrices and (iv) determine whether water concentrations and profiles of EDCs can be used to predict corresponding concentrations and profiles in sediments.

Sediment and water was collected from Hunting Creek (northern Virginia, USA) in 2014 (August through September), and analyzed for selected EDCs. The present study is in continuation of the 2013 collaboration between the Potomac Environmental Research and Education Center (PEREC) at George Mason University and the Alexandria Renew Enterprises. Hunting Creek is formed by confluence of Cameron Run and Hooff run in northern Virginia. The high in-stream concentrations of anthropogenically-derived chemicals in northern Virginia are due to the dense urban population and high WWTP discharge into the Potomac River tributaries.[108]

Materials and Methods

Field Sites

Hunting Creek is a tributary embayment of the Potomac River south of the city of Alexandria, VA, formed by the confluence of Cameron Run and Hooff Run

(Figure 2.1). Jones Point and Dyke Marsh are the boundaries of Hunting Creek on the north end and the south end respectively (Figure 2.2). The Alexandria Renew Enterprises wastewater treatment plant is located on the east side of the Hunting Creek (Figure 2.2). Alexandria Renew discharges an average of 150,000 m^3 wastewater daily[2], which would categorize the plant as a major WWTP (>2 MGD, mega gallons daily).[4]

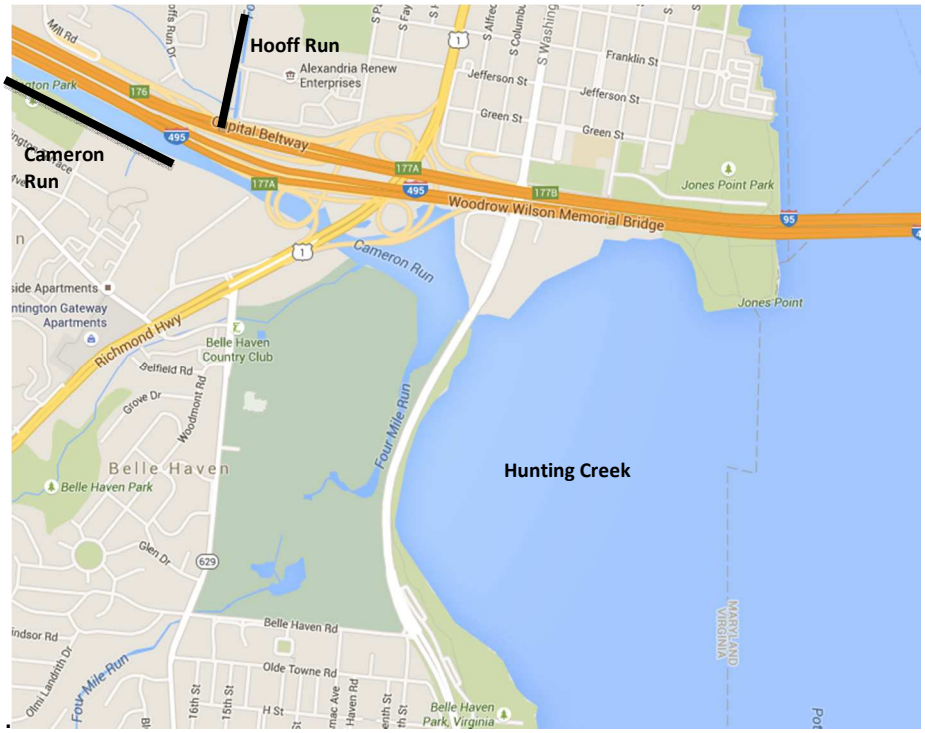


Figure 2.1. Locations of Cameron Run, Hooff Run and Hunting Creek.

Hunting Creek as a tributary embayment to the Potomac River, is an important spawning and nursery ground for both migratory and resident fish.[109] Maryland Department of the Environment lists 67 major (>2 MGD) municipal

WWTPs discharging to the Potomac River[110] and Metropolitan Washington Council of Governments (MWCOG) indicates 17 major (>2 MGD) municipal WWTPs discharging into the Potomac River.[4]

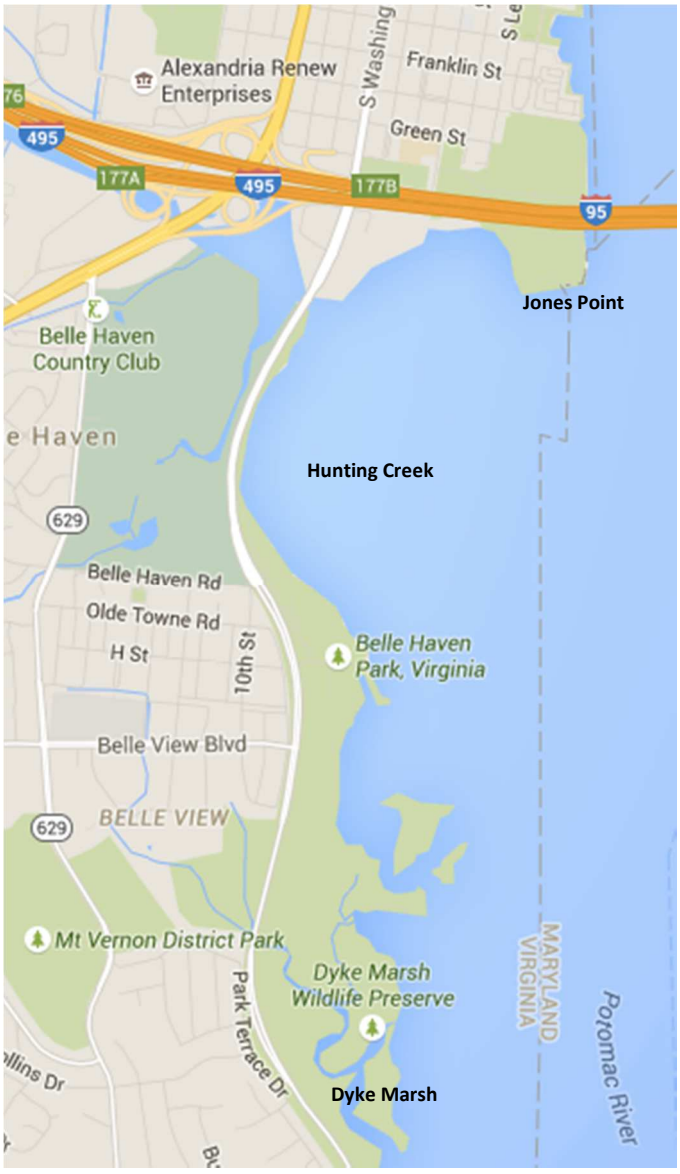


Figure 2.2. Hunting Creek location relative to WWTP and its geographic boundaries.

Hunting Creek was segmented into four hydrological zones with 5-10 sampling stations within each zone (Figure 2.3). The four zones were designed to identify distinct hydrographic regions in the watershed and spatial distributions and gradients in EDC concentrations surrounding the Alexandria Renew Enterprises facility. A total of 37 and 38 samples (sediments and water, Tables 2.2 and 2.3 respectively) were collected from the Hunting Creek watershed (Table 2.1).

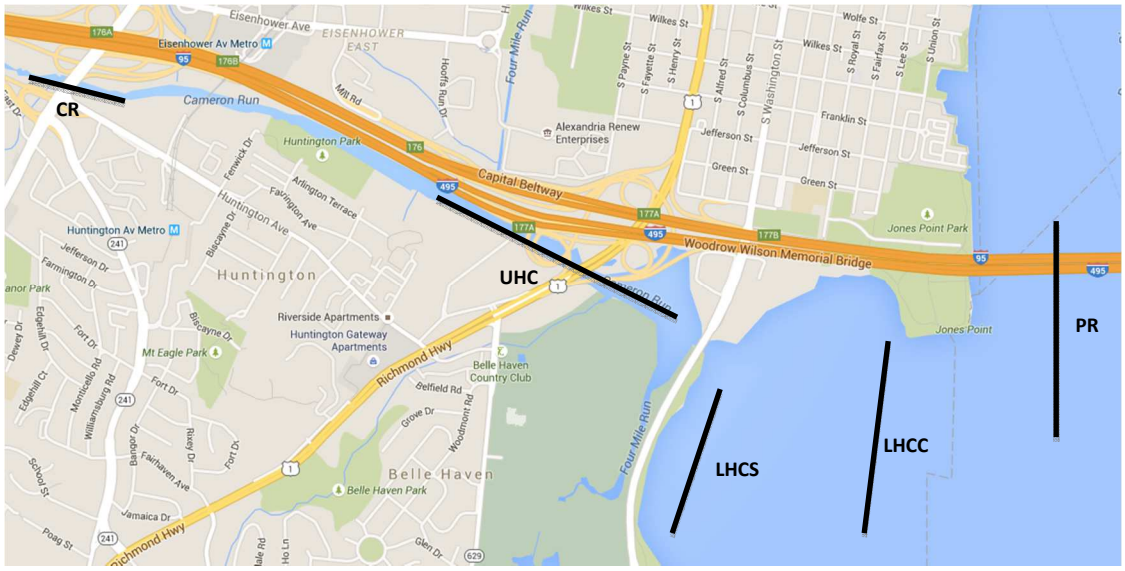


Figure 2.3. Hunting Creek was segmented into four hydrological zones. Zone 1 is Cameron Run (CR), Zone 2 is upper Hunting Creek (UHC), Zone 3 is lower Hunting Creek, with two distinct collection sites; (LHCS: lower Hunting Creek shoreline and LHCC: lower Hunting Creek channel), and Zone 4 is mainstem Potomac River.

The zones are regions of similar hydrology in the watershed where the sediment and water concentrations were averaged to provide mean regional concentrations of EDCs. Hydrologic zone 1 is upstream above the fall line and

corresponds to Cameron Run. Zone 2 is within the fall line of upper Hunting Creek, near Alexandria Renew outfall. Zone 3 is lower Hunting Creek tributary-embayment serving as the transition between upper Hunting Creek and the mainstem Potomac River, located downstream of Alexandria Renew outfall. Zone 4 is mainstem Potomac River.

Table 2.1. Hunting Creek number of samples from the four hydrological zones..

Locations	**Abbv.**	**No. Samples**
Cameron Run, Zone 1	CR	7
Upper Hunting Creek, Zone 2	UHC	7
Lower Hunting Creek[a], Zone 3	LHCC, LHCS[b]	17
Potomac River, Zone 4	PR	6, 7[c]

[a]This segment had two sites sampled in triplicate due to large number of samples.
[b] LHCC: lower hunting Creek channel, LHCS: lower Hunting Creek shoreline.
[c] Number Potomac River main stem water samples.

Table 2.2. Coordinates of sampling sites for sediment in Hunting Creek

Collection Site	Abbrev.	Lat (deg min) N	Long (deg min) W
Cameron Run 1.1	CR 1.1	38 47.885	77 4.268
Cameron Run 1.2	CR 1.2	38 47.885	77 4.268
Cameron Run 1.3	CR 1.3	38 47.885	77 4.268
Cameron Run 2	CR 2	38 47.856	77 4.140
Cameron Run 3	CR 3	38 47.790	77 3.965
Cameron Run 4	CR 4	38 47.746	77 3.876
Cameron Run 5	CR 5	38 47.687	77 3.712
Upper Hunting Creek 1.1	UHC 1.1	38 47.598	77 3.495
Upper Hunting Creek 1.2	UHC 1.2	38 47.598	77 3.495
Upper Hunting Creek 1.3	UHC 1.3	38 47.598	77 3.495
Upper Hunting Creek 2	UHC 2	38 47.543	77 3.385
Upper Hunting Creek 3	UHC 3	38 47.526	77 3.348
Upper Hunting Creek 4	UHC 4	38 47.494	77 3.287
Upper Hunting Creek 5	UHC 5	38 47.407	77 3.102
Lower Hunting Creek C1.1	LHCC 1.1	38 47.342	77 2.471
Lower Hunting Creek C1.2	LHCC 1.2	38 47.342	77 2.471
Lower Hunting Creek C1.3	LHCC 1.3	38 47.342	77 2.471
Lower Hunting Creek C2	LHCC 2	38 47.246	77 2.491
Lower Hunting Creek C3	LHCC 3	38 47.023	77 2.504
Lower Hunting Creek C4	LHCC 4	38 46.890	77 2.488
Lower Hunting Creek C5	LHCC 5	38 46.753	77 2.498
Lower Hunting Creek C6	LHCC 6	38 46.629	77 2.539
Lower Hunting Creek Ctr	LHC-Ctr	38 47.246	77 2.931
Lower Hunting Creek S1.1	LHCS 1.1	38 47.344	77 3.047
Lower Hunting Creek S1.2	LHCS 1.2	38 47.344	77 3.047
Lower Hunting Creek S1.3	LHCS 1.3	38 47.344	77 3.047
Lower Hunting Creek S2	LHCS 2	38 47.183	77 3.103
Lower Hunting Creek S3	LHCS 3	38 47.074	77 3.117
Lower Hunting Creek S4	LHCS 4	38 46.960	77 3.119
Lower Hunting Creek S5	LHCS 5	38 46.796	77 2.956
Lower Hunting Creek S6	LHCS 6	38 46.585	77 2.778
Potomac River 1.1	PR 1.1	38 47.842	77 2.255
Potomac River 1.2	PR 1.2	38 47.842	77 2.255
Potomac River 1.3	PR 1.3	38 47.842	77 2.255
Potomac River 2	PR 2	38 47.650	77 2.302
Potomac River 3	PR 3	38 47.359	77 2.283
Potomac River 4	PR 4	38 47.048	77 2.265

Replicate samples are denoted by numbers .1 to .3 following the site number.

One station in each of the four zones (Table 2.1) was sampled in triplicate to characterize sampling variability, the exact site of which was determined during sampling according to the most desirable sediment properties (fine-grained texture being the optimal property).

Sample Collection

Sediments were obtained onboard a boat (skiff and jon boat) using a Petite Ponar grab sampler (Wildco, Saginaw, MI). Once contained within the Ponar, the sediments were expelled into a stainless-steel tray where 10 g of the top surface layer was placed directly into a cleaned glass jar, placed on ice for transport to the laboratory, and stored at -20 ^{o}C until chemical analysis.

Surface water grab samples were obtained onboard a boat (skiff and jon boat) or on foot in shallow areas, using a portable Fultz submersible pump (Fultz Pumps, Inc. PA). The water depth of the station was determined and a 1-L water sample was collected in a vertically integrated fashion. Vertical integration was performed along a vertical depth profile from below the surface (starting at 0.5 m depth) to 1-m from the river bottom. The Fultz pump head was lowered from surface to depth at a constant rate while filling the 1-L bottle. Water was collected in a 1-L amber glass bottle with Teflon lined caps, labeled and stored in an ice chest for transportation to the Environmental Chemistry Laboratory at George Mason University. At the laboratory, the water samples were stored for <48 hours at 6 ^{o}C in a walk–in chiller.

A total of 38 surface water samples were collected from Hunting Creek grouped into four hydrological zones (Figure 2.3, Table 2.1). An effort was made to perform surface water sampling during ebb tide as close to the crest as possible to provide hydrologic conditions favoring both downstream flow and maximum water depth. For each sample station GPS coordinates (Table 2.3) and water conditions were measured.

Table 2.3. Coordinates of sampling sites for water in Hunting Creek

Collection Site	Abbrev.	Lat (deg min) N	Long (deg min) W
Holmes Run 1.1	HR 1.1	38 48.536	77 6.734
Holmes Run 1.2	HR 1.2	38 48.536	77 6.734
Backlick Run 1.1	BR 1.1	38 48.445	77 6.759
Backlick Run 1.2	BR 1.2	38 48.445	77 6.759
Cameron Run 1.1	CR 1.1	38 48.322	77 6.449
Cameron Run 1.2	CR 1.2	38 48.322	77 6.449
Cameron Run 1.3	CR 1.3	38 48.322	77 6.449
Upper Hunting Creek 1.	UHC 1	38 47.598	77 3.495
Upper Hunting Creek 2	UHC 2	38 47.543	77 3.385
Upper Hunting Creek 3.1	UHC 3.1	38 47.526	77 3.348
Upper Hunting Creek 3.1	UHC 3.2	38 47.526	77 3.348
Upper Hunting Creek 3.1	UHC 3.3	38 47.526	77 3.348
Upper Hunting Creek 4	UHC 4	38 47.494	77 3.287
Upper Hunting Creek 5	UHC 5	38 47.407	77 3.102
Lower Hunting Creek C1.1	LHCC 1.1	38 47.342	77 2.471
Lower Hunting Creek C1.2	LHCC 1.2	38 47.342	77 2.471
Lower Hunting Creek C1.3	LHCC 1.3	38 47.342	77 2.471
Lower Hunting Creek C2	LHCC 2	38 47.246	77 2.491
Lower Hunting Creek C3	LHCC 3	38 47.023	77 2.504
Lower Hunting Creek C4	LHCC 4	38 46.890	77 2.488
Lower Hunting Creek C5	LHCC 5	38 46.753	77 2.498
Lower Hunting Creek C6	LHCC 6	38 46.629	77 2.539
Lower Hunting Creek Ctr	LHC-Ctr	38 47.246	77 2.931
Lower Hunting Creek S1.1	LHCS 1.1	38 47.344	77 3.047
Lower Hunting Creek S1.2	LHCS 1.2	38 47.344	77 3.047
Lower Hunting Creek S1.3	LHCS 1.3	38 47.344	77 3.047
Lower Hunting Creek C2	LHCS 2	38 47.183	77 3.103
Lower Hunting Creek C3	LHCS 3	38 47.074	77 3.117
Lower Hunting Creek C4	LHCS 4	38 46.960	77 3.119
Lower Hunting Creek C5	LHCS 5	38 46.796	77 2.956
Lower Hunting Creek C6	LHCS 6	38 46.585	77 2.778
Potomac River 1	PR 1	38 47.842	77 2.255
Potomac River 2	PR 2	38 47.650	77 2.302
Potomac River 3	PR 3	38 47.359	77 2.283
Potomac River 4.1	PR 4.1	38 47.048	77 2.265
Potomac River 4.2	PR 4.4	38 47.048	77 2.265

Potomac River 4.3	PR 4.3	38 47.048	77 2.265
Potomac River 5	PR 5	38 46.701	77 2.240

Replicate samples are denoted by numbers 1 to 3 following the site number.

Materials

Sodium sulfate (ACS grade) was supplied by VWR International Inc. (Bridgeport, NJ). Florisil was purchased from J.T. Baker (Philipsburg, NJ). GCMS standard solutions of the EDC analytes and C13 isotopic surrogate standards, used in water analysis, (atrazine-13C3 and naproxen-13C,d3) are obtained from AccuStandard (New Haven, CT). The internal standards (acenaphthene-d10, phenanthrene-d10 and chrysene-d12) and deuterated surrogate standard triclosan-d3, used in water analysis, are purchased from C/D/N Isotopes Inc. (Quebec, Canada). Working standards are prepared in toluene and stored at 4^{o}C, while the surrogate standards are prepared in methanol. Final dilutions of the GCMS calibration standards are made in toluene.

Derivatization agent, N-Methyl-N-(trimethylsilyl) trifluoroacetamide (MSTFA), is purchased from Pierce (Rockford, IL) in 1 mL ampoules. Ethyl acetate (EtOAc), dichloromethane (DCM) and methanol (MeOH) are all HPLC-grade and supplied by Fisher Scientific. Sample containers and all glassware used for sample and preparation are cleaned by washing with soap, rinsing with distilled water followed by double distilled water (DDW), and ashed at 400 ^{o}C overnight. All laboratory materials are made of glass, stainless steel or Teflon to avoid sample contamination. The Teflon materials are cleaned the same way as glass but without

ashing. All materials both made of glass and Teflon are rinsed with methanol and air dried before use. DDW is produced in the laboratory in a Corning Megapure quartz element immersion still.

Sample Processing

The concentrations of EDCs (Table 2.4) in sediments were quantified using microwave-assisted extraction (MAE), 100 ^{0}C, 1500 watts for 15 minutes (MARS, CEM Corp, Matthews, NC), Florisil (60-100 mesh, J.T. Baker, Philipsburg, NJ) clean up and gas chromatography-mass spectrometry quantification (Agilent GC 7890A coupled with MS 5975C Series).

The thawed wet sediment was initially centrifuged for 10 minutes at 1500 rpm (Du Pont Sorval RC-5B, New Town, CT) to dewater prior to extraction. Approximately 1.0 g of dewatered sediment was desiccated by mixing in a mortar and pestle with about 10.0 g of anhydrous sodium sulfate. The dry powder was spiked with 50 µL of EDC Surrogate standards (Table 2.4). The dry powder was transferred to a GreenChem extraction vessel and subjected to MAE, using 15 mL of 1:1:1 Dichloromehtane:Ethyl Acetate:Methanol (DCM:EtOAc:MeOH). Sediment extraction was performed in triplicate and the extracts were combined in a 200 mL TurboVap cell and evaporated to 0.5 mL using a TurboVap evaporator (Zymark, Hopkinton, MA). The sediment analysis method is summarized in Figure 2.4.

Table 2.4. List of EDCs analyzed in Hunting Creek sediment and water samples.

EDC Analyte	Category	Use
Acetaminophen	Pain reliever and fever reducer	OTC[a] Drug
Atrazine	Pesticide	Broadleaf Weed Control
Bisphenol A	Plasticizer	Plastics
Caffeine	CNS stimulant	Coffee, soda
Carbamazepine	Anticonvulsant	Prescription Drug
Coprostanol	Sterol	Fecal Biomarker
Coumestrol	Sterol	Phytoestrogen
D(-)-Norgestrel	Synthetic Progesterone	Oral Contraceptive
Dextromethorphan	Cough suppressant	Delsym
Dichlofenac	NSAID[b]	Prescription Drug
Diphenhydramine	Antihistamine	OTC Drug
Equilin	Hormone replacement therapy	Prescription Drug
Escitalopram	SSRI[c]-Lexapro	Prescription Drug
Estrone	Estrogenic hormone	Oral Contraceptive
Fluoxetine	SSRI-Prozac	Prescription Drug
Gemfibrozil	Cholesterol reducer	Prescription Drug
Ibuprofen	NSAID	OTC Drug
Naproxen	NSAID	OTC Drug
Progesterone	Menopausal hormonal therapy	Prescription Drug
Testosterone	Steroid hormone	Prescription Drug
Triclosan	Antibacterial, Antifungal	Soap Additive
Trimethoprim	Antibacterial	Prescription Drug
Vinclozolin	Fungicide	Prescription Drug
4-*tert*-Octylphenol	Surfactant	Detergent
4-Nonylphenol	Surfactant	Detergent
17 α-Estradiol	Estrogenic hormone	Oral Contraceptive
Mestranol	Estrogenic hormone	Oral Contraceptive
19-Norethindrone	Estrogenic hormone	Oral Contraceptive
17β-Ethynlestradiol	Synthetic steroid	Oral Contraceptive

[a]Over the counter
[b]Non-steroidal anti-inflammatory drug
[c]Selective serotonin reuptake inhibitor

The concentrated extracts were subjected to Florisil chromatography for extract cleanup. The Florisil columns were comprised of 6.0 g of 5% (wt/wt) water-deactivated Florisil sandwiched between 2.0 g of anhydrous sodium sulfate. The columns were conditioned with 50 mL 1:1:1 (v/v/v) DCM:EtOAc:MeOH. The extracts were loaded on Florisil and eluted with 75 mL of 1:1:1 (v/v/v) solution of DCM:EtOAc:MeOH. The Florisil eluents were collected in 250-mL glass TurboVap tubes. The eluents were concentrated to 0.5 mL using the TurboVap. During solvent evaporation the Florisil eluent was solvent-exchanged with toluene. The concentrated fractions were transferred to 1.0-mL GCMS vials for analysis.

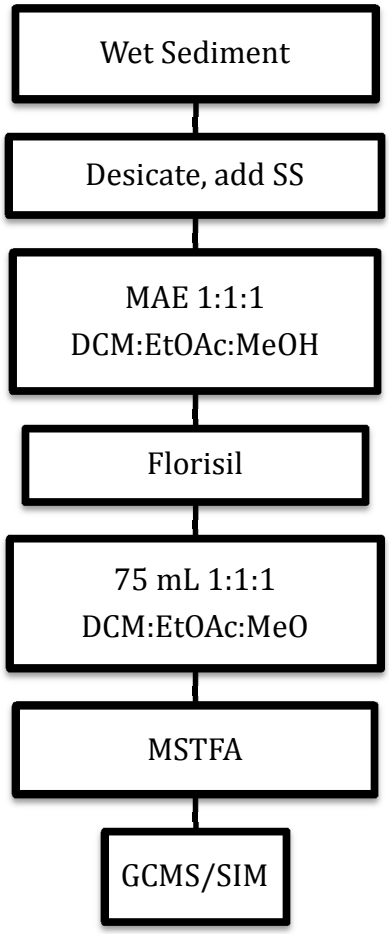


Figure 2.4. Summary of sediment analysis method.

The river water samples were initially filtered through Whatman GF/F (Sigma Aldrich, MO) glass fiber filters to separate the particles from water. The EDCs were extracted from filtered water by using Waters Oasis HLB (Waters, MA) solid phase extraction (SPE) followed by chromatography-mass spectrometry (GCMS) identification and quantitation as summarized in Figure 2.5.

The filter was immediately sealed in an aluminum envelope and stored at -20 ^{o}C until further analysis. Establishing the concentrations of EDCs (Table 2.4) in

surface water was performed for both the particle (filter) and dissolved phases using procedures previously described by Shala and Foster.[111] The filtered water was initially spiked with surrogate standards (Appendix B) prior to extraction. 500 mL of the filtered water was extracted and adjusted to pH 7.0 with 10 mL of 0.5 M phosphate (NaH_2PO_4) buffer and processed.

The SPE cartridges (3 mL capacity) were pre-conditioned with 3 mL hexane, 3 mL ethyl acetate, 3 mL methanol and 5 mL of 10 mM pH 7 phosphate buffer. Solid phase extraction was performed using a 12-fold vacuum extraction box (Supelco Inc., PA). Extractions were performed under vacuum at a flow rate of 5-10 mL/min.

Following extraction, cartridges were rinsed initially with 10 mL of phosphate buffer and aspirated under vacuum for 30 min. To pre-elude lipophilic compounds and help remove any residual water, cartridges were washed with 2 mL of hexane. The analytes were eluted with 2x2.5 mL aliquots of ethyl acetate, followed by 2x2.5 mL methanol. The eluents were combined in a 250 mL TurbVac tube and evaporated to 0.5 mL under dry nitrogen in the TurboVap evaporator. The analytes were spiked with internal standards (Appendix B) and derivatized with MSTFA prior to GCMS analysis.

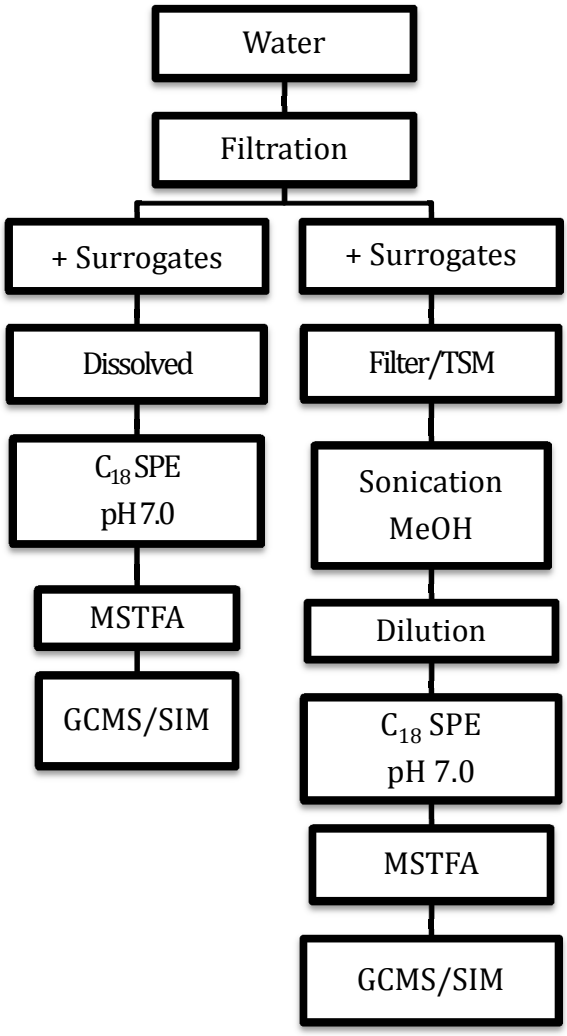


Figure 2.5. Summary of water analysis method

The GF/F filters (Figure 2.6) were spiked with 240 ng triclosan-d3 surrogates, then sonicated four time with 15 mL aliquots methanol (MeOH), diluted to 1 L with double distilled water (DDW) to which 10 mL of pH 7.0 phosphate buffer was added. Eluents were concentrated to 0.5 mL in a TurboVap tube under a gentle stream of nitrogen using a TurboVap nitrogen evaporator.

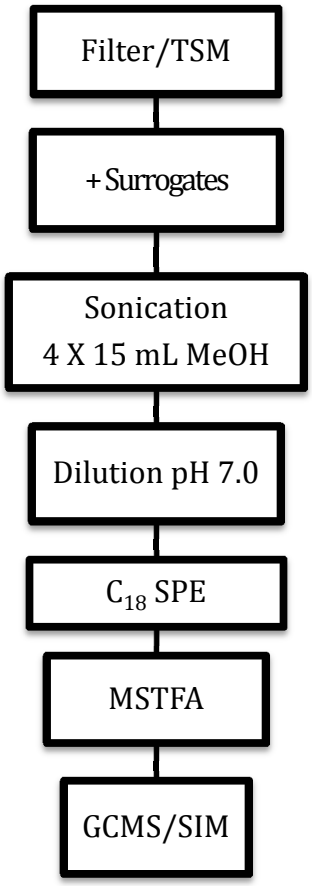


Figure 2.6. Summary of filter analysis method.

GCMS Analysis

GCMS analysis was performed using an Agilent 7890A series gas chromatograph (Agilent Technologies, Santa Clara, CA) interfaced to an Agilent 5975C mass-selective detector. Retention time locking, data acquisition, processing, and instrumental control was performed by the Agilent MSD ChemStation software (Version E.02.02.1431). An MMI inlet was used in the solvent vent mode, enabling

large volume injection (LVI) of 10 µL. The EDC analytes are separated using an Agilent HP-5MS capillary column (5%biphenyl/95%dimethylsiloxane) with the following dimensions: 30 m x 0.25 mm i.d., 0.25 µm thick film. An Agilent model 7673A automated liquid sampler (auto sampler) was used to provide 10 µL injections into the GC. An Agilent ultra-inert 2 mm dimpled inlet liner was used at the inlet for inert performance. The GC operating conditions were as follows for the EDC analysis: the initial column temperature of 70 °C for 15 seconds, programmed to 300 °C at 600 °C/min, and kept at this temperature for 2 minutes. The helium carrier gas flow is maintained at a constant pressure of 17.3 psi a retention time locked method, adopted from the Agilent Pesticide and Endocrine Disruptor database, using the locked retention time of chlorpyriphos methyl (16.596 min) was used as a retention time reference.

Electron impact (EI) mass spectra, in both full-scan mode and selected ion mode (SIM), are obtained at 70 eV with monitoring from 50 m/z to 510 m/z for full-scan mode. In SIM mode the quantifying ion and two additional qualifying ions were used for each target analyte, table presented in Appendix C. The quadruple analyzer and the ion source temperatures were held constant at 150 and 230 °C respectively. All calibration and quantitation was accomplished employing ChemStation software (Version E.02.02.1431).

All extracts were spiked with 50 µL of Internal standards (Table 2.3) prior to derivitization. The endocrine distruptors are derivatized using N-Methyl-N-(trimethylsilyl) trifluoroacetamide (MSTFA). Prior to injection, the extracts

contained in GCMS vials were amended with 50 μL of MSTFA and held in a heating block for 30 min at 80 ^{o}C to form trimethyl silyl derivatives with carboxyl, hydroxyl and amine functional groups, thereby enhancing GCMS identification and quantitation. Not all of the EDC analytes were derivatized.

Calibration standards were evaluated through ChemStation (version E.02.02.1431) using internal standard injections standards at 6 concentrations using acenaphthene-d10, phenanthrene-d10 and chrysene-d12 as the internal injection standards. Chemical identification was performed by matching (i) retention times of sample GCMS peaks relative to the authentic analytical standards, (ii) the retention time of the primary (quantifier) SIM extracted ion, and (iii) matching the correct relative ratios of GCMS-SIM secondary and tertiary ions relative to the primary (quantifier) ion. Quantitation of the EDC concentrations was performed by using the quantifier ion ratio to the internal injection standard in calibration,. The quantifier ion was a prominent ion in the mass spectrum of the pure EDC standard with a m/e ratio unique from other EDCs.

Quality Assurance

Analytical limits of detection (LODs) for Hunting Creek water and sediment samples were determined by multiplying the standard deviation of 10 replicate runs of the medium concentrated calibration standard (320.0 ng/vial) by the Student t-test for the 95% confidence level. The values were then divided by the approximate sample mass (1 g) for sediment and by 0.5 L for water samples to determine the

method detection limits (MDLs) in ng/g for bed sediment and in ng/L for water samples and are presented in Appendix B. Method Detection Limits varied from 16 to 390 ng/g in sediment and 10 to 505 ng/L in water for the analytes of interest.

Method recoveries were tested for extraction, cleanup efficiency and analyte recovery through surrogate and matrix spike experiments in sediments and water, in triplets. The mean surrogate recovery for triclosan-d3, used in water samples was 83% ± 2%. Sediment surrogate recoveries were 106% ± 15% for atrazine-13C, and 83% ± 20% for naproxen-13C. The Hunting Creek matrix spike recovery percentages, as well as QA recoveries in bed sediment (MAE method) and water (filtration method) are presented in Appendix B. The laboratory blanks were below detection limits. Sample chromatograms from calibration standard, water and sediment are presented in Figures 2.7, 2.8 and 2.9 respectively.

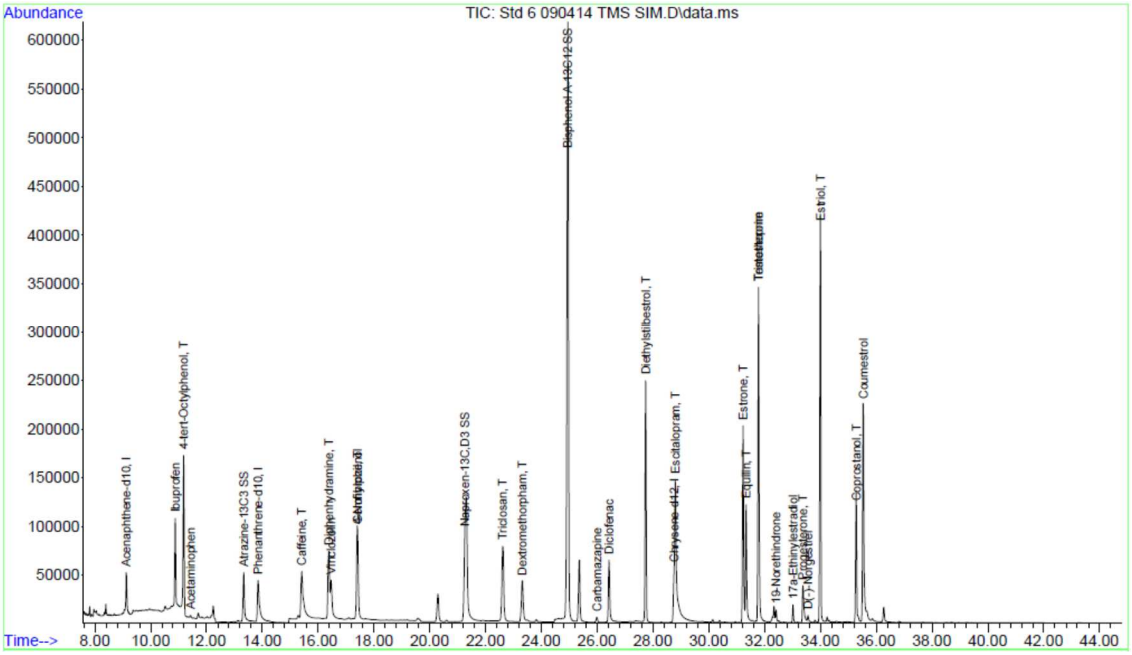


Figure 2.7. Chromatogram of calibration standard, highest concentration.

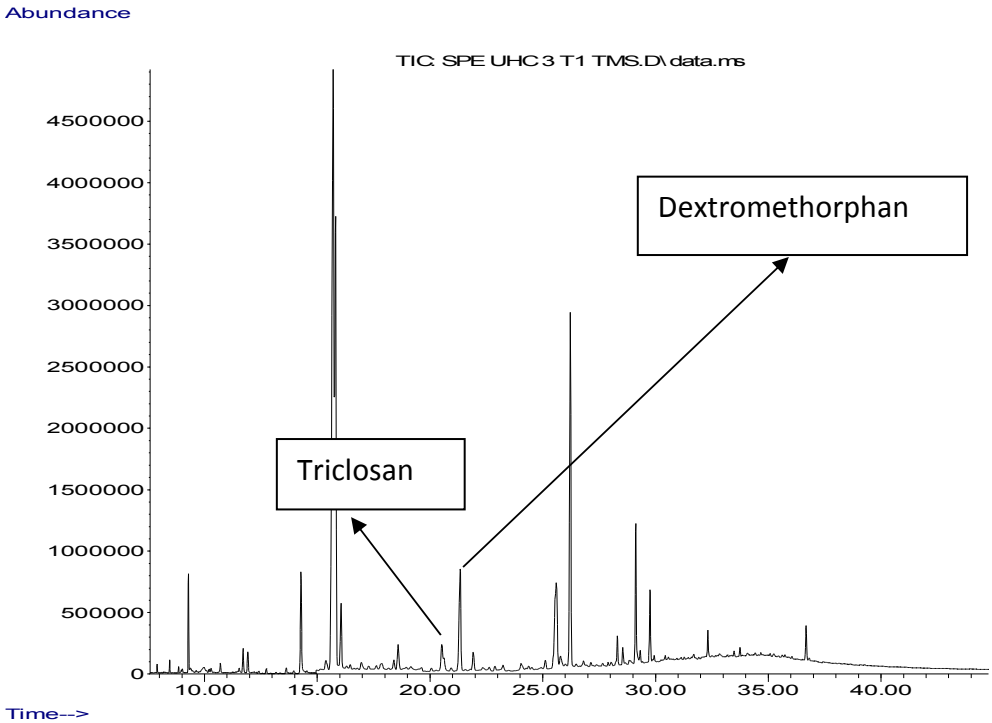


Figure 2.8. Water sample chromatogram

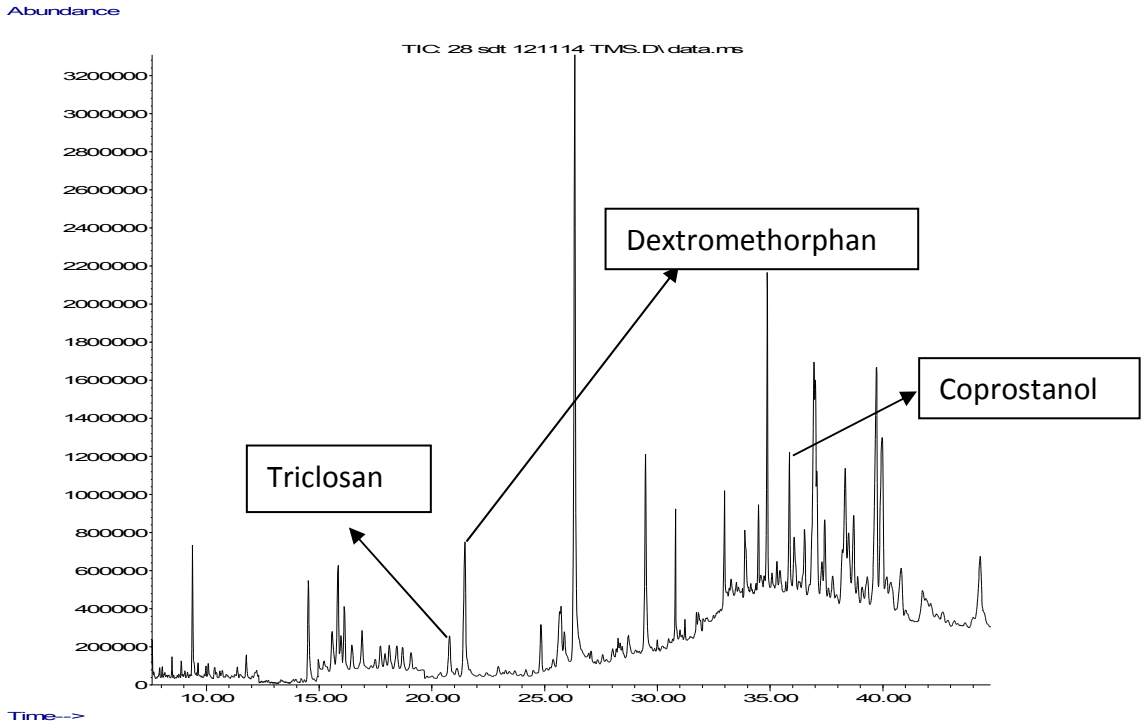


Figure 2.9. Sediment sample chromatogram

Ancillary Measurements

The moisture content of sediment was determined gravimetrically.[74] Sediment (5 g) was pre-weighed, added to a porcelain thimble and heated to constant weight (48 hrs) at 60 °C. The dried sediments were reweighed to yield moisture loss. Moisture content was used to convert the wet mass of sediment determined prior to quantitation to dry mass (dry weights).

Grain size was determined as percent sand using wet sieving through a 64 μm brass sieve followed by gravimetric analysis (± 0.1 mg) of the size fractions.[74] Sediment organic carbon and nitrogen contents were determined using a soil CN

Analyzer (Flash 2000, ThermoScientific, Waltham, MA). Approximately 1 g of sediment was oven dried and ground to a fine powder using mortar and pestle. The ground sample was transferred to a silver combustion cup and treated with 1 M HCl to degas carbon dioxide. Degassed sediment was re-dried and analyzed for organic carbon content using aspartic acid as the reference standard. All sediments were analyzed in triplicate.

Total suspended matter (TSM) of water collected was determined by vacuum filtering the water samples through Whatman GF/F (0.7 μm pore diameter; Sigma Aldrich, MO) glass fiber filters to separate the particles from water and TSM amount was determined gravemetrically.[112] Organic content of TSM were determined using soil CN Analyzer (Flash 2000, ThermoScientific, Waltham, MA). Once TSM was determined the filters were placed in a desiccator with 1 M HCl for 24 hours to remove unwanted inorganic carbon from the filter, leaving only the organic carbon on the filter.[113] The filters were then folded and placed into silver cups in preparation for organic carbon analysis using the CN Analyzer.

Fugacity Estimates and Equilibrium Assessment

To determine the equilibrium distribution of EDCs between water and sediment fugacity theory was used. Fugacity (F) is defined as the escaping tendency of a chemical in a phase, or its vapor pressure, and is calculated in pressure units (atm) for sediments and water using phase-specific Henry's law constants (atm-kg/mol) in conjunction with mean individual detected EDC concentrations (kg/mol)

measured by GCMS (Equation 2.1).[43] Mean concentrations of EDCs in sediments and water were converted from ng/g to mol/kg by using the molar mass.

$$F_{ij} = K_{H\,i,j} \times C_{i,j} \qquad \text{Equation 2.1}$$

$F_{i,j}$ = Fugacity of i^{th} chemical in j^{th} phase (atm), phase equivalent vapor pressure
$K_{H\,i,j}$ = Henry's Law Constant of i^{th} chemical in j^{th} phase (atm.kg/ml)
$C_{i,j}$ = Concentration (mol/kg) of i^{th} chemical in j^{th} phase measured by GCMS

Phase specific Henry's law constants (atm.kg/mol) for sediment and water were estimated using equation 1.2, adapted from Schwarzenbach et al.[43].

$$K_{H\,i,j} = \frac{K_{H_i}}{K_{d\,i,j}} = \frac{K_{H_i}}{(\alpha_j \beta_j) K_{ow_i}} \qquad \text{Equation 2.2}$$

$K_{H\,i,j}$ = Henry's Law Constant of i^{th} chemical in j^{th} phase (atm.kg/ml)
$K_{d\,i,j}$ = Distribution constant of i^{th} chemical in j^{th} phase (L/kg)
α_j = Constant for j^{th} phase (water=1, sediments=0.41)
β_j = Phase constant (β_j water=1, sediment=f_{oc}= organic carbon fraction)
$K_{ow,i}$ = Water octanol partition coefficient (K_{ow}) of i^{th} chemical
$K_{H,i}$ = Henry's law constant of i^{th} chemical (atm.kg/mol)

Individual K_H and K_{ow} values were determined from EPA EPI suite[10] and presented in Appendix D. The phase constant (β_j) and constant for the j^{th} phase (α_j) are not necessary since water is in liquid phase.

To determine equilibrium or direction of flow, sediment fugacity was divided by water fugacity of the corresponding sampling location. A fugacity ratio ($F_{sediment}/F_{water}$) of 1 is accepted as equilibrium partitioning. In this study a ratio between 0.5 to 1.5 was considered equilibrium. A >1.5 ratio indicates fish to sediment flow of analytes and a <0.5 ratio indicates sediments to fish flow.

Results

Ancillary Data

Sand content from Hunting Creek sediments varied from 3% to 81%, and organic carbon content range was 0%-5% (Table 2.5). Organic carbon content indicated a positive correlation with clay content (Figure 2.10). Sediments from lower Hunting Creek channel and mainstem Potomac River sampling sites showed lower sand content 10%-18% and 2%-26% respectively; in contrast Cameron Run and upper Hunting Creek had the highest sand content varying from 50% to 81%.

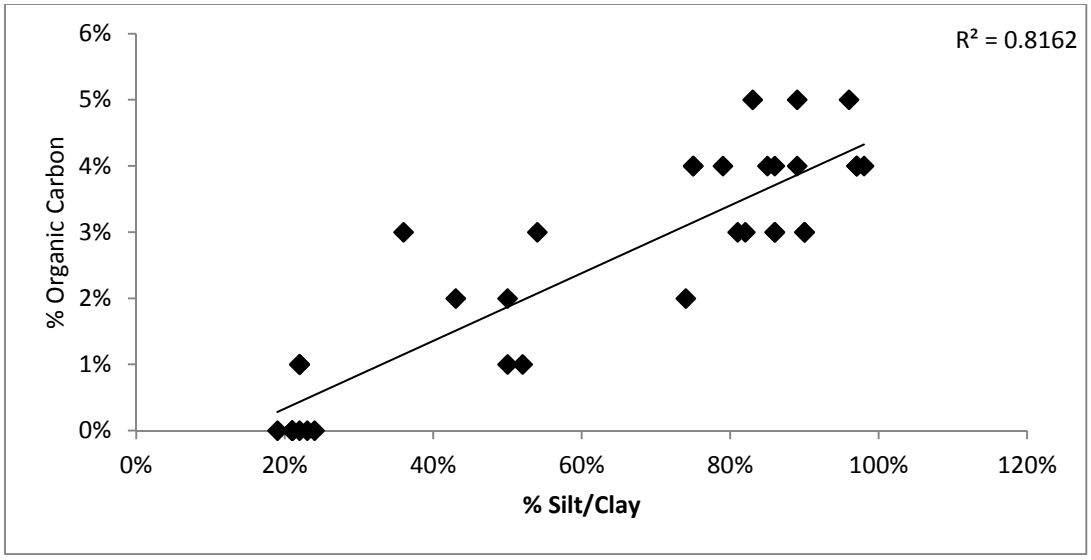


Figure 2.10. Regression graph correlating clay content to organic carbon content. Silt/Clay content was determined by subtracting sand content from 100.

Table 2.5. Ancillary data for Hunting Creek Sediments

Collection Site	% Sand	% Silt/Clay	% Moisture	% OC
Cameron Run 1.1	48%	52%	69%	1.2%
Cameron Run 1.2	50%	50%	63%	0.9%
Cameron Run 1.3	50%	50%	56%	1.9%
Cameron Run 2	78%	22%	77%	1.0%
Cameron Run 3	78%	22%	77%	0.0%
Cameron Run 4	79%	21%	78%	0.1%
Cameron Run 5	79%	21%	79%	0.1%
Upper Hunting Creek 1.1	79%	21%	78%	0.1%
Upper Hunting Creek 1.2	79%	21%	78%	0.1%
Upper Hunting Creek 1.3	81%	19%	79%	0.1%
Upper Hunting Creek 2	76%	24%	76%	0.3%
Upper Hunting Creek 3	77%	23%	83%	0.1%
Upper Hunting Creek 4	78%	22%	78%	1.0%
Upper Hunting Creek 5	78%	22%	76%	1.1%
Lower Hunting Creek C1.1	14%	86%	54%	3.0%
Lower Hunting Creek C1.2	14%	86%	45%	4.3%
Lower Hunting Creek C1.3	11%	89%	44%	5.3%
Lower Hunting Creek C2	17%	83%	42%	5.4%
Lower Hunting Creek C3	10%	90%	54%	3.4%
Lower Hunting Creek C4	10%	90%	56%	3.0%
Lower Hunting Creek C5	14%	86%	54%	3.0%
Lower Hunting Creek C6	18%	82%	59%	2.7%
Lower Hunting Creek Ctr	25%	75%	38%	4.4%
Lower Hunting Creek S1.1	46%	54%	62%	2.8%
Lower Hunting Creek S1.2	64%	36%	70%	3.0%
Lower Hunting Creek S1.3	57%	43%	65%	1.6%
Lower Hunting Creek S2	25%	75%	51%	3.7%
Lower Hunting Creek S3	15%	85%	43%	4.4%
Lower Hunting Creek S4	11%	89%	45%	3.7%
Lower Hunting Creek S5	3%	97%	39%	4.3%
Lower Hunting Creek S6	4%	96%	39%	4.5%
Potomac River 1.1	26%	74%	58%	2.4%
Potomac River 1.2	19%	81%	52%	3.3%
Potomac River 1.3	21%	79%	57%	3.6%
Potomac River 2	3%	97%	49%	3.8%
Potomac River 3	2%	98%	48%	4.0%
Potomac River 4	3%	97%	48%	3.6%

Highest TSM value was observed from mainstem Potomac River water samples (24.6 ± 26.5 mg/L) and lowest TSM was observed in Cameron Run (1.86 ± 0.60 mg/L) (Table 2.6).

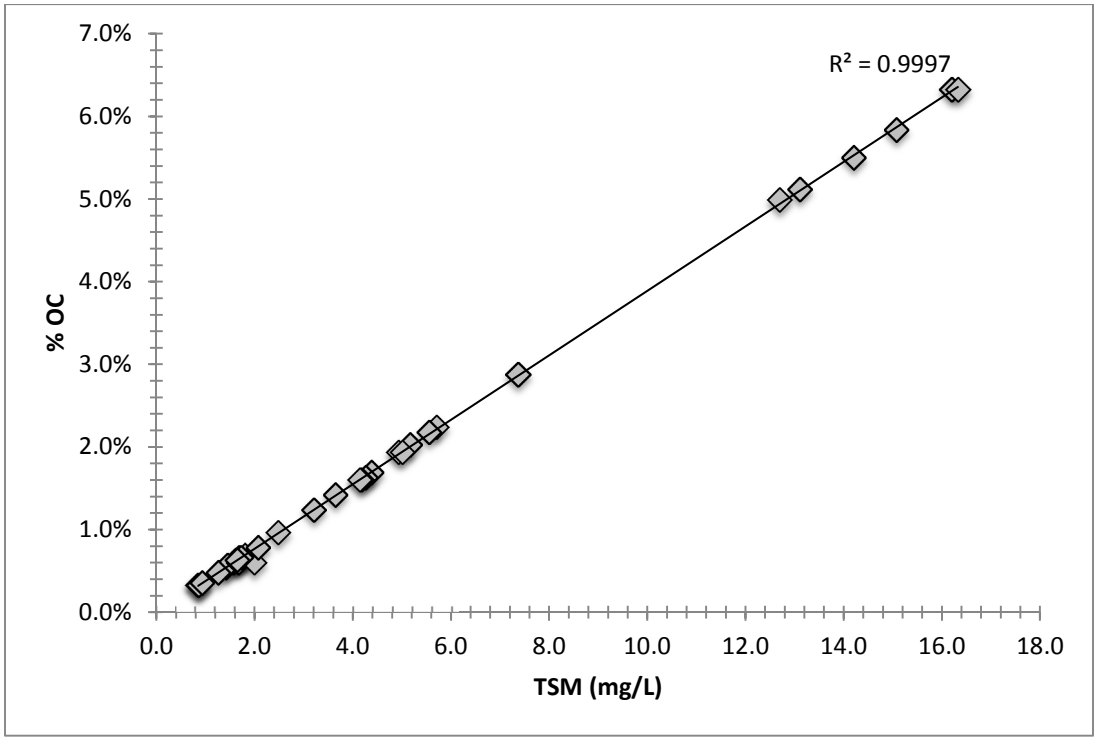


Figure 2.11. Regression graph correlating total suspended matter (TSM) and percent organic carbon (%OC).

The organic carbon determined from TSM filters demonstrated a strong correlation (Figure 2.11). The highest organic carbon content was observed in mainstem Potomac River ranging from 5.0% to 6.3% and lowest was observed in Cameron Run water samples ranging from 0.5% to 1.2%.

Table 2.6. Total suspended matter (TSM) and corresponding percent organic carbon content (%OC) for water samples.

Collection Site	Abbrev.	TSM (mg/L)	% OC
Holmes Run 1.1	HR 1.1	3.20	1.2%
Holmes Run 1.2	HR 1.2	1.57	0.6%
Backlick Run 1.1	BR 1.1	1.69	0.7%
Backlick Run 1.2	BR 1.2	1.71	0.7%
Cameron Run 1.1	CR 1.1	1.43	0.5%
Cameron Run 1.2	CR 1.2	1.63	0.6%
Cameron Run 1.3	CR 1.3	1.81	0.7%
Upper Hunting Creek 1.	UHC 1	1.46	0.6%
Upper Hunting Creek 2	UHC 2	2.48	1.0%
Upper Hunting Creek 3.1	UHC 3.1	5.15	2.0%
Upper Hunting Creek 3.1	UHC 3.2	7.36	2.9%
Upper Hunting Creek 3.1	UHC 3.3	5.69	2.2%
Upper Hunting Creek 4	UHC 4	4.38	1.7%
Upper Hunting Creek 5.1	UHC 5.1	5.54	2.2%
Upper Hunting Creek 5.2	UHC 5.2	4.92	1.9%
Upper Hunting Creek 5.3	UHC 5.3	5.00	1.9%
Lower Hunting Creek C1	LHCC 1	ND	ND
Lower Hunting Creek C2.1	LHCC 2.1	1.68	0.6%
Lower Hunting Creek C2.2	LHCC 2.2	0.85	0.3%
Lower Hunting Creek C2.2	LHCC 2.3	0.87	0.3%
Lower Hunting Creek C3	LHCC 3	2.00	0.6%
Lower Hunting Creek C4	LHCC 4	ND	ND
Lower Hunting Creek C5	LHCC 5	ND	ND
Lower Hunting Creek C6	LHCC 6	0.87	0.3%
Lower Hunting Creek Ctr	LHC-Ctr	3.64	1.4%
Lower Hunting Creek S1.1	LHCS 1.1	4.25	1.6%
Lower Hunting Creek S1.2	LHCS 1.2	4.17	1.6%
Lower Hunting Creek S1.3	LHCS 1.3	4.14	1.6%
Lower Hunting Creek C2	LHCS 2	2.08	0.8%
Lower Hunting Creek C3	LHCS 3	1.73	0.7%
Lower Hunting Creek C4	LHCS 4	1.65	0.6%
Lower Hunting Creek C5	LHCS 5	1.27	0.5%
Lower Hunting Creek C6	LHCS 6	0.94	0.4%
Potomac River 1	PR 1	ND	ND
Potomac River 2	PR 2	16.2	6.3%
Potomac River 3	PR 3	15.1	5.8%
Potomac River 4.1	PR 4.1	14.2	5.5%

Potomac River 4.2	PR 4.4	12.7	5.0%
Potomac River 4.3	PR 4.3	13.1	5.1%
Potomac River 5	PR 5	16.3	6.3%

EDCs in Sediments

Of the 31 EDC analytes analyzed, triclosan, dextromethorphan and coprostanol were detected in Hunting Creek sediments above analytical detection limits; Ibuprofen, acetaminophen, 4-tert-octylphenol, diphenhydramine, vinclozolin, 4-nonylphenol, gemfibrozil, diethylstilbestrol, escitalopram, trimethoprim, 19-norethindrone and coumestrol were initially detected but were below detection limits.

The greatest sediment concentration of triclosan was detected in Cameron Run with the mean concentration of 293 ± 67.1 (ng/g dwt) and lowest concentration was observed in the mainstem Potomac River with a mean concentration of 51.3 ± 11.3 (ng/g dwt) (Figure 2.12). The highest concentration of dextromethorphan was detected in lower Hunting Creek (81.3 ± 44.1 ng/g dwt) and lowest in Potomac River mainstem (42.2 ± 8.00 ng/d dwt) (Figure 2.12).

The detection frequencies of triclosan varied across the four hydrological zones, with the lowest (47%) determined in the Potomac River mainstem and the greatest in Cameron Run and upper Hunting Creek (57%). The lowest detection frequency of dextromethorphan (33%) occurred in Potomac River mainstem and highest (43%) was in upper Hunting Creek, with no detection in Cameron Run. Coprostanol, a biomarker for human fecal matter, was not detected in Cameron Run,

but showed 100% detection frequency in all of the downstream sites. Cameron Run is upstream of the discharge location of Alexandria Renew plant, while upper Hunting Creek is the location of the discharge and the mean concentrations of the two most prominent analytes did not indicate a concentration gradient.

To assess sample variability replicates of sediments were collected. The variability between samples is actual field variability because the standard deviation between replicates (0.00 to 0.46) is much lower than field samples (0.00 to 59.2).

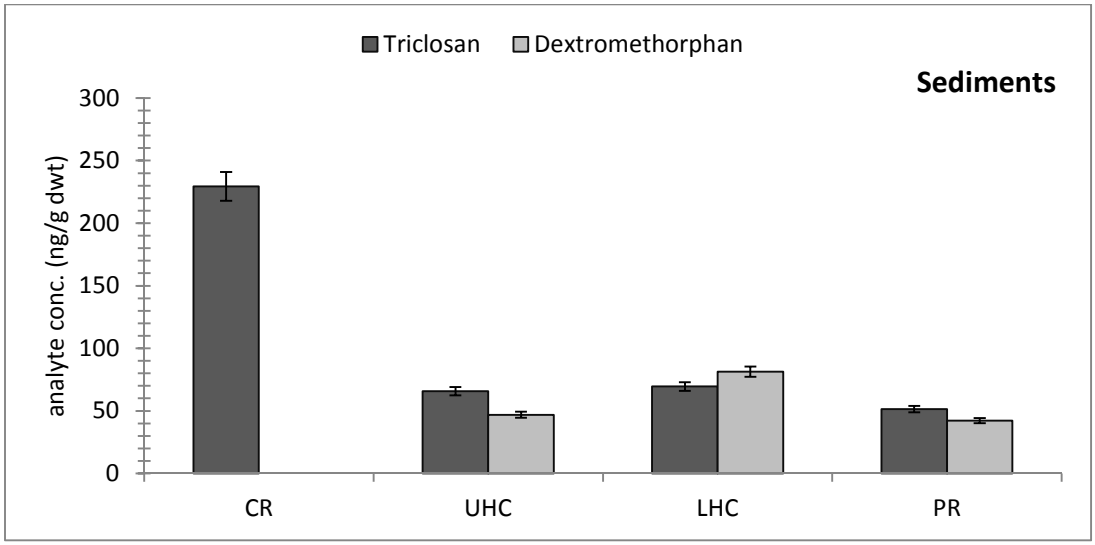


Figure 2.12. Mean concentrations (± sd) of triclosan and dextromethorphan in Hunting Creek sediments. CR: Cameron Run, Zone 1, UHC: upper Hunting Creek, Zone 2, LHC: lower Hunting Creek, Zone 3, PR: Potomac River, Zone 4.

EDCs in Water

Three of the 31 target EDCs were detected in Hunting Creek water samples, consistent with the EDCs detected in sediments, triclosan and dextromethorphan and coprostanol. Coprostanol is a fecal matter biomarker.

Triclosan was detected only in Cameron Run and upper Hunting Creek, with concentrations of 18.1 ± 4.76 ng/L and 12.9 ± 1.66 ng/L, respectively (Figure 2.13). Upper Hunting Creek was the only region where both triclosan and dextromethorphan were detected with mean concentrations of 12.9 ± 1.66 ng/L and 64.0 ± 6.71 ng/L respectively.

Dextromethorphan was detected in upper and lower Hunting Creek as well as Potomac River mainstem, with highest dextromethorphan concentration in Potomac River, 87.5 ± 2.3 ng/L and lowest in upper Hunting Creek (64.0 ± 6.71 ng/L) (Figure 2.13). Dextromethorphan was not detected in Cameron Run water, while triclosan was detected in water from all four zones.

Statistical analysis indicated significant differences in Hunting Creek water concentrations (ng/L) across the four hydrodynamic zones (one-way, ANOVA, $p<0.05$). The filters used to collect total suspended matter (TSM) had such a small mass that it precluded analysis in the solid phase.

Detection frequency of triclosan varied among the four zones, with no detections recorded in lower Hunting Creek and the mainstem Potomac River to the greatest detection frequency in Cameron Run (57%) and lowest in upper Hunting Creek (38%). Highest detection frequency of dextromethorphan (84%) occurred in Potomac River mainstem and lowest (50%) was in upper Hunting Creek, with no detection in Cameron Run.

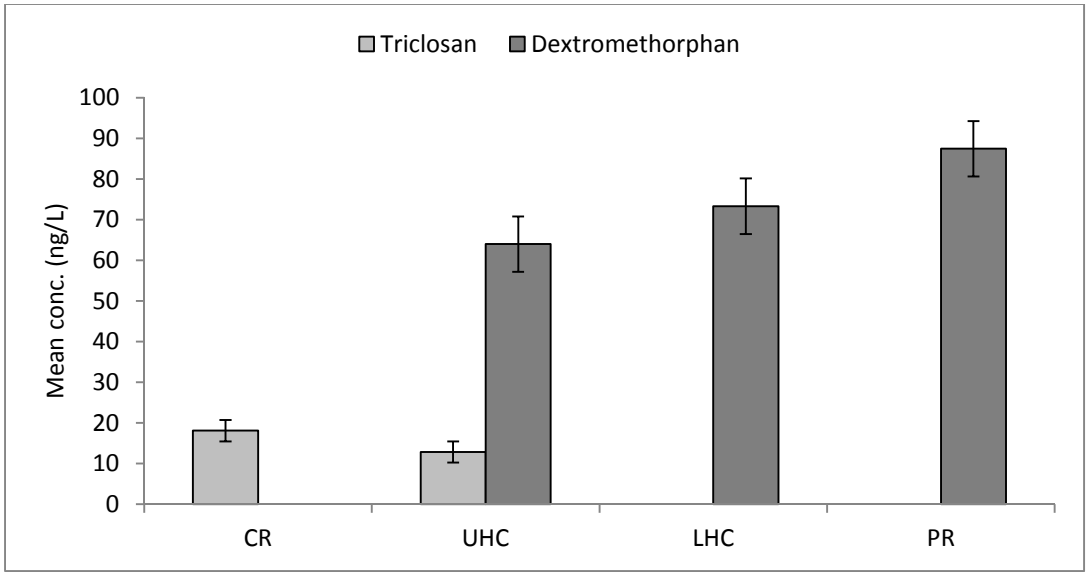


Figure 2.13. Mean concentrations (± sd) of Triclosan and Dextromethorphan measured in Hunting Creek water samples. CR: Cameron Run, Zone 1, UHC: upper Hunting Creek, Zone 2, LHC: lower Hunting Creek, Zone 3, PR: Potomac River, Zone 4.

To assess sample variability, replicates of stream water were collected (Figure 2.14). The variability between samples is actual field variability because the standard deviation between replicates (0.00 to 0.83) is much lower than field samples (0.00 to 67.4).

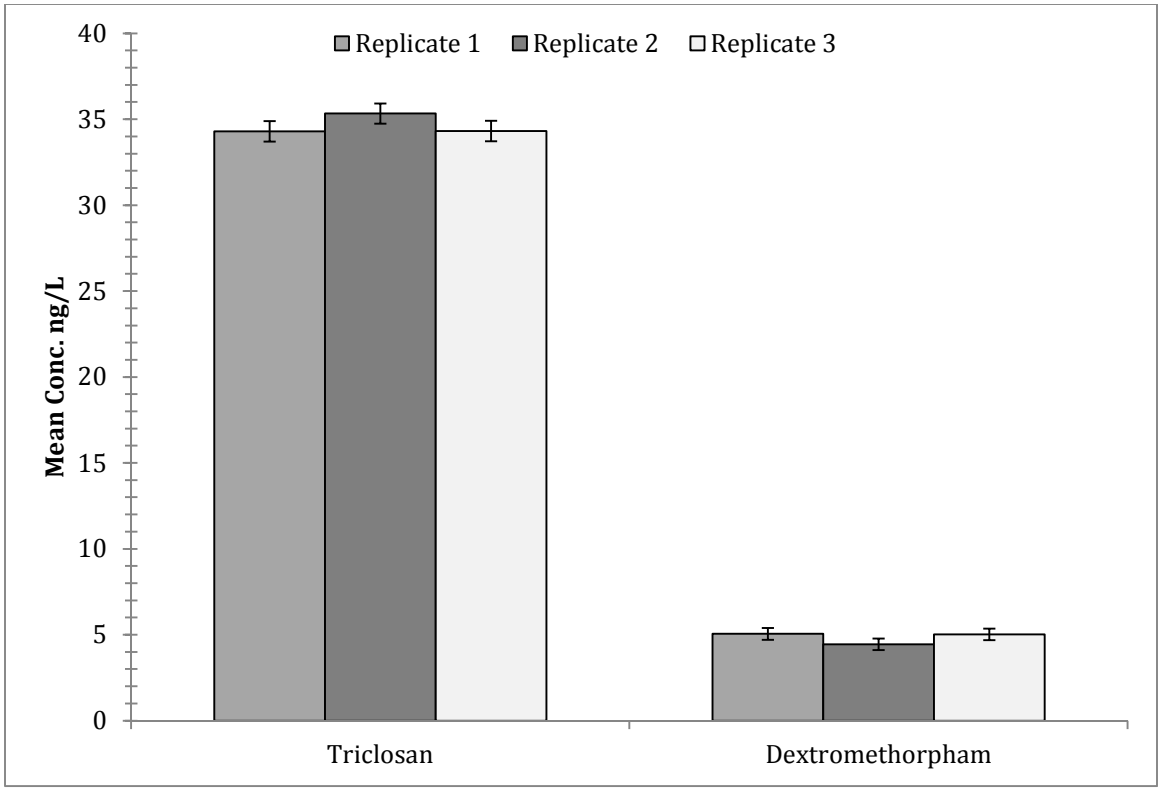


Figure 2.14. Water replicates (n=3) of upper Hunting Creek, indicating minimal variability and standard deviation range of 0.00 to 0.83.

Distribution and Fugacity of EDCs in Water and Sediments

Since only triclosan and dextromethorphan were detected in water and sediments the direction of flux was determined only for them. Fugacity ratios of TCS and DXM for Hunting Creek water and sediments (Figure 2.15) showed a consistent direction of flux from sediments to water.

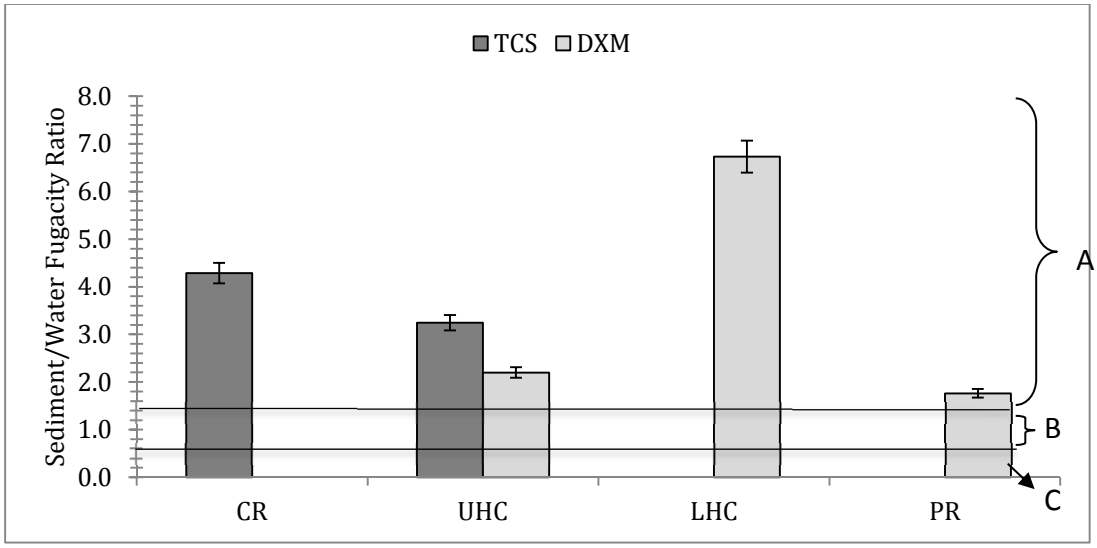


Figure 2.15. Fugacity ratios of Hunting Creek sediments and water samples. A ratio of 0.5-1.5 indicates equilibrium (**B**), while a <0.5 ratio is water to sediment flux (**C**) and >1.5 is sediments to water (**A**).

Discussion

Analyzing endocrine disruptors, using GCMS (gas chromatography, mass spectrometry) is inherently difficult, due to their low volatility. To increase volatility all extracts, spikes and calibration standards were derivatized using N-Methyl-N-(trimethylsilyl) trifluoroacetamide (MSTFA). MSTFA replaces the hydrogen of the hydroxyl functional group, with a trimethylsilyl (TMS) group (Figure 2.16).

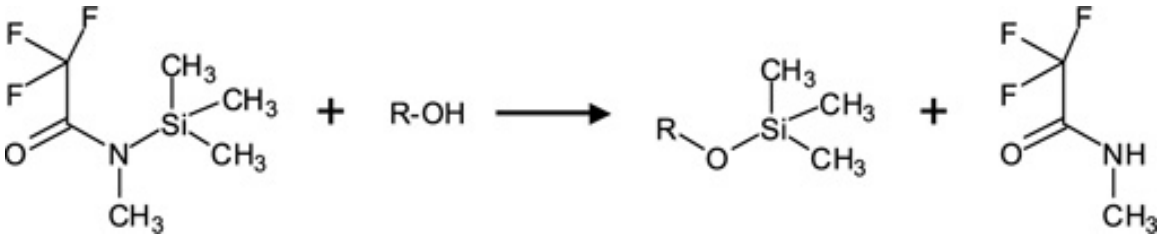


Figure 2.16. Derivatizing using MSTFA.

The TMS derivatives have a lower boiling point than the parent molecule, which allows GC (Gas chromatography) analysis at lower temperatures.[114]

Due to the difficulty of GCMS analysis of endocrine disruptors only two out of a list of 31 target EDCs were positively detected above detection limits. Ibuprofen, acetaminophen, 4-tert-octylphenol, diphenhydramine, vinclozolin, 4-nonylphenol, gemfibrozil, diethylstilbestrol, escitalopram, trimethoprim, 19-norethindrone and coumestrol were initially detected but were screened as below method detection limits.

Both water and sediment concentrations were significantly different from one another, (one-way ANOVA, $p<0.05$) indicating geospatial differences among the hydrodynamic zones. Analysis of Hunting Creek revealed three interesting points, (i) triclosan was ubiquitous in Hunting Creek sediments, and was detected in two segments of the stream water and showed opposite concentration gradient from the WWTP, with highest triclosan concentration above the WWTP outfall (ii) with the exception of Cameron Run, dextromethorphan was also detected in both water and sediments from Hunting Creek, and also showed reverse concentration gradient away from the WWTP, with highest dextromethorphan concentration in mainstem

Potomac River (iii) triclosan concentration in sediments (ng/g dwt) from Cameron Run was fivefold greater than the water concentration (ng/L).

Significant differences existed among the sediment concentrations of dextromethorphan and triclosan (one-way, ANOVA, $p<0.05$), indicating geospatial differences exists within the hydrological zones of the Hunting Creek, and since the concentration gradient indicates higher concentration of triclosan in Cameron Run which is located upstream of Alexandria Renew outfall and it decreases downstream of the outfall, Alexandria Renew would not be regarded as a primary source of contamination for triclosan, and another source could be the primary source of triclosan in Hunting Creek sediments and water, ruling out the WWTP as the primary source.

The abundance of triclosan in the sediments and water (Figure 2.17) is consistent with a study done between 1999 and 2000 by the US Geological Survey, which tested a network of 139 streams across 30 states for 95 different chemicals, indicating triclosan as one of the most frequently detected (57.6%) with the highest concentrations (2.3 μg/L).[98]

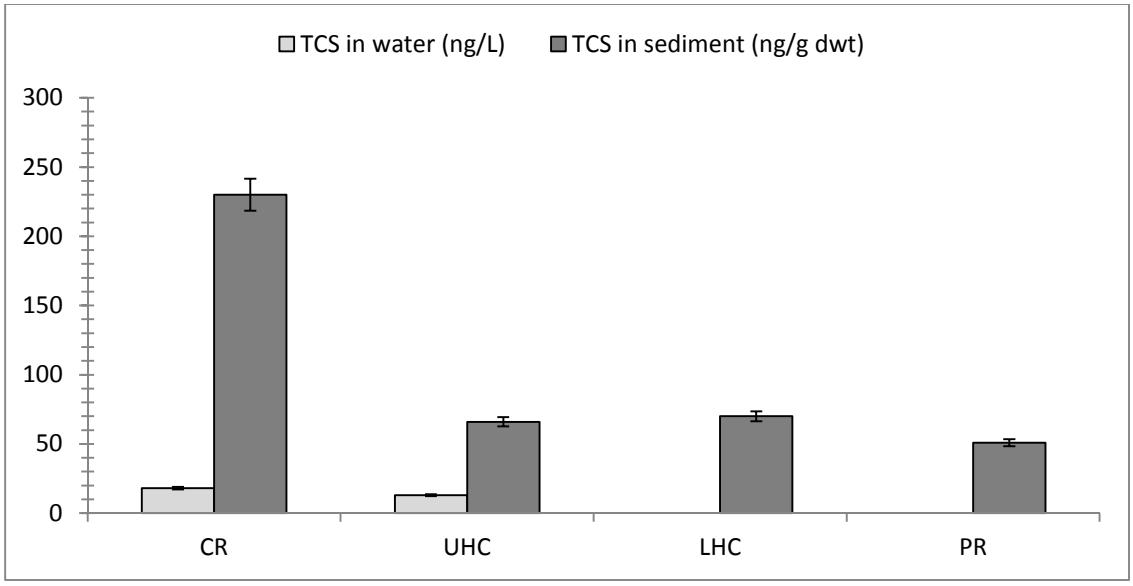


Figure 2.17. Mean concentrations of triclosan in water and sediment from Hunting Creek.

Triclosan is antibacterial added to a wide variety of products such as clothing, kitchenware, furniture and toys, as well as an active ingredient in antibacterial soaps, body washes, toothpaste and some cosmetics.[115] The research on potential health effects of triclosan utilizes animal models, and the results are not always applicable to humans.[115] Studies have identified thyroid and androgenic effects due to exposure to triclosan[116-119], and endocrine distruption[120, 121] as potential effects to animals. Meeker et al. reported a positive association between Triclosan concentrations detected in pregnant women's urine and the use of certain personal products[122] and that the detection of maternal urinary triclosan concentrations persisted throughout the pregnancy as well as postpartum.[123]

Due to lack of sufficient data on triclosan toxicity in humans FDA does not recommend changing consumer use of triclosan containing products but advises concerned consumers of not using triclosan in light of lack evidence that triclosan provides extra health benefits over regular soap and water.[115]

Organic carbon normalized triclosan concentration in Cameron Run sediments is alarmingly high, 1071 ± 262 μg/g OC, compared to 36.9 ± 25.1, 5.42±5.26 and 3.39±2.63 (μg/g OC) from upper Hunting Creek, lower Hunting Creek and Potomac River respectively. The organic carbon normalized concentrations show a pattern of decreasing concentration in Potomac River < lower Hunting Creek < upper Hunting Creek < Cameron Run.

High water and sediment concentrations of triclosan in Cameron Run (upstream of WWTP) is surprising and is an indication of triclosan having other sources of introduction into the aquatic environment other than discharge through WWTPs. A possible explanation for this high concentration could be related to septic tanks, which by design leach, in Cameron Run. Virginia Department of Environmental Quality has reported 221 septic tanks in Cameron Run, and none in Hunting Creek.[124] The high triclosan concentration in Cameron Run cannot be attributed to stormwater runoff since triclosan is an antibacterial and would have no application on farmland.

Dextromethorphan is a non-opioid antittusive OTC cough suppressant with a history of abuse recorded in many countries[125, 126], its abuse induces psychiatric symptoms such as depression[125-127] and the abuse is on the rise in the US,

demanding close monitoring due to the risks of severe medical complications, addictions as well as psychiatric effects.[128, 129] A recent study showed repeated, high dose DXM treatment of rats decreases neurogenesis and also results in depression like behavior in rats.[130]

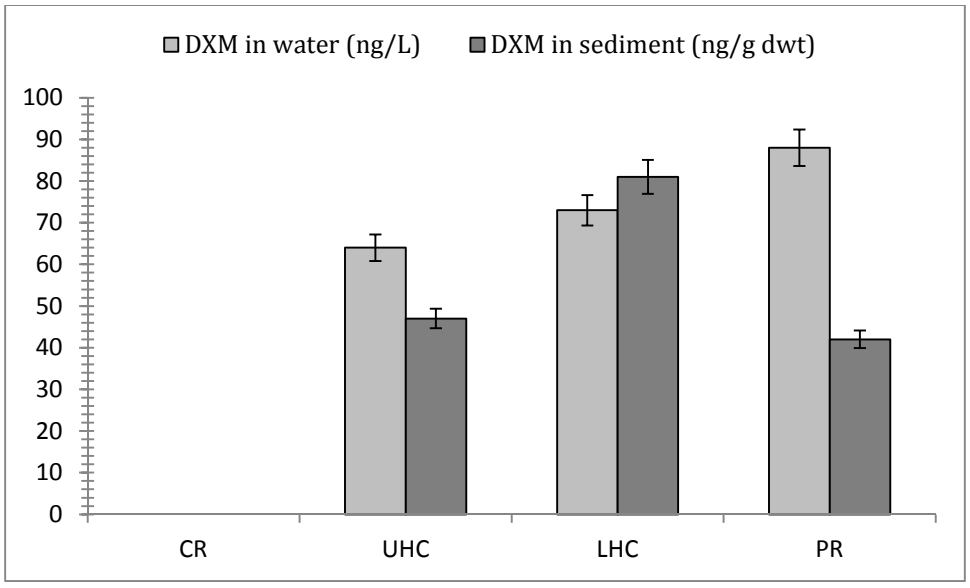


Figure 2.18. Mean concentrations of dextromethorphan in water and sediments from Hunting Creek.

Interestingly dextromethorphan was detected in three out of the four hydrodynamic zones, with the exception of Cameron Run zone (Figure 2.18). Organic carbon normalized concentrations of dextromethorphan in Hunting Creek sediments did not indicate a concentration gradient and was not detected in Cameron Run, while the highest concentration was detected in lower Hunting Creek (16.6±13.4 μg/g OC).

Acute toxicity of triclosan has been investigated in several species[131], none of which included the species analyzed for this study, with the exception of bluegill (*Lepomis macrochirus*), with reported LC_{50} (lethal concentration resulting in 50% mortality) of 370 µg/L, with test duration of 96 hours.[132]; the mean concentrations of triclosan detected from Cameron Run and upper Hunting Creek (18 and 13 ng/L) fall well below this threshold.

Dextromethorphan toxicity research on fish is limited, with reported LC_{50} of 4.0 mg/L for 96 hours in rainbow trout (*Oncorhynchus mykiss*)[133] and the mean concentrations of dextromethorphan in all four regions is well below this value.

Triclosan detection in sediment was not a good indicator for possibility of detection in water samples (Figure 2.16); TCS was detected only in Cameron Run and upper Hunting Creek water, while it was detected in the sediments of all four zones. Dextromethorphan detection in sediments was a good predictor for detecting DXM in water samples.

The fugacity calculations for TCS and DXM in water and sediments indicated sediments to water flux indicating higher concentrations of TCS and DXM in bed sediments. Fugacity values can be utilized as a tool to profile distribution patterns of contaminants across ecosystem and biota. Fugacity is analogous to partial pressure of an ideal gas and directly related to concentration.[60] Fugacity ratios of analytes in different media can be calculated and the magnitude of the ratios will determine equilibrium or direction of flow.

Conclusion

Through the study of Hunting Creek water and sediments, contemporary EDCs in water and sediments were quantified and the most prominent chemicals were determined to be triclosan (TCS), a broad spectrum antibacterial agent, and dextromethorphan (DXM), cough suppressant agent readily available in OTC cold medicines. Geospatial differences between collection sites were observed, implicating the WWTP as a possible source for DXM, and with a possibility of leaking septic tanks as possible source for TCS. Resemblance of dextromethorphan detection and concentration profiles in water and sediments indicate one could predict occurrence and concentration of DXM; however triclosan did not reveal a consistent pattern of occurrence in water samples.

The most interesting find of this study was the very high water and sediment concentration of triclosan in Cameron Run. Cameron Run is upstream of the Alexandria Renew outfall and indicates that WWTP is not the only source for introduction of triclosan into the aquatic environment, and further research is warranted to determine the exact cause of increased water and sediments concentration of TCS upstream of WWTP discharge point. Also DXM had a reverse concentration gradient, with lowest mean DXM concentration in upper Hunting Creek zone coinciding with the WWTP outfall and highest DXM concentration was

observed in the mainstem Potomac River showing Alexandria Renew could not be the introduction point of DXM into Hunting Creek.

CHAPTER 3 EDCS IN FISH FROM HUNTING CREEK, ACCOTINK CREEK AND POHICK CREEK: FUGACITY, BIOACCUMULATION, TISSUE DISTRIBUTION AND POTENTIAL TRANSFER TO OFFSPRING

Introduction

Scientific understanding of the relationship between exposure to toxic chemicals and health, both in humans and wildlife, has advanced rapidly over the last decade. One particular class of toxic chemicals in the aquatic environment of interest by environmental managers is endocrine disrupting chemicals (EDCs). Endocrine disruptor chemicals (EDCs) are a large group of micropollutants that include certain pharmaceuticals, personal care products and flame retardants according to the International Programme on Chemical Safety (IPCS), who define EDCs as "exogenous substances or mixtures that alter the function(s) of the endocrine system and consequently causes adverse health effects in an intact organism, or its progeny, or (sub) populations."[7]

The term "endocrine disruptor" was introduced for the first time in 1991 after a working session on "chemically-induced alterations in sexual development."[134]The ubiquitous presence of EDCs has been well documented in different matrices across the globe.[72] Fetal and postnatal life stage vulnerability in

humans has been observed when exposed to endocrine disrupting chemicals (EDCs), causing strong and often irreversible effects on developing organs, whereas exposure of adults causes lesser or no effect.[135] Increased incidence of reproductive abnormalities has been documented in Potomac River smallmouth bass over the past 10 years, most likely stemming from increased levels of EDCs in the environment.[66, 67, 71]

EDCs are emitted to the aquatic environment through industrial waste discharge, urban stormwater runoff and wastewater treatment discharges. These chemicals found in wastewater discharge are from every day practices of hygiene[136] (triclosan in antibacterial soap), excreting ingested medicine (dextromethorphan)[137] and also flushing of unused prescriptions.[138]

EDCs are chemically diverse, are primarily synthetic and are used in a wide range of items and goods and tend to bioaccumulate in aquatic biota.[135] "Bioaccumulation is defined as the accumulation of chemicals in the tissue of organisms through any route, including respiration, ingestion, or direct contact with contaminated water, sediment, and pore water in sediment".[139]

Bioaccumulation plays an important role in fate pathway of EDCs in aquatic systems as well as the toxicity of EDCs in the organisms, due to several reasons;[140] (i) it may enhance the persistence of chemicals in the ecosystems, since they can be stored in the tissue of the organisms, (ii) stored chemicals are not exposed to direct physical and chemical degradation, (iii) increased risk of biomagnification, which is

an increase of chemical concentration up the food chain, and finally (iv) the probability of direct health effects on the organism.

An increased number of reproductive abnormalities, such as presence of feminine features in male fish and disease related mortality of the young-of-the-year, has been observed and well documented in Potomac River fish, most likely stemming from increased levels of EDCs in the environment[66, 67, 71, 109], signifies the importance of studying the river's tributary embayments. The Potomac River receives wastewater discharge from 17 and 67 major (> 2 MGD, mega gallons daily) municipal WWTPs from greater Metropolitan Washington and Maryland, respectively.[4, 110]

Understanding bioaccumulation as a fate process is important to protecting ecological and public health in aquatic systems; also understanding the relation and direction of transfer between water, sediment and fish is critical to determining how sediment accumulation can affect the bioaccumulation of micropollutants in the biota.

In the 1960s and early 1970, it was discovered that some organic chemicals such as DDT and PCBs were bioaccumulated from the environment into birds and fish, inspiring concern for the environment and the fate process of contaminants.[43] Distribution of a chemical in water, sediment and biota is a function of its chemical and physical properties, most important of which are molecular weight, volatility and molecular structure.

The predictive capability in assessing the bioaccumulation of EDCs is important in managing water quality. Howard and Muir rated 92 out of 275 drugs detected in the environment as potentially bioaccumulative using quantitative structure property relationships (QSPR).[141] The rate of bioaccumulation in an organism depends on the availability of the pollutant as well as the ability of the organism to excrete the pollutant or store it.[142]

Study Objectives

The goal of the present study was to assess the bioaccumulation of endocrine disruptors in fish from Hunting Creek, Accotink Creek and Pohick Creek. The primary objectives of the present study were to (i) determine the distribution of TCS and DXM between water, sediments and fish, (ii) to quantify EDCs in four fish species in the upper Potomac River, and determine the distribution of detected EDCs between water, sediments and fish (iii) assess geospatial differences between collection sites within the creek, (iv) analyze tissue distribution of contaminants, and (v) assess the potential transfer to offspring.

Fish were collected from Hunting Creek, Accotink Creek and Pohick Creek (northern Virginia, USA) in 2014 (March through May) and were analyzed for selected EDCs.

Materials and Methods

Fish Species Selected

Several species of fish were captured from Hunting Creek, Accotink Creek and Pohick Creek for EDC analysis in tissues (Figure 3.1). All three of the streams represent tributaries of the tidal-freshwater Potomac River that have watersheds draining highly developed urban/suburban landscapes (Chapter 2) in northern Virginia. In addition, each of the streams drains into tidal embayments, which represent important nurseries and food sources for fish species. Fish species collected in Hunting Creek included white perch (*Morone Americana*) and banded killifish (*Fundulus diaphanous*); these were collected in April-May 2014. Both white perch and banded killifish were analyzed as whole fish only, due to size constraints.

Table 3.1. Fish species collected from Hunting Creek, Accotink Creek and Pohick Creek and their trophic levels.

Fish Species		**Trophic level**
Common name	Scientific name	
Gizzard shad	*Dorosoma cepedianum*	2.4 ± 0.21
fugAlewife	*Alosa pseudoharengus*	3.4 ± 0.32
White perch	*Morone Americana*	3.1 ± 0.35
Banded killifish	*Fundulus diaphanous*	3.3 ± 0.17

Trophic levels obtained from FishBase[38]

White perch are an important commercial and popular game fish in eastern North America, and is the most common fish found in Hunting Creek. White Perch are ubiquitous in freshwater estuaries.[35] The diet of white perch is age dependent.

Adults prey on crabs, shrimps and small fish while juveniles feed on aquatic insects and small crustaceans.[35] White perch species collected from Hunting Creek were small, ranging from 2-5 cm, and represented juveniles feeding on a mixed diet of small benthic organisms and zooplankton.[35-37]

Banded killifish are also abundant in Hunting Creek, but although this species has no commercial value it serves as a food source for other fish and wading birds.[35] Banded killifish, as an intermediary trophic level II, would provide contrast for higher trophic level species. The diet of banded killifish consists of small crustaceans, mollusks and worms.[35, 36]

Fish were collected in Accotink and Pohick Creek from March-May 2014 and consisted of gizzard shad (*Dorosoma cepedianum*) and alewife (*Alosa pseudoharengus*). Historically the alewife fishery has been very important in the greater Chesapeake Bay area, but has declined recently due to few reasons, one of which is degradation and destruction of spawning and nursery habitats in the area.[35] Adult alewife feed on copepods, ctenophores, mysid shrimps, amphipods and small fish.[35] Adult alewives can be found in the lower Chesapeake Bay in later winter and early spring. During spawning season (March-April), they ascend to the freshwater tributaries such as Accotink and Pohick Creeks. Gizzard shads are common in freshwater and slightly brackish habitats in the bay, and spawn in shallow freshwater with a peak from April-June[35]. Gizzard shad are filter-feeding planktivores, consuming phytoplankton, zooplankton and bottom detritus.[35] Fish tissue, skin and gonads were processed for contaminant analysis.

Sample Sites

Fish analyzed were sampled from three sites, Hunting Creek, Accotink Creek and Pohick Creek (Figure 3.1), which are three tributaries to the tidal Potomac River. Hunting Creek sampling sites were in the tidal region, while those in Accotink Creek and Pohick Creek were located in non-tidal regions. Hunting Creek is formed by the confluence of Cameron Run and Hooff Run (Figure 3.2). Jones Point forms the northern boundary of Hunting Creek, and the southern boundary is Dyke Marsh. Alexandria Renew Enterprises wastewater treatment plant is located on the east side of the Hunting Creek, and discharges 150,000 m^3 (average) of wastewater daily[2] into upper Hunting Creek.

The Accotink Creek watershed has an area of 132 km^2 spanning the cities of Vienna and Fairfax, VA, and the Fort Belvoir military installation. Accotink Creek is a 40 km long tributary stream of the freshwater tidal Potomac River in Fairfax County (Virginia, USA).[27] Accotink Creek is characterized by heavy development throughout most of the watershed, with the exception of the portion flowing through the Fort Belvoir Military Reservation, which in comparison to other areas is the only relatively underdeveloped land in the entire watershed.[27] The fish species from Accotink Creek were harvested from the Fort Belvoir section of the creek.

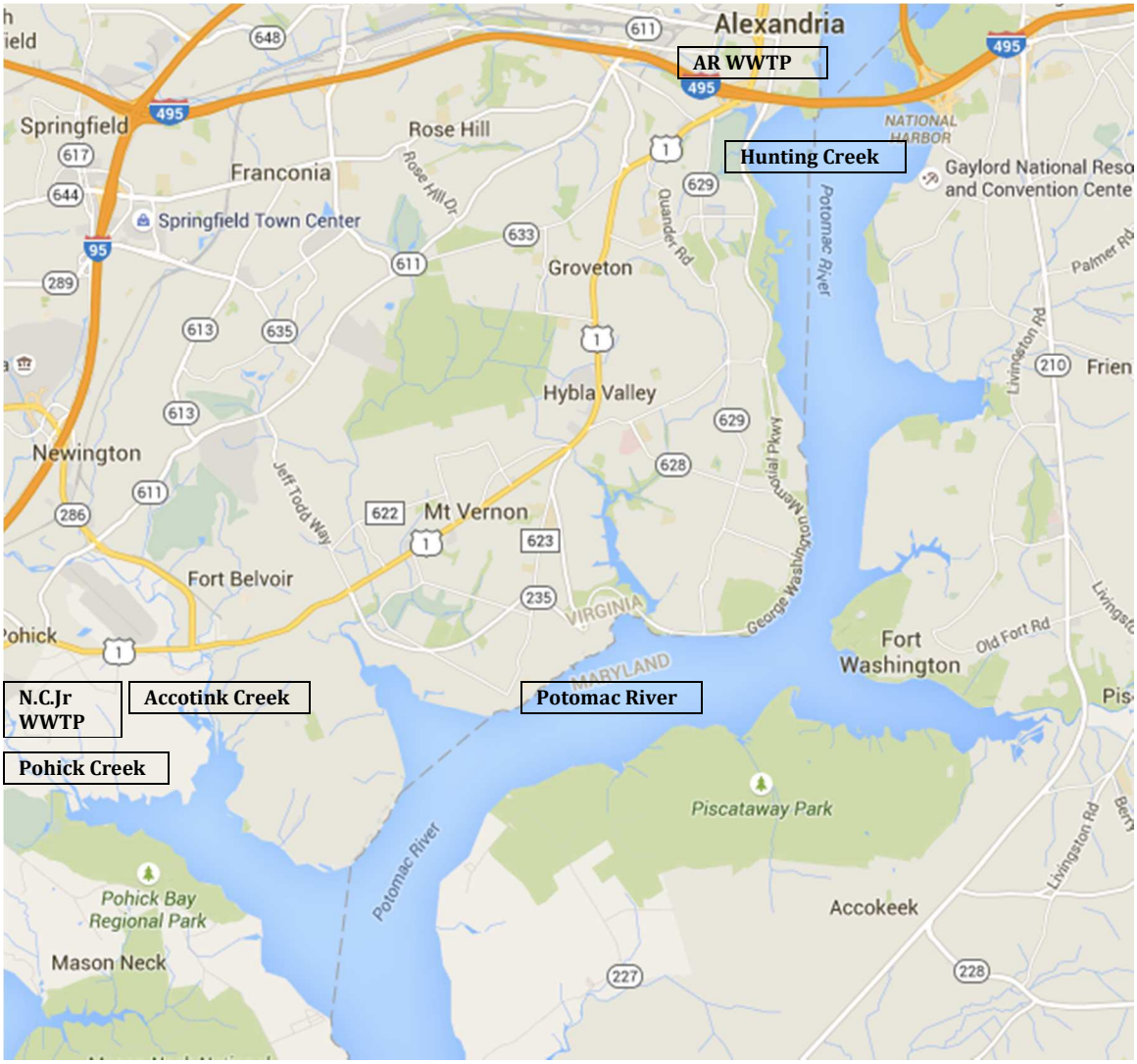


Figure 3.1. Hunting Creek, Pohick Creek and Accotink Creek relative to Potomac River.

Pohick Creek is also a tributary stream of the Potomac River, stretching for 22 km, across the 95 square kilometers watershed located in the central southern portion of Fairfax County (Virginia, USA) and drains into the Potomac River.[28] The Pohick Creek fish were also harvested from the portion of the stream flowing

through the Fort Belvoir military installation, which is relatively undeveloped compared to the rest of the watershed.[28]

Figure 3.2 Hunting Creek and its geographic boundaries.

Field Sampling

Hunting Creek fish were collected using a 16.7 mm seine net at site 6 and by otter trawl net at site 1 from April to May of 2014 (Figure 3.2). The fish were sorted and stored according to the protocol stated in Chapter 1, field-sampling section. The fish collected from Pohick and Accotink Creeks were harvested using a hoop net, left over night, from March to May of 2014. The individuals captured were adult fish and were placed on ice and wrapped in ashed aluminum foil and stored in the -20 °C freezer upon arrival in the laboratory.

Materials

GCMS standard solutions of the EDC analytes, as mixtures and single analyte solutions, and carbon-13 isotopic surrogate standards (atrazine-13C3 and naproxen-13C,d3) are obtained from AccuStandard (New Haven, CT) as well as the internal injection standards (acenaphthene-d10, phenanthrene-d10 and chrysene-d12) used in gas chromatography/mass spectrometry (GCMS) analysis. Working standards (1,000 ng/uL) were prepared in toluene and stored at 4^{o}C.

The derivatizating reagent used in GCMS analysis was N-methyl-N-(trimethylsilyl) trifluoroacetamide (MSTFA), and was purchased from Pierce (Rockford, IL) in 1 mL ampoules. Acetonitrile (ACN) and Toluene (TOL) solvents were all HPLC-grade and supplied by Fisher Scientific (Hanover, IL). QuEChERS method specific reagents including sodium acetate, magnesium sulfate, primary secondary amines, acetic acid and endcapped C_{18} sorbent (used for separation of

non-polar to moderately polar compounds such as fatty acids) were all pesticide grade solvents and ACS grade sorbents, purchased from Fisher Scientific (Hanover, IL).

Sample containers and all glassware used for sample preparation are cleaned by washing with soap, rinsing with distilled water followed by double distilled water (DDW), and ashed at 400 ^{o}C overnight. All laboratory containers were made of glass, stainless steel or Teflon to avoid sample contamination. The Teflon materials were cleaned the same way as glass but without ashing. All materials both made of glass and Teflon were rinsed with methanol and air dried before use. DDW was produced in the laboratory in a Corning Megapure quartz element immersion still.

Sample Processing

Collected fish were processed for GCMS analysis using a modified QuEChERS method developed by Lehotay et al.,[143] QuEChERS is based on the salting out effect and solid phase clean up of the intact lipids from the tissue extracts (Figure 3.3). The term QuEChERS stands for "Quick, Easy, Cheap, Effective, Rugged, and Safe. Preserved whole fish (white perch and banded killifish from HC) were thawed and then homogenized in a die-cast blender (Oster, Boca Raton, FL). Fish from Accotink Creek and Pohick Creek were dissected to obtain isolated tissues consisting of gonads, filet muscle, skin and stomach. The various tissues were initially homogenized as previously described for whole fish. Approximately 1 g of whole

fish or tissue homogenate was added to a 50 mL glass centrifuge tube at which point both the surrogate standards and internal injection standards were added to the tissue matrix (Table 3.2). Addition of internal injection standards in the first step was necessary to lock in the analyte:standard ratio before an aliquot was removed for further processing. The analytes were extracted from tissue with 15 mL of 1% (v/v) acetic acid in acetonitrile[144, 145], which was added to the glass tube and subsequently shaken on a vortex mixer for 1 minute. Following extraction, NaCl (0.2 g), anhydrous $MgSO_4$ (0.3 g) and anhydrous Na_2SO_4 (1.7 g) were added, in solid form, as salting-out agents, and the mixture was again shaken for 1 minute.

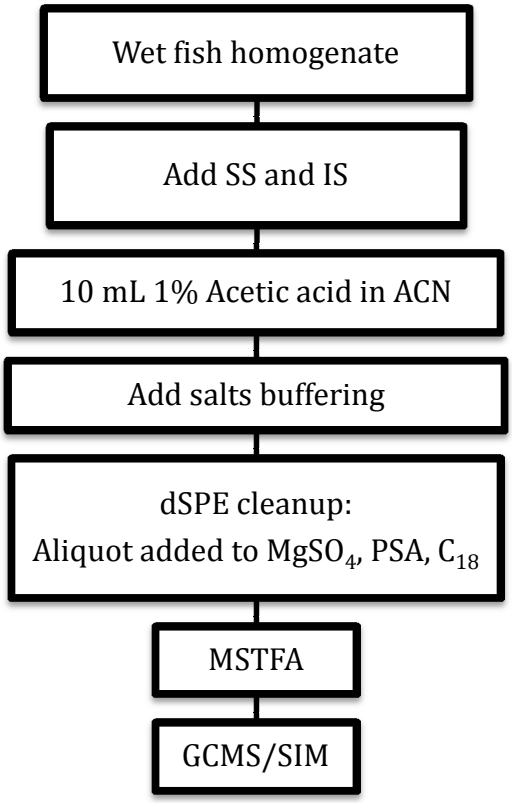


Figure 3.3. QuEChERS method flow chart

Following this procedure, the liquid organic phase was separated from the residual solids using centrifugation for 8 minutes at 3400 rpm, and the supernatant was decanted into a clean 15 mL conical extraction tube.

Table 3.2. Internal and Surrogate Standards used in GCMS analysis.

Internal Injection Standards	**Surrogate Standards**
Acenaphthene-d10 (IS[a])	Naproxen-13C-d3 (SS[d])
Phenanthrene-d10 (IS[b])	Atrazine-13C3 (SS[e])
Chrysene-d12 (IS[c])	

The next step involved dispersive solid phase extraction (dSPE), used as the sample extract cleanup step. The above supernatant was added to a new extraction

tube along with $MgSO_4$ (0.5 g), PSA (primary secondary amine, 0.1 g) and C_{18} sorbents (0.4 g). The extraction tube was centrifuged for 8 minutes at 3400 rpm, and the extract was decanted into a 200 mL TurboVap (Biotage, Sweden) tube. The non-polar fish lipids partition into the C_{18} solid phase while the analytes remain in the solution phase. The solution phase was decanted and solvent volume reduced, while undergoing solvent exchange with toluene, to a final volume of 0.5 mL. Prior to GCMS analysis a 100 µL aliquot of the toluene extract was derivitized with 15 µL of N-methyl-N(trimethylsilyl) trifluoroacetamide (MSTFA) in a heater block for 30 minutes at 80 °C immediately prior to GCMS analysis. This final step increased the volatility of the otherwise mostly non-volatile analytes of concern (Table 3.3) by replacing the hydrogen on the hydroxyls with a trimethylsilyl group [$(CH_3)_3$-Si] to facilitate GCMS identification.

Table 3.3. List of EDCs analyzed in Hunting Creek, Accotink Creek and Pohick Creek fish.

Analyte	**Category**	**Use/Origin**
Acetaminophen	Pain reliever and fever reducer	OTC[a] Drug
Atrazine	Pesticide	Broadleaf Weed Control
Bisphenol A	Plasticizer	Plastics
Caffeine	CNS stimulant	Coffee, Soda
Carbamazepine	Anti-convulsant	Prescription Drug
Coprostanol	Sterol	Fecal Biomarker
Coumestrol	Sterol	Phytoestrogen
D(-)-Norgestrel	Synthetic Progesterone	Oral Contraceptive
Dextromethorphan	Cough suppressant	Delsym
Dichlofenac	NSAID[b]	Prescription Drug
Diphenhydramine	Antihistamine	OTC Drug
Equilin	Hormone replacement therapy	Prescription Drug
Escitalopram	SSRI[c]-Lexapro	Prescription Drug
Estrone	Estrogenic hormone	Oral Contraceptive
Fluoxetine	SSRI-Prozac	Prescription Drug
Gemfibrozil	Cholesterol reducer	Prescription Drug
Ibuprofen	NSAID	OTC Drug
Naproxen	NSAID	OTC Drug
Progesterone	Menopausal hormonal therapy	Prescription Drug
Testosterone	Steroid hormone	Prescription Drug
Triclosan	Antibacterial, Antifungal	Soap Additive
Trimethoprim	Antibacterial	Prescription Drug
Vinclozolin	Fungicide	Prescription Drug
4-*tert*-Octylphenol	Surfactant	Detergent
4-Nonylphenol	Surfactant	Detergent
17 α-Estradiol	Estrogenic hormone	Oral Contraceptive
Mestranol	Estrogenic hormone	Oral Contraceptive
19-Norethindrone	Estrogenic hormone	Oral Contraceptive
17β-Ethynlestradiol	Synthetic steroid	Oral Contraceptive

[a]Over the counter
[b]Non-steroidal anti-inflammatory drug
[c]Selective serotonin reuptake inhibitor

Analytical Conditions

GCMS analysis was performed using an Agilent 7890A series gas chromatograph (Agilent Technologies, Santa Clara, CA) interfaced to an Agilent 5975C mass-selective detector. Retention time locking, data acquisition, processing, and instrumental control were performed by the Agilent MSD ChemStation software (Version E.02.02.1431). An MMI inlet was used in the solvent vent mode, enabling large volume injections (LVI) of 10 μL directly into the MMI inlet.

The analytes were separated and identified using an Agilent HP-5MS capillary column (5%biphenyl/95%dimethylsiloxane) with the following dimensions: 30 m x 0.25 mm i.d., 0.25 μm thick film. An Agilent model 7673A automated liquid sampler (auto sampler) was used to provide 10 μL injections into the GC. An Agilent ultra-inert 2 mm dimpled inlet liner was used at the inlet for optimal inert performance. The GC operating conditions were as follows for EDCs: the initial column temperature of 70 °C for 15 seconds, programmed to 300 °C at 600 °C/min, and kept at this temperature for 2 minutes. The helium carrier gas flow is maintained at a constant pressure of 17.3 psi, a retention time locked method, adopted from the Agilent Pesticide and Endocrine Disruptor database, using the locked retention time of chlorpyriphos methyl (16.596 min) was used as the retention time reference standard.

Electron impact (EI) mass spectra, in both full-scan mode and selected ion mode (SIM), are obtained at 70 eV with monitoring from 50 m/z to 510 m/z for full-scan mode.

In SIM mode the quantifying ion and three additional qualifying ions were used for each target EDC. The quadrupole analyzer and the ion source temperatures were held constant at 150 and 230 °C, respectively. All calibration and quantitation was accomplished employing Agilent MSD ChemStation software (Version E.02.02.1431).

Calibration standards were evaluated through ChemStation using internal standard injections standards at 6 concentrations, using acenaphthene-d10, phenanthrene-d10 and chrysene-d12 as the internal injection standards. Quantitation of the individual EDCs was performed using one quantifier ion, or target ion and two or three qualifier ions. Target ion (quantifier ion) is an ion characteristic of the target compound that distinguishes this compound from any others with similar retention times. The extracted ion chromatogram for the quantifier ion was used for quantitation in the tissue extracts. Qualifier ions, selected from the mass spectrum of the target compound, in the correct ratio relative to the target ion gives evidence of correct target compound identification as a confirmatory step.

Quality Assurance

Analytical limits of detection (LODs) for the samples from all three sites were determined by multiplying the standard deviation of 10 replicate runs of the medium concentrated calibration standard (320.0 ng/vial) by the Student's t-test for the 95% confidence level. The values were then divided by the approximate

sample mass (1 g) to determine the method detection limits (MDLs) in ng/g wet weight (wwt) and are presented in Appendix C. Method Detection Limits varied from 16.1 to 270 ng/g for the analytes of interest. All of the analytes were detected in their derivatized form, with the exception of fluoxetine and D(-)-Norgestrel.

Method recoveries were tested for extraction, cleanup efficiency and analyte recovery through surrogate, QA and matrix spike experiments in fish, in triplets. The mean surrogate recovery (N=98), for naproxen-13C-d3 was 79.3% ± 0.6% and for atrazine-13C3 was 114% ± 2% (Appendix C). The laboratory blanks were below detection limits. A sample blank chromatogram is presented below (Figure 3.4).

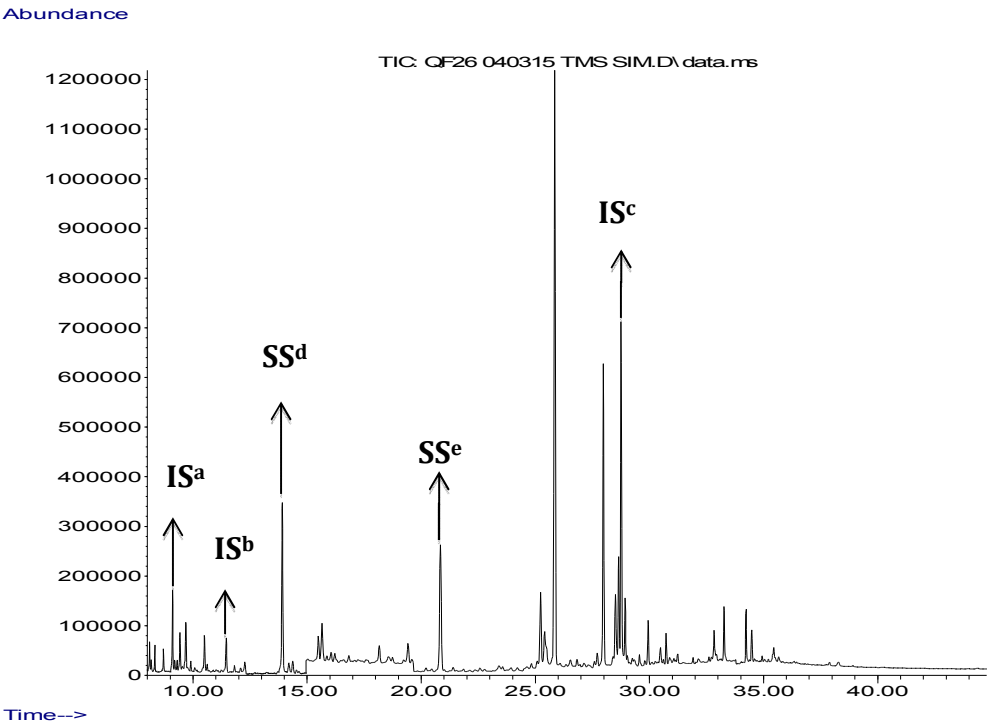


Figure 3.4. Blank chromatogram. Internal and surrogate standards in table 3.2.

Lipid Analysis

The lipid content of whole fish from Hunting Creek as well as specific tissues including muscle, skin, and gonads of the collected fish from Pohick Creek and Accotink Creek were determined using the microwave extraction method (MAE) (Chapter 1). Approximately10 g of fish homogenate was extracted twice, with 15 mL dichloromethane (DCM):methanol (MeOH) (2:1, v/v) using MAE. Both extracts were combined in a 125 mL separatory funnel (Fisher Scientific, Hampton, NH) for back extraction. To remove non-lipid extractables the solution was salt washed with 3x50 mL aqueous sodium chloride (0.05 M in DDW) to remove small organic acids. The organic phase containing the lipids was collected in a pre-weighted 200 mL TurboVap tube and evaporated to dryness using TurboVap evaporator (Zymark, Hopkinton, MA). Lipid mass was determined on the residual solid material remaining in the tube following solvent evaporation using gravimetric analysis. This method provides a total extractable lipid determination and % lipid is determined by dividing the extracted lipid mass by the fish homogenate mass, multiplied by 100.

Bioaccumulation and Fugacity Theory

"Bioaccumulation is the uptake of organic compounds by biota from either water or food".[146] Many of these compounds are toxic and attain concentrations in biota several orders of magnitude greater than concentrations in aquatic environment, thus rendering bioaccumulation as a serious threat to both biota and the humans consuming them as food.[146]

Bioaccumulation can occur as passive uptake of dissolved chemicals from water (bioconcentration), or get taken up as sorbed molecules on sediment particles or present in their food (bioaccumulation).[43]

Determining bioaccumulation requires relating the concentration in the organism to the concentration in medium of the environment which the organism inhibits[43]. Bioaccumulation factor (BAF, kg/L) is utilized to calculate the net result of concentration distribution between organism and environmental medium (Equation 3.1).

$$BAF = \frac{C_{fish}}{C_{water}} \qquad \text{Equation 3.1}$$

C_{fish} is the concentration of the contaminant of concern in fish and C_{water} is the concentration of that contaminant in water. The BCBAF (version 3.01) program in Estimation Programs Interface Suite (EPI Suite, verison 4.10) offered by the U. S. Environmental Protection Agency utilizes a screening-level tool which includes the Arnot-Gobas quantitative structure-activity relationship model (QSAR) to estimate BAFs for organic chemicals in fish.

Fugacity is analogous to partial pressure of an ideal gas and directly related to concentration.[60] Fugacity ratios of analytes in different media can be calculated and the magnitude of the ratios will determine equilibrium or direction of flow. Fugacity values can be utilized as a tool to profile distribution patterns of

contaminants across ecosystem and biota. A fugacity ratio of 0.5-1.5 is defined as equilibrium. A >1.5 fish:sediment fugacity ratio indicates fish to sediment flow of analytes and a <0.5 ratio indicates the reverse flow. The following equations were utilized in determining fugacity for DXM and TCS.

$$F_{ij} = K_{H\,i,j} \times C_{i,j} \qquad \text{Equation 3.2}$$

$F_{i,j}$ = Fugacity of i^{th} chemical in j^{th} phase (atm), phase equivalent vapor pressure
$K_{H\,i,j}$ = Henry's Law Constant of i^{th} chemical in j^{th} phase (atm.kg/ml)
$C_{i,j}$ = Concentration (mol/kg) of i^{th} chemical in j^{th} phase measured by GCMS

Phase specific Henry's law constants (atm.kg/mol) for sediment and fish were estimated using equation 1.2, adapted from Schwarzenbach et al.[43]

$$K_{H\,i,j} = \frac{K_{H_i}}{K_{d\,i,j}} = \frac{K_{H\,i}}{(\alpha_j \beta_j) K_{ow_i}} \qquad \text{Equation 3.3}$$

$K_{H\,i,j}$ = Henry's Law Constant of i^{th} chemical in j^{th} phase (atm.kg/ml)
$K_{d\,i,j}$ = Distribution constant of i^{th} chemical in j^{th} phase (L/kg)
α_j = Constant for j^{th} phase (fish=1, sediments=0.41)
β_j = Phase constant (fish=f_L=lipid fraction, sediment=f_{oc}= organic carbon fraction)
$K_{ow,i}$ = Water octanol partition coefficient (K_{ow}) of i^{th} chemical
$K_{H,i}$ = Henry's law constant of i^{th} chemical (atm.kg/mol)

Individual K_H and K_{ow} values were either determined from EPA EPI suite[10] and are presented in Appendix D.

Results

Lipid Content

Lipid content of whole white perch from Hunting Creek was 2.5% by mass and banded killifish displayed a much smaller amount of lipid at 0.21% (Figure 3.5). Lipid content of different tissues for gizzard shad and alewife varied from 0.28% to 2.67%, with alewife displaying an overall higher percentage of lipids (Figure 3.6).

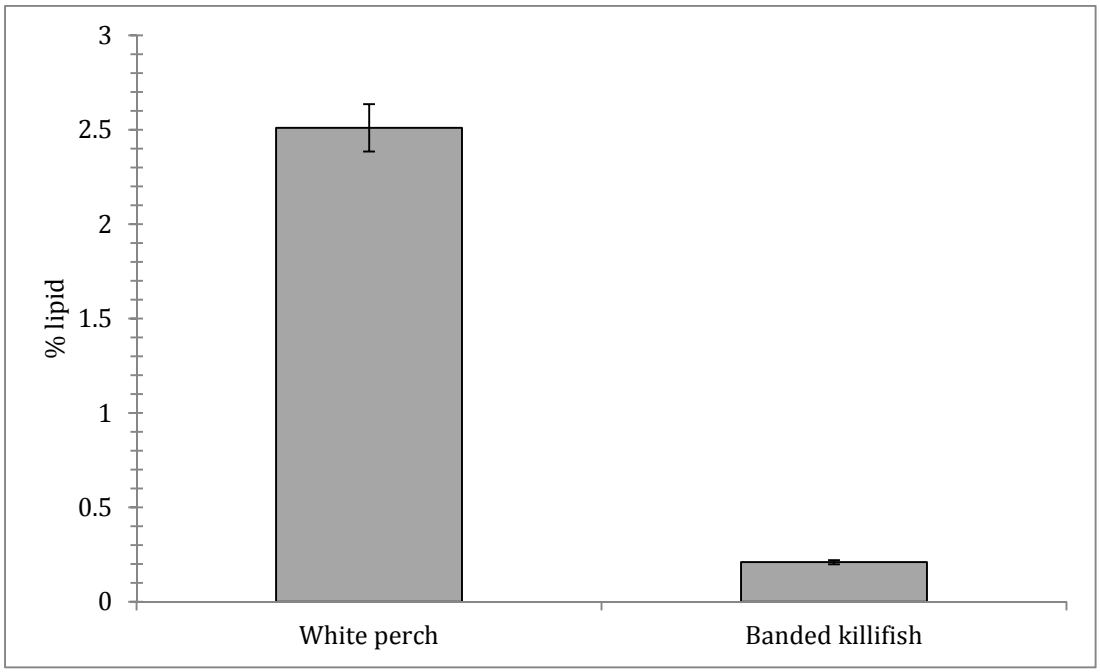


Figure 3.5. Extractable lipid content of white perch and banded killifish from Hunting Creek.

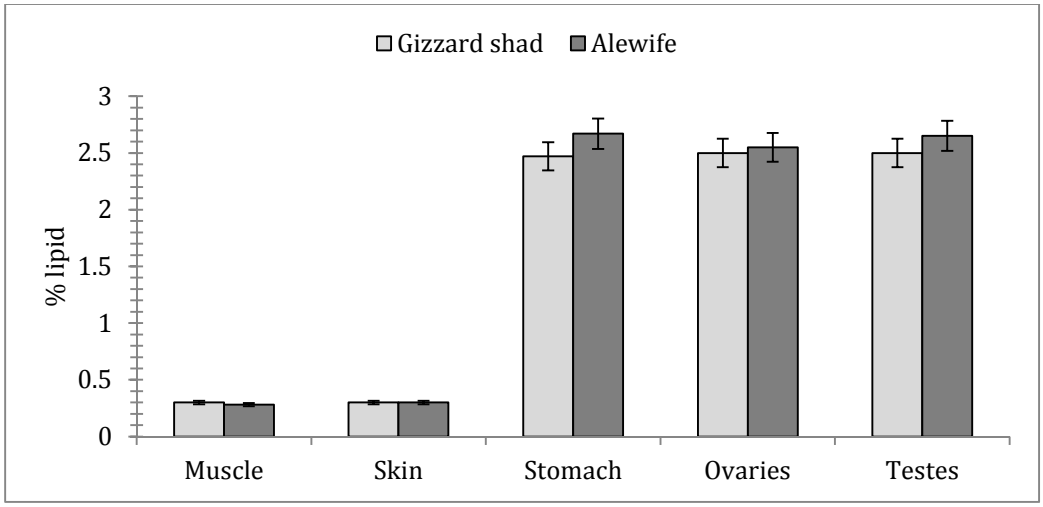


Figure 3.6. Extractable lipids in tissues of alewife and gizzard shad from Accotink and Pohick creeks.

No strong correlation (Figure 3.7) was observed between lipid content and analyte concentration (ng/g wwt), thus lipid normalization of concentrations was not warranted as was performed in PCB analysis (Chapter 1).

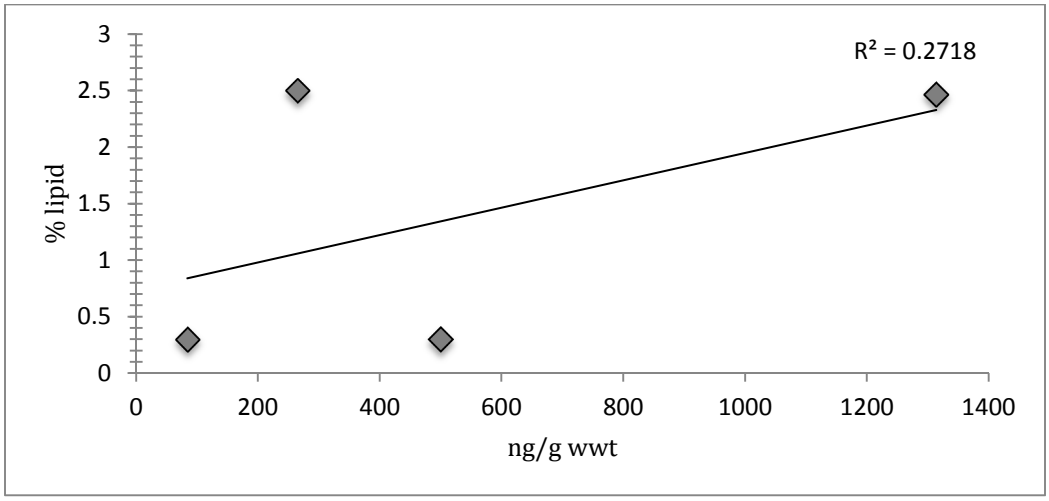


Figure 3.7. Lipid content vs. analyte concentration correlation.

EDC Concentrations in Fish from Hunting Creek

Only two EDCs were detected in Hunting Creek fish, triclosan (TCS) and dextromethorphan (DXM) (Figure 3.8). TCS mean concentrations ranged from 72.2 ± 14.6 to 81.8 ± 15.8 (ng/g wwt) in banded killifish and white perch respectively. DXM mean concentrations ranged from 305 ± 43.3 to 469 ± 57.1 (ng/g wwt) in white perch and banded killifish respectively.

Consistent with the EDCs detected in Hunting Creek sediments and water concentrations (Chapter 2) only TCS and DXM were observed in fish. But in contrast to Hunting Creek sediments, DXM concentrations were consistently higher than triclosan for both species and sampling locations.

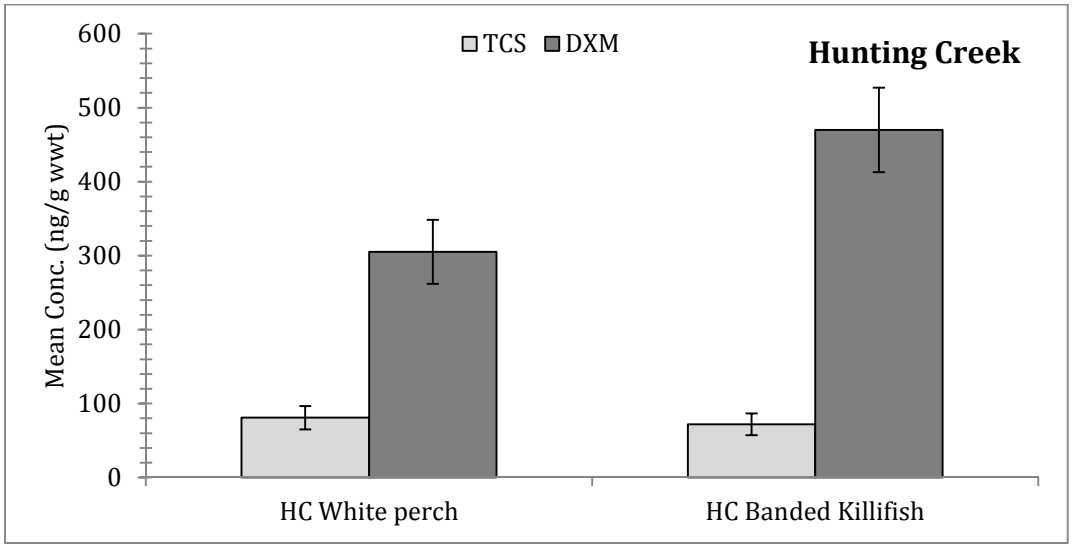


Figure 3.8. Mean concentrations of TCS and DXM in white perch and banded killifish from Hunting Creek.

DXM showed 100% detection frequency in both species, white perch and banded killifish in Hunting Creek. TCS detection frequency varied from 33% to 50% in Hunting Creek white perch and banded killifish respectively. A sample chromatogram of white perch (whole fish homogenate) from Hunting Creek is presented below (Figure 3.9) illustrating the GCMS peaks for TCS and DXM typically found in fish extracts.

Statistical analysis indicated significant differences between the concentrations of triclosan and dextromethorphan (ng/g wwt) of fish species collected from Hunting Creek (one-way ANOVA, $p<0.05$).

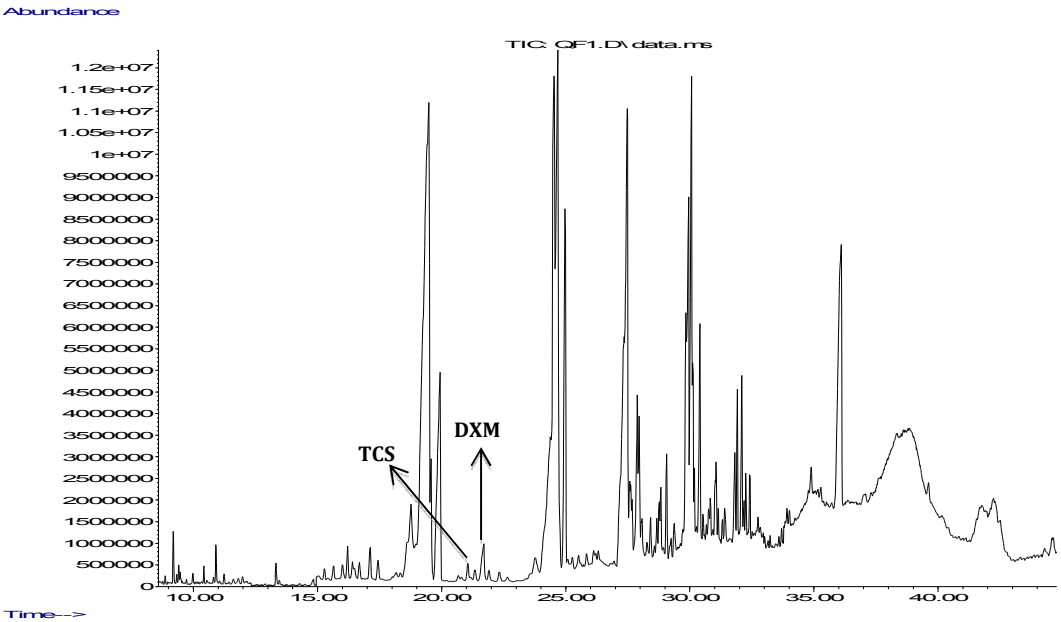


Figure 3.9. Sample chromatogram, white perch from Hunting Creek.

EDC Concentrations in Fish and Tissues from Accotink Creek

Consistent with EDCs detected in Hunting Creek water, sediments and fish, triclosan and dextromethorphan were the only EDCs detected in Accotink Creek fish (alewife and gizzard shad). Triclosan mean concentrations in whole fish varied from 45.5 ± 7.04 to 76.1 ± 9.13 (ng/g wwt) in gizzard shad and alewife respectively (Figure 3.10). Dextromethorphan mean concentrations in whole fish varied from 45.3 ± 9.12 to 101 ± 11.1 (ng/g wwt) in alewife and gizzard shad respectively (Figure 3.10).

Student's-t test, with 95% confidence level, indicated TCS concentrations (ng/g wwt) in whole alewife homogenate (± 3.78, range: 70.2-76.8) and whole gizzard shad homogenate (± 2.55, range: 42.2-48.8) are significantly different (p=0.000). Student's-t test, with 95% confidence level, indicated DXM concentrations (ng/g wwt) in whole alewife homogenate (±3.57, range: 42.6-48.9) and whole gizzard shad homogenate (± 2.39, range: 98.2-104) are significantly different as well (p=0.000).

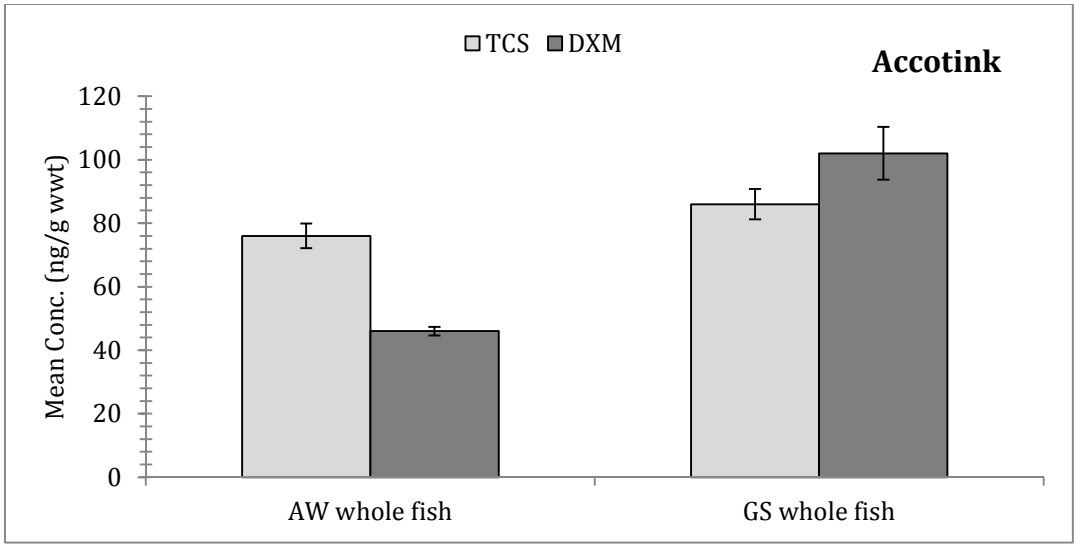


Figure 3.10. Mean concentrations in whole fish, alewife (AW) and gizzard shad (GS)

Triclosan and dextromethorphan detection frequency in Accotink alewife and gizzard shad whole fish homogenate were 100% and 67% respectively. Statistical analysis of TCS and DXM concentrations (ng/g wwt) in alewife and gizzard shad tissues indicated significant differences between tissue concentrations and fish species (two-way ANOVA, $p<0.05$). Dextromethorphan (DXM) was observed in both gizzard shad and alewife tissues, with 100% detection frequency. Triclosan detection frequency was 30% and only detected in alewife muscle, and gizzard shad ovaries and testes.

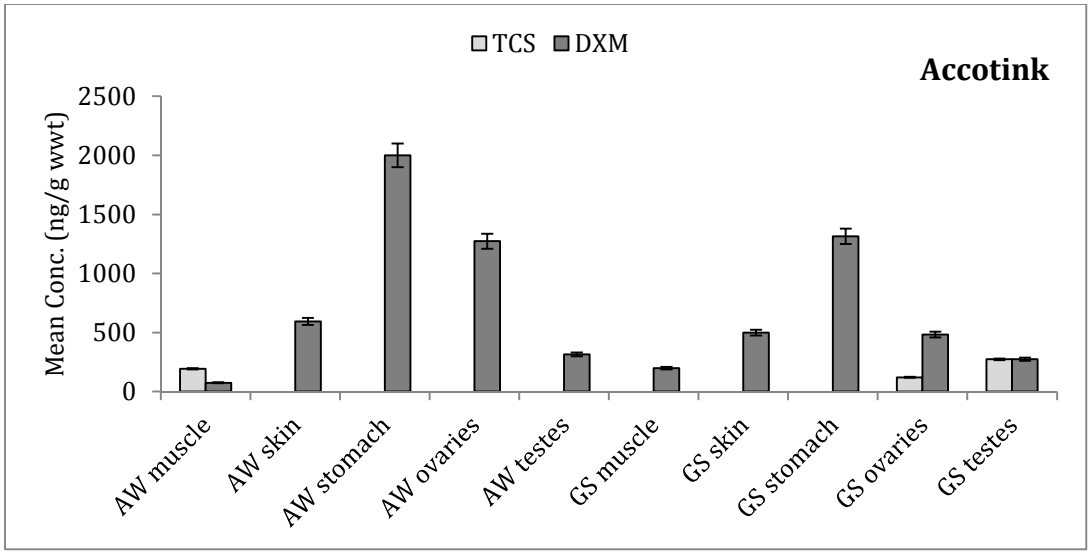


Figure 3.11. Mean concentrations of TCS and DXM in fish tissue.

Fish from Accotink were also analyzed as muscle, skin, stomach, ovaries and testes to determine EDC distribution profiles across different tissues. Overall DXM had the highest detection frequency and with concentrations much greater than TCS (Figure 3.11).

The greatest DXM concentration was observed in alewife stomach (2000 ± 156 ng/g wwt) and lowest in alewife muscle (74.3.5 ± 5.34 ng/g wwt). The greatest TCS concentration was observed in gizzard shad testes (274 ± 60.5 ng/g wwt) and lowest concentration was observed in alewife muscle (192 ± 87.4 ng/g wwt). Sample chromatograms of muscle, whole fish, ovaries and testes are presented in figures 3.12, 3.13, 3.14 and3.15 respectively, illustrating the GCMS peaks for TCS and DXM typically found in fish extracts.

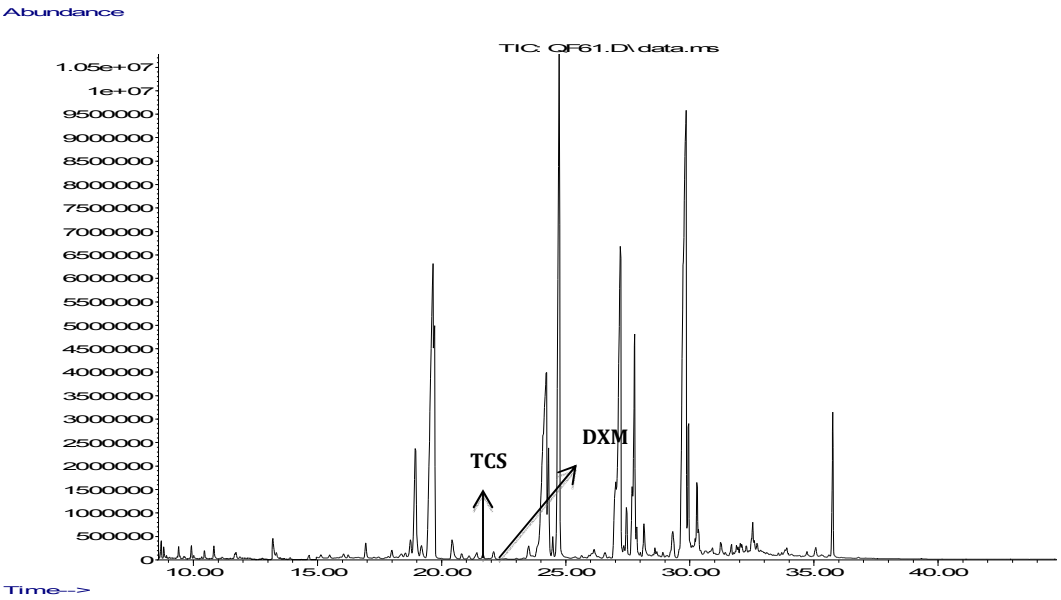


Figure 3.12. Alewife muscle tissue GCMS analysis chromatogram.

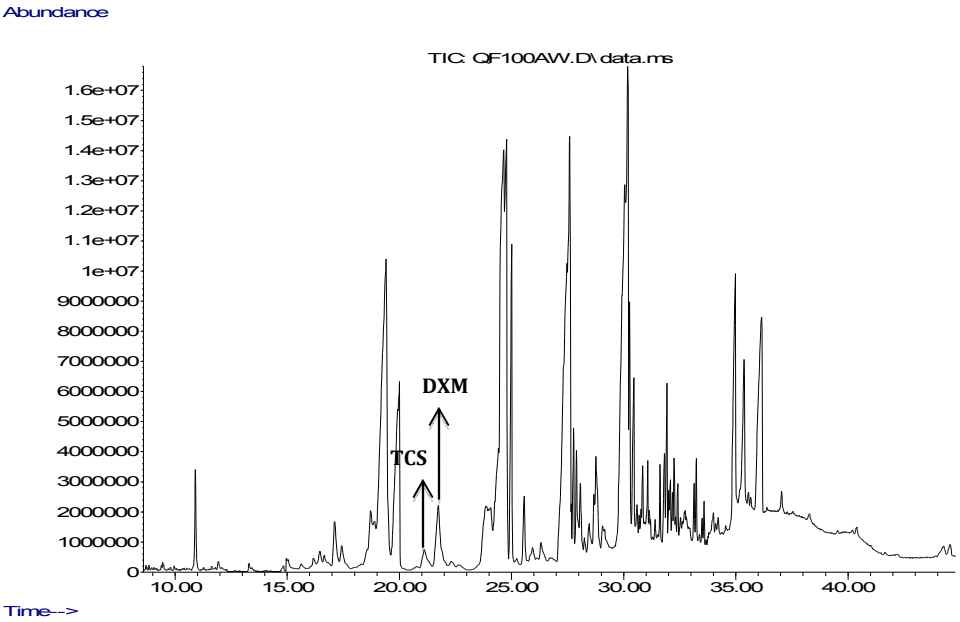


Figure 3.13. Alewife whole fish homogenate GCMS analysis chromatogram.

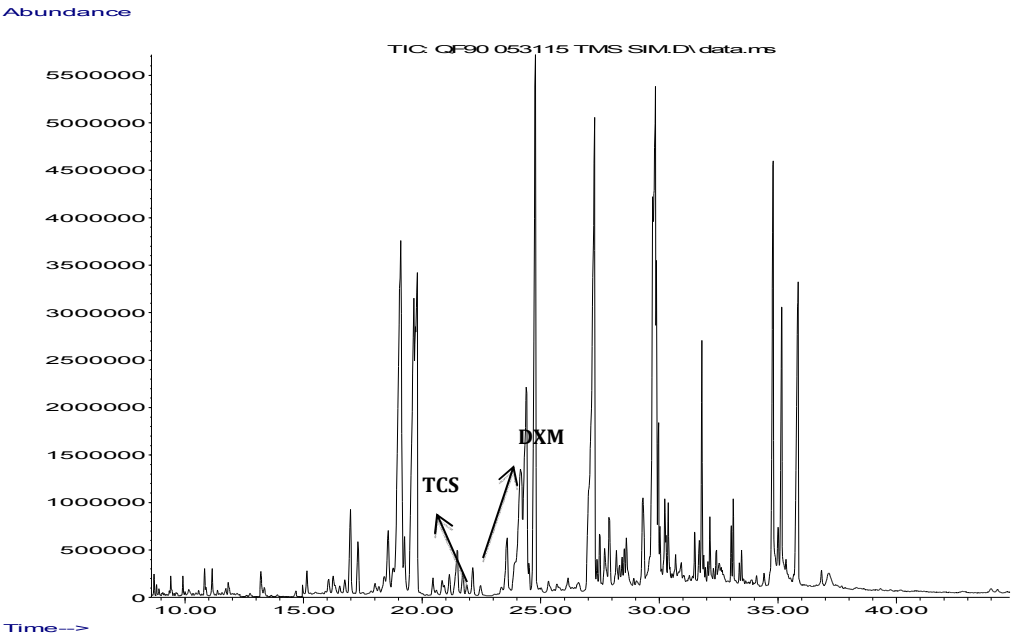


Figure 3.14. Gizzard shad ovaries GCMS analysis chromatogram.

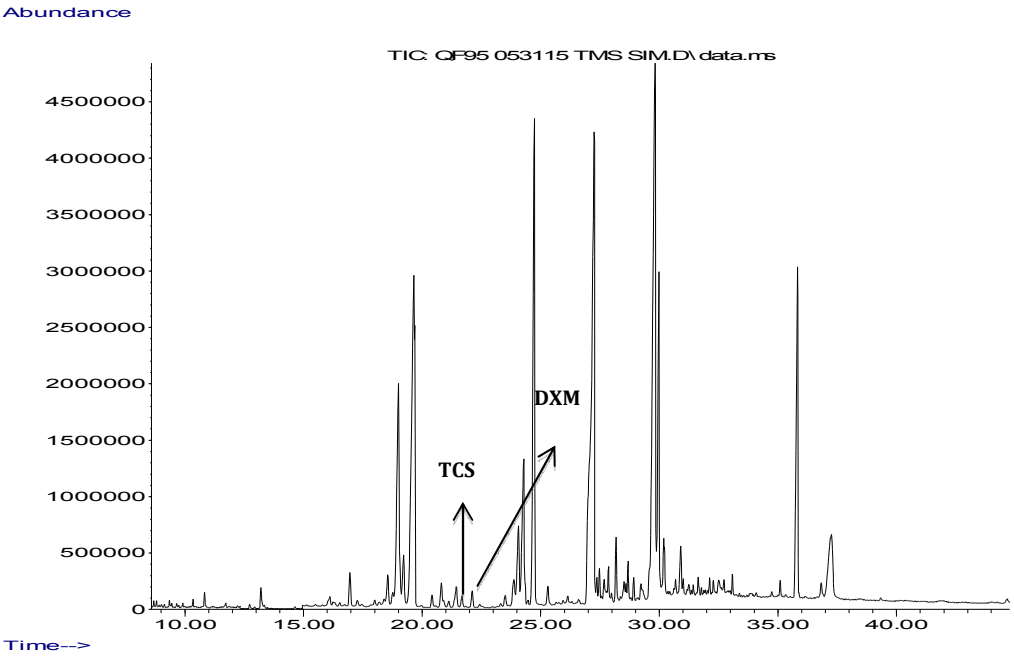


Figure 3.15. Gizzard shad testes GCMS analysis chromatogram.

EDC concentration in Fish and Tissues from Pohick Creek

Triclosan mean concentrations in whole fish varied from 47.9 ± 6.89 to 51.7 ± 5.32 (ng/g wwt) in alewife and gizzard shad respectively (Figure 3.16). Dextromethorphan mean concentrations in whole fish varied from 49.1 ± 9.68 to 134 ± 11.78 (ng/g wwt) in alewife and gizzard shad respectively (Figure 3.16).

Student's-t test, with 95% confidence level, indicated TCS concentrations (ng/g wwt) in whole alewife homogenate (± 3.57, range: 42.6-48.9) and whole gizzard shad homogenate (± 2.39, range: 98.2-104) are significantly different (p=0.000). Student's-t test, with 95% confidence level, indicated DXM concentrations (ng/g wwt) in whole alewife homogenate (±3.57, range: 42.6-48.9) and whole gizzard shad homogenate (± 2.39, range: 98.2-104) are significantly different (p=0.000).

Triclosan detection frequency in Pohick Creek whole fish homogenate ranged from 67% to 100% in alewife and gizzard shad respectively. Dextromethorphan detection frequency was 100% in alewife and gizzard shad whole fish homogenate.

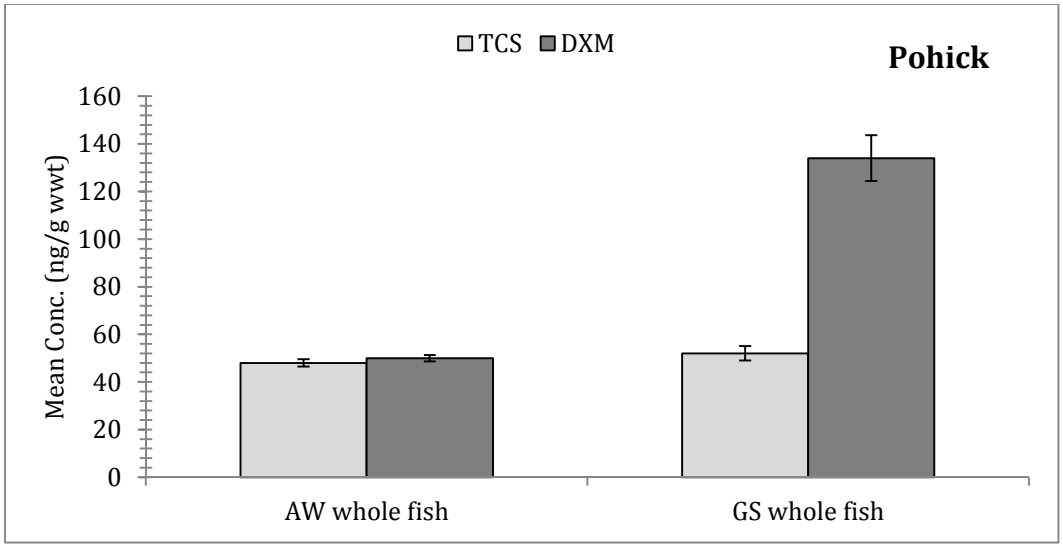


Figure 3.16. Mean concentrations in whole fish, alewife (AW) and gizzard shad (GS)

Statistical analysis of TCS and DXM concentrations (ng/g wwt) in alewife and gizzard shad tissues indicated significant differences between tissue concentrations and fish species (two-way ANOVA, $p<0.05$).

Fish from Pohick Creek were analyzed as muscle, skin, stomach, ovaries and testes to determine EDC distribution profiles across different tissue. Consistent with Accotink results, DXM had the highest detection frequency in fish tissue with concentrations much greater than TCS (Figure 3.17).

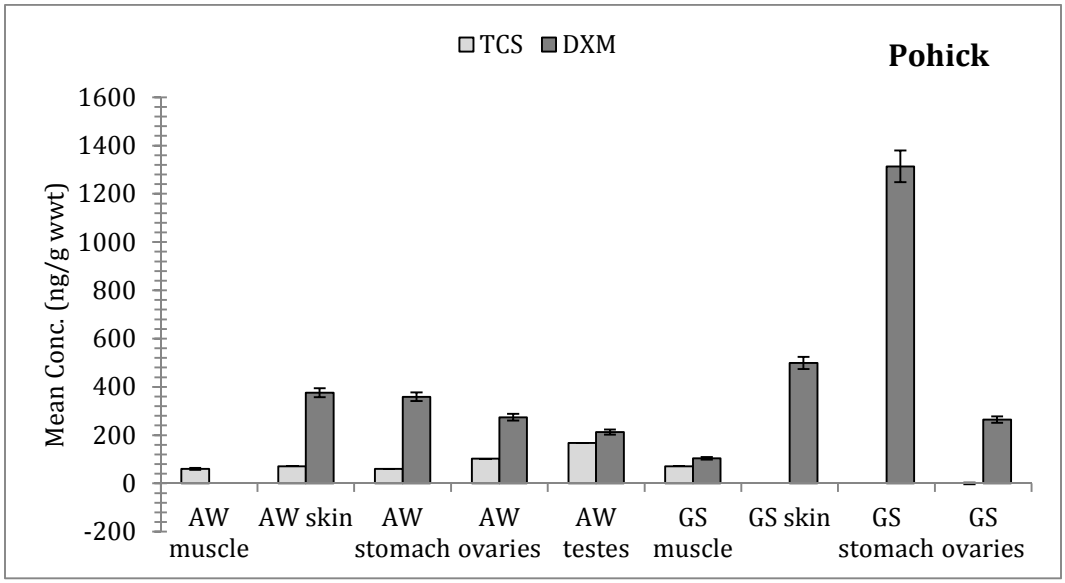


Figure 3.17. Mean concentrations of TCS and DXM in analyzed alewife (AW) and gizzard shad (GS) from Pohick Creek.

Highest DXM concentration was observed in gizzard shad stomach (1314 ± 39.6 ng/g wwt) and lowest in gizzard shad muscle (84.3 ± 8.30 ng/g wwt). The highest TCS concentration was observed in alewife testes (167 ± 22.8 ng/g wwt) and lowest concentration was observed in alewife muscle (59.8 ± 10.5 ng/g wwt).

Dextromethorphan (DXM) was detected in both gizzard shad and alewife, in selected tissue homogenates with 89% detection frequency. Triclosan (TCS) was observed in fish tissue with 67% detection frequency.

Bioaccumulation and Fugacity Assessment

To determine the relationship between fish and sediment triclosan and dextromethorphan relationship, fugacity of each contaminant in fish species and tissues were calculated, as previously discussed in Chapter 1. A fugacity ratio of <0.5 indicated sediments to fish flow, 0.5-1.5 indicated equilibrium and >1.5 indicated fish to sediments flux (Figure 3.18).

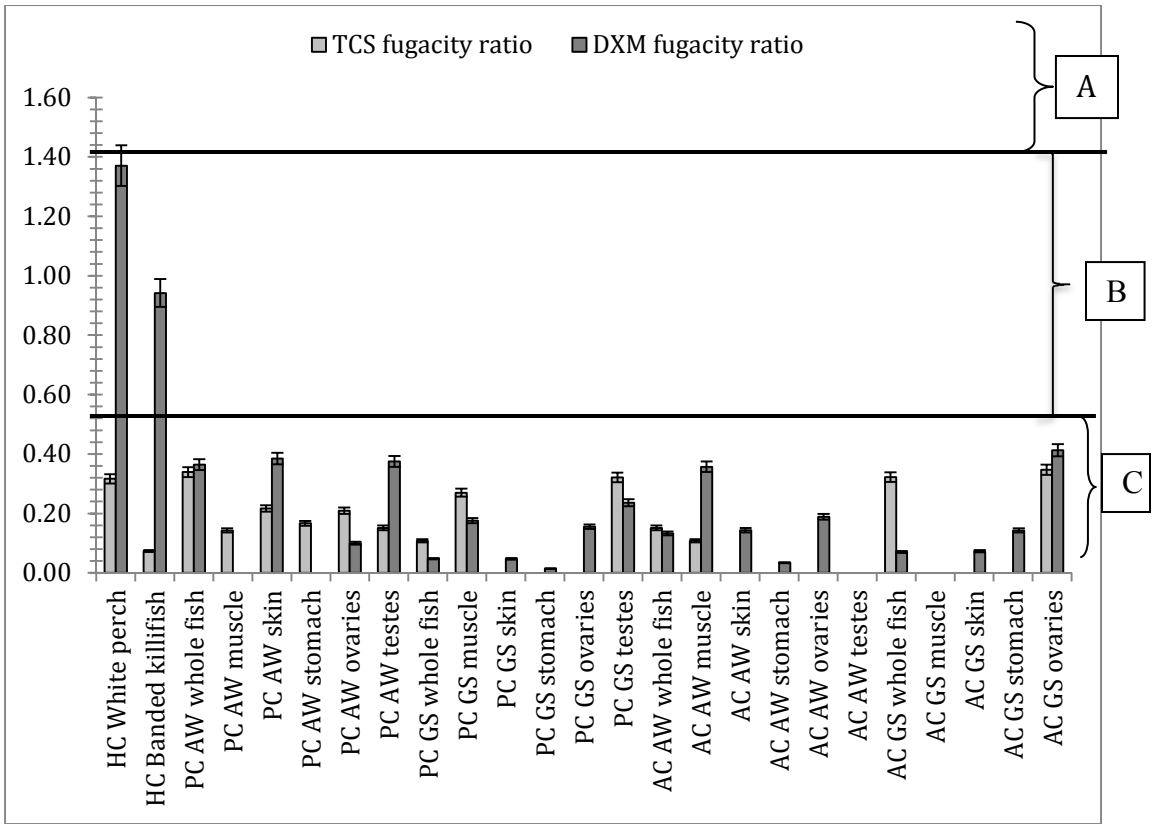


Figure 3.18. Fugacity ratios of triclosan (TCS) and dextromethorphan (DXM) from Hunting Creek (HC), Pohick Creek (PC) and Accotink Creek (AC) for alewife (AW), gizzard shad (GS), white perch and banded killifish.
A: Fish → Sediments
B: Equilibrium
C: Sediment → Fish

Triclosan and dextromethorphan in al fish samples (whole fish and tissue) from Pohick Creek and Accotink Creek showed sediments to fish flux indicating higher concentrations in sediments. In contrast whole fish samples from Hunting Creek showed equilibrium for dextromethorphan between fish and sediment concentrations indicating equal fugacities between the sediments and fish. Triclosan direction of flux for Hunting Creek fish were sediments to fish indicating higher TCS concentrations in sediments.

Calculating field BAFs for analytes requires detection in both fish and water samples; therefore it was only determined for dextromethorphan since DXM was the only analyte with detection in both water and fish. Bioaccumulation factor (BAF), expressed as Log values, of DXM for white perch and banded killifish in Hunting Creek were calculated to be 3.6 and 3.8 respectively (Figure 3.19).

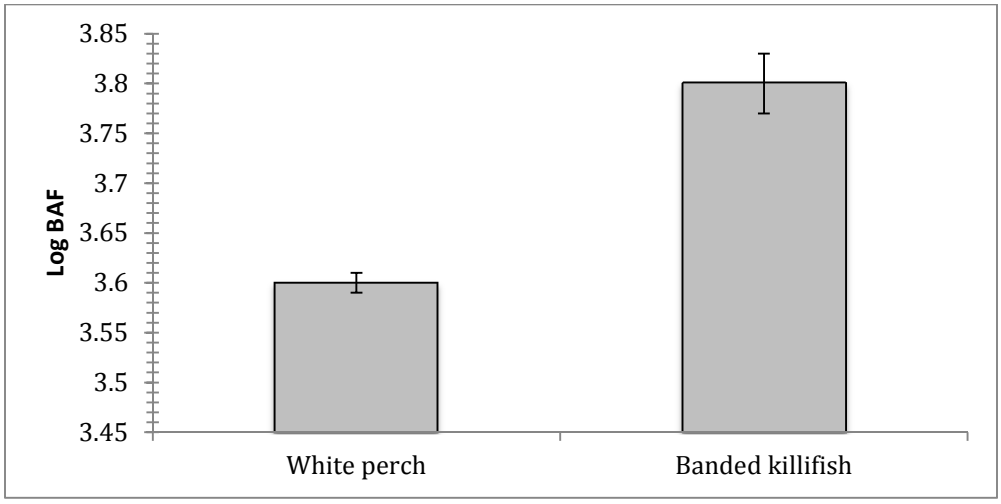


Figure 3.19. Log BAFs for DXM observed in white perch and banded killifish in Hunting Creek.

Discussion

Contemporary endocrine disruptors in four fish species in the upper Potomac River were quantified by GCMS. Out of 31 selected EDCs only 2 of them (triclosan and dextromethorphan) were quantified in whole fish and tissue homogenates. However, 12 additional EDCs were tentatively identified in fish but fell below method detection limits, and included ibuprofen, acetaminophen, 4-tert-octylphenol, diphenhydramine, vinclozolin, 4-nonylphenol, gemfibrozil, diethylstilbestrol, escitalopram, trimethoprim, 19-norethindrone and coumestrol, indicating low parts-per-trillion environmental concentrations. Clearly, lowering method detection limits (MDLs) by a factor of less than five would provide many more quantifiable EDCs in fish samples. A new method will be required to achieve lower MDLs because the QuEChERS-GCMS method used in the present study was only applicable to 1 g sample masses. Perhaps methods involving liquid chromatography mass spectrometry would achieve the desired low parts per trillion limits because of the ability to avoid MSFTA derivatization, thus simplifying the analysis

Fugacity ratios indicated dextromethorphan distribution has reached equilibrium in white perch and banded killifish, while triclosan flux was from sediments to fish (Hunting Creek). Two interesting facts were observed comparing fugacity ratios: (i) there was a clear pattern of sediments to fish flux for triclosan in

all the samples, whole fish and tissue, across the three sampled tributaries, (ii) consistently higher fugacity ratios were observed for DXM.

The fugacity distribution pattern of TCS and DXM did not show any fish to sediment flow, indicating sediments having higher concentrations of TCS and DXM. Fugacity values can be utilized as a tool to profile distribution patterns of contaminants across ecosystem and biota. Ecologically, flux from tissues to water/sediments implies that food is an important source of chemical, and the important aspect is to relate food consumption to the pelagic or benthic food webs. Fugacity is analogous to partial pressure of an ideal gas and directly related to concentration.[60] Fugacity ratios of analytes in different media can be calculated and the magnitude of the ratios will determine equilibrium or direction of flow.

Fugacity distribution was not determined for water and sediments from Accotink Creek and Pohick Creek due to lack of water samples from those two tributaries. Hunting Creek water sediments fugacity calculations determined water to sediments flow for both TCS and DXM in Hunting Creek. TCS water sediment fugacity ratio could only be determined in Cameron Run and upper Hunting Creek zone since those zones were the only ones with TCS detection in water samples.

Bioaccumulation factor (BAF) is another parameter used in predicting fate of organic chemicals in biota, and the field-measured bioaccumulation factor (BAF) for DXM in white perch and banded killifish (trophic level 3, mid-level), expressed as Log BAF, were 3.6 and 3.8 respectively. The estimated Log BAF value from EPI Suite, assuming a biotransformation rate of zero, for mid-level trophic guild is 3.3.[10]

Field-measured BAFs, calculated from fish is ecologically relevant because it takes diet and exposure into account.[147] BAF would be a better predictive tool in the fate assessment, compared to fugacity. Fugacity estimation utilizes lipid normalized fish concentrations, and lipid content and fish EDC concentration (ng/g wwt) did not indicate a correlation (R^2=0.1684).

Toxicity of triclosan has been characterized in many aquatic organisms, including algae and fish.[132] Acute triclosan toxicity data are similar across fish species, ranging from 350 to 390 μg/L.[131] Oliveira et al.,[148] concluded that TCS at concentrations ≥0.3 mg/L constitute hazardous for aquatic organisms. Orvos et al. has reported LC_{50} (lethal concentration resulting in 50% mortality) of 370 μg/L, with test duration of 96 hours for bluegill (*Lepomis macrochirus*).[132]

Dextromethorphan (DXM) is a cough suppressant readily available over the counter and its abuse is on the rise in the US[149], with high risk of severe medical complications, addictions as well as psychiatric effects.[129] Literature on DXM occurrence in human urine and plasma is abundant[150-164], with one study reporting detection in water[137], but non-existent regarding occurrence in aquatic biota. With DXM use on the rise as both therapeutic and substance abuse administration, it is expected to be more frequently observed in the aquatic environment, and thus, should be continually monitored. Dextromethorphan toxicity research on fish is limited, with reported LC_{50} of 4.0 mg/L for 96 hours in rainbow trout (*Oncorhynchus mykiss*).[133]

Triclosan is a broad spectrum antibacterial agent used in personal care products, fabrics used for sportswear and plastic additives to name a few. Due to the raised public awareness regarding disease control and personal hygiene the use of triclosan in the U.S. has increase over the past 25 years.[165] Miller et al.,[166] measured TCS along with its degradation products in sediments (30-40 year-old) and associated the increased sediment concentrations with this temporal pattern in TCS use over time. The main source of triclosan contamination in sediment and biota is thought be wastewater effluent, accumulated from different sources such as houses, and hospitals.[131] The results of the present study question that assumption. Other emission sources of TSC in the aquatic environment aside from WWTP discharge appear to be important. Statistical analysis indicated geospatial differences among whole fish and tissue concentrations (ng/g wwt), and reverse concentration gradient was observed, further indicating WWTP is not the only source of EDC introduction into the aquatic environment. Another origination source for triclosan and dextromethorphan could be leaching from septic tanks.

A comparison of mean TCS and DXM concentrations (ng/g wwt) across the four fish species (white perch, banded killifish, alewife and gizzard shad) and in relation with trophic level showed no significant dependency relating to trophic level (two-way ANOVA, $p>0.05$), although feeding habits could be a factor.

Triclosan detection in fish has been documented in several studies. Adolfsson-Erici et al.,[167] conducted a study in Sweden, and detected high TCS levels in bile of caged rainbow trout (*Oncorhynchus mykiss*) exposed to WWTP effluent (47

mg/kg wwt) and lower concentrations in wild perch (*Perca fluviatilis*) downstream of the discharge point (0.44 mg/kg wwt). Bream (*Abramis brama*) analyzed by Houtman et al.,[168] from the Netherlands, also showed high concentrations of TCS in bile (14-80 µg/mL). Valters et al.,[169] sampled 13 fish species from the Detroit River and reported TCS concentrations ranging from 1.87 to 10.3 µg/g wwt. The observed mean concentration of triclosan, in this study, in whole fish varied from 47.9 ± 6.89 ng/g wwt in alewife to 81.8 ± 5.84 ng/g wwt in white perch, which are well below the reported ranges by Valters et al.

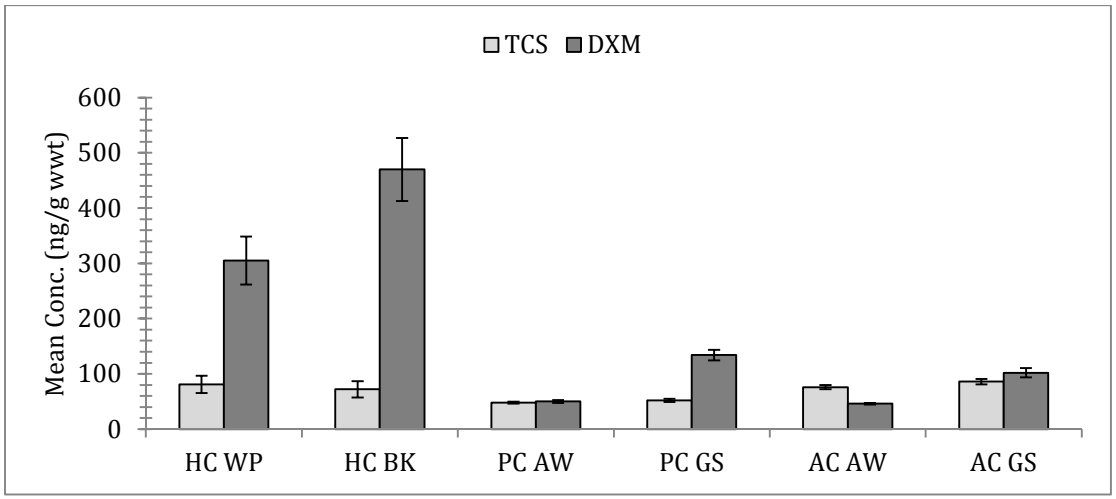


Figure 3.20. Mean TCS and DXM concentrations (ng/g wwt) in whole fish from Hunting Creek (HC), Pohick Creek (PC) and Accotink Creek (AC).
WP: white perch
BK: banded killifish
AW: alewife
GS: gizzard shad

Statistical analysis indicated significant differences among TCS and DXM whole fish concentrations among species at each creek location. Hunting Creek

DXM mean concentrations (ng/g wwt) in white perch and banded killifish were greater than DXM mean concentrations in alewife and gizzard shad from Accotink Creek and Pohick Creek by a factor of 6-10 (Figure 3.18). The Hunting Creek watershed had the lowest percentage of forested land (13.7%) compared to Pohick Creek (50.5%) and Accotink Creek (37.6%). It also has the largest percentage of high intensity residential area (36.6%) compared to minimal values of Pohick Creek and Accotink Creek, 0.06% and 0.02% respectively.[27, 29, 34] The lower forested land area and largest percentage of high intensity residential area in Hunting Creek is reflective in higher mean concentrations of TCS and DXM in Hunting Creek fish (Figure 3.20).

Statistical analysis indicated significant differences between fish species and tissue concentrations (ng/g wwt) from Accotink Creek and Pohick Creek (two-way ANOVA, $p<0.05$). The species differences are most likely from trophic guild interactions and dietary habits. Gizzard shad is intermediary trophic level II fish while alewife, white perch and banded killifish are intermediary trophic level III. The tissue concentrations differences are due to several factors, one of which are lipid content in ovaries and testes. The higher DXM concentration in stomach tissue relative to the DXM concentration in water implies that DXM is bioaccumulated through food sources rather than just uptake from water.

This study revealed two interesting points. The first is that DXM concentrations in fish tissue (ng/g wwt) were consistently greater than TCS concentrations in fish tissue (ng/g wwt) by a factor of 2 to 5, and the highest

concentrations (ng/g wwt) of TCS and DXM were detected in stomach, ovaries and skin. Interestingly, skin has very low lipid content (0.3%) compared to stomach, ovaries and testes with lipid content ranging from 2.5% to 2.7%, yet DXM concentration (ng/g wwt) in skin was greater than DXM concentration in muscle tissue by a factor of 2-8. This increase in skin DXM concentration might be due to the constant contact of skin with water. Skin is the organ that is in touch with the DXM pollution from the water.

There was no deterministic trend for triclosan detection frequency and concentrations across different tissues, in contrast DXM indicated a clear pattern of detection frequency and concentration in three specific tissue types of skin, stomach and ovaries. Lack of TCS detection in tissue homogenates and also the considerable lower concentrations in whole fish homogenate might be due to the fact that TCS transforms into methyl-triclosan during WWTP treatment.[170, 171]

The possibility of parental transfer of TCS and DXM to offspring was analyzed in this study. Several studies have demonstrated the parental transfer of a thyroid endocrine distrupter (DE-71, a commercial mixture of polychlorinated biphenyl ethers, PBDEs) to progeny in zebrafish (*Danio rerio)* [172], rats[173] and ranch mink (*Mustela vison*).[174] Parental changes induced by EDCs can be passed to the progeny, and only affect them physiologically[175] due to the fact that the majority of toxins, including EDCs, do not have the ability to cause genetic mutations.[176-178]

Schwindt states that the mechanism of parental transfer to offspring is through the ability of EDCs to turn genes on and off and affect how genes are read; a

process referred to as epigenetics.[175] Through this mechanism EDCs induce changes in parents, which hinder offspring development (epigenetics). Another proposed mechanism for transfer is the actual uptake (bioaccumulation) of EDCs in ovaries.[179]

Miller demonstrated a strong correlation between p,p'-DDE and PCB muscle concentration in gravid fish to egg concentrations in those fish; study analyzed two species, chinook salmon (*Oncorthynchus tshawytscha*) and lake trout (Salvelinus namaycush).[180]

Considering those studies and the high concentrations in ovaries and testes, ranging from 102 to 274 ng/g wwt (for TCS) and 265 to 1,273 ng/g wwt (for DXM) parental transfer to offspring is possible, warranting further research and analysis.

Conclusion

This study determined the prominent analytes present in fish collected from Hunting Creek, Pohick Creek and Accotink. Of the 31 EDCs, 2 were detected above the analytical detection limits in Hunting Creek, Accotink and Pohick Creek fish. Consistent with chapter 2 results, which showed TCS and DXM in water and bed sediment of Hunting Creek, TCS and DXM was observed in fish (Table 3.4).

TCS and DXM concentrations increased in the following trend: fish>sediment>water. Sediment concentration is higher than concentration observed in water due to sorption of analytes to the clay/silt makeup of the bed

sediment. The significantly higher concentrations in fish are due to the bioaccumulation tendency of EDCs.

Table 3.4. Summary of DXM and TCS mean concentrations in water, sediments and fish in Hunting Creek (HC), Accotink Creek (AC) and Pohick Creek (PC).

Location	Sample Type	EDC detected	Mean Conc.	Units
HC	Fish, white perch	DXM	304 ± 43.3	ng/g wwt
HC	Fish, banded killifish	DXM	470 ± 7.06	ng/g wwt
HC	Water	DXM	74.9 ± 11.8	ng/L
HC	Sediments	DXM	103 ± 84.2	ng/g dwt
HC	Fish, white perch	TCS	81.8 ± 5.84	ng/g wwt
HC	Fish, banded killifish	TCS	72.2 ± 4.56	ng/g wwt
HC	Water	TCS	15.5 ± 3.71	ng/L
HC	Sediments	TCS	72.5 ± 9.41	ng/g dwt
AC	Fish, alewife	DXM	45.3 ± 9.12	ng/g wwt
AC	Fish, gizzard shad	DXM	101 ± 11.1	ng/g wwt
AC	Sediments	DXM	91.1 ± 3.52	ng/g dwt
AC	Fish, alewife	TCS	76.1 ± 9.13	ng/g wwt
AC	Fish, gizzard shad	TCS	45.5 ± 7.04	ng/g wwt
AC	Sediments	TCS	ND	ng/g dwt
PC	Fish, alewife	DXM	49.1 ± 9.68	ng/g wwt
PC	Fish, gizzard shad	DXM	134 ± 11.8	ng/g wwt
PC	Sediments	DXM	233 ± 48.9	ng/g dwt
PC	Fish, alewife	TCS	47.9 ± 6.89	ng/g wwt
PC	Fish, gizzard shad	TCS	51.7 ± 5.32	ng/g wwt
PC	Sediments	TCS	ND	ng/g dwt

All fish concentrations are mean concentrations detected in whole fish homogenate.

Analysis and quantitation of EDCs in fish from Hunting Creek, Accotink Creek and Pohick Creek revealed geospatial and species differences among the sampled sites and fish. Concentrations (ng/g wwt) of TCS and DXM in different tissues were

significantly different with high concentrations in ovaries and testes, indicating potential of parental transfer to offspring. Fugacity estimates demonstrated an overall higher fugacity value for DXM compared to TCS. Sediments to fish flux was observed for TCS in 100% of samples, while DXM had reached equilibrium in 8% of samples with sediment to fish flux in the remaining samples. Overall TCS and DXM showed water to sediments and sediments to fish flux reflective of the concentration gradient observed in water, sediment and fish. The consistent detection of triclosan, antibacterial agent used in soaps and hygienic products, and dextromethorphan, the active ingredient in cough/cold medicine, in water and sediments and biota warrants further research and analysis.

APPENDIX A

Gunston Cove PCB LODs and MDLs

PCBs	Limits of Detection (ng)	MDLs Fish Tissue (ng/g)	MDLs Bed Sediment (ng/g)
4,10	0.13	0.13	0.03
7,9	0.19	0.19	0.04
6	0.09	0.09	0.02
5,8	0.19	0.19	0.04
19	0.09	0.09	0.02
12	0.10	0.10	0.02
18	0.10	0.10	0.02
15	0.06	0.06	0.01
17	0.07	0.07	0.01
24,27	0.13	0.13	0.03
16,32	0.10	0.10	0.02
34	0.07	0.07	0.01
26	0.07	0.07	0.01
25	0.60	0.60	0.12
28,31	0.12	0.12	0.02
20,33	0.11	0.11	0.02
22	0.08	0.08	0.02
45	0.08	0.08	0.02
46,69,52	0.63	0.63	0.13
49	0.08	0.08	0.02
47,48	0.17	0.17	0.03
104	0.06	0.06	0.01
44	0.10	0.10	0.02
42,59	0.67	0.67	0.13
37	0.11	0.11	0.02
41,71,64	0.28	0.28	0.06

PCBs	Limits of Detection (ng)	MDLs Fish Tissue (ng/g)	MDLs Bed Sediment (ng/g)
40	0.07	0.07	0.01
67	0.08	0.08	0.02
63	0.08	0.08	0.02
74	0.10	0.10	0.02
70	0.08	0.08	0.02
66	0.15	0.15	0.03
93,95	0.13	0.13	0.03
91	0.10	0.10	0.02
56,60	0.16	0.16	0.03
84	0.07	0.07	0.01
92,101	0.41	0.41	0.08
99	0.10	0.10	0.02
119	0.05	0.05	0.01
83	0.11	0.11	0.02
97	0.04	0.04	0.01
87,115	0.13	0.13	0.03
85	0.09	0.09	0.02
136	0.06	0.06	0.01
110	0.06	0.06	0.01
77	0.08	0.08	0.02
82	0.08	0.08	0.02
151	0.06	0.06	0.01
135,144	0.15	0.15	0.03
147	0.18	0.18	0.04
109,123,118	0.22	0.22	0.04
149	0.10	0.10	0.02
134	0.86	0.86	0.17
131,146	0.18	0.18	0.04
114	0.17	0.17	0.03
132	0.20	0.20	0.04
153	0.10	0.10	0.02
105	0.10	0.10	0.02
179	0.05	0.05	0.01
141	0.07	0.07	0.01
137	0.09	0.09	0.02

PCBs	Limits of Detection (ng)	MDLs Fish Tissue (ng/g)	MDLs Bed Sediment (ng/g)
176	0.10	0.10	0.02
164,138	0.18	0.18	0.04
158	0.17	0.17	0.03
129	0.37	0.37	0.07
178	0.08	0.08	0.02
187	0.12	0.12	0.02
183	0.10	0.10	0.02
128,167	0.11	0.11	0.02
185	0.12	0.12	0.02
174	0.11	0.11	0.02
177	0.10	0.10	0.02
171	0.10	0.10	0.02
156,157	0.22	0.22	0.04
173	0.12	0.12	0.02
172	0.09	0.09	0.02
197	0.06	0.06	0.01
180	0.04	0.04	0.01
193	0.14	0.14	0.03
191	0.08	0.08	0.02
170,190	0.17	0.17	0.03
199	0.07	0.07	0.01
196,203	0.15	0.15	0.03
189	0.11	0.11	0.02
195	0.13	0.13	0.03
208	0.09	0.09	0.02
207	0.03	0.03	0.01
194	0.07	0.07	0.01
205	0.10	0.10	0.02
206	0.10	0.10	0.02

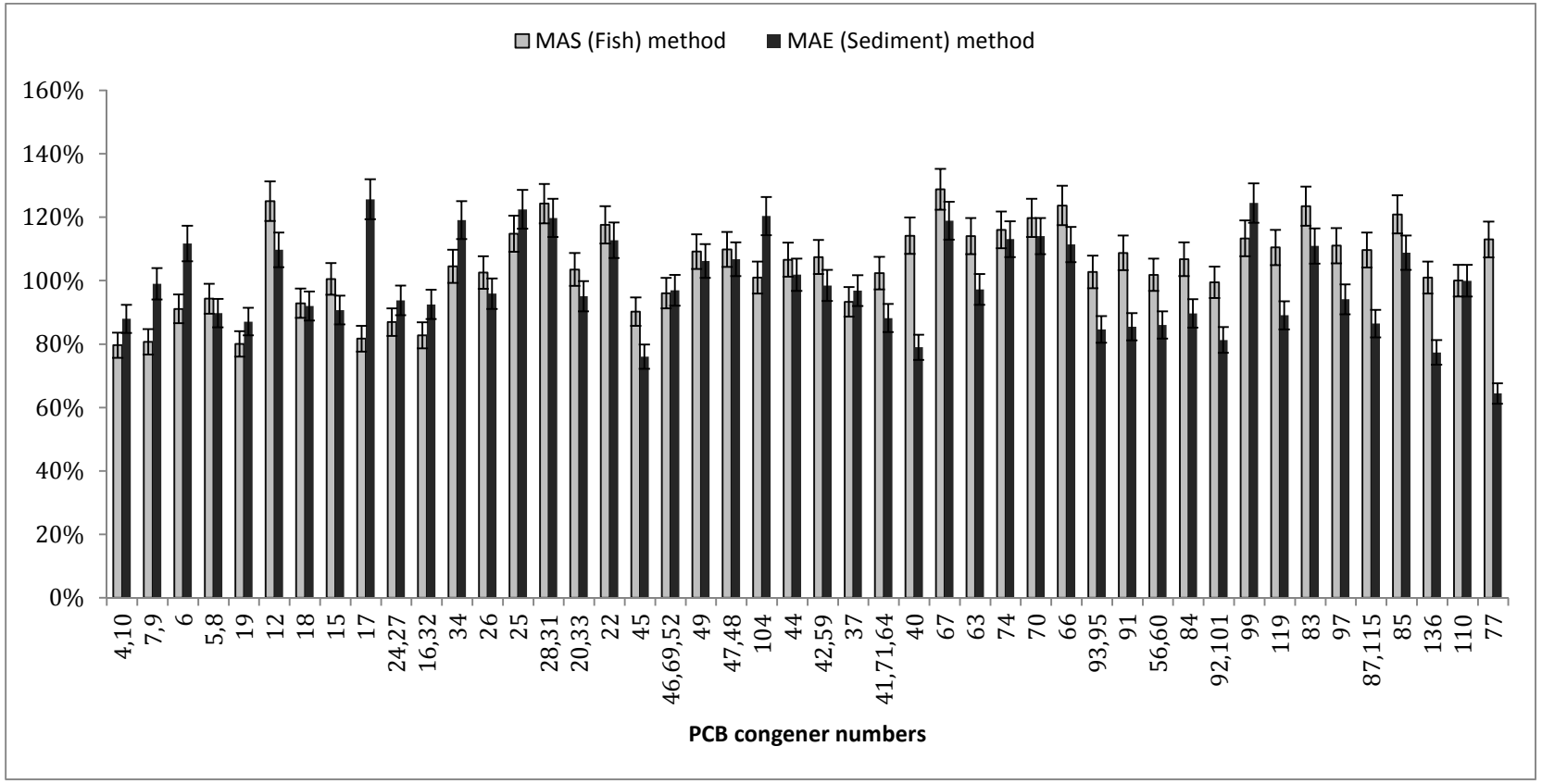
Hunting Creek PCB LODs and MDLs

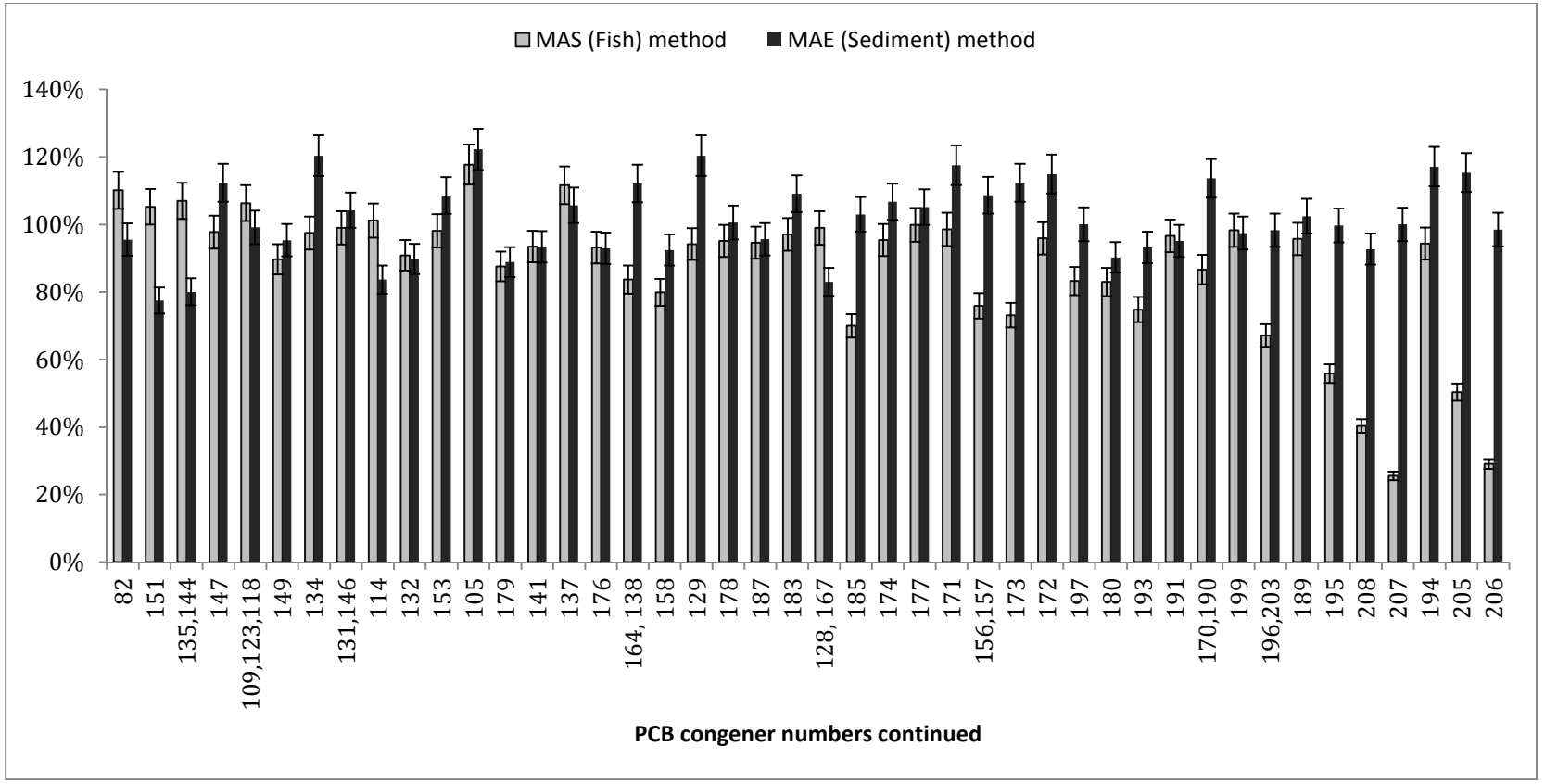
PCBs	Limits of Detection (ng)	MDLs Fish Tissue (ng/g)	MDLs Bed Sediment (ng/g)
1	0.04	0.04	0.04
2	0.10	0.10	0.10
3	0.11	0.11	0.11
4, 10	0.04	0.04	0.04
7, 9	0.06	0.06	0.06
6	0.03	0.03	0.03
5, 8	0.07	0.07	0.07
19	0.01	0.01	0.01
12	0.09	0.09	0.09
18	0.02	0.02	0.02
15	0.12	0.12	0.12
17	0.02	0.02	0.02
24, 27	0.03	0.03	0.03
16, 32	0.03	0.03	0.03
34	0.01	0.01	0.01
29	0.02	0.02	0.02
26	0.04	0.04	0.04
25	0.05	0.05	0.05
31	0.11	0.11	0.11
28	0.13	0.13	0.13
20, 33	0.08	0.08	0.08
22	0.05	0.05	0.05
45	0.03	0.03	0.03
46	0.02	0.02	0.02
69	0.02	0.02	0.02
52	0.03	0.03	0.03
49	0.02	0.02	0.02
47, 48	0.06	0.06	0.06
104	0.03	0.03	0.03
44	0.03	0.03	0.03
37	0.08	0.08	0.08
59, 42	0.06	0.06	0.06
41, 64, 71	0.07	0.07	0.07

PCBs	Limits of Detection (ng)	MDLs Fish Tissue (ng/g)	MDLs Bed Sediment (ng/g)
40	0.03	0.03	0.03
67	0.04	0.04	0.04
63	0.03	0.03	0.03
74	0.05	0.05	0.05
70	0.04	0.04	0.04
66	0.05	0.05	0.05
93, 95	0.06	0.06	0.06
91	0.02	0.02	0.02
56, 60	0.09	0.09	0.09
92	0.02	0.02	0.02
84	0.03	0.03	0.03
101	0.04	0.04	0.04
99	0.02	0.02	0.02
119	0.02	0.02	0.02
83	0.03	0.03	0.03
97	0.02	0.02	0.02
87, 115	0.05	0.05	0.05
85	0.05	0.05	0.05
136	0.02	0.02	0.02
77	0.06	0.06	0.06
110	0.02	0.02	0.02
82	0.07	0.07	0.07
151	0.02	0.02	0.02
135, 144	0.05	0.05	0.05
109	0.03	0.03	0.03
147	0.03	0.03	0.03
123	0.05	0.05	0.05
149	0.01	0.01	0.01
118	0.08	0.08	0.08
134	0.05	0.05	0.05
114	0.05	0.05	0.05
131	0.03	0.03	0.03
146	0.03	0.03	0.03
153	0.03	0.03	0.03
132	0.05	0.05	0.05

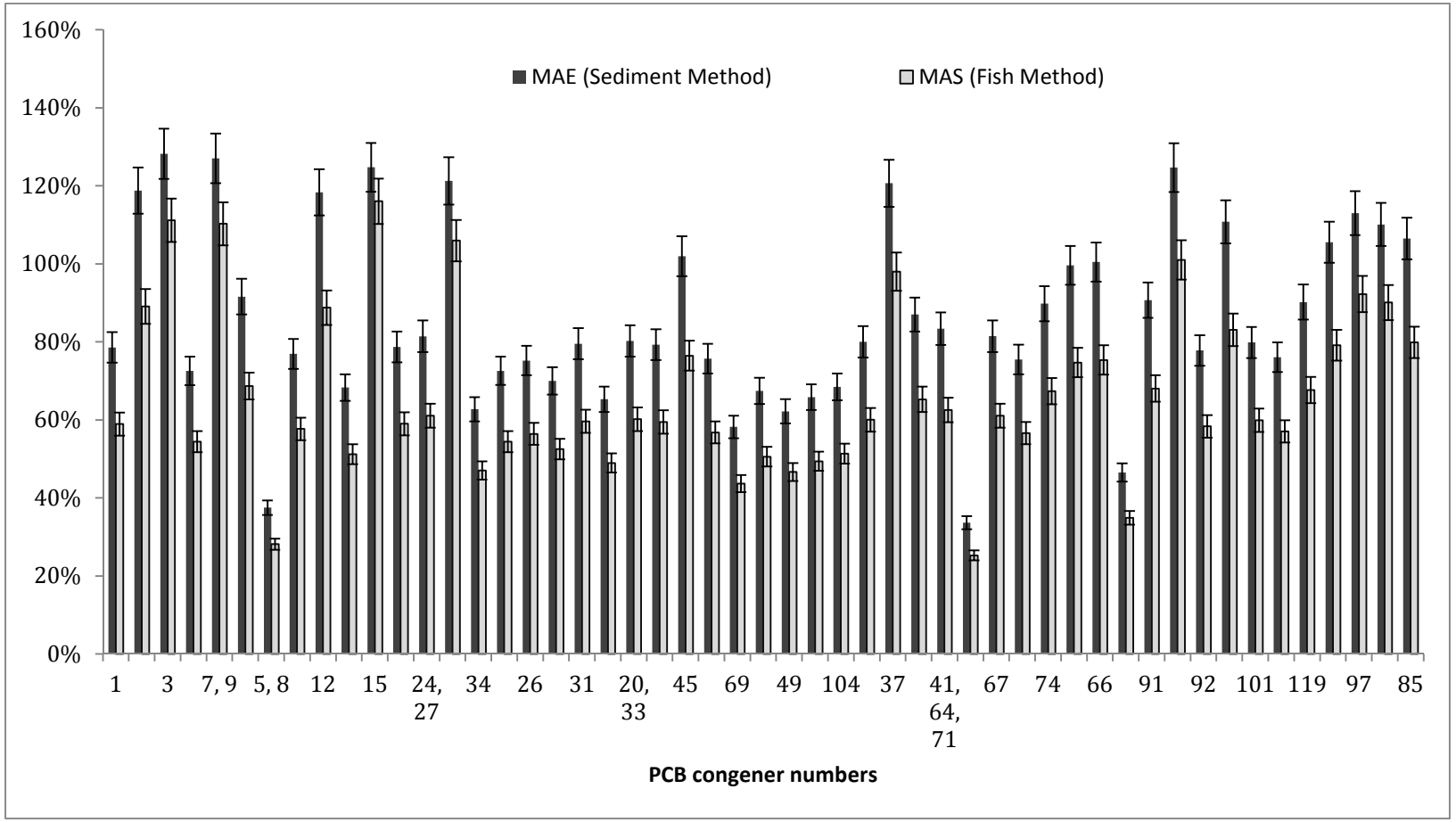
PCBs	Limits of Detection (ng)	MDLs Fish Tissue (ng/g)	MDLs Bed Sediment (ng/g)
105	0.04	0.04	0.04
141	0.05	0.05	0.05
179	0.03	0.03	0.03
137	0.03	0.03	0.03
176	0.02	0.02	0.02
138, 164	0.04	0.04	0.04
158	0.03	0.03	0.03
129	0.03	0.03	0.03
178	0.03	0.03	0.03
187	0.03	0.03	0.03
183	0.02	0.02	0.02
128, 167	0.07	0.07	0.07
185	0.03	0.03	0.03
174	0.02	0.02	0.02
177	0.02	0.02	0.02
171	0.03	0.03	0.03
156	0.05	0.05	0.05
173	0.03	0.03	0.03
157	0.08	0.08	0.08
172	0.04	0.04	0.04
197	0.03	0.03	0.03
180	0.03	0.03	0.03
193	0.03	0.03	0.03
191	0.03	0.03	0.03
170, 190	0.07	0.07	0.07
199	0.10	0.10	0.10
196, 203	0.09	0.09	0.09
189	0.05	0.05	0.05
208	0.04	0.04	0.04
195	0.14	0.14	0.14
207	0.04	0.04	0.04
194	0.07	0.07	0.07
205	0.11	0.11	0.11
206	0.09	0.09	0.09

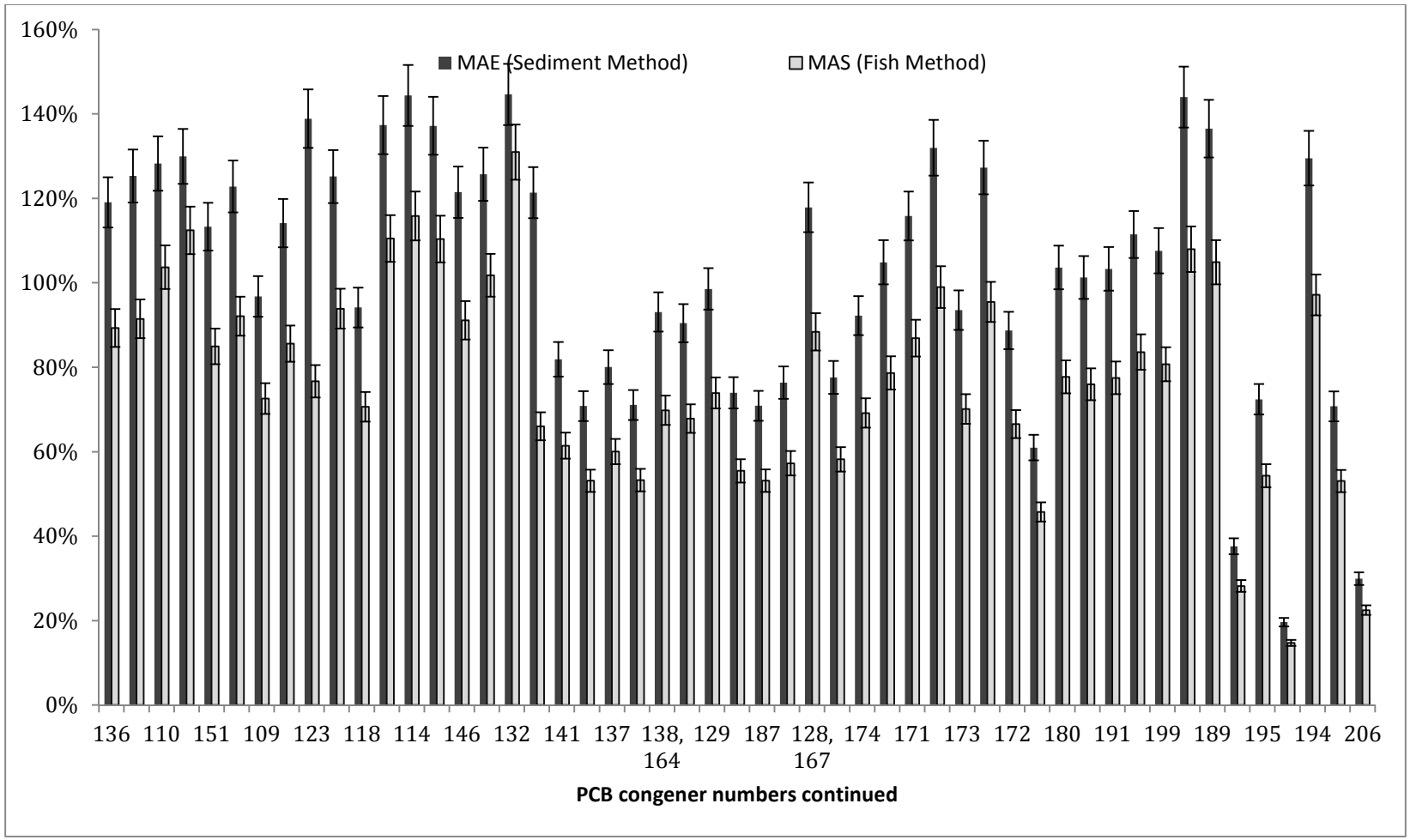
Analyte Standard Recovery Percentages in Fish Tissue and Bed Sediment Matrices, Gunston Cove



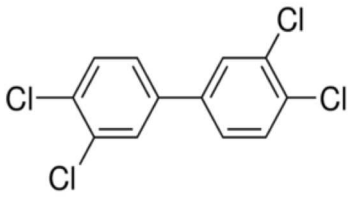
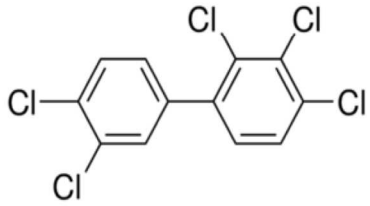
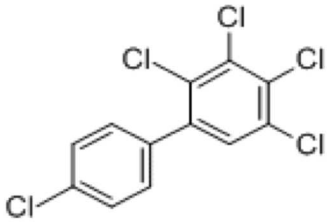
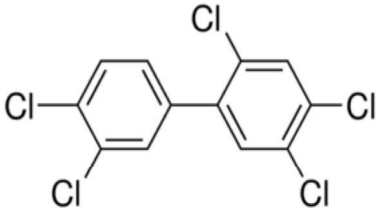
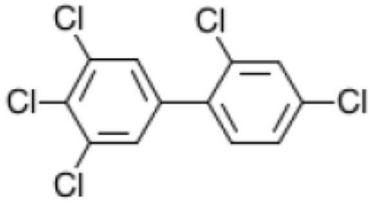

MAS (Fish) method
MAE (Sediment) method
140%
120%
100%
80%
60%
40%
20%
0%
82
151
135,144
147
109,123,118
149
134
131,146
114
132
153
105
179
141
137
176
164, 138
158
129
178
187
183
128, 167
185
174
177
171
156,157
173
172
197
180
193
191
170,190
199
196,203
189
195
208
207
194
205
206
PCB congener numbers continued

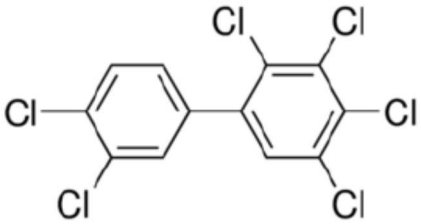
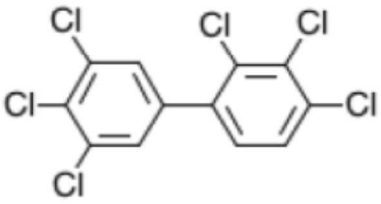
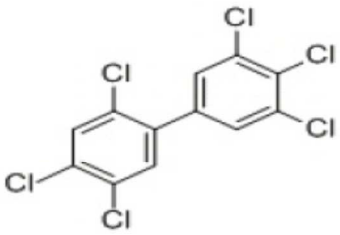
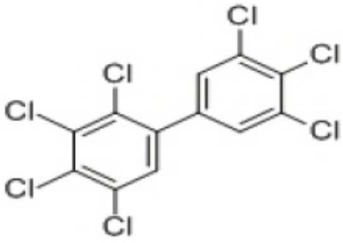
Analyte Standard Recovery Percentages in Fish Tissue and Bed Sediment Matrices, Hunting Creek




160%
140%
120%
100%
80%
60%
40%
20%
0%
MAE (Sediment Method)
MAS (Fish Method)
136 110 151 109 123 118 114 146 132 141 137 138, 164 129 187 128, 167 174 171 173 172 180 191 199 189 195 194 206
PCB congener numbers continued

Dioxin-like PCBs analyzed in the Gunston Cove and Hunting Creek study

PCB 77		CAS Number: 32598-13-3 3,3',4,4'-tetra-CB MW: 291.99 amu Log Kow: 6.63 KH: 0.952 Pa.m3/mol	non-*ortho*	tetra-CB
PCB 105		CAS Number: 32598-14-4 2,3,3',4,4'-penta-CB MW: 326.43 amu Log Kow: 6.50 KH: 28.67 Pa.m3/mol	mono-*ortho*	penta-CB
PCB 114		CAS Number: 74472-37-0 2,3,4,4',5-penta-CB MW: 326.43 amu Log Kow: 6.50 KH: 28.67 Pa.m3/mol		
PCB 118		CAS Number: 31508-00-6 2,3',4,4',5-penta-CB MW: 326.43 amu Log Kow: 7.12 KH: 9.47 Pa.m3/mol*		
PCB 123		CAS Number: 65510-44-3 2,3',4,4',5'-penta-CB MW: 323.88 amu Log Kow: 9.83* KH: 22.1 Pa.m3/mol*		

PCB 156		CAS Number: 38380-08-4 2,3,3',4,4',5-hexa-CB MW: 360.88 amu Log Kow: 7.60 KH: 14.49 Pa.m3/mol*	mono-*ortho*	hexa-CB
PCB 157		CAS Number: 69782-90-7 2,3,3',4,4',5'-hexa-CB MW: 357.84 Log Kow: 7.60 KH: 5.58 Pa.m3/mol*		
PCB 167		CAS Number: 52663-72-6 2,3',4,4',5,5'-hexa-CB MW: 360.88 Log Kow: 7.50 KH: 6.94 Pa.m3/mol*		
PCB 189		CAS Number: 39635-31-9 2,3,3',4,4',5,5'-hepta-CB MW: 391.81 Log Kow: 8.27* KH: 5.14 Pa.m3/mol*		hepta-CB

K_H and K_{ow} values were obtained from EPA's EPI suite[10]
* These values were obtained from MacKay et al., 2006[44]

APPENDIX B

List of Internal Standards and corresponding retention times (RT) and quantifying ion and qualifying ions.

RT	Compound	Quant Ion	Qual 1	Qual 2	Qual 3
9.292	Acenaphthene-d10	162	164	160	163
14.099	Phenanthrene-d10	188	189	80	184
28.907	Chrysene-d12	240	236	241	120

List of Surrogate Standards and corresponding retention times (RT) and quantifying ion and qualifying ions.

RT	Compuond	Quant Ion	Qual 1	Qual 2	Qual 3
13.279	Atrazine-13C3 SS[a]	203	218	205	70
21.649	Naproxen-13C, D3 SS[a]	189	243	306	291
25.152	Bisphenol A-13C12 SS[a]	369	370	384	
21.966	Naproxen-d3 SS[b]	188	246	305	290
23.088	Triclosan-d3 SS[b]	200	350	365	185
25.497	Bisphenol A-d4 SS[b]	361	376	73	209

[a] C-13 Isotopic surrogate standards, used for water samples.

[b] Deuterated surrogate standards, used for sediment samples.

List of EDCs and corresponding retention times (RT) and quantifying ion and qualifying ions.

RT	Compound	Quant Ion	Qual 1	Qual 2	Qual 3
11.146	Acetaminophen	206	280	295	73
11.197	Ibuprofen	160	263	117	73
11.535	4-tert-octylphenol	207	208	57	73
13.589	Atrazine	200	215	173	
15.638	Caffeine	194	109	67	55
16.811	Diphenhydramine	58	165	73	152
16.868	Vinclozolin	212	285	187	198
17.397	Fluoxetine unTMS	44	104	42	91
17.961	Gemfibrozil	201	83	179	73
17.967	4-Nonylphenol	179	292	180	73
21.426	Fluoxetine (prozac)	116	219	262	73
21.852	Naproxen Sodium Salt	185	243	302	73
23.174	Triclosan (Irgasan)	200	345	347	73
23.809	Dextromethorpham	59	271	150	214
25.485	Bisphenol A	357	358	372	73
26.807	Diclofenac sodium salt	214	242	367	73
28.180	Diethylstilbestrol	412	413	383	73
28.912	Carbamazepine	193	194	165	73
29.090	Escitalopram oxalate	58	208	238	324
30.749	Trimethoprim	419	434	420	73
31.510	Estrone	342	257	218	73
31.619	Equilin	340	216	283	73
32.094	D(-)-Norgestrel unTMS	91	79	245	110
32.100	Testosterone	129	360	270	73
32.123	17b-Estradiol	416	285	129	73
32.435	Mestranol	367	227	174	73
33.104	17a-Ethinylestradiol	425	285	196	73
33.576	Progesterone	43	124	314	272
33.636	D(-)-Norgestrel	355	317	125	73
33.834	19-Norethindrone	355	125	153	73
34.366	Estriol	311	129	345	73
35.699	Coprostanol	370	355	215	75
35.859	Coumestrol	412	397	413	73

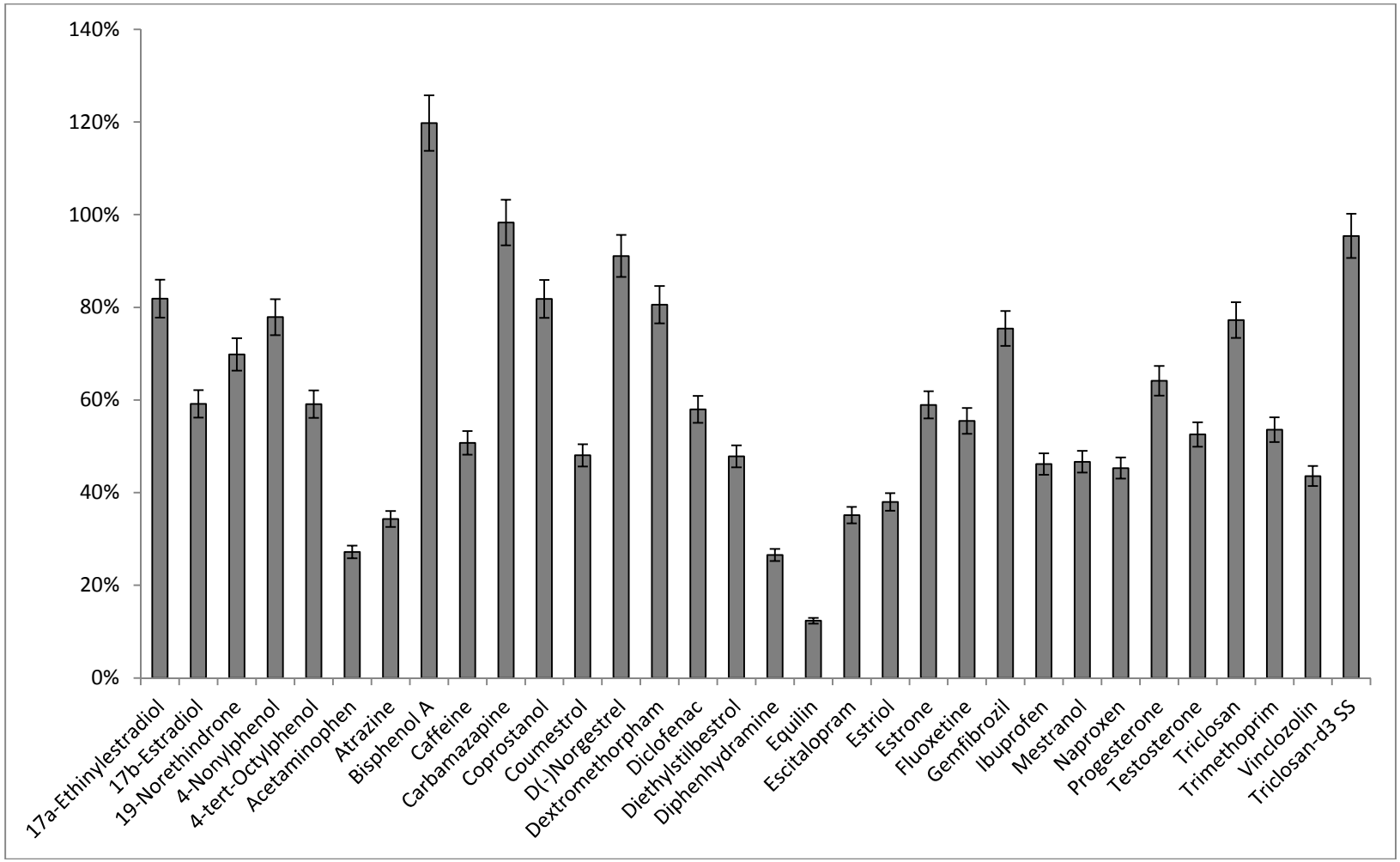
Hunting Creek Sediments EDC LODs and MDLs

EDC analytes	**Limit of Detection (LD) (ng)**	**Method Limit Detection (MDL) (ng/g)**
Ibuprofen	16.09	16.09
Acetaminophen	202.62	202.62
4-*tert*-Octylphenol	21.50	21.50
Atrazine	30.49	30.49
Caffeine	100.00	100.00
Fluoxetine	176.24	176.24
Diphenhydramine	43.69	43.69
Vinclozolin	37.42	37.42
4-Nonylphenol	27.47	27.47
Gemfibrozil	29.42	29.42
Naproxen	75.00	75.00
Triclosan	42.06	42.06
Dextromethorpham	34.11	34.11
Bisphenol A	35.89	35.89
Carbamazapine	34.73	34.73
Diclofenac	48.52	48.52
Diethylstilbestrol	31.25	31.25
Escitalopram	31.02	31.02
Trimethoprim	33.95	33.95
Estrone	31.81	31.81
Equilin	40.31	40.31
17β-Estradiol	35.00	35.00
Testosterone	56.00	56.00
Mestranol	65.21	65.21
19-Norethindrone	269.75	269.75
D(-)Norgestrel	53.26	53.26
17α-Ethinylestradiol	63.98	63.98
Progesterone	156.12	156.12
Estriol	36.94	36.94
Coprostanol	50.00	50.00
Coumestrol	42.77	42.77

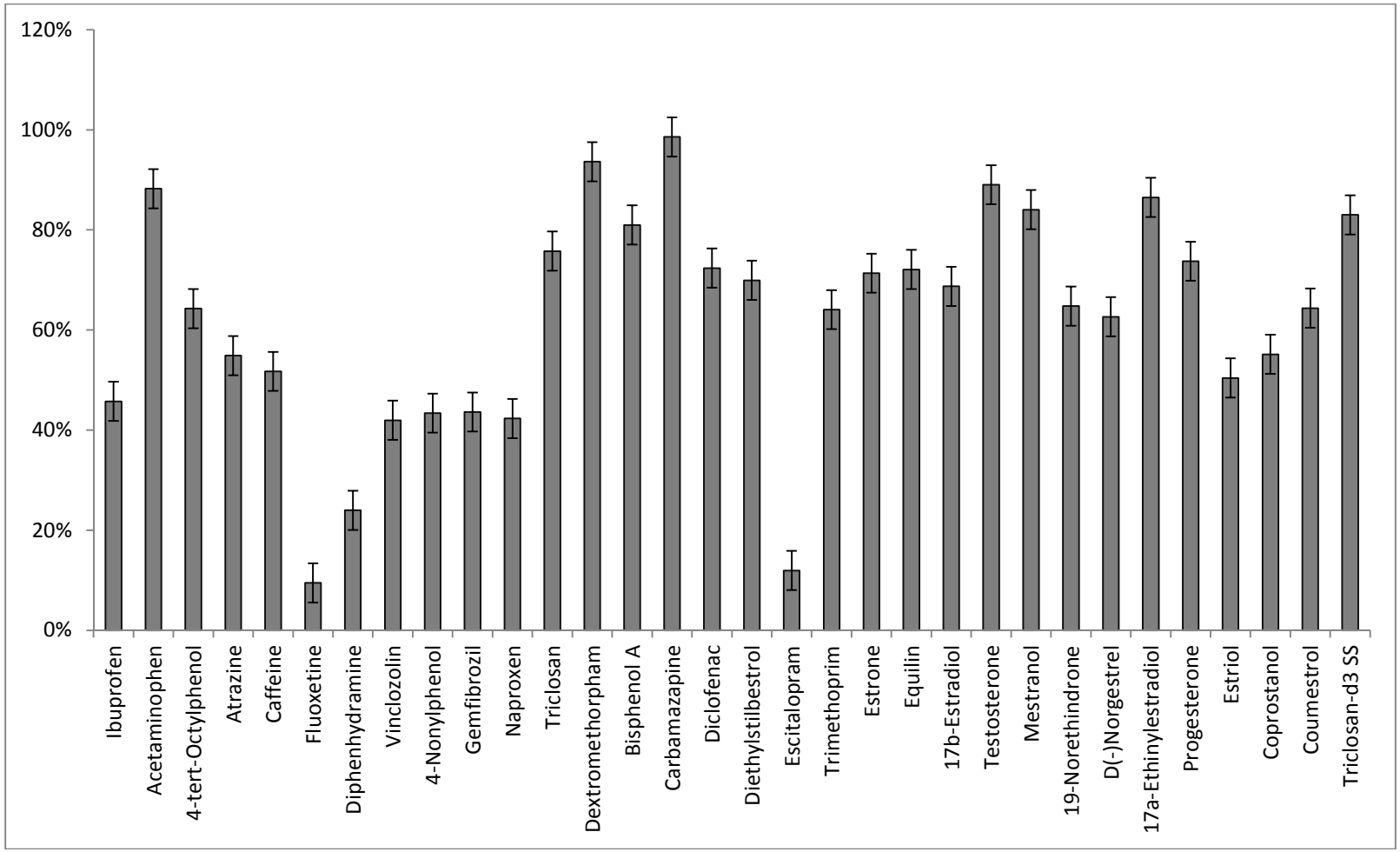
Hunting Creek water EDC LODs and MDLs

EDC analytes	Limit of Detection (LD) (ng)	Method Limit Detection (MDL) (ng/L)
Ibuprofen	42.53	42.53
Acetaminophen	170.00	170.00
4-tert-Octylphenol	14.27	14.27
Atrazine	57.61	57.61
Caffeine	60.28	60.28
Fluoxetine	335.91	335.91
Diphenhydramine	10.34	10.34
Vinclozolin	19.00	19.00
4-Nonylphenol	18.87	18.87
Gemfibrozil	172.02	172.02
Naproxen	88.37	88.37
Triclosan	11.44	11.44
Dextromethorpham	42.04	42.04
Bisphenol A	51.90	51.90
Carbamazapine	76.75	76.75
Diclofenac	308.11	308.11
Diethylstilbestrol	137.46	137.46
Escitalopram	15.16	15.16
Trimethoprim	183.45	183.45
Estrone	206.45	206.45
Equilin	46.20	46.20
17b-Estradiol	21.15	21.15
Testosterone	10.08	10.08
Mestranol	39.09	39.09
19-Norethindrone	504.94	504.94
D(-)Norgestrel	72.25	72.25
17a-Ethinylestradiol	21.90	21.90
Progesterone	62.55	62.55
Estriol	40.38	40.38
Coprostanol	14.99	14.99
Coumestrol	51.17	51.17

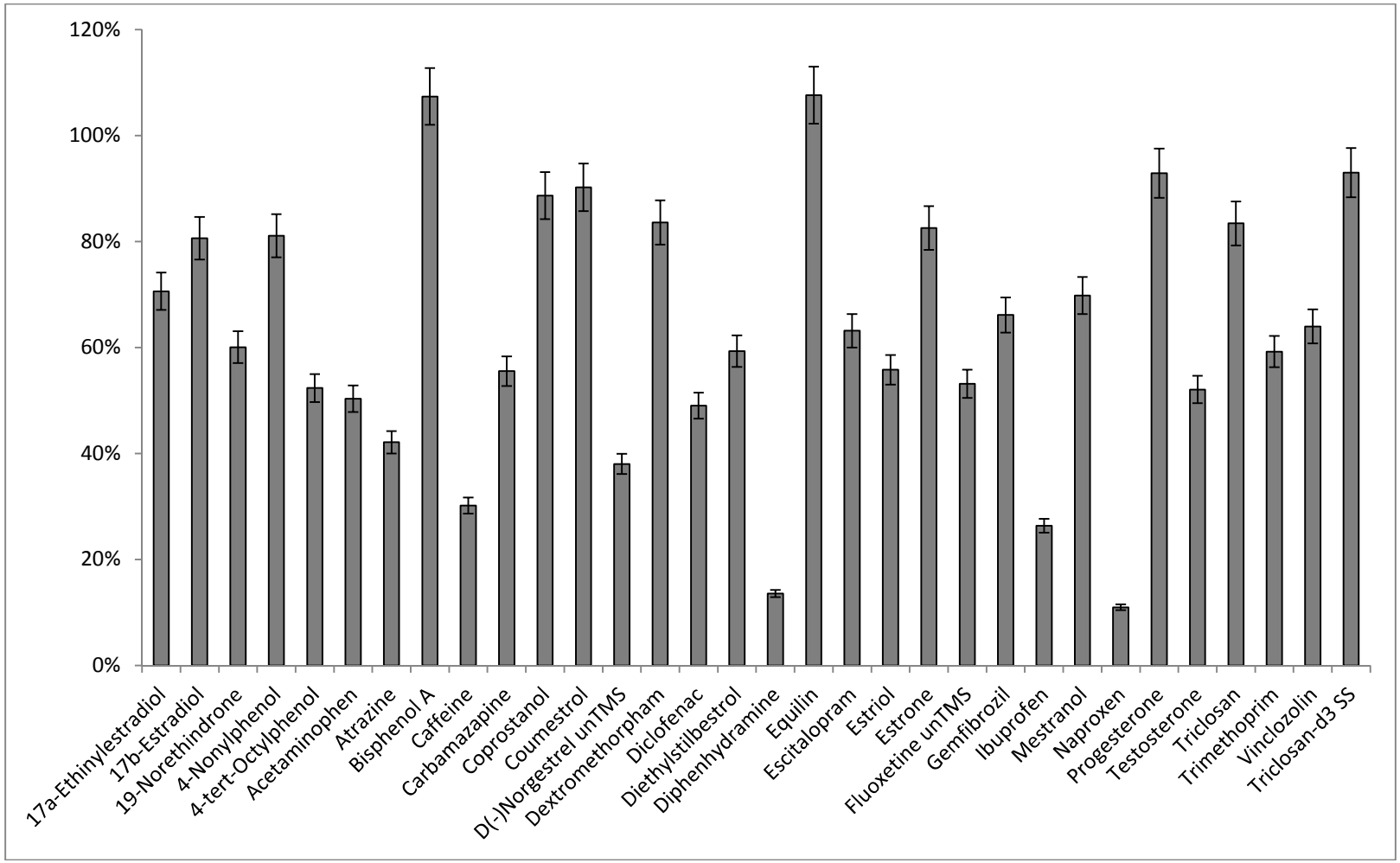
Hunting Creek sediments QA recoveries.



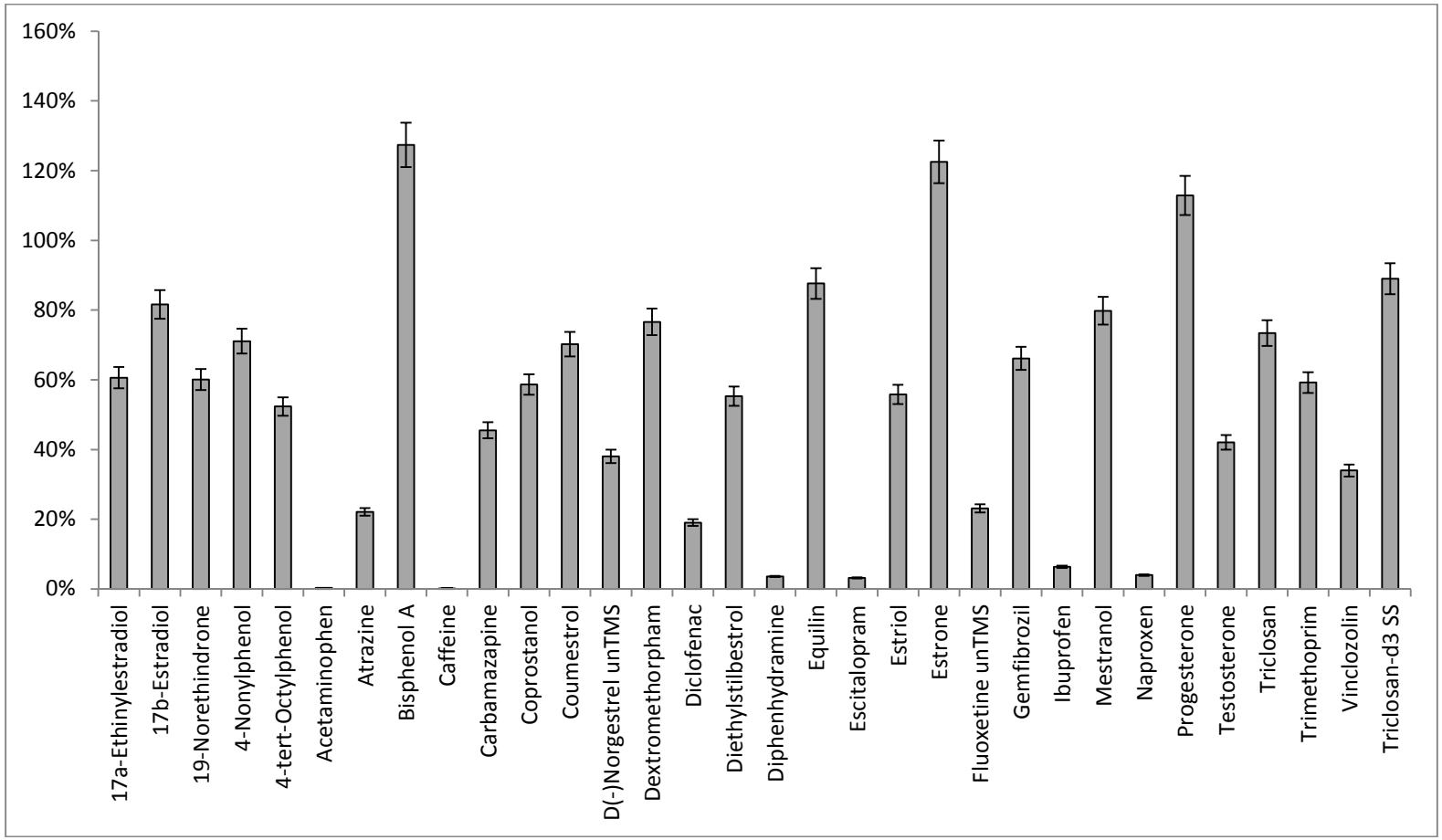
Hunting Creek sediments matrix spike recoveries



Hunting Creek water QA recoveries



Hunting Creek water matrix spike recoveries.



APPENDIX C

List of EDCs and corresponding retention times (RT) and quantifying ion and qualifying ions.

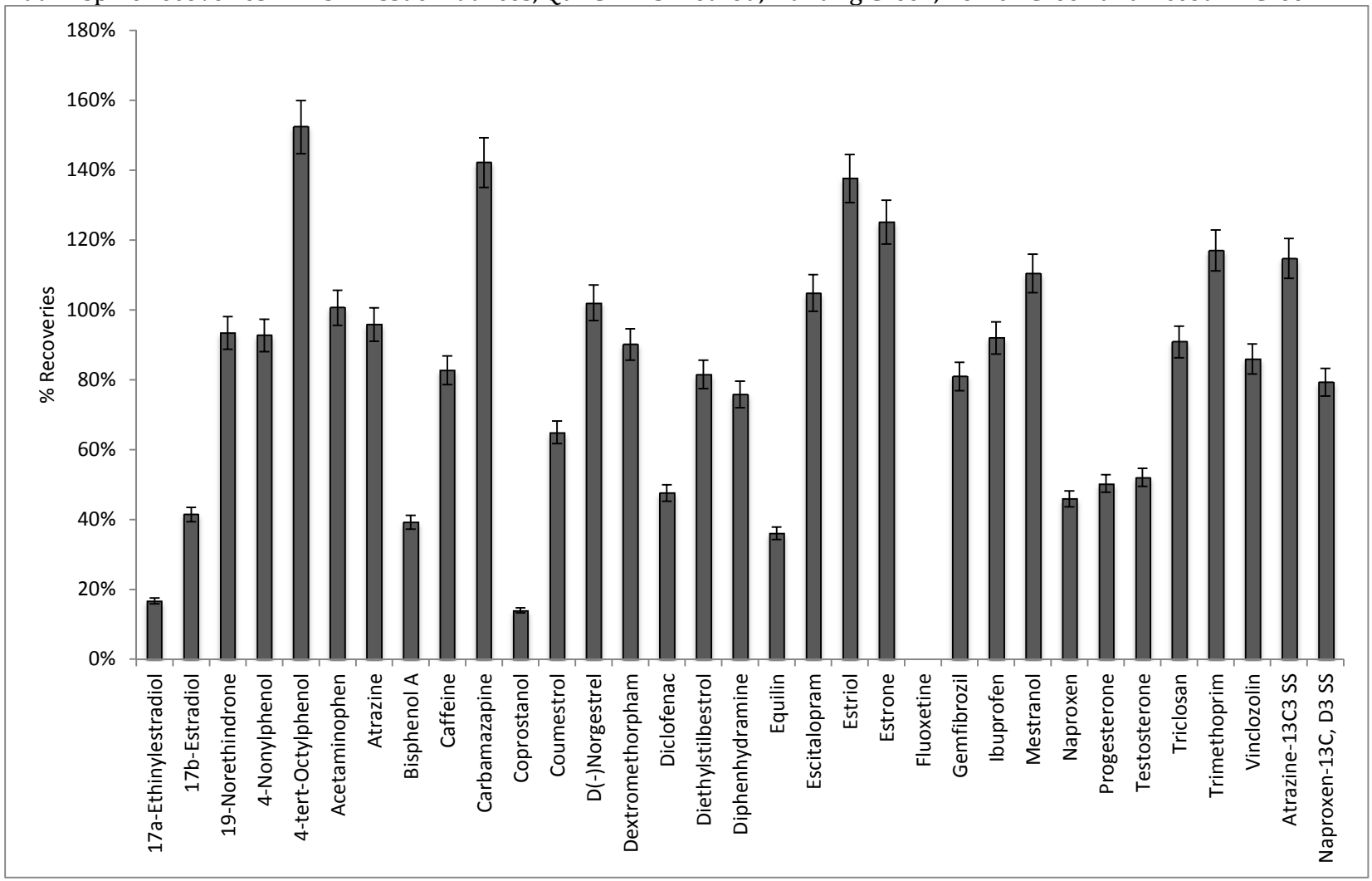
RT	Compuond	Quant Ion	Qual 1	Qual 2	Qual 3
11.146	Acetaminophen	206	280	295	73
11.197	Ibuprofen	160	263	117	73
11.535	4-tert-octylphenol	207	208	57	73
13.589	Atrazine	200	215	173	
15.638	Caffeine	194	109	67	55
16.811	Diphenhydramine	58	165	73	152
16.868	Vinclozolin	212	285	187	198
17.397	Fluoxetine unTMS	44	104	42	91
17.961	Gemfibrozil	201	83	179	73
17.967	4-Nonylphenol	179	292	180	73
21.426	Fluoxetine (prozac)	116	219	262	73
21.852	Naproxen Sodium Salt	185	243	302	73
23.174	Triclosan (Irgasan)	200	345	347	73
23.809	Dextromethorpham	59	271	150	214
25.485	Bisphenol A	357	358	372	73
26.807	Diclofenac sodium salt	214	242	367	73
28.180	Diethylstilbestrol	412	413	383	73
28.912	Carbamazepine	193	194	165	73
29.090	Escitalopram oxalate	58	208	238	324
30.749	Trimethoprim	419	434	420	73
31.510	Estrone	342	257	218	73
31.619	Equilin	340	216	283	73
32.094	D(-)-Norgestrel unTMS	91	79	245	110
32.100	Testosterone	129	360	270	73
32.123	17b-Estradiol	416	285	129	73
32.435	Mestranol	367	227	174	73
33.104	17a-Ethinylestradiol	425	285	196	73
33.576	Progesterone	43	124	314	272
33.636	D(-)-Norgestrel	355	317	125	73
33.834	19-Norethindrone	355	125	153	73

RT	Compuond	Quant Ion	Qual 1	Qual 2	Qual 3
34.366	Estriol	311	129	345	73
35.699	Coprostanol	370	355	215	75
35.859	Coumestrol	412	397	413	73

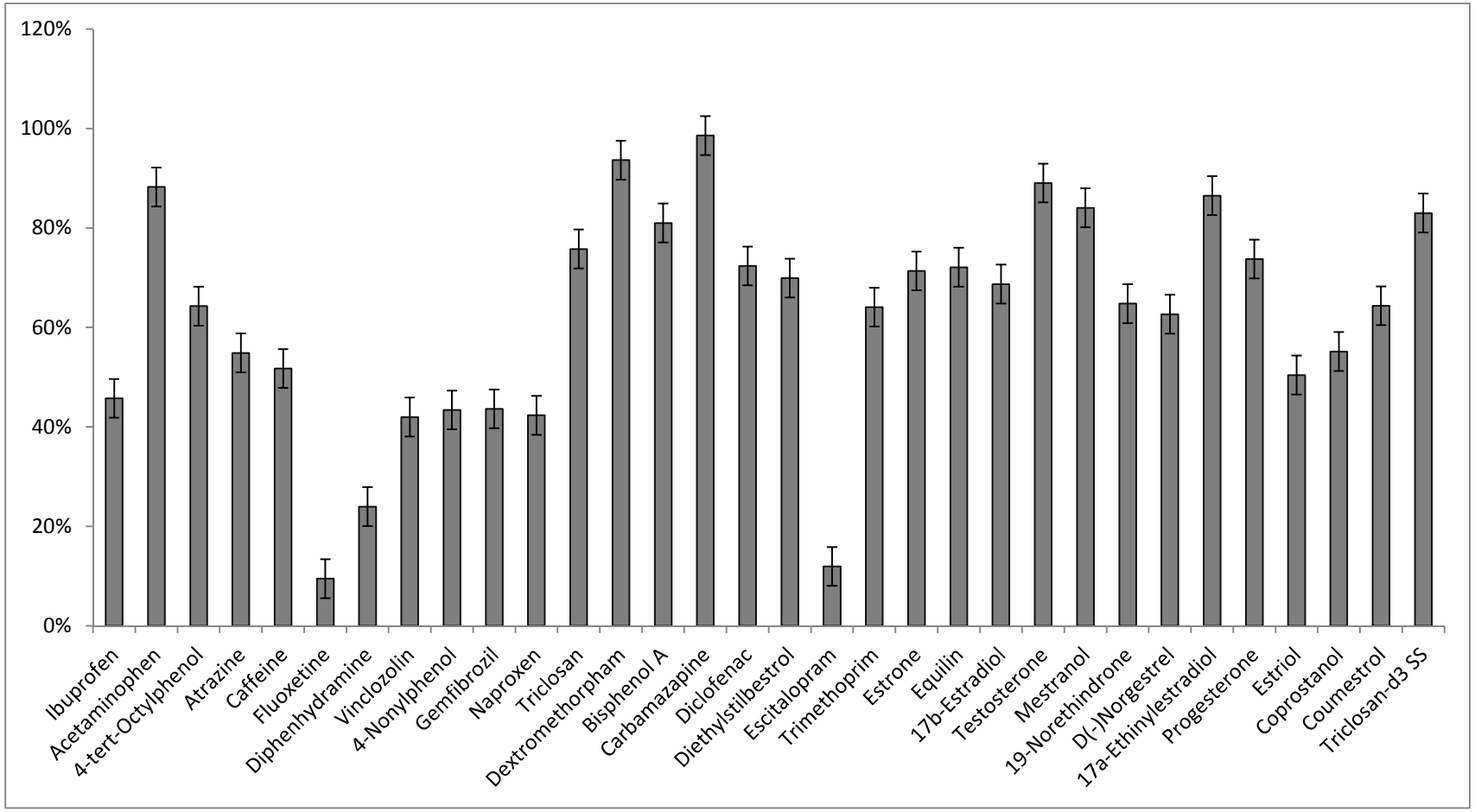
QuEChERS method EDC LODs and MDLs

EDC analytes	**Limit of Detection (LD) (ng)**	**Method Limit Detection (MDL) (ng/g)**
Ibuprofen	16.09	16.09
Acetaminophen	202.62	202.62
4-*tert*-Octylphenol	21.50	21.50
Atrazine	30.49	30.49
Caffeine	100.00	100.00
Fluoxetine	176.24	176.24
Diphenhydramine	43.69	43.69
Vinclozolin	37.42	37.42
4-Nonylphenol	27.47	27.47
Gemfibrozil	29.42	29.42
Naproxen	75.00	75.00
Triclosan	42.06	42.06
Dextromethorpham	34.11	34.11
Bisphenol A	35.89	35.89
Carbamazapine	34.73	34.73
Diclofenac	48.52	48.52
Diethylstilbestrol	31.25	31.25
Escitalopram	31.02	31.02
Trimethoprim	33.95	33.95
Estrone	31.81	31.81
Equilin	40.31	40.31
17β-Estradiol	35.00	35.00
Testosterone	56.00	56.00
Mestranol	65.21	65.21
19-Norethindrone	269.75	269.75
D(-)Norgestrel	53.26	53.26
17α-Ethinylestradiol	63.98	63.98
Progesterone	156.12	156.12
Estriol	36.94	36.94
Coprostanol	50.00	50.00
Coumestrol	42.77	42.77

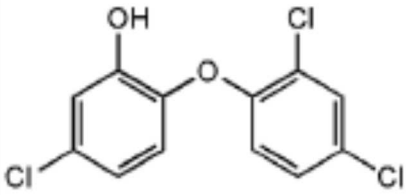
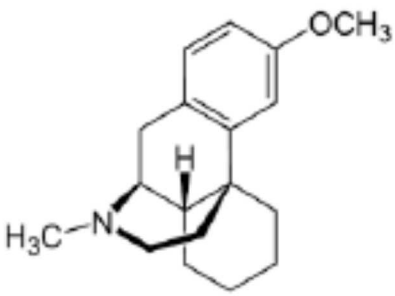
Matrix spike recoveries in Fish Tissue Matrices, QuEChERS method, Hunting Creek, Pohick Creek and Accotink Creek



QA/QC recoveries for Hunting Creek, Accotink Creek and Pohick Creek sediment analysis .



APPENDIX D

	Triclosan CAS No: 003380-34-5 K_H: 4.99E-9 (atm.m^3/mol) Log Kow: 4.66 289.54 g/mol
	Dextromethorphan CAS No: 000125-71-3 K_H: 1.20E-7 (atm.m^3/mol) Log Kow: 3.97 271.397 g/mol

Henry's Law constant and octanol-water partition coefficient values for triclosan and dextromethorphan, obtained from EPA's EPI Suite.

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BIOGRAPHY

Golala Arya was born in Mariwan, Kurdistan in 1979, coinciding with the death of freedom in Iran. In 1995, along with her family she was sent to the United States via United Nations High Commissioner for Refugees. She received a Bachelor of Arts in Chemistry from Michigan State University in 2005. In 2009, she received a Master of Science in Chemistry from George Mason University.