Validated Methods for the Identification of New Halogenated Polycyclic Aromatic Compounds in the Canadian Environment

by

Zhe Xia

A thesis submitted to the Faculty of Graduate Studies of the
University of Manitoba
in partial fulfilment of the requirements of the degree of

DOCTOR OF PHILOSOPHY

Department of Chemistry
University of Manitoba
Winnipeg, Manitoba, Canada

Abstract

Polycyclic aromatic compounds (PACs) represent a complex and structurally diverse group of aromatic organic contaminants of both petrogenic and pyrogenic origins. These compounds are released into the environment from anthropogenic and natural sources as complex mixtures, encompassing thousands of different aromatic, alkyl-aromatic, and heterocyclic hydrocarbons containing N-, S-, or O- atoms. Overwhelmingly, most of the research in this field has focused on the sixteen 16 US Environmental Protection Agency priority polycyclic aromatic hydrocarbons (PAHs). However, there is still a large knowledge gap on the identification and quantitation of other PACs, for instance halogenated PAHs (HPAHs), which has led to the overarching hypothesis of my thesis: additional PACs are present in the environment and gas chromatography coupled to mass spectrometry can be used to accurately identify and quantify them. The specific class of PACs that I was interested in studying were ones containing halogen atoms. The rationale for this is that the addition of a halogen on the PAC backbone will increase their environmental persistent and bioaccumulative potential relative to non-halogenated species. The first advancement I made was the detection of HPAHs in environmental samples from the Athabasca Oil Sands Region (AOSR). To my knowledge, this is the first report on the detection of HPAHs from AOSR. The second major advancement I made was the validation of two extraction methods for PACs for abiotic (sediment) and biotic (avian egg) samples, respectively. In both studies, I was able to significantly streamline the sample preparation process of PACs. I also compared various quantification methods for PAC analysis which has led to improved measurement accuracy and precision. The overall results of my work will impact future studies on the development of even faster and more cost-effective analytical monitoring techniques, tools designed to delineate sources of crude oil exposures, and forensic studies leading to new areas of analytical research.

Preface

This thesis is an original work entirely written by Zhe Xia. Due to the style of the "sandwich method", there might be some redundancies in the introductory paragraphs of the five manuscripts as well as the introductory chapter.

Chapter 3 in this thesis is a manuscript published in Chemosphere. I was responsible for performing the analyses, processing the data, and writing this manuscript. Dr. Ifeoluwa Idowu, Wesley Johnson, and Olga Francisco helped in method development conceptualization. Drs. Chris Marvin, Philippe J. Thomas, Jörg Stetefeld, Bernard Crimmins, and Mark Fry reviewed the document and provided help with drafting of the manuscript. Dr. Gregg Tomy, as the primary investigator of this study, was responsible for acquisition of the funds, project administration, supervision, experiment conceptualization and manuscript review.

Chapter 4 in this thesis is a manuscript published in Chemosphere. I was responsible for performing the formal analysis, processing the data, and writing this manuscript. Dr. Ifeoluwa Idowu, Evan Kerr, Nicole Klaassen, Harsimran Assi, and Harley Bray provided help in method development conceptualization and analyses. Drs. Chris Marvin, Philippe J. Thomas and Jörg Stetefeld helped with drafting of the manuscript. Dr. Gregg Tomy, as the primary investigator of this study, was responsible for acquisition of the funds, project administration, supervision, experiment conceptualization and manuscript review.

Chapter 5 in this thesis is a manuscript published in Chemosphere. I was responsible for performing the formal analysis, processing the data, and writing this manuscript. Dr. Ifeoluwa Idowu, Thor Halldorson, Amica-Mariae Lucas, Claire Stein, Manpreet Kaur, Thane Tomy helped in method development conceptualization and analyses. Drs. Chris Marvin, Philippe J. Thomas,

Craig E. Hebert, Jennifer Provencher and Reyd A. Smith and Frederic Dwyer-Samuel reviewed and edited the document. Seabird egg samples were collected by Dr. Jennifer Provencher's team and collaboators. Dr. Gregg Tomy, as the primary investigator of this study, was responsible for acquisition of the funds, project administration, supervision, experiment conceptualization and manuscript review.

Chapter 6 in this thesis is a manuscript published in Journal of Chromatography A. I was responsible for reviewing the manuscript and providing help in methodology. The lead author, Dr. Ifeoluwa G. Idowu, was primarily responsible for interpreting data and performing the analyses, writing the manuscript. Dr. Courtney D. Sandau reviewed the document and provided expertise. Michelle Misselwitz provided assistance in data curation and visualization. Dr. Chris Marvin reviewed the document. Philippe Thomas provided help in funding acquisition and supervision. Dr. Gregg Tomy, as the primary investigator of this study, was responsible for acquisition of the funds, validation and manuscript review.

Chapter 7 in this thesis is a manuscript published in Environmental Toxicology and Chemistry. The toxicity analyses were conducted at the University of Lethbridge. The chemical analyses were conducted at the Centre for Oil and Gas Research and Development (COGRAD), University of Manitoba. I was responsible for reviewing the manuscript and performing the chemical analyses. The lead author, Justin Dubiel, was primarily responsible for interpreting data, performing the toxicological analyses, writing and reviewing the manuscript. Derek Green contributed on methodology, validation, and reviewing the manuscript. Yamin Raza and Hunter M. Johnson contributed on methodology. Dr. Gregg T. Tomy reviewed the manuscript and performed the chemical analyses. Dr. Alice Hontela provided help on supervision and funding acquisition. Dr. Jon A. Doering contributed to conceptualization, write and review the manuscript. Dr. Steve B.

Wiseman, as the primary investigator of this study, was responsible for funding acquisition, validation and manuscript review.

List of Publications

1. Xia, Z.; Idowu, I.; Marvin, C.; Thomas, P.J.; Johnson, W.; Francisco, O.; Stetefeld, J.; Crimmins, B.; Fry, M.; Tomy, G.T. Identification of Halogenated Polycyclic Aromatic Compounds in Biological Samples from Alberta Oil-Sands Region. *Chemosphere*, **2019**, *215*, 206-213.

2. Brown, A.K.; Xia, Z.; Bulloch, P.; Idowu, I.; Francisco, O.; Stetefeld, J.; Stout, J.; Zimmer, J.; Marvin, C.; Letcher, R.J.; Tomy, G.T. Validated quantitative cannabis profiling for Canadian regulatory compliance-Cannabinoids, aflatoxins, and terpenes. *Analytica Chimica Acta*, **2019**, *1088*, 79-88.

3. Bulloch, P.; Schur, S.; Muthumuni, D.; Xia, Z.; Johnson, W.; Palace, V.; Letcher, R.; Tomy, G.T. F_2-Isoprostanes in fish mucus: A new, non-invasive analysis of oxidative stress biomarkers. *Chemosphere*, **2019**, *239*, 124797.

4. Marvin, C.; Tomy, G.; Thomas, P.; Holloway, A.; Sandau, C.; Idowu, I.; Xia, Z. Considerations for Prioritization of Polycyclic Aromatic Compounds as Environmental Contaminants. *Environmental Science & Technology*, **2020**, *54*, 14787–14789.

5. Thomas, P.J.; Newell, E.; Eccles, K.; Holloway, A.C.; Idowu, I.; Xia, Z.; Hassan, E.; Tomy, G.; Quenneville, C. Co-exposures to trace elements and polycyclic aromatic compounds (PACs) impacts North American river otter (*Lontra canadensis*) baculum. *Chemosphere*, **2021,** *265*, 128920.

6. Fujita, K. K.; Xia, Z.; Tomy, G.; Montina, T.; Wiseman, S. ^{1}H NMR based metabolomic profiling of early life stage zebrafish (Danio rerio) exposed to a water-soluble fraction of weathered sediment-bound diluted bitumen. *Aquatic Toxicology*, **2021**, *232*, 105766.

7. Xia, Z.; Idowu, I.G.; Kerr, E.; Klaassen, N.; Assi, H.; Bray, H.; Marvin, C.; Thomas, P.J.; Stetefeld, J.; Tomy, G.T. New Approaches to Reduce Sample Processing Times for the Determination of Polycyclic Aromatic Compounds in Environmental Samples. *Chemosphere*, **2021,** *274*, 129738.

8. Idowu, I.G., Xia, Z., Sandau, C.D., Misselwitz, M., Thomas, P., Marvin, C., Tomy, G.T. Comparison of different approaches to quantify substituted polycyclic aromatic compounds. *Journal of Chromatography A*, **2021**, *1651*, 462317.

9. Dubiel, J., Green, D., Raza, Y., Johnson, H.M., Xia, Z., Tomy, G.T., Hontela A., Wiseman, S. Alkylation of Benz[*a*]anthracene Affects Toxicity to Early-Life Stage Zebrafish and In

Vitro Aryl Hydrocarbon Receptor 2 Transactivation in a Position-Dependent Manner. *Environmental Toxicology and Chemistry*, **2022**, *41*(8), 1993-2002.

10. Moradi, V., Halldorson, T., Idowu, I., Xia, Z., Vitharana, N., Marvin, C., Thomas, P., Tomy, G.T. Microbead-Beating Extraction of Polycyclic Aromatic Compounds from Seabird Plasma and Whole Blood. *Separations*, **2023**, *10*(1), 48.

11. Xia, Z., Idowu, I., Halldorson, T., Lucas, A.M., Stein, C., Kaur, M., Tomy, T., Marvin, C., Thomas, P.J., Hebert, C. E., Smith, R.A., Dwyer-Samuel, F., Provencher, J.F., Tomy, G.T. Microbead beating extraction of avian eggs for polycyclic aromatic compounds. *Chemosphere*, **2023**, *335*, 139059.

12. Moradi, V., Halldorson, T., Xia, Z., Vitharana, N., Marvin, C., Thomas, P.J., Sorais, M. Crossin, G.T., Tomy, G. Determining Polycyclic Aromatic Compounds in Bird Feathers Using Pressurized Fluid Extraction. *Separations*, **2023**, *10*(9), 503.

Acknowledgements

The completion of this work would not be possible without the huge support from many people. I would like to take this opportunity to acknowledge their help and encouragement.

First, I would like to express my deepest gratitude to my supervisor, Dr. Gregg T. Tomy, for his endless assistance, guidance, and support throughout the course of my learning journey. Gregg has not only shared with me his knowledge of environmental and analytical chemistry but also encouraged me to stay enthusiastic as a young experimentalist. I am very appreciative of the sound contributions, constructive criticism and advice from my Ph.D. committee members, Drs. Hélène Perreault, Jörg Stetefeld and Mark Fry. My appreciation also goes to my external examiner, Dr. Stuart Harrad, from the University of Birmingham, thank you for accepting to be part of my committee and for carefully reading through my thesis.

Second, I am grateful for the help for all the lab members throughout my program: Dr. Ifeoluwa Idowu, Thor Halldorson, Olga Fransisco, Wesley Johnson, Dr. Alistair Brown, Lisa Oswald, and Dr. Lisa Peters. A huge thank you goes to Dr. Ifeoluwa Idowu for her help and instruction during the starting days of my program. I would like to thank all the graduate students from the Centre for Oil and Gas Research and Development (COGRaD): Patrique Bulloch, Jamie Dearnley, Jade Wish, Vida Moradi, Nipuni Vitharana, Saarankah Sambanthan, and all undergraduate students worked with me: Sara Schur, Brett Starosilec, Nicholas Blandford, Nicole Klaassen, Harley Bray, Harsimran Assi, Kathleen Sawatzky, Evan Kerr, Oliver Peters, Amica-Mariae Lucas, Ben Van Bastelaere, Claire Stein, Manpreet Kaur, Thane Tomy, Erin Liebzeit, Kara Loudon, and Jonas Pathammavong.

Third, I am especially appreciative to the Department of Chemistry at the University of Manitoba for the assistance and help during my program. I also thank Dr. Takeshi Ohura for sending me those precious analytical standards.

To my loving parents, Changqing Gong and Zhi Xia, I am deeply grateful for their eternal support and their unconditional and continuous love to me. I would not be even closed to here without them. To my dearest friends in Winnipeg, my second home city, I wish to express my gratitude for their tremendous help and support over the past decade (in the order of time I met them): Siyao Liu, Songhao Liao, Yi Xie, Runqing Mao, Rui Zhong, Shuai Tan, Yue Zhao and Yuanheng Liu — thanks for sharing your joy and celebrating every milestone with me. I am also extremely appreciative for the love and encouragement from my amazing friends that I met in China (in alphabetical order): Kai He, Lan Lan, Yulin Li, Hanhan Wang, Lu Xu, Jize Zhang and Qian Zhang. They are always there whenever I needed them. I remember those long phone calls back in the early days when I felt lonely or lost. I feel so blessed and honored to have them as my friends. In addition, my thanks to friends I met virtually during the COVID lockdowns, who have accompanied me online for that special period.

Finally, I would like to express my appreciation and love to the two cutest and most beautiful cats that I have ever met, Ranger and Poco. I was fortunate to have the chance to cat-sit them during the COVID lockdowns as they provided me important emotional support. They are highly self-sustainable and have become wonderful companions to me.

Dedication

This thesis is dedicated to my loving parents.

Table of Contents

List of Figures

List of Tables

Appendix

List of Abbreviations

Abbreviations	Definition
2D TLC	Two-dimensional thin layer chromatography
Ace	Acenaphthene
Acy	Acenaphthylene
AHH	Aryl hydrocarbon hydroxylase
AhR	Aryl hydrocarbon receptor
Ant	Anthracene
AOSR	Alberta Oil Sands Region
APAH	Alkylated polycyclic aromatic hydrocarbon
APCI	Atmospheric-pressure chemical ionization
ARRF	Average relative response factor
ASE	Accelerated solvent extraction
ATSDR	Agency for Toxic Substances and Disease Registry
B	Magnetic sector detector
BaA	Benzo[*a*]anthracene
BaP	Benzo[*a*]pyrene
BbF	Benzo[*b*]fluoranthene
BghiP	Benzo[*g,h,i*]perylene
BkF	Benzo[*k*]fluoranthene
BMF	Biomagnification factor
BP	Boiling point
BrPAHs	Brominated PAHs
CALA	Canadian Association for Laboratory Accreditation
CAS	Chemical Abstracts Service registry
cDNA	Complementary DNA
Chr	Chrysene
CI	Chemical ionization
ClPAHs	Chlorinated PAHs
COGRaD	Centre for Oil and Gas Research and Development
COVID	Corona virus disease
CYP	Cytochrome P
DahA	Dibenz[*a,h*]anthracene
DART	Direct analysis in real time
DCM	Dichloromethane
DE	Diatomaceous earth
DESI	Desorption electrospray ionization
DLC	Dioxin-like compound
DMBAA	Dimethylbenz[*a*]anthracene

DMSO	Dimethylsulfoxide
DNA	Deoxyribonucleic acid
dpf	Days postfertilization
dSPE	Dispersive solid phase extraction
dw	Dry weight
e/m	Charge to mass ratio
ECD	Electron capture detector
ECOSAR™	Ecological Structure Activity Relationships™
EI	Electron ionization
EPI Suite™	Estimation Programs Interface Suite™
EROD	Ethoxyresorufin O-deethylase
ESI	Electrospray ionization
eV	Eletron volt
FAB	Fast atom bombardment
FBF	Flat bottom flask
FFP™	Folded flight path
FID	Flame ionization detector
Fle	Fluorene
Flu	Fluoranthene
FPD	Flame photometric detector
FTICR	Fourier transform ion cyclotron resonance
GC	Gas chromatography
GC × GC-HR-MS	Two-dimensional gas chromatography–high resolution mass spectrometry
GC-HRMS	Gas chromatography–high resolution mass spectrometry
GC-MS	Gas chromatography–mass spectrometry
GC-MS/MS	Gas chromatography–tandem mass spectrometry
GC × GC-HRToFMS	Comprehensive two-dimensional GC high resolution time of flight mass spectrometry
GDP	Gross Domestic Product
GFP	Green fluorescent protein
GPC	Gel permeation chromatography
HCl	Hydrogen Chloride
HepG2	Human-derived hepatoma
HPAH	Halogenated polycyclic aromatic hydrocarbon
hpf	Hours postfertilization
HPLC	High performance liquid chromatography
ICP	Inductively coupled plasma
IDIS	Isotope dilution internal standar
IEC	International Electrotechnical Commission
Ind	Indeno[1,2,3-*c*,*d*]pyrene

IPIS	Instrument performance internal standard
ISO	International Organization for Standardization
IUPAC	International Union of Pure and Applied Chemistry
LC_{50}	Median lethal concentration
LD_{50}	Median lethal dose
LLE	Liquid-liquid extraction
LOD	Limit of detection
log BCF	Log bioconcentration factor
log K_{OC}	Log soil adsorption coefficient
log K_{OW}	Log octanol / water partition coefficient
LOQ	Limit of Quantification
lw	Lipid weight
M	Analyte molecule
m/z	Mass-to-charge ratio
$M^{+\bullet}$	Molecular ion
MALDI	Matrix-assisted laser desorption/ionization
MBAA	Methylbenz[*a*]anthracene
MBET	Microbead extraction tubes
MDL	Method detection limit
mmu	Milli mass unit
MP	Melting point
MRM	Multiple reaction monitoring
MS	Mass spectrometer
MW	Molecular weight
Nap	Naphthalene
ND	No detect
NIST	National Institute of Standards and Technology
NPD	Nitrogen phosphorus detector
NR	Neutral red
O	Orbitrap detector
OXPHOS	Oxidative phosphorylation
PAC	Polycyclic aromatic compound
PAH	Polycyclic aromatic hydrocarbon
PCB	Polychlorinated biphenyl
PCDDs	Dibenzo-p-dioxins
PCDFs	Dibenzofurans
p-chem	Physiochemical
PCNs	Polychlorinated naphthalenes
PFTBA	Perfluorotributylamine

pH	Acidity
Phe	Phenanthrene
PID	Photoionization detector
PLE	Pressurized liquid extraction
PM	Particulate mater
ppm	Parts per million
psi	Pound per square inch
PT	Performance testing
Pyr	Pyrene
Q	Quadrupole detector
qAOP	Quantitative adverse outcome pathway
qPCR	Quantitative polymerase chain reaction
QqQ	Triple quadrupole detector
QSAR	Quantitative structure–activity relationship
Q-TOF	Quadrupole time-of-flight detector
QuEChERS	Quick Easy Cheap Effective Rugged Safe
R	Resolution
R^2	Correlation coefficient
R_{2D}	Resolution of 2D separation
RIS	Recovery internal standard
RP	Resolving power
RRF	Relative response factor
RSD	Relative standard deviation
s_0	Standard deviations
s_0’	Adjusted standard deviation
SIM	Single ion monitoring
SLE	Solid-liquid extraction
SRM	Standard Reference Material
TCD	Thermal conductivity detector
TOF	Time-of-flight detector
t_r	Retention time
UHP	Ultra-high purity
US EPA	United states environmental protection agency
VP	Vapor pressure
W	Width of chromatographic peaks
WS	Water solubility
WWTP	Wastewater treatment plant
XICs	Extracted ion chromatograms
Δm	Mass accuracy

Σ	Total

Chapter 1: Introduction

1.1 Chemical Constituents of Fossil Fuels

Fossil fuels, such as petroleum and coal, are formed naturally from the remains of dead plants or animals in the Earth's crust. Organic molecules, such as polysaccharides, glycosides, lipids, and proteins, break apart into insoluble organic matter, kerogens, under near-to-ambient temperature and pressure with the aid of microbial activities in a process known as diagenesis.[1-4] Kerogens are then transformed to fossil fuel by the heat and pressure of Earth's crust over very long periods, ranging from thousands to millions of years in a process known as catagenesis.[1,4]

Petroleum-based fossils, including oil and gas, are an important part of Canada's economy. Annually, this sector generates more than 52 billion Canadian dollars or 4.2% of the total Canada's gross domestic Product (GDP) and employs *ca*. 70,000 Canadians. On a global scale, one quarter of total Canadian exports are attributable to the oil and gas sector. Today, Canada exports *ca*. 3 million barrels per day of oil and projected to double by 2030.[5,6]

Crude oil contains numerous compounds, including primarily hydrocarbons and polycyclic compounds with or without heterocyclic atoms (*e.g.*, nitrogen, sulphur, oxygen). Metals, mineral and inorganic sulphur can also be found in crude oil. There are four major chemical classes for crude oil constituents, namely saturates, aromatics, resins and asphaltenes.[7]

The term polycyclic aromatic compounds (PACs) encompasses compounds that have multiple fused aromatic rings and can be categorized into different sub-classes. These include polycyclic aromatic hydrocarbons (PAHs), alkylated polycyclic aromatic hydrocarbons (APAHs),

halogenated polycyclic aromatic hydrocarbons (HPAHs) and heterocyclic compounds which can contain either S-, N- and O- incorporated into the cyclic ring.

Among all the different classes of PACs, PAHs are one of the most studied group of chemicals in the aromatic group and 16 PAHs have been identified as priority pollutants by the United States Environmental Protection Agency (US EPA) and are listed under Schedule 1 of the Canadian Environmental Protection Act.[7-11] The structure of the 16 PAHs is shown in Figure 1.1.

While my research involves understanding the behaviour of PACs in the environment, my primary focus is on HPAHs.[7,12] Specifically, I studied HPAHs that contain chlorine and bromine atoms attached to the aromatic backbone.

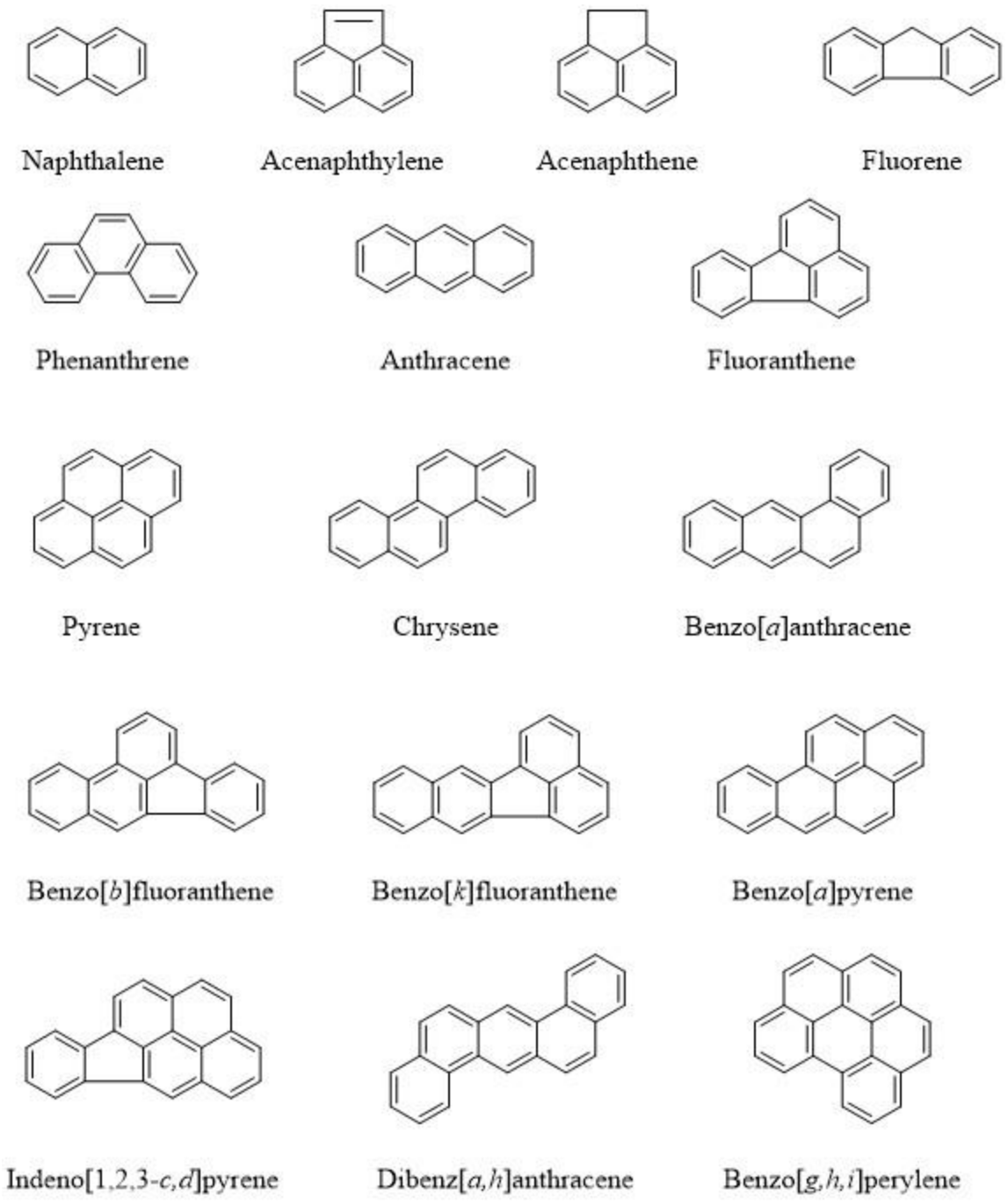

Figure 1.1: *Structure of 16 US EPA priority polycyclic aromatic hydrocarbons.*

1.2 Formation of HPAHs

In Canada, the Alberta Oil Sands Region (AOSR), represents the third largest oil reserve in the world, containing approximately 1.8 trillion barrels of bitumen (thick crude oil), which is rich in PACs.[13-16] Emissions of these compounds into the surrounding environment occur primarily from industrial activities related to oil and gas sector.[17-20] In addition, PACs are also known to be a by-product from fossil fuels combustion, such as wood, coal and petroleum.[21,22]

The AOSR is also rich in elemental chlorine and bromine as it was covered by sea water millions of years ago.[23] Forcing conditions like elevated pressures and temperatures which occur during oil sand formation, including diagenesis and catagenesis, is likely to facilitate the formation of HPAHs with elements Al and Fe as catalysts.[24]

To date, HPAHs have been assumed to be generated from the combustion of fossil fuels and industrial thermal activities.[22,25-27] However, I hypothesised that HPAHs can be formed *in situ* in oil deposits as both PACs and halogen atoms are available for their formation.

Due to the natural abundance and environmental availability of halogen elements, chlorinated PAHs (ClPAHs) and brominated PAHs (BrPAHs) have only recently been the focus of environmental chemistry research and monitoring activities.[28,29] With the exception of polychlorinated naphthalenes (PCNs), other HPAHs have received little attention.[7,30,31] The lack of research is due in part to the lack of analytical methods and availability of analytical standards. The challenge with the synthesis, however, is ensuring the addition of a halogen to a specific carbon atom.[31] Because of the large number of potential sites of halogen additions, numerous theoretically possible constitutional isomers can arise.[32]

Research on HPAHs can be traced back to the 1980s; they were obtained by chlorination of matrices containing PAHs, such as coal tar, contaminated water, fly ash, carbon black and PAH standards by various chlorine-containing reagents like bleach, chlorine gas, and HCl gas.[33-38] Halogenated PAHs can be emitted during combustion processes and as by-products of industrial activities;[26,39-41] they can also be formed in photochemical reactions by exposing PAHs with halogens under strong sun light.[42]

Due to the structural similarity of HPAHs and PAHs, HPAHs are likely to be persistent in the environment.[7] Interestingly, the toxicity of some of the HPAHs has been shown similar to that of dibenzo-p-dioxins and dibenzofurans (PCDD/Fs).[43,44] Some studies have also shown that HPAHs are a class of potential carcinogens and genotoxicants.[12,45] Thus, although HPAHs are not purposely synthesized and emitted into the environment, they should not be ignored when it comes to the evaluation of carcinogenic risk and monitoring environmental related pollutants. Current research pertaining to HPAHs is concentrated on their environmental occurrence. Recently, HPAHs have been detected in various environmental matrices, abiotic samples and biota, such as soil,[46] fly ash,[47] electronic waste,[48] air,[49] sediment,[50] seafood from southern China,[51] and semi-aquatic organisms from the Alberta oil sands region.[52] The physicochemical properties of HPAHs, including vapour pressure, solubility, and partition coefficient, and photostability are well compiled by Sun *et al.*,[29] and hence will not be discussed in this thesis. The toxicology and formation mechanisms of HPAHs have received less attention; in fact there are only a few papers discussing the formation of HPAHs, and most of these papers do not propose a plausible mechanism of toxicity.[24,26,40,42,47,53-57] Most studies use toxic equivalents (TEQs) to estimate the risk arising from human exposure and only a few studies have conducted toxicological research.[26,44,58-65]

1.3 Physicochemical Properties of HPAHs

Considering the negative biological effects and because so little is known about their environmental fate and distribution, recent research has focused on understanding the fate and behaviour of these compounds in the environment. The physicochemical (p-chem) properties calculated by Estimation Programs Interface Suite™ (EPI Suite™) of a few HPAHs, including vapor pressure, solubility, and partition coefficient, and photostability were compiled by Gonzales;[66] however, a full suite of the congeners of HPAHs is not listed.

EPI Suite™, a software developed by the US EPA, can predict p-chem properties, and estimate the environmental fate of chemical compounds.[67] EPI Suite™ uses mathematical algorithms and fragment-based method that can calculate p-chem properties by summing the contribution of all fragments.[68] Ecological Structure Activity Relationships™ (ECOSAR™) can be used to predict aquatic toxicity, which includes various endpoints, such as acute (short-term) toxicity and chronic (long-term or delayed) toxicity for different organisms, including fish, aquatic invertebrates, and green algae, salt water and terrestrial species.[69] In addition, EPI Suite™ also includes the following estimation programs, *viz.*, KOWWIN™, AOPWIN™, HENRYWIN™, MPBPWIN™, BIOWIN™, BioHCwin™, KOCWIN™, WSKOWWIN™, WATERNT™, BCFBAF™, HYDROWIN™, KOAWIN™, and AEROWIN™.[67]

HPAHs examined in this thesis were limited to congeners containing four halogen atoms due to their environmental relevance and availability. For simplicity, one constitutional isomer of HPAH has been used to represent each class of congeners: 1-Cl/Br-PAH, 1,2-Cl_2/Br_2-PAH, 1,2,3-Cl_3/Br_3-PAH, and 1,2,3,4-Cl_4/Br_4-PAH are used to represent mono-halogenated to tetra-halogenated PAHs, respectively.

The predicted p-chem and environmental fate properties of HPAHs are shown in Table 1.1. Melting point, boiling point, and vapor pressure are related to the molecular weight; all HPAHs are in the solid phase at room temperature and the majority of HPAHs, especially 2 – 4 rings HPAHs, are semi-volatile. Most HPAHs are not very soluble in water (log octanol / water partition coefficient (log K_{OW}) > 4] and are readily bioavailable (log K_{OW} > 4); all HPAHs are strongly bound to soil or sediment (log soil adsorption coefficient (log K_{OC}) > 3.5), which suggests there is negligible transportation to underground water. The majority of HPAHs have moderate to high potential for bioconcentration (bioconcentration factor log BCF > 3). Although evidence suggest HPAH are not highly toxic, they may still be a risk for biological organisms because of the high potential for bioconcentration.

With an increase in halogen content and molecular weight, congeners of HPAHs are less soluble in water, less volatile, more toxic, and partitioning more into soil or sediment. Congeners of mono- and di-halogenated PAHs with more halogen atoms generally have higher potential for bioconcentration, while some tri- and tetra-halogenated PAHs have lower potential for bioconcentration, which may be due to their higher molecular weight and lower bioavailability. It seems that plants (*e.g.*, green algae) are estimated to be less sensitive to the toxic effect of HPAHs compared to animals (*e.g.*, fish and daphnid). Not surprisingly, the chemical properties also depend on the PAH skeleton.

Comparing Cl-PAHs and Br-PAHs, Br-PAHs are less soluble and less volatile than Cl-PAHs, but this difference decreases with increasing number of halogens. Br-PAHs are predicted to be more toxic than Cl-PAHs, and this difference increases with increasing number of halogens. Due to their high predicted toxicity, the predicted LC_{50} data for Br-PAHs are similar between fish and daphnid.

Although EPI Suite™ is useful for estimating p-chem and environmental fate properties of the HPAHs, constitutional isomers cannot be differentiated based on the mathematical algorithms and fragment-based method. Constitutional isomers have two different types, resulted by substitution position of halogen atoms or structural isomers due to PAH skeletons, for instance Cl-Phe and Cl-Ant.

Table 1.1: *Predicted p-chem and environmental fate properties of the HPAHs by EPI Suite™ and ECOSAR™.*[67,69]

Name	MW (g mol^{-1})	Molecular Formula	MP[a] (°C)	BP[b] (°C)	VP[c] (Pa)	Log K_{OW}[d]	WS (mg L^{-1})[e]	Henry's Law Constant (atm m^3 mol^{-1})[f]	Log K_{OC}[g]	Log BCF[h]	Toxicity (mg L^{-1})[i]		
											Fish, 96h, LC_{50}	Daphnid, 48h, LC_{50}	Green Algae, 96h, EC_{50}
Nap	128.18	$C_{10}H_8$	5.01	231.64	5.38E+00	3.17	1.42E+02	3.70E-04	3.19	1.84	9.39	5.94	6.91
Cl-Nap	162.62	$C_{10}H_7Cl$	34.55	260.14	2.24E+00	3.81	2.18E+01	3.15E-04	3.40	2.36	3.14	2.11	3.14
Cl_2-Nap	197.07	$C_{10}H_6Cl_2$	63.40	286.30	1.88E-01	4.46	7.06E+00	2.68E-04	3.61	3.19	1.00	0.72	1.36
Cl_3-Nap	231.51	$C_{10}H_5Cl_3$	83.52	310.11	4.19E-02	5.10	1.31E+00	2.28E-04	4.17	3.62	0.31	0.24	0.57
Cl_4-Nap	265.95	$C_{10}H_4Cl_4$	106.02	331.57	7.21E-04	5.75	2.28E-01	1.94E-04	4.40	4.05	0.094	0.076	0.24
Br-Nap	207.07	$C_{10}H_7Br$	54.47	277.21	7.09E-01	4.06	1.38E+01	1.54E-04	3.40	2.35	2.41	1.65	2.70
Br_2-Nap	285.97	$C_{10}H_6Br_2$	91.76	316.45	2.48E-02	4.95	8.79E-01	6.43E-05	3.62	3.52	0.53	0.39	0.90
Br_3-Nap	364.86	$C_{10}H_5Br_3$	119.15	349.50	2.11E-03	5.84	5.19E-02	2.68E-05	3.83	4.11	0.11	0.087	0.28
Br_4-Nap	443.76	$C_{10}H_4Br_4$	142.21	380.20	2.18E-04	6.73	2.92E-03	1.12E-05	4.05	4.69	0.021	0.018	0.082
Acy	152.20	$C_{12}H_8$	60.35	294.42	1.64E-01	3.76	3.56E+00	4.25E-05	3.70	2.15	3.30	2.21	3.21
Cl-Acy	186.64	$C_{12}H_7Cl$	88.30	317.45	2.52E-02	4.40	6.91E-01	3.62E-05	3.92	2.57	1.07	0.76	1.41
Cl_2-Acy	221.09	$C_{12}H_6Cl_2$	104.96	338.14	1.06E-02	5.05	1.29E-01	3.08E-05	4.14	3.58	0.33	0.25	0.60
Cl_3-Acy	255.53	$C_{12}H_5Cl_3$	118.56	357.04	1.42E-03	5.69	2.36E-02	2.62E-05	4.70	4.01	0.10	0.081	0.25
Cl_4-Acy	289.98	$C_{12}H_4Cl_4$	137.35	375.74	3.16E-04	6.34	4.24E-03	2.23E-05	4.91	4.43	0.030	0.026	0.10
Br-Acy	231.09	$C_{12}H_7Br$	98.00	331.01	9.65E-03	4.65	2.51E-01	1.77E-05	3.92	2.73	0.80	0.58	1.18

Br_2-Acy	309.99	$C_{12}H_6Br_2$	125.21	362.36	8.97E-04	5.54	1.56E-02	7.39E-06	4.14	3.91	0.17	0.13	0.38
Br_3-Acy	388.89	$C_{12}H_5Br_3$	148.56	393.06	9.06E-05	6.43	9.04E-04	3.08E-06	4.35	4.49	0.034	0.029	0.12
Br_4-Acy	467.78	$C_{12}H_4Br_4$	176.99	423.76	7.69E-06	7.32	5.03E-05	1.28E-06	4.57	4.55	0.006	0.006	0.034
Ace	154.21	$C_{12}H_{10}$	53.64	272.72	1.72E-01	4.15	2.53E+00	5.87E-05	3.70	2.25	1.48	1.03	1.74
Cl-Ace	188.66	$C_{12}H_9Cl$	74.79	301.18	8.25E-02	4.33	7.74E-01	9.95E-05[j]	3.92	2.53	1.25	0.88	1.59
Cl_2-Ace	223.10	$C_{12}H_8Cl_2$	91.42	308.61	3.76E-03	5.25	8.40E-02	3.50E-05[j]	4.08	3.13	1.02	0.73	1.41
Cl_3-Ace	257.55	$C_{12}H_7Cl_3$	108.28	343.57	3.81E-03	5.62	2.64E-02	5.46E-05[j]	4.34	2.53	0.12	0.094	0.28
Cl_4-Ace	291.99	$C_{12}H_6Cl_4$	124.09	365.02	1.62E-03	5.80	1.18E-02	1.92E-05[j]	4.57	3.49	0.092	0.075	0.24
Br-Ace	233.11	$C_{12}H_9Br$	86.64	316.83	2.71E-02	4.42	3.81E-01	3.25E-05[j]	3.92	2.58	1.28	0.91	1.71
Br_2-Ace	312.01	$C_{12}H_8Br_2$	118.54	350.62	2.01E-03	5.43	1.86E-02	3.75E-06[j]	4.08	3.25	0.98	0.71	1.49
Br_3-Ace	390.90	$C_{12}H_7Br_3$	139.20	380.53	2.31E-04	6.20	1.37E-03	5.17E-06[j]	4.34	3.76	0.054	0.045	0.17
Br_4-Ace	469.80	$C_{12}H_6Br_4$	166.32	414.78	1.69E-05	6.47	2.58E-04	5.95E-07[j]	4.57	3.94	0.037	0.032	0.13
Fle	166.22	$C_{13}H_{10}$	63.69	292.57	8.21E-02	4.02	1.34E+00	3.46E-05	3.96	2.42	2.11	1.45	2.33
Cl-Fle	200.67	$C_{13}H_9Cl$	84.70	315.79	3.00E-02	4.66	3.53E-01	2.94E-05	4.18	2.74	0.67	0.49	1.01
Cl_2-Fle	235.11	$C_{13}H_8Cl_2$	100.94	325.77	1.19E-02	4.83	1.65E-01	2.08E-05[j]	4.36	2.86	0.55	0.41	0.89
Cl_3-Fle	269.56	$C_{13}H_7Cl_3$	119.87	355.62	1.48E-03	5.95	1.19E-02	2.13E-05	4.95	3.59	0.063	0.051	0.17
Cl_4-Fle	304.00	$C_{13}H_6Cl_4$	138.67	374.32	3.30E-04	6.59	2.11E-03	1.81E-05	5.17	4.02	0.019	0.016	0.070
Br-Fle	245.12	$C_{13}H_9Br$	99.98	329.46	1.00E-02	4.91	1.26E-01	1.44E-05	4.18	2.90	0.50	0.37	0.83
Br_2-Fle	324.02	$C_{13}H_8Br_2$	127.09	365.29	7.28E-04	5.01	3.60E-02	2.22E-06 [j]	4.36	2.97	0.52	0.39	0.93
Br_3-Fle	402.91	$C_{13}H_7Br_3$	149.45	391.64	9.59E-05	6.69	4.45E-04	2.50E-06	4.60	4.08	0.021	0.018	0.080

Br_4-Fle	481.81	$C_{13}H_6Br_4$	176.32	422.34	8.48E-06	7.58	2.47E-05	1.04E-06	4.83	3.84	0.004	0.004	0.023
Phe	178.24	$C_{14}H_{10}$	78.09	327.31	5.75E-03	4.34	6.77E-01	2.56E-05	4.22	3.27	1.15	0.81	1.48
Cl-Phe	212.68	$C_{14}H_9Cl$	105.05	346.99	3.42E-03	4.99	1.60E-01	2.18E-05	4.44	3.62	0.36	0.27	0.63
Cl_2-Phe	247.13	$C_{14}H_8Cl_2$	121.11	365.68	8.27E-04	5.63	2.94E-02	1.86E-05	4.65	4.63	0.11	0.088	0.26
Cl_3-Phe	281.57	$C_{14}H_7Cl_3$	134.59	384.38	2.10E-04	6.28	5.30E-03	1.58E-05	5.21	5.06	0.033	0.028	0.11
Cl_4-Phe	316.02	$C_{14}H_6Cl_4$	153.39	403.07	4.57E-05	6.92	9.40E-04	1.34E-05	5.43	5.48	0.010	0.009	0.043
Br-Phe	257.13	$C_{14}H_9Br$	114.32	359.00	1.41E-03	5.24	5.67E-02	1.07E-05	4.44	3.78	0.26	0.20	0.52
Br_2-Phe	336.03	$C_{14}H_8Br_2$	141.25	389.70	1.32E-04	6.13	3.43E-03	4.45E-06	4.65	4.96	0.054	0.045	0.16
Br_3-Phe	414.92	$C_{14}H_7Br_3$	164.58	420.40	1.29E-05	7.02	1.96E-04	1.86E-06	4.86	5.36	0.011	0.010	0.049
Br_4-Phe	493.82	$C_{14}H_6Br_4$	189.76	451.10	1.16E-06	7.91	1.08E-05	7.73E-07	5.09	4.93	0.002	0.002	0.014
Ant	178.24	$C_{14}H_{10}$	78.09	327.31	2.89E-04	4.35	6.91E-01	2.56E-05	4.21	2.60	1.15	0.81	1.48
Cl-Ant	212.68	$C_{14}H_9Cl$	105.05	346.99	5.72E-03	4.99	1.60E-01	2.18E-05	4.43	2.96	0.36	0.27	0.63
Cl_2-Ant	247.13	$C_{14}H_8Cl_2$	121.11	365.68	8.27E-04	5.63	2.94E-02	1.86E-05	4.65	3.97	0.11	0.088	0.26
Cl_3-Ant	281.57	$C_{14}H_7Cl_3$	134.59	384.38	2.10E-04	6.28	5.30E-03	1.58E-05	5.21	4.40	0.033	0.028	0.11
Cl_4-Ant	316.02	$C_{14}H_6Cl_4$	153.39	403.07	4.57E-05	6.92	9.40E-04	1.34E-05	5.43	4.82	0.010	0.009	0.043
Br-Ant	257.13	$C_{14}H_9Br$	114.32	359.00	1.41E-03	5.24	5.67E-02	1.07E-05	4.43	3.12	0.26	0.20	0.52
Br_2-Ant	336.03	$C_{14}H_8Br_2$	141.25	389.70	1.32E-04	6.13	3.43E-03	4.45E-06	4.65	4.29	0.054	0.045	0.16
Br_3-Ant	414.92	$C_{14}H_7Br_3$	164.58	420.40	1.29E-05	7.01	1.96E-04	1.86E-06	4.86	4.70	0.011	0.010	0.049
Br_4-Ant	493.82	$C_{14}H_6Br_4$	189.76	451.10	1.16E-06	7.90	1.08E-05	7.73E-07	5.07	4.27	0.002	0.002	0.014
Flu	202.26	$C_{16}H_{10}$	119.90	371.85	4.17E-04	4.93	1.30E-01	2.94E-06	4.74	3.07	0.39	0.29	0.66

Cl-Flu	236.70	$C_{16}H_9Cl$	134.58	390.55	1.49E-04	5.58	3.75E-02	2.50E-06	4.96	3.35	0.12	0.094	0.28
Cl_2-Flu	271.15	$C_{16}H_8Cl_2$	153.38	409.24	3.23E-05	6.22	6.79E-03	2.13E-06	5.17	4.36	0.036	0.030	0.11
Cl_3-Flu	305.59	$C_{16}H_7Cl_3$	164.98	427.94	8.33E-06	6.87	1.21E-03	1.81E-06	5.74	4.78	0.011	0.010	0.045
Cl_4-Flu	340.04	$C_{16}H_6Cl_4$	182.21	446.63	1.83E-06	7.51	2.13E-04	1.54E-06	5.95	4.46	0.003	0.003	0.018
Br-Flu	281.15	$C_{16}H_9Br$	147.31	402.56	5.50E-05	5.82	1.30E-02	1.23E-06	4.96	3.51	0.085	0.069	0.22
Br_2-Flu	360.05	$C_{16}H_8Br_2$	170.93	433.26	5.27E-06	6.71	7.73E-04	5.11E-07	5.17	4.68	0.017	0.015	0.068
Br_3-Flu	438.95	$C_{16}H_7Br_3$	195.76	463.96	4.71E-07	7.60	4.36E-05	2.13E-07	5.39	4.41	0.003	0.003	0.020
Br_4-Flu	517.84	$C_{16}H_6Br_4$	210.10	494.66	1.56E-07	8.49	2.38E-06	8.88E-08	5.61	3.98	0.0006	0.0006	0.006
Pyr	202.26	$C_{16}H_{10}$	119.90	371.85	4.59E-05	4.93	2.25E-01	2.94E-06	4.74	2.89	0.39	0.29	0.66
Cl-Pyr	236.70	$C_{16}H_9Cl$	134.58	390.55	1.49E-04	5.58	3.75E-02	2.50E-06	4.95	3.35	0.12	0.094	0.28
Cl_2-Pyr	271.15	$C_{16}H_8Cl_2$	153.38	409.24	3.23E-05	6.22	6.79E-03	2.13E-06	5.16	4.36	0.036	0.030	0.11
Cl_3-Pyr	305.59	$C_{16}H_7Cl_3$	164.98	427.94	8.33E-06	6.87	1.21E-03	1.81E-06	5.73	4.78	0.011	0.010	0.045
Cl_4-Pyr	340.04	$C_{16}H_6Cl_4$	182.21	446.63	1.83E-06	7.51	2.13E-04	1.54E-06	5.94	4.46	0.003	0.003	0.018
Br-Pyr	281.15	$C_{16}H_9Br$	147.31	402.56	5.50E-05	5.82	1.34E-02	1.23E-06	4.95	3.50	0.085	0.069	0.22
Br_2-Pyr	360.05	$C_{16}H_8Br_2$	170.93	433.26	5.27E-06	6.71	7.73E-04	5.11E-07	5.16	4.68	0.017	0.015	0.068
Br_3-Pyr	438.95	$C_{16}H_7Br_3$	195.76	463.96	4.71E-07	7.60	4.36E-05	2.13E-07	5.39	4.41	0.003	0.003	0.020
Br_4-Pyr	517.84	$C_{16}H_6Br_4$	210.10	494.66	1.56E-07	8.49	2.38E-06	8.88E-08	5.60	3.98	0.0006	0.0006	0.006
BaA	228.30	$C_{18}H_{12}$	135.96	399.19	3.62E-05	5.52	2.91E-01	1.77E-06	5.25	3.47	0.13	0.10	0.29
Cl-BaA	262.74	$C_{18}H_{11}Cl$	150.62	417.89	2.14E-05	6.17	8.46E-03	1.51E-06	5.46	3.73	0.039	0.033	0.12
Cl_2-BaA	297.19	$C_{18}H_{10}Cl_2$	163.79	436.58	5.26E-06	6.81	1.51E-03	1.28E-06	5.69	4.75	0.012	0.010	0.048

Cl_3-BaA	331.63	$C_{18}H_9Cl_3$	181.01	455.28	1.15E-06	7.45	2.67E-04	1.09E-06	6.23	4.49	0.003	0.003	0.019
Cl_4-BaA	366.08	$C_{18}H_8Cl_4$	198.23	473.97	2.47E-07	8.10	5.96E-05	9.30E-07	6.46	4.17	0.001	0.0001	0.008
Br-BaA	307.19	$C_{18}H_{11}Br$	163.35	429.89	7.78E-06	6.41	2.90E-03	7.39E-07	5.46	3.90	0.028	0.024	0.094
Br_2-BaA	386.09	$C_{18}H_{10}Br_2$	186.95	460.59	7.23E-07	7.30	1.69E-04	3.08E-07	5.69	4.56	0.006	0.005	0.029
Br_3-BaA	464.98	$C_{18}H_9Br_3$	208.53	491.29	6.86E-08	8.19	9.40E-06	1.28E-07	5.89	4.13	0.001	0.001	0.008
Br_4-BaA	543.88	$C_{18}H_8Br_4$	222.87	521.99	7.72E-09	9.08	5.09E-07	5.35E-08	6.11	3.69	0.0002	0.0002	0.002
Chr	228.30	$C_{18}H_{12}$	135.96	399.19	2.08E-07	5.52	2.64E-02	1.77E-06	5.26	3.50	0.13	0.10	0.29
Cl-Chr	262.74	$C_{18}H_{11}Cl$	150.62	417.89	2.14E-05	6.17	8.46E-03	1.51E-06	5.47	3.73	0.039	0.033	0.12
Cl_2-Chr	297.19	$C_{18}H_{10}Cl_2$	163.79	436.58	5.26E-06	6.81	1.51E-03	1.28E-06	5.68	4.75	0.012	0.010	0.048
Cl_3-Chr	331.63	$C_{18}H_9Cl_3$	181.01	455.28	1.15E-06	7.45	2.67E-04	1.09E-06	6.24	4.49	0.003	0.003	0.019
Cl_4-Chr	366.08	$C_{18}H_8Cl_4$	198.23	473.97	2.47E-07	8.10	4.66E-05	9.30E-07	6.47	4.17	0.001	0.0001	0.008
Br-Chr	307.19	$C_{18}H_{11}Br$	163.35	429.89	7.78E-06	6.41	2.90E-03	7.39E-07	5.47	3.90	0.028	0.024	0.094
Br_2-Chr	386.09	$C_{18}H_{10}Br_2$	186.95	460.59	7.23E-07	7.30	1.69E-04	3.08E-07	5.68	4.56	0.006	0.005	0.029
Br_3-Chr	464.98	$C_{18}H_9Br_3$	208.53	491.29	6.86E-08	8.19	9.40E-06	1.28E-07	5.90	4.13	0.001	0.001	0.008
Br_4-Chr	543.88	$C_{18}H_8Br_4$	222.87	521.99	7.72E-09	9.08	5.09E-07	5.35E-08	6.12	3.69	0.0002	0.0002	0.002
BbF	252.32	$C_{20}H_{12}$	169.41	442.75	3.32E-06	6.11	2.07E-02	2.03E-07	5.78	3.48	0.042	0.035	0.13
Cl-BbF	286.76	$C_{20}H_{11}Cl$	181.72	461.45	7.92E-07	6.75	1.94E-03	1.73E-07	5.99	4.12	0.013	0.011	0.051
Cl_2-BbF	321.21	$C_{20}H_{10}Cl_2$	198.94	480.14	1.70E-07	7.40	3.44E-04	1.47E-07	6.21	4.51	0.004	0.004	0.020
Cl_3-BbF	355.65	$C_{20}H_9Cl_3$	212.05	498.84	4.02E-08	8.04	6.02E-05	1.25E-07	6.77	4.20	0.001	0.001	0.008
Cl_4-BbF	390.10	$C_{20}H_8Cl_4$	220.79	517.53	1.06E-08	8.69	1.04E-05	1.07E-07	6.99	3.88	0.0003	0.0003	0.003

Br-BbF	331.21	$C_{20}H_{11}Br$	193.31	473.45	2.91E-07	7.00	6.57E-04	8.48E-08	5.99	4.28	0.009	0.008	0.040
Br_2-BbF	410.11	$C_{20}H_{10}Br_2$	214.54	504.15	2.76E-08	7.89	3.77E-05	3.54E-08	6.21	4.27	0.0017	0.0017	0.012
Br_3-BbF	489.01	$C_{20}H_9Br_3$	228.88	534.85	3.07E-09	8.78	2.08E-06	1.47E-08	6.43	3.84	0.0003	0.0003	0.003
Br_4-BbF	567.90	$C_{20}H_8Br_4$	243.22	565.55	3.34E-10	9.67	1.12E-07	6.14E-09	6.64	3.40	0.00006	0.00007	0.00097
BkF	252.32	$C_{20}H_{12}$	169.41	442.75	1.05E-07	6.11	1.08E-02	2.03E-07	5.77	3.70	0.042	0.035	0.13
Cl-BkF	286.76	$C_{20}H_{11}Cl$	181.72	461.45	7.92E-07	6.75	1.94E-03	1.73E-07	5.98	4.12	0.013	0.011	0.051
Cl_2-BkF	321.21	$C_{20}H_{10}Cl_2$	198.94	480.14	1.70E-07	7.40	3.44E-04	1.47E-07	6.20	4.51	0.004	0.004	0.020
Cl_3-BkF	355.65	$C_{20}H_9Cl_3$	212.05	498.84	4.02E-08	8.04	6.02E-05	1.25E-07	6.76	4.20	0.001	0.001	0.008
Cl_4-BkF	390.10	$C_{20}H_8Cl_4$	220.79	517.53	1.06E-08	8.69	1.04E-05	1.07E-07	6.98	3.88	0.0003	0.0003	0.003
Br-BkF	331.21	$C_{20}H_{11}Br$	193.31	473.45	2.91E-07	7.00	6.57E-04	8.48E-08	5.98	4.28	0.009	0.008	0.040
Br_2-BkF	410.11	$C_{20}H_{10}Br_2$	214.54	504.15	2.76E-08	7.89	3.77E-05	3.54E-08	6.20	4.27	0.0017	0.0017	0.012
Br_3-BkF	489.01	$C_{20}H_9Br_3$	228.88	534.85	3.07E-09	8.78	2.08E-06	1.47E-08	6.42	3.84	0.0003	0.0003	0.003
Br_4-BkF	567.90	$C_{20}H_8Br_4$	243.22	565.55	3.34E-10	9.67	1.12E-07	6.14E-09	6.63	3.40	0.00006	0.00007	0.00097
BaP	252.32	$C_{20}H_{12}$	169.41	442.75	1.31E-07	6.11	1.04E-02	2.03E-07	5.77	3.71	0.042	0.035	0.13
Cl-BaP	286.76	$C_{20}H_{11}Cl$	181.72	461.45	7.92E-07	6.75	1.94E-03	1.73E-07	5.98	4.12	0.013	0.011	0.051
Cl_2-BaP	321.21	$C_{20}H_{10}Cl_2$	198.94	480.14	1.70E-07	7.40	3.44E-04	1.47E-07	6.20	4.51	0.004	0.004	0.020
Cl_3-BaP	355.65	$C_{20}H_9Cl_3$	212.05	498.84	4.02E-08	8.04	6.02E-05	1.25E-07	6.76	4.20	0.001	0.001	0.008
Cl_4-BaP	390.10	$C_{20}H_8Cl_4$	220.79	517.53	1.06E-08	8.69	1.04E-05	1.07E-07	6.98	3.88	0.0003	0.0003	0.003
Br-BaP	331.21	$C_{20}H_{11}Br$	193.31	473.45	2.91E-07	7.00	6.57E-04	8.48E-08	5.98	4.28	0.009	0.008	0.040
Br_2-BaP	410.11	$C_{20}H_{10}Br_2$	214.54	504.15	2.76E-08	7.89	3.77E-05	3.54E-08	6.20	4.27	0.0017	0.0017	0.012

Br_3-BaP	489.01	$C_{20}H_9Br_3$	228.88	534.85	3.07E-09	8.78	2.08E-06	1.47E-08	6.42	3.84	0.0003	0.0003	0.003
Br_4-BaP	567.90	$C_{20}H_8Br_4$	243.22	565.55	3.34E-10	9.67	1.12E-07	6.14E-09	6.63	3.40	0.00006	0.00007	0.00097
Ind	276.34	$C_{22}H_{12}$	199.66	486.31	1.67E-08	6.70	2.49E-03	2.34E-08	6.29	4.09	0.014	0.012	0.054
Cl-Ind	310.79	$C_{22}H_{11}Cl$	214.94	505.01	2.59E-08	7.34	4.43E-04	1.99E-08	6.50	3.96	0.004	0.004	0.022
Cl_2-Ind	345.23	$C_{22}H_{10}Cl_2$	223.67	523.70	6.83E-09	7.99	7.78E-05	1.69E-08	6.73	4.23	0.0012	0.0012	0.009
Cl_3-Ind	379.68	$C_{22}H_9Cl_3$	232.40	542.40	1.78E-09	8.63	1.35E-05	1.44E-08	7.29	3.91	0.0003	0.0004	0.003
Cl_4-Ind	414.12	$C_{22}H_8Cl_4$	241.13	561.09	4.62E-10	9.27	2.33E-06	1.23E-08	7.50	3.60	0.0001	0.0001	0.0013
Br-Ind	355.24	$C_{22}H_{11}Br$	220.55	517.01	1.1E-08	7.59	1.48E-04	9.74E-09	6.59	3.84	0.003	0.003	0.017
Br_2-Ind	434.13	$C_{22}H_{10}Br_2$	234.89	547.71	1.21E-09	8.48	8.39E-06	4.06E-09	6.73	3.99	0.0005	0.0006	0.005
Br_3-Ind	513.03	$C_{22}H_9Br_3$	249.23	578.41	1.31E-10	9.37	4.59E-07	1.69E-09	6.94	3.55	0.0001	0.0001	0.0014
Br_4-Ind	591.92	$C_{22}H_8Br_4$	263.57	609.11	1.38E-11	10.26	2.45E-08	7.05E-10	7.16	3.11	0.00002	0.00002	0.0004
DahA	278.36	$C_{22}H_{14}$	180.52	470.09	1.85E-09	6.70	3.30E-03	1.23E-07	6.28	4.64	0.014	0.012	0.054
Cl-DahA	312.80	$C_{22}H_{13}Cl$	197.74	488.78	1.06E-07	7.34	4.31E-04	1.04E-07	6.50	3.96	0.004	0.004	0.022
Cl_2-DahA	347.25	$C_{22}H_{12}Cl_2$	214.96	507.48	2.24E-08	7.99	7.56E-05	8.88E-08	6.71	4.23	0.0012	0.0012	0.009
Cl_3-DahA	381.69	$C_{22}H_{11}Cl_3$	224.82	526.17	5.72E-09	8.63	1.31E-05	7.56E-08	7.27	3.91	0.0003	0.0004	0.003
Cl_4-DahA	416.14	$C_{22}H_{10}Cl_4$	233.56	544.87	1.49E-09	9.27	2.26E-06	6.43E-08	7.49	3.60	0.0001	0.0001	0.0013
Br-DahA	357.25	$C_{22}H_{13}Br$	209.33	500.79	3.87E-08	7.59	1.44E-04	5.11E-08	6.50	3.84	0.003	0.003	0.017
Br_2-DahA	436.15	$C_{22}H_{12}Br_2$	227.31	531.49	3.91E-09	8.48	8.15E-06	2.13E-08	6.71	3.99	0.0005	0.0006	0.005
Br_3-DahA	515.04	$C_{22}H_{11}Br_3$	241.65	562.19	4.26E-10	9.37	4.46E-07	8.88E-09	6.92	3.55	0.0001	0.0001	0.0014
Br_4-DahA	593.94	$C_{22}H_{10}Br_4$	255.99	592.89	4.55E-11	10.26	2.38E-08	3.70E-09	7.15	3.11	0.00002	0.00002	0.0004

BghiP	276.34	$C_{22}H_{12}$	199.66	486.31	1.17E-07	6.70	2.49E-03	2.34E-08	6.29	4.09	0.014	0.012	0.054
Cl-BghiP	310.79	$C_{22}H_{11}Cl$	214.94	505.01	2.59E-08	7.34	4.43E-04	1.99E-08	6.50	3.96	0.004	0.004	0.022
Cl_2-BghiP	345.23	$C_{22}H_{10}Cl_2$	223.67	523.70	6.83E-09	7.99	7.78E-05	1.69E-08	6.72	4.23	0.0012	0.0012	0.009
Cl_3-BghiP	379.68	$C_{22}H_9Cl_3$	232.40	542.40	1.78E-09	8.63	1.35E-05	1.44E-08	7.28	3.91	0.0003	0.0004	0.003
Cl_4-BghiP	414.12	$C_{22}H_8Cl_4$	241.13	561.09	4.62E-10	9.27	2.33E-06	1.23E-08	7.50	3.60	0.0001	0.0001	0.0013
Br-BghiP	355.24	$C_{22}H_{11}Br$	220.55	517.01	1.1E-08	7.59	1.48E-04	9.74E-09	6.59	3.84	0.003	0.003	0.017
Br_2-BghiP	434.13	$C_{22}H_{10}Br_2$	234.89	547.71	1.21E-09	8.48	8.39E-06	4.06E-09	6.72	3.99	0.0005	0.0006	0.005
Br_3-BghiP	513.03	$C_{22}H_9Br_3$	249.23	578.41	1.31E-10	9.37	4.59E-07	1.69E-09	6.93	3.55	0.0001	0.0001	0.0014
Br_4-BghiP	591.92	$C_{22}H_8Br_4$	263.57	609.11	1.38E-11	10.26	2.45E-08	7.05E-10	7.16	3.11	0.00002	0.00002	0.0004

[a] *melting point, mean value or weighted method.*
[b] *boiling point, adapted Stein and Brown method.*
[c] *vapor pressure, by modified Grain method or mean of Antoine and Grain methods.*
[d] *octanol/water partition coefficient, estimated by KOWWIN™.*
[e] *water solubility, estimated by WSKOW™.*
[f] *estimated by group method of HENRYWIN™.*
[g] *soil adsorption coefficient, estimated by MCI method of KOCWIN™.*
[h] *bioconcentration factor, estimated by regression-based method by BCFBAF™.*
[I] *estimated by ECOSAR™ as neutral organic compound.*
[j] *estimated by bond method of HENRYWIN™.*

1.4 Literature Review of Environmental Occurrence of Halogenated Polycyclic Aromatic Hydrocarbons

1.4.1 Abiotic Matrices

The detection of HPAHs can be traced back to the late 1980s. They were detected in automobile exhaust, snow and urban air, in both gas and particulate phases.[70] Most studies on HPAHs are focused on abiotic matrices, especially air samples due to their volatility, however, considering the complexity of abiotic matrices, the occurrence of HPAHs can be categorized according to the different types of sample matrices.

1.4.1.1 Air

In 1993, Nilsson and Östman reported the detection of HPAHs in air samples from traffic heavy street areas and road tunnels in Sweden. The concentrations of HPAHs in air samples from tunnels were higher than street areas in both gas phase and particulate phase.[71] Ohura *et al.* reported mean concentrations of total ClPAHs (ΣClPAHs) of 31 pg/m^3 and the temporal trend of ClPAHs in particulate maters (PM) in Shizuoka (Japan); most HPAHs were more abundant in winter than in summer.[72] Kitazawa *et al.* and Ohura *et al.* followed this study and reported similar temporal trends. Mean total ClPAHs concentration in Shizuoka fluctuated in various studies from 1992 – 2020 as shown in Table 1.2.[63,73-76] In addition, a study by Ohura *et al.* in 2008 also suggested that the volatile HPAHs were more abundant than HPAHs adsorbed by PM.[74]

Air samples from other Japanese cities have also been investigated. In a study by Kakimoto *et al.*, total concentrations of 19 ClPAHs (Σ_{19}ClPAHs) were reported for four cities, namely Sapporo, Sagamihara, Kanazawa, and Kitakyushu, where they were lower than those in

Shizuoka.[25] In Nagoya, the mean concentration of Σ_{24}ClPAHs was determined to be 70 – 93 pg/m^3;[62] in Tokyo Bay, Σ_{15}ClPAHs of ambient air was determined to be 54 pg/m^3.[77] The Σ_{23}ClPAHs ranges from 17.2 to 204 pg/m^3 in summer to 47.3 to 209 pg/m^3 in winter of various sampling sites in Japan.[78]

Ma *et al.*[61] demonstrated that HPAHs are ubiquitous in inhalable fine particulate matter (PM) in Shanghai (China) and that concentrations of Σ_{12}ClPAHs are greater than in Japanese cities. Kakimoto *et al.*[25] showed that both summer and winter concentrations of HPAHs in Beijing were higher than in Japanese and Korean cities; in Shenzhen, the HPAH concentration was comparable and even higher than in Beijing.[79] Gao *et al.* determined that traffic-related emissions contribute more HPAH than exhaust pipe emission.[80] Jin *et al.* reported HPAHs in Beijing,[22,81] and on the Tibetan Plateau.[82] The air on the Tibetan Plateau was lower in HPAHs than urban areas.[49] However, Cao *et al.*[83] and Wang *et al.*[84] reported higher HPAHs concentration in different cities in China.

Kakimoto *et al.*[25], Voung *et al.*[85] and Sei *et al.*[76] have shown that HPAHs concentrations in Busan (South Korea), Ulsan (South Korea) and Dhaka (Bangladesh) are similar to those in other cities in China and Japan. Jin *et al.*[86]and Kawatsu *et al.*[87] also reported similar concentration of HPAHs in Czech Republic and India to Cao *et al.*[83] and Wang *et al.*[84].

In Guangzhou (China), HPAHs in air samples near E-waste recycling sites were higher in concentration than that in air samples in urban areas, suggesting the E-waste is a significant source of HPAHs.[88] HPAHs have been found in secondary copper smelters,[26,89,90] waste incinerators,[91] secondary zinc smelters,[92] and steelmaking factories[93] which suggests that combustion of fossil fuels, industrial thermal processes, and waste incinerators can be sources of HPAHs. Xu *et al.* also reported metallurgical plants, like iron ore sinter plants, secondary aluminum smelters, and

secondary lead smelters are possible sources for HPAHs.[94] Automobile exhaust was also found to be another important source of HPAHs into the atmosphere.[80,95]

In summary, combustion of fossil material and industrial combustion have played an important role in formation of HPAHs.

Table 1.2: *Mean total concentrations of ClPAHs (ΣClPAH), BrPAHs (ΣBrPAH) and HPAHs (ΣHPAH) in air samples.*

Sample[a]	Location	Extraction method	Analytical instrument[b]	Concentration (pg m^{-3})			N[c]	Reference
				ΣClPAH	ΣBrPAH	ΣHPAH		
Air, PM (A)	Shizuoka, Japan	Sonication	GC-MS (Q)	31	-	-	12+0	Ohura *et al.* 2005[72]
Air, PM (A)	Shizuoka, Japan	Sonication	GC-MS (Q)	32	-	-	12+0	Kitazawa, *et al.* 2006[73]
Air, GP (A)	Shizuoka, Japan	Soxhlet extraction	GC-HRMS (B)	90	-	-	26+0	Ohura *et al.* 2008[74]
Air, PM (A)		Sonication		17	-	-		
Air, PM (A)	Shizuoka, Japan	Sonication	GC-HRMS (B)	15.22	8.57	23.79	15+11	Ohura *et al.* 2009[63]
Air (A)	Shizuoka, Japan	Soxhlet extraction	GC-HRMS (B)	132.66 (winter)	-	-	20+0	Ohura *et al.* 2013[75]
				32.15 (summer)	-	-		
Air, PM (A)	Sapporo, Japan	Sonication	GC-HRMS (B)	1.28 (summer)	-	-	19+0	Kakimoto *et al.* 2014[25]
				8.51 (winter)	-	-		
	Sagamihara, Japan			1.54 (summer)	-	-		
				8.43 (winter)	-	-		
	Kanazawa, Japan			0.76 (summer)	-	-		
				3.29 (winter)	-	-		
	Kitakyushu, Japan			1.38 (summer)	-	-		
				14.3 (winter)	-	-		
	Busan, Korea			1.17 (summer)	-	-		
				14.2 (winter)	-	-		
	Beijing, China			12.76 (summer)	-	-		
				211.6 (winter)	-	-		
Air, PM (A)	Shenzhen, China	N/A	GC-MS (Q)	397 (PM_{10})	-	-	3+6	Sun *et al.* 2015[79]
				190 ($PM_{2.5}$)	-	-		
Urban air, PM	Guangzhou, China	Accelerated solvent extraction (ASE)	GC-MS (Q)	22	6	28	16+11	Chen *et al.* 2016[88]
E-waste site air, PM				33	17	50		

Air, PM (A)	Nagoya, Japan	N/A	GC-MS (Q)	70.29-92.63	-	-	24+0	Ohura *et al.* 2016[62]
Air, whole sampling time (A)	Beijing, China	ASE	GC-HRMS (B)	166.6 (GP)	5.8 (GP)	-	19+19	Jin *et al.* 2017[22]
				92.6 (PM)	18.3 (PM)	-		
Air, heating period (A)				89.0 (GP)	5.3 (GP)	-		
				312.4 (PM)	34.7 (PM)	-		
Air, non-heating period (A)				95.8 (GP)	6.3 (GP)	-		
				33.0 (PM)	3.2 (PM)	-		
Air, Secondary Copper Smelters (A)	China	ASE	GC-HRMS	5760 – 271000	590 – 52400	-	19+19	Jin *et al.* 2017[26]
Ambient air (A)	China	ASE	GC-HRMS (B)	7.0 – 219	3.0 – 126	-	19+19	Xu *et al.* 2018[94]
Stack gas (A)				68300 – 156000	2900 – 13500	-		
Air, GP (A)	Tokyo Bay and inland, Japan	Soxhlet extraction	GC-MS (Q)	40	-	-	18+0	Ohura *et al.* 2018[77]
Air, PM (A)				14	-	-		
Air, GP, Entrance of tunnel (A)	Tanglang hill tunnel, Shenzhen, China	Soxhlet extraction	GC-MS (Q)	1050	670	-	3+6	Gao *et al.* 2018[80]
Air, PM10, Entrance of tunnel (A)				27800	320	-		
Air, PM, Entrance of tunnel (A)				2850	1820	-		
Air, GP, Exit of tunnel (A)				620	480	-		

Air, PM10, Exit of tunnel (A)				9220	130	-		
Air, PM, Exit of tunnel (A)				4950	2090	-		
Air, GP (A)	Japan	Soxhlet extraction	GC-MS (Q)	61.72 (summer)	-	-	24+0	Ohura *et al.* 2019[78]
				33.31 (winter)	-	-		
Air, PM (A)				27.10 (summer)	-	-		
				64.50 (winter)	-	-		
Air (A)	Ulsan, Korea	Soxhlet extraction	GC-MS (Q)	207	84	-	24+11	Vuong *et al.* 2020[85]
Air (P)	Tibet, China	ASE	GC-HRMS (B)	0.78–4.16	0.15–0.59	-	19+19	Jin *et al.* 2020[49]
Air, PM (A)	Beijing, China	Sonication	GC-MS (Q)	520	-	-	5+0	Cao *et al.* 2020[83]
	Zhengzhou, China			670	-	-		
	Xinxiang, China			380	-	-		
Air, near metallurgical plants (A)	n/a	ASE	GC-HRMS (Q-TOF)	818.9	194.9	-	13+17	Yang *et al.* 2020[96]
Exhaust from Grilling (A)	Japan	Soxhlet extraction	GC-HRMS (B)	19000	-	-	20+0	Masuda *et al.* 2020[97]
Air, e-waste dismantling workshops (A)	South China	Soxhlet extraction	GC-MS/MS (QqQ)	32.3–364	8.29–1130	-	15+16	Liu *et al.* 2020[98]
Air, GP (P)	Ulsan, South Korea	Soxhlet extraction	GC-MS (Q)	8.64	11.6	-	24+11	Vuong *et al.* 2020[99]
Air, PM (P)				9.64	1.62	-		
Air, Deposition (P)	Košetice, Czech Republic	Soxhlet extraction,	GC-MS/MS (QqQ)	580	494	-	20+21	Jin *et al.* 2021[86]

	Praha-Libuš, Czech Republic	vortex shaking		547	449	-		
Air, $PM_{2.5}$ (A)	Shizuoka, Japan	Soxhlet extraction	GC-MS/MS (QqQ)	33.9	2.95	-	21+16	Sei *et al.* 2021[76]
	Dhaka, Bangladesh.			131	42.8	-		
Ambient air (A)	Japan	Soxhlet extraction	GC-MS/MS (QqQ)	74.0	509	-	22+21	Sei *et al.* 2021 [95]
Vehicle exhaust (A)	Mongolia			130000	22300	-		
Air, secondary copper smelters (A)	China	Soxhlet extraction	GC-HRMS (B)	-	16300–599000	-	0+18	Lin *et al.* 2022[89]
Air, secondary copper smelters (A)	China	Soxhlet extraction	GC-HRMS (B)	126300–4147200	-	-	16+0	Lin *et al.* 2022[90]
Stack gas, secondary zinc smelters (A)	Jiangxi, Hunan and Yunnan, China	n/a	GC-HRMS	9388150	164880	-	19+19	Yang *et al.* 2022[92]
Stack gas, electric arc furnace for steelmaking (A)	n/a	Soxhlet extraction	GC-HRMS	25850–4191000	1020–341000	-	18+18	Yang *et al.* 2022[93]
Air, $PM_{2.5}$ (A)	Mumbai, India, Urban	Sonication	GC-MS (O)	540	250	-	30+15	Kawatsu *et al.* 2022[87]
	Mumbai, India, Suburban			160	20	-		

Air, GP, heating period (P)	Dongjiakou, China	Soxhlet extraction	GC-MS (Q)	295 (industrial)	614 (industrial)	-	8+16	Wang *et al.* 2022[84]
				194 (rural)	500 (rural)	-		
Air, GP, Non-heating period (P)				288 (industrial)	484 (industrial)	-		
				126 (rural)	301 (rural)	-		

[a] *A: Active sampling; P: Passive sampling.*

[b] *GC-MS: gas chromatography–mass spectrometry; GC-HRMS: gas chromatography–high resolution mass spectrometry; GC-MS/MS: gas chromatography–tandem mass spectrometry; Q: quadrupole detector; B: magnetic sector detector; O: orbitrap detector; QqQ: triple quadrupole detector: Q-TOF: quadrupole time-of-flight detector.*

[c] *N: number of HPAH analytical standard, denoted as number of Cl-PAHs + number of Br-PAHs.*

Overall, there appears to be a consistent seasonal trend of HPAHs in the environment. Several sources of atmospheric HPAHs have been determined, such as combustion, industrial thermal processes, waste incinerators. It is hard to draw a conclusive message of geographical differences among different cities as there is no standard sample extraction and processing method for HPAHs.

1.4.1.2 Soil, Dust and Sediments

There are a few studies examining occurrence of HPAHs in soil as shown in Table 1.3. Ma *et al.* measured Σ_{20}HPAHs concentrations in various types of soils and found a significant difference in HPAHs concentrations between contaminated soil (surface soil in e-waste recycling facility and near a chemical industrial complex) and reference soil (urban area, rural area and agricultural area).[43] The concentration of ΣHPAHs in soil collected from Shenzhen was similar to that in reference soils (Ma *et al.*[43]) while sediment samples from Japan showed a higher concentration of ClPAHs[100,101] On the Tibetan Plateau, 0.61 – 72.3 pg/g of HPAHs have been detected in soil samples, which is lower than in any other soil sample by at least 50-fold.[49] Wang *et al.* also reported the concentration of Σ_{31}HPAHs in soil from various industrial parks are slightly higher than soil samples from their surroundings.[102]

Table 1.3: *Mean total concentrations of ClPAHs (ΣClPAHs), BrPAHs (ΣBrPAHs) and HPAHs (ΣHPAHs) in soil, dust and sediments.*

Sample type	Sample details	Location	Extraction method	Analytical instrument[a]	Concentration (ng/g)			N[b]	Reference
					ΣClPAH	ΣBrPAH	ΣHPAH		
Soil	E-waste recycling facility (*n = 10*)	China	Soxhlet extraction	GC-MS (Q)	26.8	-	-	20+0	Ma *et al.* 2009[43]
	Chemical industrial complex (*n = 12*)				59.1	-	-		
	Urban area (*n = 3*)				88.0	-	-		
	Rural area (*n = 2*)				ND[c]	-	-		
	Agricultural area (*n = 7*)				0.15	-	-		
	Topsoil (*n = 181*)	Shenzhen, China	Soxhlet extraction	GC-MS (Q)	0.50	7.67	8.17	3+6	Ni and Zeng, 2012[100]
	Soil after 1997 Plastimet Inc. Fire	Hamilton, Ontario, Canada	Accelerated solvent extraction (ASE)	GC × GC-HR-MS (TOF)	-	-	261 800	5+2	Fernando *et al.* 2014[103]
	Black ash	Soil after 2013 Rim Fire, California	Soxhlet extraction	GC-MS (Q)	3.63	9.26	-	3+6	Chen *et al.* 2018[58]
	White ash				1.03	2.66	-		
	Non-burned soil				3.58	16.33	-		
	Tibetan Plateau	Tibet, China	ASE	GC-HRMS	0.00311–0.297	0.00061–0.072.3	-	19+18	Jin *et al.* 2020[49]
	Surface soil	Multiple sites, Japan	Soxhlet extraction	GC-MS/MS (QqQ)	14.4	-	-	24+0	Imaeda *et al.* 2021[101]
	Petrochemical industrial park (PIP)	China (Gansu,	Ultrasonic extraction	GC-MS/MS (QqQ)	3.12	7.45	-	14+17	Wang *et al.* 2022[102]

	Brominated flame-retardant manufacturing park (BFRP)	Shandong, Guangdong)			1.48	21.6	-		
	Electronic waste dismantling park (EWDP)				0.26	6.68	-		
	Surrounding area of the PIP				3.23	6.27	-		
	Surrounding area of the BFRP				0.48	5.60	-		
	Surrounding area of the EWDP				2.57	2.99	-		
Dust	Workshop-floor dust (*n = 5*)	China	Soxhlet extraction	GC-MS (Q)	103	-	-	20+0	Ma *et al.* 2009[43]
	Fly ash, waste incinerators (*n = 11*)	South Korea	Soxhlet extraction	GC-MS (Q)	< 0.06–6992	< 0.14–1235	-	20+11	Horii *et al.* 2008 [60]
	Bottom ash, waste incinerators (*n = 11*)				< 0.06–68	< 0.14–0.65	-		
	Fly ash (*n = 4*)	n/a	Soxhlet extraction	GC-HRMS (B)	67.688	-	-	9+0	Fan *et al.* 2017[40]
	Fly ash, municipal solid waste incineration (*n = 5*)	China	Soxhlet extraction	GC-HRMS (B)	851	-	-	9+0	Fan *et al.* 2019[39]
	Fly ash, iron ore sintering (*n = 5*)				61.7	-	-		
	Fly ash, steel smelting (*n = 5*)				174	-	-		

	Office building (*n = 2*)	China	Soxhlet extraction	GC-MS/MS (QqQ)	7.91	27.0	-	16+18	Tang *et al.* 2020[65]
	E-waste dismantling workshop (*n = 5*)				137.0	60.0	-		
	Raw materials crushing workshop (*n = 3*)				35.1	399	-		
	Secondary copper blast furnace workshop (*n = 3*)				53.8	152	-		
	Residential area (*n = 2*)				137	25.7	-		
	Indoor dust, Japanese residence	Japan	Soxhlet extraction	GC-MS/MS (QqQ)	3.450	1.030	-	22+21	Sei *et al.* 2021 [95]
	Secondary copper smelters	China	Soxhlet extraction	GC-HRMS (B)	-	5.09–39.3	-	0+18	Lin *et al.* 2022[89]
	Secondary copper smelters	China	Soxhlet extraction	GC-HRMS (B)	24.7–1279.0	-	-	16+0	Lin *et al.* 2022[90]
	Electric arc furnace for steelmaking	n/a	Soxhlet extraction	GC-HRMS	73.64–162.0	3.2–14	-	18+18	Yang *et al.* 2022[93]
Sediment	Sediment, 7 sampling sites	Ariake Bay, Japan	Ultrasonification	GC-HRMS	0.700–6.100	-	-	21+0	Sankoda *et al.* 2013[24]
	Tokyo Bay (*n = 12*)	Japan	Soxhlet extraction	GC-MS (Q)	0.584	-	-	20+0	Horii, *et al.* 2009[104]
	Saginaw River (*n = 10*)	US			1.140	-	-		
	New Bedford Harbor (*n = 2*)				1.880	-	-		

Former chlor-alkali plant (*n = 3*)				8.820	-	-		
Surface sediments	Shenzhen, China	Soxhlet extraction	GC-MS (Q)	27.6	11.9	-	3+6	Sun *et al.* 2011[105]
Yellow sea,	Asia	Soxhlet extraction	GC-MS/MS (QqQ)	0.731	0.0327	0.518	20+11	Ohura *et al.* 2015[106]
Negombo				0.761	0.0466	0.618		
Kandy				1.147	0.058	0.410		
Maowei Sea	Guangxi, China	ASE	GC-MS (Q)	3.29	-	-	20+0	Wang *et al.* 2017[50]
Surface sediment, Pearl River Estuary (*n = 27*)	China	ASE	GC-MS (Q)	13.14	8.00	-	8+9	Yuan *et al.* 2020[107]
Sea sediment	Tokyo Bay, Japan	Soxhlet extraction	GC-MS/MS (QqQ)	1.060	6.570	-	22+21	Sei *et al.* 2021 [95]
Surface sediment, Chaobai River	Beijing, China	Soxhlet extraction	GC-HRMS (B)	0.336	0.038	-	18+19	Du *et al.* 2022[108]
	Tianjin, China			1.018	0.075	-		

[a] *GC-MS: gas chromatography–mass spectrometry; GC-HRMS: gas chromatography–high resolution mass spectrometry; GC-MS/MS: gas chromatography–tandem mass spectrometry; GC × GC-HR-MS: two-dimensional gas chromatography–high resolution mass spectrometry; Q: quadrupole detector; B: magnetic sector detector; QqQ: triple quadrupole detector; TOF: time-of-flight detector.*

[b] *N: number of HPAH analytical standard, denoted as number of Cl-PAHs + number of Br-PAHs.*

[c] *ND, no detect.*

Soil after a wildfire showed a much higher concentration of HPAHs. Fernando *et al.* reported ΣHPAHs in soil from the 1997 Plastimet Inc. Fire in Hamilton, Ontario to be 261.8 μg/g as determined by targeted and untargeted analysis.[103] A study by Chen *et al.* seems to disagree with Fernando *et al.*; ClPAHs in soil samples impacted by the 2013 Rim Fire (California) were not significantly higher than non-burnt soil. However, the latter only used targeted analysis and included a significant fewer number of analytical standards.[58]

Sankoda *et al.* detected HPAHs in the tidal flat sediments from Domen River and the Yabe River (Fukuoka Prefecture, Kyushu, Japan) with concentration from 700 to 6100 pg/g.[24] HPAHs concentrations were lower in sediments from Tokyo Bay comparing to sediment samples from the former chlor-alkali plant in 2006 due to the availability of halogens.[104] Sun *et al.* found HPAH concentrations in surface sediments in Shenzhen contained the highest concentration of HPAHs among all sediment samples.[105] Ohura *et al.* analyzed HPAHs from sediments of various sites, namely Yellow sea, Negombo, and Kandy, which were comparable to Tokyo Bay and Ariake Bay.[106] A few studies have shown the HPAHs concentrations in sediment from the Pearl River Estuary are higher than from the Maowei Sea and the Chaobai River,[50,107,108] which also agreed with findings from Sun *et al.*[105] In addition, concentrations of ClPAHs in sea sediment samples from Tokyo Bay were higher than they were in sediment core samples from the Tokyo Bay, while the concentrations of BrPAHs were similar to those in sediment from the Pearl River Estuary.[95,104,107]

Examining indoor dust samples collected from floors of various industries, Ma *et al.*, reported a mean concentration of ΣHPAHs of 103 ng/g.[43] Concentration of HPAHs in indoor dust decreased in the order of raw materials crushing workshop, secondary copper blast furnace workshop, e-waste dismantling workshop, residential area and office building in southern China.[65]

HPAHs concentrations in dust samples from industrial areas, such as secondary copper smelters, waste incinerators, and electric arc furnaces for steelmaking, have been determined to be much more abundant than them from natural environment.[39,40,60,89,90,93] The indoor dust from residences had significantly lower concentrations of HPAHs compared to industrial-related dust samples.[95]

1.4.1.3 Water

Limited studies have reported the occurrence of HPAHs in water (see Table 1.4) Concentrations of 33, 18, 17 ng/L and 44, 21, 23 ng/L for influent, secondary treatment effluent and tertiary treatment of wastewater treatment plant (WWTP), respectively, in Beijing were reported by Qiao *et al.* and suggests secondary treatment effectively removes HPAHs.[109] In a follow up study, Qiao *et al*. measured HPAHs in effluent water to be 60 ng/L.[110] In another study in Guangdong, China, HPAHs concentrations ranged from 2 – 3 ng/L in both influent and effluent.[111] Effluents of WWTP in Beijing also have similar concentration of HPAHs to those in Guangdong.[112] However, wastewater effluent from Henan contained the highest concentration (140.8 ng/L) of HPAHs.[113]

Shiraishl *et al.* reported the detection of ClPAHs in tap water samples with concentrations of 0.01 – 0.1 ng/L;[114] More recent studies have reported that concentrations of HPAHs are at least 1 – 2 orders of magnitude higher in tap water, *i.e.*, 1 – 10 ng/L.[113,115,116] The disagreement among these studies is likely due, in part, to the inclusion of BrPAHs and the commercial availability of more HPAH standards compared to the 1980s.

Yuan *et al.* determined that concentrations of HPAHs in water samples from the Pearl River Estuary (China) were greater compared to effluent receiving river in Guangdong, China.[107,111] In addition, HPAHs were found to partition to suspended particulate matter greater than the aqueous

phase due to hydrophobicity.[107] In Chaobai River from 2017 – 2018, 30 ng/L HPAHs were detected, which were comparable to levels of HPAH in water samples from the Pearl River Estuary and Shaoping Lake.[107,113,117]

Table 1.4: *Mean total concentrations of ClPAHs (ΣClPAH), BrPAHs (ΣBrPAH) and HPAHs (ΣHPAH) in water samples.*

Sample type	Location	Extraction method	Analytical instrumentation[a]	Concentration (ng/L)			N[b]	Reference
				ΣClPAHs	ΣBrPAHs	ΣHPAHs		
Tap water	Tsukuba, Japan	Liquid-liquid extraction	GC-MS (B)	0.01–0.1	-	-	10 + 0	Shiraishl *et al.* 1985[114]
Tap water	Henan, China	Solid phase extraction (SPE)	GC-MS (Q)	7.2	-	-	8 + 0	Wang *et al.* 2016[113]
Surface water, Shaoping Lake				77.2	-	-		
Wastewater, industrial plant				140.8	-	-		
Sewage treatment plant (STP) effluent				68.9	-	-		
Influent, wastewater treatment plant (WWTP)	Beijing, China	SPE	GC-MS (Q)	33 (Apr)	-	-	4 + 0	Qiao *et al.* 2017[109]
				44 (Nov)	-	-		
Secondary treatment effluent, WWTP				18 (Apr)	-	-		
				21 (Nov)	-	-		
Tertiary treatment effluent, WWTP				17 (Apr)	-	-		
				23 (Nov)	-	-		
WWTPs effluent and rivers, total phase (aqueous + particulate)	Beijing, China	SPE	GC-MS (Q)	60	-	-	9 + 0	Qiao *et al.* 2018[110]
Ecological WWTP (EWWTP) influent	Guangdong, China	SPE	GC-MS (Q)	2–3	-	-	9 + 0	Qiao *et al.* 2019[111]

EWWTP effluent				2–2	-	-		
Effluent receiving river				2–2	-	-		
Tap water	Beijing, China	SPE	GC-MS (Q)	-	-	2.90–3.04	16 + 10	Liu *et al.* 2019[116]
Chaobai River	China	SPE	GC-MS (Q)	30	-	-	8 + 0	Qiao *et al.* 2020[117]
Water, aqueous	Pearl River Estuary, China	SPE	GC-MS (Q)	-	15.7–45.6	-	7 + 10	Yuan *et al.* 2020[107]
Water, suspended particulate matter				-	-	475.6		
Tap water	Beijing, China	SPE	GC-MS (Q)	2.1	1.84	-	21 + 13	Liu *et al.* 2021[115]
Effluents of WWTP	Beijing, China	SPE	GC-MS (Q)	1.41	1.81	-	21 + 16	Liu *et al.* 2021[112]

[a] *GC-MS: gas chromatography–mass spectrometry; Q: quadrupole detector; B: magnetic sector detector.*
[b] *N: number of HPAH analytical standard, denoted as number of Cl-PAHs + number of Br-PAHs.*

1.4.2 Biota and Food Matrices

Biotic matrices are combined with food matrices together as shown in Table 1.5. There are only a few reports of HPAHs in botanical samples. Ma *et al.* determined ΣHPAHs in leaves from trees and shrubs from an e-waste recycling facility to be 87.5 ng/g (dry weight, dw), which was higher than in most of the biotic matrices, including vegetable samples from Shenzhen. This supports the theory that e-waste is a contributing source of HPAHs in the environment.[43,59] In the rural regions of the Tibetan Plateau, 33.5 – 64.9 pg/g, and 20.5 – 72.5 pg/g were measured in lichens and mosses.[49]

ΣHPAHs in New Bedford Harbor mussels from 1993 – 1997 were 21 ng/g lipid weight (lw),[104] which were higher than ΣHPAHs in seafood, fish samples, and raptor eggs from China and Europe.[51,118-120] Wickrama-Arachchige *et al.* studied fish samples from the Indian Ocean, which contained a higher concentration of HPAHs than fish samples from Japan.[121,122] HPAHs were also detected in insects, and because of their low biotransformation ability, could be a potential bioindicator of persistent organic chemicals, such as PACs.[123,124]

Imaeda *et al.* developed an analytical method to detect ClPAHs in barn swallow feces, which could be used as a non-destructive sampling method for biomonitoring.[101] Concentrations were found to range from 4.7 – 24.3 ng/g. The trophodynamics of HPAHs are also studied by Wickrama-Arachchige *et al.* and Xia *et al.*[52,122]

HPAHs were also detected from multiple food samples. Ding *et al.* reported concentrations of HPAHs in both rice and pork (from China) to be lower than 5 ng/g (dw).[59,125] Schörnick *et al.* validated and applied an extraction method for ClPAHs from fat, and fat-free or vegetable matrices, in which the concentration of ClPAHs were determined to be relatively similar to that in grilled

food.[120 95,126] Masuda *et al*. revealed that ClPAHs can be unintentionally generated by cooking fish, and the concentrations of ClPAHs were different between gas-grilled and charcoal-grilled meat samples.[126] Yan *et al.* developed an analytical method for determination of PACs from milk matrices and detected ClPAHs in infant formula and adult milk powder.[127] In addition, Zhao *et al*. detected HPAHs in human serum, and found that they were higher in coal-fired power plant workers than people without occupational exposure.[128,129]

Table 1.5: *Mean total concentrations of ClPAHs (ΣClPAHs), BrPAHs (ΣBrPAHs) and HPAHs (ΣHPAHs) in biota and food.*

Sample details	Location	Extraction method	Analytical instrumentation[a]	Concentration (ng/g)			Unit	N[b]	Reference
				ΣClPAHs	ΣBrPAHs	ΣHPAHs			
Leaves, tree, and shrub (*n = 6*)	China	Soxhlet extraction	GC-MS (Q)	87.5	-	-	dry weight (dw)	20+0	Ma *et al.* 2009[43]
Blue mussel, (*n = 4*)	New Bedford Harbor, USA	n/a	n/a	21	-	-	dw	20+0	Horii *et al.* 2009[104]
Rice (*n = 360*)	18 provinces, China	Solid-liquid extraction	GC-MS (Q)	1.01	2.09	-	dw	3+5	Ding *et al.* 2012[125]
Shrimp (*n = 43*, 6 species)	Guangdong, China	Soxhlet extraction	GC-MS (Q)	-	-	1.58	wet weight	3+6	Ni and Gao, 2013[51]
Crab (*n = 29*, 2 species)				-	-	1.35			
Shellfish (*n = 66*, 10 species)				-	-	1.39			
Rice (*n = 40*)	Shenzhen, China	Solid-liquid extraction	GC-MS (Q)	-	-	2.75	n/a	3+6	Ding *et al.* 2013[59]
Pork (*n = 52*)		Liquid-liquid extraction		-	-	4.97			
Vegetable (*n = 52*)		Soxhlet extraction		-	-	0.56			
young nymph (*n = 20*)	Xuzhou, Jiangsu, China	Soxhlet extraction	GC-MS (Q)	-	-	2.44	dw	3+5	Sun *et al.* 2015[124]
old nymph (*n = 20*)				-	-	3.05	dw		
adults (*n = 20*)				-	-	2.54	dw		

corn flour (*n = 24*)				-	-	1.68	n/a		
wheat bran (*n = 23*)				-	-	1.17	n/a		
American cockroaches (*n = 30*)	Shenzhen, China	Soxhlet extraction	n/a	-	-	49.6	dw	3+6	Wang *et al.* 2015[123]
German cockroaches (*n = 29*)				-	-	74.9			
Lake whitefish (*n = 4*)	Alberta Oil-Sands Region, Canada	ASE	GC-HRMS (TOF)	16.3	-	-	lipid weight (lw)	7+9	Xia *et al.* 2019[52]
Snails (*n = 3*)				-	281.9	-			
River otter (*n = 6*)				5.5	-	-			
Northern pike (*n = 4*)				-	39.2	-			
Fish (*n = 22*)	Liaoning, China	ASE	GC-MS (Q)	0.0059–0.206	-	-	dw	11+0	Tan *et al.* 2019[118]
Raw fish skin (Pacific saury) (*n = 2*)	Japan	Soxhlet extraction	GC-HRMS (B)	0.021	-	-	n/a	20+0	Masuda *et al.* 2019[126]
Gas-grilled fish skin without salt (Pacific saury) (*n = 3*)				0.17	-	-			
Gas-grilled fish skin with salt (Pacific saury) (*n = 4*)				0.30	-	-			
Gas-grilled pork rib (*n = 1*)				0.084	-	-			

Gas-grilled chicken drumstick (*n = 1*)			0.013	-	-			
Gas-grilled beef rib (*n = 1*)			0.075	-	-			
Charcoal-grilled fatty pork (*n = 1*)			< LOQ	-	-			
Charcoal-grilled pork rib (*n = 1*)			0.018	-	-			
Charcoal-grilled pork belly (*n = 1*)			< LOQ	-	-			
T. albacares (large) white muscle (*n = 3*)	Indian Ocean near ASE Sri Lanka	GC-MS (O)	529	776	-	lw	30+21	Wickrama-Arachchige. *et al.* 2020[122]
T. albacares (Small) white muscle (*n = 3*)			65.8	45.6	-			
T. albacares (small) red muscle (*n = 3*)			17.6	6.17	-			
K. pelamis (small) white muscle			36.8	9.51	-			
K. pelamis (small) red muscle (*n = 3*)			53.4	40.1	-			
A. thazard (white & red muscle mixed) (*n = 1*)			34.1	4.73	-			

Lichen	Tibet, China	ASE	GC-HRMS (B)	0.260–0.741	0.0335–0.0649	-	n/a	19+19	Jin *et al.* 2020[49]
Moss				0.338–0.934	0.0205–0.0725	-			
Milk (*n = 43*)	China	Sonication	GC-MS/MS (QqQ)	Nq[c]	-	-	n/a	2+0	Yan *et al.* 2021[127]
Infant formula (*n = 31*)				nq-3.63	-	-			
Adult milk powder (*n = 8*)				0.23	-	-			
Mussels	Irish Sea, UK	ASE	GC-HRMS (B)	3.164	-	-	lw	8+0	Schörnick *et al.* 2021[120]
	Perth, UK			0.492	-	-	lw		
Gun powder green Tea	China			0.396	-	-	n/a		
Jasmine tea	Thailand			0.579	-	-	n/a		
Rice	Thailand			0.0018–0.009	-	-	n/a		
Pork belly	n/a			0.0538	-	-	lw		
Pork belly–marinated	n/a			0.848	-	-	lw		
Marinated pork comb	n/a			0.0677	-	-	lw		
Eels (*Anguilla anguilla*)	Berlin, Germany			0.016-0.026	-	-	lw		
Peregrine falcon egg	Germany			0.0565	-	-	lw		
Eagle owl egg	Germany			0.0368	-	-	lw		
Osprey egg	Germany			0.0305	-	-	lw		
Barn swallow feces	Mutiple sites, Japan	Soxhlet extraction	GC-MS/MS (QqQ)	14.6	-	-	n/a	24+0	Imaeda *et al.* 2021[101]
Grilled chicken	Japan	Soxhlet extraction	GC-MS/MS (QqQ)	0.647	0.0493	-	n/a	22+21	Sei *et al.* 2021[95]

Fish and shellfish, offshore pelagic species (*n = 19*)	Sri Lanka	ASE	GC-MS (O)	2.58–27.1	0.30–9.53	-	dw	30+21	Wickrama-Arachchige. *et al.* 2021[121]
Fish and shellfish, coastal and estuarine species (*n = 11*)	Sri Lanka			3.87–56.5	0.44–8.51	-	dw		
Fish and shellfish, marine species (*n = 10*)	Japan			0.28–34.3	0.35–18.3	-	dw		
Human blood, female (*n = 8*)	Shandong, China	Sonication, SPE	GC-MS/MS (QqQ)	-	-	23	lw	9+14	Zhao *et al.* 2022[129]
Human blood, male (*n = 8*)				-	-	21	lw		
Human blood, female, exposure to coal-fired power (*n = 32*)	Shandong, China	Sonication, SPE	GC-MS/MS (QqQ)	-	-	40.9	lw	9+14	Zhao *et al.* 2022[128]
Human blood, male, exposure to coal-fired power (*n = 125*)				-	-	42.1	lw		
Human blood, female, control (*n = 7*)				-	-	19.1	lw		
Human blood, male, control (*n = 8*)				-	-	27.0	lw		
Shredded squid (*n = 16*)	Multiple sea areas, China	Sonication, QuEChERS	GC-MS/MS (QqQ)	-	-	5.09	n/a	11+7	Li *et al.* 2022[119]

Cod fillet (*n = 16*)	-	-	0.62	n/a

[a] *GC-MS: gas chromatography–mass spectrometry; GC-HRMS: gas chromatography–high resolution mass spectrometry; GC-MS/MS: gas chromatography–tandem mass spectrometry; Q: quadrupole detector; B: magnetic sector detector; O: orbitrap detector; QqQ: triple quadrupole detector; TOF: time-of-flight detector.*

[b] *N: number of HPAH analytical standard, denoted as number of Cl-PAHs + number of Br-PAHs.*

[c] *nq: not quantified.*

1.4.3 Analytical Methods and Instrumentation

It is not uncommon for there to be multiple analytical steps for the determination of persistent organic pollutants in environmental samples. Traditional extraction methods, *e.g.*, Soxhlet extraction, ultrasonic extraction, and solid phase extraction, have all been widely applied to various matrices for HPAHs. Accelerated solvent extraction has received more attention in recent decades.

There are no unified methods for the analysis of HPAHs in air samples. Both active and passive sampler have been employed (*see* Table 1.2); however, details such as flow rate for active sampling are often missing. In addition, there is a lack of authentic commercially available HPAHs standards (*see* Table 1.2, Table 1.3, Table 1.4, and Table 1.5). Not only the total number of HPAHs standards are difference in each study, different congeners for HPAHs have also been selected to serve as standards. Taken together, this makes comparisons of HPAHs in air samples difficult to compare amongst studies.

Likewise, there is a variety of analytical instrumentation used for the detection and quantitation of HPAHs. Gas chromatography (GC) coupled to a variety of mass spectrometer types is the most common analytical instrumentation used for PAC analysis. Due to the presence of halogen atom(s), negative chemical ionization has been used to characterize HPAHs as well.[70,71,130] However, other instrumentation has been used for HPAH detection, for example normal phase high performance liquid chromatography (HPLC),[131], and reverse phase HPLC coupled with ultraviolet absorption spectroscopy and APCI-MS (atmospheric-pressure chemical ionization, APCI).[53,132-134]

1.5 Toxicity, Mutagenicity and Carcinogenicity of HPAHs

The mutagenicity of HPAHs on *Salmonella typhimurium* TA98 and TA100 has was studied back in the 1980s.[45] Chlorinated pyrenes have shown mutagenicity with and without the presence of S9 activation enzyme system, which is related to the substitution position for halogen atoms.[45] Löfroth *et al.* studied S9-depended mutagenicity for 8 monochlorinated PAHs, and have shown the mutagenicity of HPAHs is structure dependent.[135] Bhatia *et al.* have concluded that there is no toxic effect but strong mutagenic effect for Cl_n-Flu, Cl_n-Pyr, and Cl_n-Chr, including frameshift mutation and base pair alterations.[136] In addition, Cl_n-BaA, *e.g.*, 7-Cl-BaA, was found to be mutagenic on *Salmonella typhimurium* TA100 with S9 mix, but chlorinated BaA was determined to be less potent than the parent PAH, BaA.[137]

The embrylethality of chlorinated chrysenes in chicken embryos has been investigated by Gustafsson *et al.*, as well as EROD (ethoxyresorufin O-deethylase) and AHH (aryl hydrocarbon hydroxylase) induction and showed that this group of HPAHs can cause anomalies, including edema and beak defects.[138]

Ohura *et al.* studied the aryl hydrocarbon receptor (AhR)-mediated activities of ClPAHs by yeast assay (*Saccharomyces cerevisiae* YCM3) and found that AhR-mediated activities of ClPAHs are positively related with the size of the PAH skeleton. The AhR activities and cytochrome P450 1A1 expression seem to increase with number of chlorine atoms for lower molecular weight ClPAHs (3 – 4 ring ClPAHs), whereas they tend to decrease with number of chlorine atoms for higher molecular weight ClPAHs (> 4 ring ClPAHs), which can be related to the spatial dimension of ClPAHs.[44] Horii *et al.* also investigated *in vitro* AhR activities of HPAHs by using a recombinant rat hepatoma cell (H4IIE-*luc*) assay. A similar relationship to Ohura *et al.*

of potency and chlorination degree was concluded, and BrPAHs were found to be more potent than ClPAHs with the same PAH skeleton.[139]

Pinto *et al.* investigated the cytotoxicity and genotoxicity of Cl-PAHs, namely 6-Cl-BaP, 1,3-Cl_2-Flu, and 3-Cl-Flu in a human-derived hepatoma (HepG2) cell line using the neutral red (NR) uptake and comet assays, respectively. 6-Cl-BaP has more potent genotoxicity but 1,3-Cl_2-Flu, and 3-Cl-Flu did not show significant genotoxicity (with survival of 60 – 80% of cells).[55]

Additionally, Ohura *et al.* found that ClPAHs can enhance cytochrome P (CYP) 1A1 and 1B1 activities in human breast cancer MCF-7 cells compared to parent PAHs, especially Cl-Phe. 6-Cl-BaP showed the greatest capacity of inducing the expression of CYP1B1 but not CYP1A1, with the presence of 17β-estradiol. 6-Cl-BaP can also induce the expression of estrogen response elements-regulated green fluorescent protein (GFP) and estrogen receptor (ER)-responsive genes.[140]

Kakimoto *et al.* investigated the effect of human cytochrome P450 enzymes on 1-Cl-Pyr and its oxidative metabolites, which are detected in human urine matrix and can be a possible biomarker for estimating human exposure to ClPAHs. CYP P450 1A1 enzyme was determined to be the most efficient enzyme to metabolize 1-Cl-Pyr; 1-Cl-Pyr and its metabolites have agonist activity for the human AhR.[141]

Huang *et al.* conducted two *in vitro* assays for HPAHs, namely EROD assay in rat hepatoma (H4IIE) cells and the SOS/*umu* test (*S. typhimurium* TA1535/pSK1002). ClPAHs enhanced the AhR activities in EROD assay and some have shown the DNA-damaging effects in the SOS/*umu* test with the addition of S9 mix.[142]

Luo *et al.* studied 1-Cl-Pyr potential to induce metabolic perturbation to L02 cells. The most significantly perturbed pathway was glycerophospholipid metabolism after exposure to 1-Cl-Pyr, indicating its potential damage to the cell membrane. Other energy production-related pathways were also affected, such as oxidative phosphorylation (OXPHOS), glycolysis, and fatty acid β oxidation.[143] 6-Cl-BaP can also cause stronger toxicity to human hepatic cells, a stronger metabolomic perturbation, a stronger oxidative stress, a stronger inhibition effect, wider perturbations to metabolic pathways, a stronger inhibition effect on mitochondrial β oxidation of fatty acid and weaker transcriptomic perturbation compared to BaP. Finally, 6-Cl-BaP produced a stronger inhibition on the activities of complexes I and V, while BaP suppressed the expression of 20 genes regulating mitochondrial ETC mainly via AhR activation.[144]

In vivo toxicology studies have also been conducted for HPAHs. Sakakibara *et al.* found that concentrations of 7-Cl-BaA in F334 rats' organs with 14-day oral administration of 7-Cl-BaA and BaA (*e.g.*, liver, kidney, duodenum, heart, and lung) were higher than those of BaA, and regulated some CYP genes in organs, especially the heart.[145] Su *et al.* investigated embryo- and cardiotoxicity of 3-Br-Flu in zebrafish and found that 3-Br-Flu exhibits cytotoxicity through apoptosis and necrosis in vascular endothelial cells, SVEC4-10 cells.[146] 2-Br-methyl Nap have been tested for acute and developmental effects on zebrafish (*Danio rerio*) embryos. Moderate acute toxicity and strong developmental effects were observed. In addition, 2-bromomethyl Nap also induced abnormal phenotypes, including pericardial edema, spine curvature, and shortened body length moderate lethal effects.[147]

M. Putz and A. Putz reviewed the *in silico* toxicological and/or biological study of ClPAHs based on their quantitative structure–activity relationship (QSAR) and basic chemical principles. The structural information of HPAHs has been combined with chemical reactivity principles, such

as electronegativity and hardness, and to generate the logistic framework of ligand-receptor interaction (Michaelis–Menten enzyme kinetics) and predict the toxicity of HPAHs.[148]

1.6 Scope of my Research

My research is aimed to individually detect, separate and accurately quantify HPAHs and other PACs in environmental samples using GC-MS/MS and comprehensive two-dimensional GC high resolution time of flight mass spectrometry (GCxGC-HRToFMS).

1.7 Hypotheses

The hypotheses tested in this thesis are as follows:

Chapter 3: HPAHs are present in biological samples from the AOSR.

Chapter 4: An *in situ* non-layered ASE approach with judicious selection of extraction solvent(s) would enable exhaustive extraction of a broad suite of PACs.

Chapter 5: PACs can be efficiently extracted from avian eggs using microbead beating tissue homogenization with concomitant solvent extraction.

Chapter 6: PACs in environmental media can be accurately and precisely quantified by different mathematical approaches.

Chapter 7: The alkylation effects of the *in vivo* toxicity of BaA in early life stages of the zebrafish can be characterized.

1.8 Aim and Objectives

The overarching aim of my thesis is to accurately identify and quantify HPAHs and other PACs in various environmental matrices. My work will contribute to identification and separation of these compounds and enable toxicology studies on newly identified PACs.

1.9 Thesis Structure

My thesis is divided into eight (8) chapters.

Chapter 1 provides an overview of the properties, occurrence, and toxicity of analytes of interest, HPAHs, and the hypothesis, scope, aim and objectives of the thesis. **Chapter 2** includes a brief description of the methodology for sample processing and instrumental analysis of analytes of interest. This chapter serves as an introduction to the background study of the extraction method and gas chromatography mass spectrometric analysis in the following chapters. **Chapter 3** presents the detection of the presence of a novel class of PACs, HPAHs, in Canadian environment from biota samples. **Chapter 4** presents the validation of two sample clean-up methods, namely one-step extraction and dispersive solid phase extraction (dSPE). **Chapter 5** describes the validation of sample extraction method, microbead extraction of PACs in avian egg matrix. **Chapter 6** focusses on data analysis and quantification of PACs. **Chapter 7** shows the toxicity research of PACs. **Chapter 8** gives an overall conclusion of the study. Suggestions and recommendations for future research in relation to the application of this work are also mentioned.

1.10 References

(1) Schobert, H. H. *Chemistry of fossil fuels and biofuels*; Cambridge University Press, 2013.

(2) Dow, W. G. Kerogen studies and geological interpretations. *Journal of Geochemical Exploration* **1977**, *7*, 79-99. DOI: 10.1016/0375-6742(77)90078-4

(3) Albrecht, P.; Vandenbroucke, M.; Mandengué, M. Geochemical studies on the organic matter from the Douala Basin (Cameroon)—i. Evolution of the extractable organic matter and the formation of petroleum. *Geochimica et Cosmochimica Acta* **1976**, *40* (7), 791-799. DOI: 10.1016/0016-7037(76)90031-4

(4) Schwarzbauer, J.; Jovančićević, B. Origin and transformations of organic matter in geosphere. In *Fossil matter in the geosphere*, Fundamentals in organic geochemistry, Springer International Publishing, 2015; pp 1-18.

(5) Canada, P. H. R. C. o. *Oil sands construction, maintenance and operations labour demand outlook to 2023.*; 2014.

(6) Regulator, A. E. *St98: Alberta's energy reserves and supply/demand outlook.*; Alberta Energy Regulator, Calgary, Alberta, Canada, 2018.

(7) Lee, K.; Boufadel, M.; Chen, B.; Foght, J.; Hodson, P.; Swanson, S.; Venosa, A. *Expert panel report on the behaviour and environmental impacts of crude oil released into aqueous environments*; Royal Society of Canada, 2005.

(8) *Toxicological profile for polycyclic aromatic hydrocarbons*; U.S. Dept. of Health and Human Services, Public Health Service, Agency for Toxic Substances and Disease Registry, 1995.

(9) Yan, J.; Wang, L.; Fu, P. P.; Yu, H. Photomutagenicity of 16 polycyclic aromatic hydrocarbons from the US EPA priority pollutant list. *Mutat Res* **2004**, *557* (1), 99-108. DOI: 10.1016/j.mrgentox.2003.10.004

(10) Davis, E.; Walker, T. R.; Adams, M.; Willis, R. Characterization of polycyclic aromatic hydrocarbons (PAHs) in small craft harbour (SCH) sediments in Nova Scotia, Canada. *Mar Pollut Bull* **2018**, *137*, 285-294. DOI: 10.1016/j.marpolbul.2018.10.043

(11) Achten, C.; Andersson, J. T. Overview of polycyclic aromatic compounds (PAC). *Polycycl Aromat Compd* **2015**, *35* (2-4), 177-186. DOI: 10.1080/10406638.2014.994071

(12) Fu, P. P.; Von Tungeln, L. S.; Chiu, L. H.; Own, Z. Y. Halogenated-polycyclic aromatic hydrocarbons: A class of genotoxic environmental pollutants. *Journal of Environmental Science and Health, Part C* **1999**, *17* (2), 71-109. DOI: 10.1080/10590509909373510

(13) Swart, N. C.; Weaver, A. J. The Alberta Oil Sands and climate. *Nature Climate Change* **2012**, *2* (3), 134-136. DOI: 10.1038/nclimate1421

(14) Kelly, E. N.; Short, J. W.; Schindler, D. W.; Hodson, P. V.; Ma, M.; Kwan, A. K.; Fortin, B. L. Oil sands development contributes polycyclic aromatic compounds to the Athabasca River and its tributaries. *Proc Natl Acad Sci U S A* **2009**, *106* (52), 22346-22351. DOI: 10.1073/pnas.0912050106

(15) Kurek, J.; Kirk, J. L.; Muir, D. C.; Wang, X.; Evans, M. S.; Smol, J. P. Legacy of a half century of Athabasca Oil Sands development recorded by lake ecosystems. *Proc Natl Acad Sci U S A* **2013**, *110* (5), 1761-1766. DOI: 10.1073/pnas.1217675110

(16) Thienpont, J. R.; Desjardins, C. M.; Kimpe, L. E.; Korosi, J. B.; Kokelj, S. V.; Palmer, M. J.; Muir, D. C. G.; Kirk, J. L.; Smol, J. P.; Blais, J. M. Comparative histories of polycyclic aromatic compound accumulation in lake sediments near petroleum operations in western Canada. *Environ Pollut* **2017**, *231* (Pt 1), 13-21. DOI: 10.1016/j.envpol.2017.07.064

(17) Tevlin, A.; Galarneau, E.; Zhang, T.; Hung, H. Polycyclic aromatic compounds (PACs) in the Canadian environment: Ambient air and deposition. *Environ Pollut* **2021**, *271*, 116232. DOI: 10.1016/j.envpol.2020.116232
(18) Berthiaume, A.; Galarneau, E.; Marson, G. Polycyclic aromatic compounds (PACs) in the Canadian environment: Sources and emissions. *Environ Pollut* **2021**, *269*, 116008. DOI: 10.1016/j.envpol.2020.116008
(19) Muir, D. C. G.; Galarneau, E. Polycyclic aromatic compounds (PACs) in the Canadian environment: Links to global change. *Environ Pollut* **2021**, *273*, 116425. DOI: 10.1016/j.envpol.2021.116425
(20) Provencher, J. F.; Thomas, P. J.; Pauli, B.; Braune, B. M.; Franckowiak, R. P.; Gendron, M.; Savard, G.; Sarma, S. N.; Crump, D.; Zahaby, Y.; O'Brien, J.; Mallory, M. L. Polycyclic aromatic compounds (PACs) and trace elements in four marine bird species from northern Canada in a region of natural marine oil and gas seeps. *Sci Total Environ* **2020**, *744*, 140959. DOI: 10.1016/j.scitotenv.2020.140959
(21) Abdel-Shafy, H. I.; Mansour, M. S. M. A review on polycyclic aromatic hydrocarbons: Source, environmental impact, effect on human health and remediation. *Egyptian Journal of Petroleum* **2016**, *25* (1), 107-123. DOI: 10.1016/j.ejpe.2015.03.011
(22) Jin, R.; Liu, G.; Jiang, X.; Liang, Y.; Fiedler, H.; Yang, L.; Zhu, Q.; Xu, Y.; Gao, L.; Su, G.; Xiao, K.; Zheng, M. Profiles, sources and potential exposures of parent, chlorinated and brominated polycyclic aromatic hydrocarbons in haze associated atmosphere. *Sci Total Environ* **2017**, *593-594*, 390-398. DOI: 10.1016/j.scitotenv.2017.03.134
(23) Musial, G.; Reynaud, J.-Y.; Gingras, M. K.; Féniès, H.; Labourdette, R.; Parize, O. Subsurface and outcrop characterization of large tidally influenced point bars of the Cretaceous Mcmurray Formation (Alberta, Canada). *Sedimentary Geology* **2012**, *279*, 156-172. DOI: 10.1016/j.sedgeo.2011.04.020
(24) Sankoda, K.; Kuribayashi, T.; Nomiyama, K.; Shinohara, R. Occurrence and source of chlorinated polycyclic aromatic hydrocarbons (Cl- PAHs) in tidal flats of the Ariake Bay, Japan. *Environ Sci Technol* **2013**, *47* (13), 7037-7044. DOI: 10.1021/es3044483
(25) Kakimoto, K.; Nagayoshi, H.; Konishi, Y.; Kajimura, K.; Ohura, T.; Hayakawa, K.; Toriba, A. Atmospheric chlorinated polycyclic aromatic hydrocarbons in east Asia. *Chemosphere* **2014**, *111*, 40-46. DOI: 10.1016/j.chemosphere.2014.03.072
(26) Jin, R.; Liu, G.; Zheng, M.; Jiang, X.; Zhao, Y.; Yang, L.; Wu, X.; Xu, Y. Secondary copper smelters as sources of chlorinated and brominated polycyclic aromatic hydrocarbons. *Environ Sci Technol* **2017**, *51* (14), 7945-7953. DOI: 10.1021/acs.est.7b02031
(27) Jin, R.; Yang, L.; Zheng, M.; Xu, Y.; Li, C.; Liu, G. Source identification and quantification of chlorinated and brominated polycyclic aromatic hydrocarbons from cement kilns co-processing solid wastes. *Environ Pollut* **2018**, *242* (Pt B), 1346-1352. DOI: 10.1016/j.envpol.2018.08.025
(28) Jin, R.; Zheng, M.; Lammel, G.; Bandowe, B. A. M.; Liu, G. Chlorinated and brominated polycyclic aromatic hydrocarbons: Sources, formation mechanisms, and occurrence in the environment. *Progress in Energy and Combustion Science* **2020**, *76*, 100803. DOI: 10.1016/j.pecs.2019.100803
(29) Sun, J. L.; Zeng, H.; Ni, H. G. Halogenated polycyclic aromatic hydrocarbons in the environment. *Chemosphere* **2013**, *90* (6), 1751-1759. DOI: 10.1016/j.chemosphere.2012.10.094

(30) Liu, G.; Cai, Z.; Zheng, M. Sources of unintentionally produced polychlorinated naphthalenes. *Chemosphere* **2014**, *94*, 1-12. DOI: 10.1016/j.chemosphere.2013.09.021
(31) Ohura, T. Environmental behavior, sources, and effects of chlorinated polycyclic aromatic hydrocarbons. *ScientificWorldJournal* **2007**, *7*, 372-380. DOI: 10.1100/tsw.2007.75
(32) Johnson, W.; Idowu, I.; Francisco, O.; Marvin, C.; Thomas, P. J.; Stetefeld, J.; Tomy, G. T. Enumeration of the constitutional isomers of environmentally relevant substituted polycyclic aromatic compounds. *Chemosphere* **2018**, *202*, 9-16. DOI: 10.1016/j.chemosphere.2018.03.035
(33) Alben, K. Gas chromatographic-mass spectrometric analysis of chlorination effects on commercial coal-tar leachate. *Analytical Chemistry* **2002**, *52* (12), 1825-1828. DOI: 10.1021/ac50062a011
(34) Johnsen, S.; Gribbestad, I. S.; Johansen, S. Formation of chlorinated PAH-a possible health hazard from water chlorination. *Sci Total Environ* **1989**, *81-82*, 231-238. DOI: 10.1016/0048-9697(89)90129-0
(35) Eiceman, G. A.; Hoffman, R. V.; Collins, M. C.; Long, Y. T.; Lu, M. Q. Chlorine substitution reactions of polycyclic aromatic hydrocarbons on fly ash from coal-fired power plants. *Chemosphere* **1990**, *21* (1-2), 35-41. DOI: 10.1016/0045-6535(90)90375-4
(36) Johnsen, S.; Gribbestad, I. S. Influence of humic substances on the formation of chlorinated polycyclic aromatic hydrocarbons during chlorination of polycyclic aromatic hydrocarbon polluted water. *Environ Sci Technol* **1988**, *22* (8), 978-981. DOI: 10.1021/es00173a019
(37) Nilsson, U. L.; Colmsjö, A. L. Formation of chlorinated polycyclic aromatic hydrocarbons in different chlorination reactions. *Chemosphere* **1990**, *21* (8), 939-951. DOI: 10.1016/0045-6535(90)90117-c
(38) Oyler, A. R.; Liukkonen, R. J.; Lukasewycz, M. K.; Cox, D. A.; Peake, D. A.; Carlson, R. M. Implications of treating water containing polynuclear aromatic hydrocarbons with chlorine: A gas chromatographic-mass spectrometric study. *Environ Health Perspect* **1982**, *46*, 73-86. DOI: 10.1289/ehp.824673
(39) Fan, Y.; Ren, M.; Zhang, H.; Geng, N.; Li, Y.; Zhang, N.; Zhao, L.; Gao, Y.; Chen, J. Levels and fingerprints of chlorinated aromatic hydrocarbons in fly ashes from the typical industrial thermal processes: Implication for the co-formation mechanism. *Chemosphere* **2019**, *224*, 298-305. DOI: 10.1016/j.chemosphere.2019.02.117
(40) Fan, Y.; Zhang, H.; Wang, D.; Ren, M.; Zhang, X.; Wang, L.; Chen, J. Simultaneous determination of chlorinated aromatic hydrocarbons in fly ashes discharged from industrial thermal processes. *Analytical Methods* **2017**, *9* (35), 5198-5203. DOI: 10.1039/c7ay01545c
(41) Wang, D.; Xu, X.; Chu, S.; Zhang, D. Analysis and structure prediction of chlorinated polycyclic aromatic hydrocarbons released from combustion of polyvinylchloride. *Chemosphere* **2003**, *53* (5), 495-503. DOI: 10.1016/S0045-6535(03)00507-1
(42) Sankoda, K.; Nomiyama, K.; Yonehara, T.; Kuribayashi, T.; Shinohara, R. Evidence for in situ production of chlorinated polycyclic aromatic hydrocarbons on tidal flats: Environmental monitoring and laboratory scale experiment. *Chemosphere* **2012**, *88* (5), 542-547. DOI: 10.1016/j.chemosphere.2012.03.017
(43) Ma, J.; Horii, Y.; Cheng, J.; Wang, W.; Wu, Q.; Ohura, T.; Kannan, K. Chlorinated and parent polycyclic aromatic hydrocarbons in environmental samples from an electronic waste recycling facility and a chemical industrial complex in China. *Environ Sci Technol* **2009**, *43* (3), 643-649. DOI: 10.1021/es802878w

(44) Ohura, T.; Morita, M.; Makino, M.; Amagai, T.; Shimoi, K. Aryl hydrocarbon receptor-mediated effects of chlorinated polycyclic aromatic hydrocarbons. *Chem Res Toxicol* **2007**, *20* (9), 1237-1241. DOI: 10.1021/tx700148b

(45) Colmsji, A.; Rannug, A.; Rannug, U. Some chloro derivatives of polynuclear aromatic hydrocarbons are potent mutagens in salmonella typhimurium. *Mutation Research* **1984**, *135*, 21-29.

(46) Ieda, T.; Ochiai, N.; Miyawaki, T.; Ohura, T.; Horii, Y. Environmental analysis of chlorinated and brominated polycyclic aromatic hydrocarbons by comprehensive two-dimensional gas chromatography coupled to high-resolution time-of-flight mass spectrometry. *J Chromatogr A* **2011**, *1218* (21), 3224-3232. DOI: 10.1016/j.chroma.2011.01.013

(47) Yang, L.; Wang, S.; Peng, X.; Zheng, M.; Yang, Y.; Xiao, K.; Liu, G. Gas chromatography-orbitrap mass spectrometry screening of organic chemicals in fly ash samples from industrial sources and implications for understanding the formation mechanisms of unintentional persistent organic pollutants. *Sci Total Environ* **2019**, *664*, 107-115. DOI: 10.1016/j.scitotenv.2019.02.001

(48) Mukai, K.; Fujimori, T.; Shiota, K.; Takaoka, M. Quantitative speciation of insoluble chlorine in E-waste open burning soil: Implications of the presence of unidentified aromatic-Cl and insoluble chlorides. *Chemosphere* **2019**, *233*, 493-502. DOI: 10.1016/j.chemosphere.2019.05.283

(49) Jin, R.; Bu, D.; Liu, G.; Zheng, M.; Lammel, G.; Fu, J.; Yang, L.; Li, C.; Habib, A.; Yang, Y.; Liu, X. New classes of organic pollutants in the remote continental environment - chlorinated and brominated polycyclic aromatic hydrocarbons on the Tibetan Plateau. *Environ Int* **2020**, *137* (February), 105574. DOI: 10.1016/j.envint.2020.105574

(50) Wang, Y. J.; Liao, R. Q.; Liu, W. L.; Kannan, K.; Ohura, T.; Wu, M. H.; Ma, J. Chlorinated polycyclic aromatic hydrocarbons in surface sediment from Maowei Sea, Guangxi, China: Occurrence, distribution, and source apportionment. *Environ Sci Pollut Res Int* **2017**, *24* (19), 16241-16252. DOI: 10.1007/s11356-017-9193-0

(51) Ni, H. G.; Guo, J. Y. Parent and halogenated polycyclic aromatic hydrocarbons in seafood from south China and implications for human exposure. *J Agric Food Chem* **2013**, *61* (8), 2013-2018. DOI: 10.1021/jf304836q

(52) Xia, Z.; Idowu, I.; Marvin, C.; Thomas, P. J.; Johnson, W.; Francisco, O.; Stetefeld, J.; Crimmins, B.; Fry, M.; Tomy, G. T. Identification of halogenated polycyclic aromatic hydrocarbons in biological samples from Alberta Oil-Sands Region. *Chemosphere* **2019**, *215*, 206-213. DOI: 10.1016/j.chemosphere.2018.10.050

(53) Gao, Z. Y.; Jiang, W. S.; Sun, D.; Xie, Y.; Chen, Z. L.; Yu, L. J.; Xie, S. Y.; Huang, R. B.; Zheng, L. S. Chlorination for efficient identification of polycyclic aromatic hydrocarbons by liquid chromatography-mass spectrometry. *Talanta* **2010**, *81* (1-2), 48-54. DOI: 10.1016/j.talanta.2009.11.039

(54) Miyake, Y.; Tokumura, M.; Wang, Q.; Amagai, T.; Horii, Y.; Kannan, K. Mechanism of formation of chlorinated pyrene during combustion of polyvinyl chloride. *Environ Sci Technol* **2017**, *51* (24), 14100-14106. DOI: 10.1021/acs.est.7b04854

(55) Pinto, M.; Rebola, M.; Louro, H.; Antunes, A. M. M.; José, S. S.; Rocha, M.; Silva, M. J.; Cardoso, A. S. Chlorinated polycyclic aromatic hydrocarbons associated with drinking water disinfection: Synthesis, formation under aqueous chlorination conditions and genotoxic effects. *Polycyclic Aromatic Compounds* **2014**, *34* (4), 356-371. DOI: 10.1080/10406638.2014.891143

(56) Sankoda, K.; Toda, I.; Sekiguchi, K.; Nomiyama, K.; Shinohara, R. Aqueous secondary formation of brominated, chlorinated, and mixed halogenated pyrene in presence of halide ions. *Chemosphere* **2017**, *171*, 399-404. DOI: 10.1016/j.chemosphere.2016.12.044
(57) Turner, D. E.; O'Malley, R. F.; Sardella, D. J.; Barinelli, L. S.; Kaul, P. The reaction of iodine monochloride with polycyclic aromatic compounds: Polar and electron transfer pathways. *The Journal of Organic Chemistry* **2002**, *59* (24), 7335-7340. DOI: 10.1021/jo00103a026
(58) Chen, H.; Chow, A. T.; Li, X.-W.; Ni, H.-G.; Dahlgren, R. A.; Zeng, H.; Wang, J.-J. Wildfire burn intensity affects the quantity and speciation of polycyclic aromatic hydrocarbons in soils. *ACS Earth and Space Chemistry* **2018**, *2* (12), 1262-1270. DOI: 10.1021/acsearthspacechem.8b00101
(59) Ding, C.; Ni, H. G.; Zeng, H. Human exposure to parent and halogenated polycyclic aromatic hydrocarbons via food consumption in Shenzhen, China. *Sci Total Environ* **2013**, *443*, 857-863. DOI: 10.1016/j.scitotenv.2012.11.018
(60) Horii, Y.; Ok, G.; Ohura, T.; Kannanct, K. Occurrence and profiles of chlorinated and brominated polycyclic aromatic hydrocarbons in waste incinerators. *Environ Sci Technol* **2008**, *42* (6), 1904-1909. DOI: 10.1021/es703001f
(61) Ma, J.; Chen, Z.; Wu, M.; Feng, J.; Horii, Y.; Ohura, T.; Kannan, K. Airborne PM2.5/PM10-associated chlorinated polycyclic aromatic hydrocarbons and their parent compounds in a suburban area in Shanghai, China. *Environ Sci Technol* **2013**, *47* (14), 7615-7623. DOI: 10.1021/es400338h
(62) Ohura, T.; Kamiya, Y.; Ikemori, F. Local and seasonal variations in concentrations of chlorinated polycyclic aromatic hydrocarbons associated with particles in a Japanese megacity. *J Hazard Mater* **2016**, *312*, 254-261. DOI: 10.1016/j.jhazmat.2016.03.072
(63) Ohura, T.; Sawada, K.; Amagai, T.; Shinomiya, M. Discovery of novel halogenated polycyclic aromatic hydrocarbons in urban particulate matters: Occurrence, photostability, and ahr activity. *Environ Sci Technol* **2009**, *43* (7), 2269-2275. DOI: 10.1021/es803633d
(64) Sun, J. L.; Ni, H. G.; Zeng, H. Ecological risk assessment of parent and halogenated polycyclic aromatic hydrocarbons in surface sediments from an urban river in south China. *Environ Toxicol Chem* **2012**, *31* (8), 1867-1873. DOI: 10.1002/etc.1890
(65) Tang, J.; Ma, S.; Liu, R.; Yue, C.; Li, G.; Yu, Y.; Yang, Y.; An, T. The pollution profiles and human exposure risks of chlorinated and brominated PAHs in indoor dusts from e-waste dismantling workshops: Comparison of GC–MS, GC–MS/MS and GC × GC–MS/MS determination methods. *J Hazard Mater*, **2020**, *394*, 122573. DOI: 10.1016/j.jhazmat.2020.122573.
(66) Gonzales, L. R. Trace analysis of halogenated polycyclic aromatic hydrocarbons from an electronic waste recycling area and guangzhou, china. Oregon State University, **2011**.
(67) *Estimation programs interface suite™*; Washington DC, 2012.
(68) US, E. P. A. *Sustainable futures / P2 framework manual*; 2012. http://www.epa.gov/oppt/exposure/pubs/episuite.htm.
(69) *Ecological structure activity relationships™ (ECOSAR™)*; USEPA, 2012.
(70) Haglund, P.; Alsberg, T.; Bergman, A.; Jansson, B. Analysis of halogenated polycyclic aromatic hydrocarbons in urban air, snow and automobile exhaust. *Chemosphere* **1987**, *16* (10-12), 2441-2450. DOI: 10.1016/0045-6535(87)90303-1
(71) Nilsson, U. L.; Oestman, C. E. Chlorinated polycyclic aromatic hydrocarbons: Method of analysis and their occurrence in urban air. *Environmental Science & Technology* **2002**, *27* (9), 1826-1831. DOI: 10.1021/es00046a010

(72) Ohura, T.; Kitazawa, A.; Amagai, T.; Makino, M. Occurrence, profiles, and photostabilities of chlorinated polycyclic aromatic hydrocarbons associated with particulates in urban air. *Environ Sci Technol* **2005**, *39* (1), 85-91. DOI: 10.1021/es040433s

(73) Kitazawa, A.; Amagai, T.; Ohura, T. Temporal trends and relationships of particulate chlorinated polycyclic aromatic hydrocarbons and their parent compounds in urban air. *Environ Sci Technol* **2006**, *40* (15), 4592-4598. DOI: 10.1021/es0602703

(74) Ohura, T.; Fujima, S.; Amagai, T.; Shinomiya, M. Chlorinated polycyclic aromatic hydrocarbons in the atmosphere: Seasonal levels, gas-particle partitioning, and origin. *Environ Sci Technol* **2008**, *42* (9), 3296-3302. DOI: 10.1021/es703068n

(75) Ohura, T.; Horii, Y.; Kojima, M.; Kamiya, Y. Diurnal variability of chlorinated polycyclic aromatic hydrocarbons in urban air, Japan. *Atmospheric Environment* **2013**, *81* (3), 84-91. DOI: 10.1016/j.atmosenv.2013.08.044

(76) Sei, K.; Wang, Q.; Tokumura, M.; Hossain, A.; Raknuzzaman, M.; Miyake, Y.; Amagai, T. Occurrence, potential source, and cancer risk of PM(2.5)-bound polycyclic aromatic hydrocarbons and their halogenated derivatives in Shizuoka, Japan, and Dhaka, bangladesh. *Environ Res* **2021**, *196*, 110909. DOI: 10.1016/j.envres.2021.110909

(77) Ohura, T.; Horii, Y.; Yamashita, N. Spatial distribution and exposure risks of ambient chlorinated polycyclic aromatic hydrocarbons in Tokyo Bay area and network approach to source impacts. *Environ Pollut* **2018**, *232*, 367-374. DOI: 10.1016/j.envpol.2017.09.037

(78) Ohura, T.; Suhara, T.; Kamiya, Y.; Ikemori, F.; Kageyama, S.; Nakajima, D. Distributions and multiple sources of chlorinated polycyclic aromatic hydrocarbons in the air over Japan. *Sci Total Environ* **2019**, *649*, 364-371. DOI: 10.1016/j.scitotenv.2018.08.302

(79) Sun, J. L.; Jing, X.; Chang, W. J.; Chen, Z. X.; Zeng, H. Cumulative health risk assessment of halogenated and parent polycyclic aromatic hydrocarbons associated with particulate matters in urban air. *Ecotoxicol Environ Saf* **2015**, *113*, 31-37. DOI: 10.1016/j.ecoenv.2014.11.024

(80) Gao, P. P.; Zhao, Y. B.; Ni, H. G. Incidence of real-world automotive parent and halogenated PAH in urban atmosphere. *Environ Pollut* **2018**, *237*, 515-522. DOI: 10.1016/j.envpol.2018.02.077

(81) Jin, R.; Zheng, M.; Yang, H.; Yang, L.; Wu, X.; Xu, Y.; Liu, G. Gas-particle phase partitioning and particle size distribution of chlorinated and brominated polycyclic aromatic hydrocarbons in haze. *Environ Pollut* **2017**, *231* (Pt 2), 1601-1608. DOI: 10.1016/j.envpol.2017.09.066

(82) Jin, R.; Fu, J.; Zheng, M.; Yang, L.; Habib, A.; Li, C.; Liu, G. Polychlorinated naphthalene congener profiles in common vegetation on the Tibetan Plateau as biomonitors of their sources and transportation. *Environmental Science & Technology* **2020**, *54* (4), 2314-2322. DOI: 10.1021/acs.est.9b06668

(83) Cao, Z.; Wang, M.; Shi, S.; Zhao, Y.; Chen, X.; Li, C.; Li, Y.; Wang, H.; Bao, L.; Cui, X. Size-distribution-based assessment of human inhalation and dermal exposure to airborne parent, oxygenated and chlorinated PAHs during a regional heavy haze episode. *Environ Pollut* **2020**, *263* (Pt A), 114661. DOI: 10.1016/j.envpol.2020.114661

(84) Wang, P.; Qi, A.; Huang, Q.; Wang, Y.; Tuo, X.; Zhao, T.; Duan, S.; Gao, H.; Zhang, W.; Xu, P.; Zhang, T.; Zhang, X.; Wang, W.; Yang, L. Spatial and temporal variation, source identification, and toxicity evaluation of brominated/chlorinated/nitrated/oxygenated-PAHs at a heavily industrialized area in eastern China. *Sci Total Environ* **2022**, *822*, 153542. DOI: 10.1016/j.scitotenv.2022.153542

(85) Vuong, Q. T.; Kim, S. J.; Nguyen, T. N. T.; Thang, P. Q.; Lee, S. J.; Ohura, T.; Choi, S. D. Passive air sampling of halogenated polycyclic aromatic hydrocarbons in the largest industrial city in Korea: Spatial distributions and source identification. *J Hazard Mater* **2020**, *382* (August 2019), 121238. DOI: 10.1016/j.jhazmat.2019.121238

(86) Jin, R.; Bandowe, B. A. M.; Zheng, M.; Liu, G.; Nezikova, B.; Prokes, R.; Cupr, P.; Klanova, J.; Lammel, G. Atmospheric deposition of chlorinated and brominated polycyclic aromatic hydrocarbons in central Europe analyzed by GC-MS/MS. *Environ Sci Pollut Res Int* **2021**, *28* (43), 61360-61368. DOI: 10.1007/s11356-021-15038-3

(87) Kawatsu, Y.; Masih, J.; Ohura, T. Occurrences and potential sources of halogenated polycyclic aromatic hydrocarbons associated with PM(2.5) in Mumbai, India. *Environ Toxicol Chem* **2022**, *41* (2), 312-320. DOI: 10.1002/etc.5211

(88) Chen, S. J.; Wang, J.; Wang, T.; Wang, T.; Mai, B. X.; Simonich, S. L. M. Seasonal variations and source apportionment of complex polycyclic aromatic hydrocarbon mixtures in particulate matter in an electronic waste and urban area in south China. *Sci Total Environ* **2016**, *573*, 115-122. DOI: 10.1016/j.scitotenv.2016.08.101

(89) Lin, B.; Liu, G.; Wu, G.; Chen, C.; Liang, Y.; Wang, P.; Guo, J.; Yang, L.; Jin, R.; Sun, Y.; Zheng, M. Variation in the formation characteristics of PBDD/F, brominated PAH, and PBDE congeners along the secondary copper smelting processes. *J Hazard Mater* **2022**, *439*, 129602. DOI: 10.1016/j.jhazmat.2022.129602

(90) Lin, B.; Yang, Y.; Yang, L.; Liu, G.; Li, C.; Xu, J.; Hou, S.; Zheng, M. Congener profiles and process distributions of polychlorinated biphenyls, polychlorinated naphthalenes and chlorinated polycyclic aromatic hydrocarbons from secondary copper smelting. *J Hazard Mater* **2022**, *423* (Pt B), 127125. DOI: 10.1016/j.jhazmat.2021.127125

(91) Jin, R.; Liu, G.; Zheng, M.; Fiedler, H.; Jiang, X.; Yang, L.; Wu, X.; Xu, Y. Congener-specific determination of ultratrace levels of chlorinated and brominated polycyclic aromatic hydrocarbons in atmosphere and industrial stack gas by isotopic dilution gas chromatography/high resolution mass spectrometry method. *J Chromatogr A* **2017**, *1509*, 114-122. DOI: 10.1016/j.chroma.2017.06.022

(92) Yang, Y.; Liu, G.; Zheng, M.; Liu, S.; Yang, Q.; Liu, X.; Wang, M.; Yang, L. Discovery of significant atmospheric emission of halogenated polycyclic aromatic hydrocarbons from secondary zinc smelting. *Ecotoxicol Environ Saf* **2022**, *238*, 113594. DOI: 10.1016/j.ecoenv.2022.113594

(93) Yang, L.; Shen, J.; Zheng, M.; Yang, Q.; Li, D.; Liu, G. Occurrence of chlorinated and brominated polycyclic aromatic hydrocarbons from electric arc furnace for steelmaking. *Environ Pollut* **2022**, *294*, 118663. DOI: 10.1016/j.envpol.2021.118663

(94) Xu, Y.; Yang, L.; Zheng, M.; Jin, R.; Wu, X.; Li, C.; Liu, G. Chlorinated and brominated polycyclic aromatic hydrocarbons from metallurgical plants. *Environ Sci Technol* **2018**, *52* (13), 7334-7342. DOI: 10.1021/acs.est.8b01638

(95) Sei, K.; Wang, Q.; Tokumura, M.; Miyake, Y.; Amagai, T. Accurate and ultrasensitive determination of 72 parent and halogenated polycyclic aromatic hydrocarbons in a variety of environmental samples via gas chromatography-triple quadrupole mass spectrometry. *Chemosphere* **2021**, *271*, 129535. DOI: 10.1016/j.chemosphere.2021.129535

(96) Yang, L.; Wu, J.; Zheng, M.; Cao, Z.; Li, C.; Shi, M.; Liu, G. Non-target screening of organic pollutants and target analysis of halogenated polycyclic aromatic hydrocarbons in the atmosphere around metallurgical plants by high-resolution GC/Q-TOF-MS. *Environmental Sciences Europe* **2020**, *32* (1). DOI: 10.1186/s12302-020-00376-9

(97) Masuda, M.; Wang, Q.; Tokumura, M.; Miyake, Y.; Amagai, T. Risk assessment of polycyclic aromatic hydrocarbons and their chlorinated derivatives produced during cooking and released in exhaust gas. *Ecotoxicol Environ Saf* **2020**, *197*, 110592. DOI: 10.1016/j.ecoenv.2020.110592
(98) Liu, R.; Ma, S.; Yu, Y.; Li, G.; Yu, Y.; An, T. Field study of PAHs with their derivatives emitted from E-waste dismantling processes and their comprehensive human exposure implications. *Environ Int* **2020**, *144*, 106059. DOI: 10.1016/j.envint.2020.106059
(99) Vuong, Q. T.; Thang, P. Q.; Nguyen, T. N. T.; Ohura, T.; Choi, S. D. Seasonal variation and gas/particle partitioning of atmospheric halogenated polycyclic aromatic hydrocarbons and the effects of meteorological conditions in Ulsan, south Korea. *Environ Pollut* **2020**, *263* (Pt A), 114592. DOI: 10.1016/j.envpol.2020.114592
(100) Ni, H. G.; Zeng, E. Y. Environmental and human exposure to soil chlorinated and brominated polycyclic aromatic hydrocarbons in an urbanized region. *Environ Toxicol Chem* **2012**, *31* (7), 1494-1500. DOI: 10.1002/etc.1854
(101) Imaeda, C.; Niizuma, Y.; Ohura, T. Potential of barn swallow feces as a non-destructive biomonitoring tool for anthropogenic pollutants: Site and chemical specificities and an evaluation of soil contaminants. *Environmental Pollutants and Bioavailability* **2021**, *33* (1), 317-325. DOI: 10.1080/26395940.2021.1986136
(102) Wang, Y.; Su, P.; Ge, X.; Ren, H.; Ma, S.; Shen, G.; Chen, Q.; Yu, Y.; An, T. Identification of specific halogenated polycyclic aromatic hydrocarbons in surface soils of petrochemical, flame retardant, and electronic waste dismantling industrial parks. *J Hazard Mater* **2022**, *436*, 129160. DOI: 10.1016/j.jhazmat.2022.129160
(103) Fernando, S.; Jobst, K. J.; Taguchi, V. Y.; Helm, P. A.; Reiner, E. J.; McCarry, B. E. Identification of the halogenated compounds resulting from the 1997 plastimet inc. Fire in Hamilton, Ontario, using comprehensive two-dimensional gas chromatography and (ultra)high resolution mass spectrometry. *Environ Sci Technol* **2014**, *48* (18), 10656-10663. DOI: 10.1021/es503428j
(104) Horii, Y.; Ohura, T.; Yamashita, N.; Kannan, K. Chlorinated polycyclic aromatic hydrocarbons in sediments from industrial areas in japan and the united states. *Arch Environ Contam Toxicol* **2009**, *57* (4), 651-660. DOI: 10.1007/s00244-009-9372-1
(105) Sun, J. L.; Ni, H. G.; Zeng, H. Occurrence of chlorinated and brominated polycyclic aromatic hydrocarbons in surface sediments in Shenzhen, south China and its relationship to urbanization. *J Environ Monit* **2011**, *13* (10), 2775-2781. DOI: 10.1039/c1em10465a
(106) Ohura, T.; Sakakibara, H.; Watanabe, I.; Shim, W. J.; Manage, P. M.; Guruge, K. S. Spatial and vertical distributions of sedimentary halogenated polycyclic aromatic hydrocarbons in moderately polluted areas of Asia. *Environ Pollut* **2015**, *196*, 331-340. DOI: 10.1016/j.envpol.2014.10.028
(107) Yuan, K.; Qing, Q.; Wang, Y.; Lin, F.; Chen, B.; Luan, T.; Wang, X. Characteristics of chlorinated and brominated polycyclic aromatic hydrocarbons in the Pearl River Estuary. *Sci Total Environ* **2020**, *739*, 139774. DOI: 10.1016/j.scitotenv.2020.139774
(108) Du, Y.; Xu, X.; Liu, Q.; Lin, L.; Bai, L.; Wang, D. Contribution of atmospheric deposition to halogenated polycyclic aromatic hydrocarbons in surface sediments: A validation study. *Sci Total Environ* **2022**, *815*, 152889. DOI: 10.1016/j.scitotenv.2021.152889
(109) Qiao, M.; Cao, W.; Liu, B.; Bai, Y.; Qi, W.; Zhao, X.; Qu, J. Impact of upgrading wastewater treatment plant on the removal of typical methyl, oxygenated, chlorinated and parent

polycyclic aromatic hydrocarbons. *Sci Total Environ* **2017**, *603-604*, 140-147. DOI: 10.1016/j.scitotenv.2017.06.097
(110) Qiao, M.; Bai, Y.; Cao, W.; Huo, Y.; Zhao, X.; Liu, D.; Li, Z. Impact of secondary effluent from wastewater treatment plants on urban rivers: Polycyclic aromatic hydrocarbons and derivatives. *Chemosphere* **2018**, *211*, 185-191. DOI: 10.1016/j.chemosphere.2018.07.167
(111) Qiao, M.; Fu, L.; Cao, W.; Bai, Y.; Huang, Q.; Zhao, X. Occurrence and removal of polycyclic aromatic hydrocarbons and their derivatives in an ecological wastewater treatment plant in south China and effluent impact to the receiving river. *Environ Sci Pollut Res Int* **2019**, *26* (6), 5638-5644. DOI: 10.1007/s11356-018-3839-4
(112) Liu, Q.; Xu, X.; Lin, L.; Wang, D. Occurrence, distribution and ecological risk assessment of polycyclic aromatic hydrocarbons and their derivatives in the effluents of wastewater treatment plants. *Sci Total Environ* **2021**, *789*, 147911. DOI: 10.1016/j.scitotenv.2021.147911
(113) Wang, X.; Kang, H.; Wu, J. Determination of chlorinated polycyclic aromatic hydrocarbons in water by solid-phase extraction coupled with gas chromatography and mass spectrometry. *J Sep Sci* **2016**, *39* (9), 1742-1748. DOI: 10.1002/jssc.201501286
(114) Shiraishi, H.; Pilkington, N. H.; Otsuki, A.; Fuwa, K. Occurrence of chlorinated polynuclear aromatic hydrocarbons in tap water. *Environ Sci Technol* **1985**, *19* (7), 585-590. DOI: 10.1021/es00137a001
(115) Liu, Q.; Xu, X.; Lin, L.; Yang, G.; Wang, D. Occurrence, health risk assessment and regional impact of parent, halogenated and oxygenated polycyclic aromatic hydrocarbons in tap water. *J Hazard Mater* **2021**, *413*, 125360. DOI: 10.1016/j.jhazmat.2021.125360
(116) Liu, Q.; Xu, X.; Wang, L.; Lin, L.; Wang, D. Simultaneous determination of forty-two parent and halogenated polycyclic aromatic hydrocarbons using solid-phase extraction combined with gas chromatography-mass spectrometry in drinking water. *Ecotoxicol Environ Saf* **2019**, *181* (18), 241-247. DOI: 10.1016/j.ecoenv.2019.06.011
(117) Qiao, M.; Fu, L.; Li, Z.; Liu, D.; Bai, Y.; Zhao, X. Distribution and ecological risk of substituted and parent polycyclic aromatic hydrocarbons in surface waters of the Bai, Chao, and Chaobai Rivers in northern China. *Environ Pollut* **2020**, *257*, 113600. DOI: 10.1016/j.envpol.2019.113600
(118) Tan, J.; Lu, X.; Fu, L.; Yang, G.; Chen, J. Quantification of Cl-PAHs and their parent compounds in fish by improved ASE method and stable isotope dilution GC-MS. *Ecotoxicol Environ Saf* **2019**, *186* (October), 109775. DOI: 10.1016/j.ecoenv.2019.109775
(119) Li, W.; Wu, S. Halogenated polycyclic aromatic hydrocarbons and their parent compounds in ready-to-eat seafood rich in salt: Method validation, profiles, correlation, and exposure risks. *Food Control* **2022**, *136*. DOI: 10.1016/j.foodcont.2022.108864
(120) Schörnick, C.; Lüth, A.; Wobst, B.; Rotard, W. Method development and determination of chlorinated polycyclic aromatic hydrocarbons in different matrices. *Food Anal. Methods* **2021**, *14* (6), 1150-1166. DOI: 10.1007/s12161-020-01956-4 (acccessed 2022/08/25/06:44:15).
(121) Wickrama-Arachchige, A. U.; Guruge, K. S.; Inagaki, Y.; Tani, H.; Dharmaratne, T. S.; Niizuma, Y.; Ohura, T. Halogenated polycyclic aromatic hydrocarbons in edible aquatic species of two asian countries: Congener profiles, biomagnification, and human risk assessment. *Food Chem* **2021**, *360*, 130072. DOI: 10.1016/j.foodchem.2021.130072
(122) Wickrama-Arachchige, A. U.; Hirabayashi, T.; Imai, Y.; Guruge, K. S.; Dharmaratne, T. S.; Ohura, T. Accumulation of halogenated polycyclic aromatic hydrocarbons by different

tuna species, determined by high-resolution gas chromatography Orbitrap mass spectrometry. *Environ Pollut* **2020**, *256*, 113487. DOI: 10.1016/j.envpol.2019.113487
(123) Wang, Y.-B.; Sojinu, S. O.; Sun, J.-L.; Ni, H.-G.; Zeng, H.; Zou, M. Are cockroaches reliable bioindicators of persistent organic pollutant contamination of indoor environments? *Ecological Indicators* **2015**, *50*, 44-49. DOI: 10.1016/j.ecolind.2014.10.022
(124) Sun, J. L.; Wang, Y. B.; Zeng, H.; Ni, H. G. Parent and halogenated polycyclic aromatic hydrocarbons in farmed cockroaches and implications for human exposure. *Ecotoxicol Environ Saf* **2015**, *111*, 256-262. DOI: 10.1016/j.ecoenv.2014.10.024
(125) Ding, C.; Ni, H. G.; Zeng, H. Parent and halogenated polycyclic aromatic hydrocarbons in rice and implications for human health in China. *Environ Pollut* **2012**, *168*, 80-86. DOI: 10.1016/j.envpol.2012.04.025
(126) Masuda, M.; Wang, Q.; Tokumura, M.; Miyake, Y.; Amagai, T. Simultaneous determination of polycyclic aromatic hydrocarbons and their chlorinated derivatives in grilled foods. *Ecotoxicol Environ Saf* **2019**, *178* (April), 188-194. DOI: 10.1016/j.ecoenv.2019.04.046
(127) Yan, K.; Wu, S.; Gong, G.; Xin, L.; Ge, Y. Simultaneous determination of typical chlorinated, oxygenated, and european union priority polycyclic aromatic hydrocarbons in milk samples and milk powders. *J Agric Food Chem* **2021**, *69* (13), 3923-3931. DOI: 10.1021/acs.jafc.1c00283
(128) Zhao, C.; Li, A.; Zhang, G.; Pan, Y.; Meng, L.; Yang, R.; Li, Y.; Zhang, Q.; Jiang, G. Parent and halogenated polycyclic aromatic hydrocarbons in the serum of coal-fired power plant workers: Levels, sex differences, accumulation trends, and risks. *Environ Sci Technol* **2022**, *56* (17), 12431-12439. DOI: 10.1021/acs.est.2c03099
(129) Zhao, C.; Li, C.; Wang, C.; Li, Y.; Yang, R.; Zhang, Q.; Jiang, G. Ultrasensitive determination of 39 parent and emerging halogenated polycyclic aromatic hydrocarbons in human serum. *Anal Methods* **2022**, *14* (14), 1430-1438. DOI: 10.1039/d2ay00029f
(130) Kamiya, Y.; Kameda, T.; Ohura, T.; Tohno, S. Determination of particle-associated PAH derivatives (ClPAHs, NPAHs, OPAHs) in ambient air and automobile exhaust by gas chromatography/mass spectrometry with negative chemical ionization. *Polycyclic Aromatic Compounds* **2017**, *37* (2-3), 128-140.
(131) Nilsson, U. L.; Colmsjö, A. L. Retention characteristics of chlorinated polycyclic aromatic hydrocarbons in normal phase HPLC. II. Chloro-substituted PAHs. *Chromatographia* **1992**, *34* (3-4), 115-120. DOI: 10.1007/bf02276122
(132) Peng, Y.; Xie, S. Y.; Chen, M.; Feng, Y. Q.; Yu, L. J.; Huang, R. B.; Zheng, L. S. Characterization of polychlorinated aromatic hydrocarbons by reversed-phase liquid chromatography with ultraviolet absorbance and mass spectrometric detection. *J Chromatogr A* **2003**, *1016* (1), 61-69. DOI: 10.1016/s0021-9673(03)01292-5
(133) Xie, S. Y.; Deng, S. L.; Yu, L. J.; Huang, R. B.; Zheng, L. S. Separation and identification of perchlorinated polycyclic aromatic hydrocarbons and fullerenes (C60, C70) by coupling high-performance liquid chromatography with ultraviolet absorption spectroscopy and atmospheric pressure chemical ionization mass spectrometry. *J Chromatogr A* **2001**, *932* (1-2), 43-53. DOI: 10.1016/s0021-9673(01)01252-3
(134) Xie, S. Y.; Huang, R. B.; Zheng, L. S. Separation and identification of perchlorinated polycyclic aromatic hydrocarbons by high-performance liquid chromatography and ultraviolet absorption spectroscopy. *J Chromatogr A* **1999**, *864* (1), 173-177. DOI: 10.1016/s0021-9673(99)00956-5

(135) Lofroth, G.; Nilsson, L.; Agurell, E.; Sugiyama, T. Salmonella/microsome mutagenicity of monochloro derivatives of some di-, tri- and tetracyclic aromatic hydrocarbons. *Mutat Res* **1985**, *155* (3), 91-94. DOI: 10.1016/0165-1218(85)90123-5

(136) Bhatia, A. L.; Tausch, H.; Stehlik, G. Mutagenicity of chlorinated polycyclic aromatic compounds. *Ecotoxicol Environ Saf* **1987**, *14* (1), 48-55. DOI: 10.1016/0147-6513(87)90082-0

(137) Mori, Y.; Goto, S.; Onodera, S.; Naito, S.; Matsushita, H.; Takitani, S. Changes in mutagenic properties and chemical fate of benz(*a*)anthracene in chlorine-treated water with and without bromide ion. *Chemosphere* **1993**, *27* (11), 2155-2162.

(138) Gustafsson, E.; Brunstrom, B.; Nilsson, U. Lethality and erod-inducing potency of chlorinated chrysene in chick embryos. *Chemosphere* **1994**, *29* (9-11), 2301-2308. DOI: 10.1016/0045-6535(94)90398-0

(139) Horii, Y.; Khim, J. S.; Higley, E. B.; Giesy, J. P.; Ohura, T.; Kannan, K. Relative potencies of individual chlorinated and brominated polycyclic aromatic hydrocarbons for induction of aryl hydrocarbon receptor-mediated responses. *Environ Sci Technol* **2009**, *43* (6), 2159-2165. DOI: 10.1021/es8030402

(140) Ohura, T.; Morita, M.; Kuruto-Niwa, R.; Amagai, T.; Sakakibara, H.; Shimoi, K. Differential action of chlorinated polycyclic aromatic hydrocarbons on aryl hydrocarbon receptor-mediated signaling in breast cancer cells. *Environ Toxicol* **2010**, *25* (2), 180-187. DOI: 10.1002/tox.20488

(141) Kakimoto, K.; Nagayoshi, H.; Inazumi, N.; Tani, A.; Konishi, Y.; Kajimura, K.; Ohura, T.; Nakano, T.; Tang, N.; Hayakawa, K.; Toriba, A. Identification and characterization of oxidative metabolites of 1-chloropyrene. *Chem Res Toxicol* **2015**, *28* (9), 1728-1736. DOI: 10.1021/acs.chemrestox.5b00173

(142) Huang, C.; Xu, X.; Wang, D.; Ma, M.; Rao, K.; Wang, Z. The aryl hydrocarbon receptor (AhR) activity and DNA-damaging effects of chlorinated polycyclic aromatic hydrocarbons (Cl-PAHs). *Chemosphere* **2018**, *211*, 640-647. DOI: 10.1016/j.chemosphere.2018.07.087

(143) Luo, Y.; Zhang, B.; Geng, N.; Sun, S.; Song, X.; Chen, J.; Zhang, H. Insights into the hepatotoxicity of pyrene and 1-chloropyrene using an integrated approach of metabolomics and transcriptomics. *Science of The Total Environment* **2022**, *829*, 154637.

(144) Luo, Y.; Zhang, B.; Geng, N.; Sun, S.; Song, X.; Chen, J.; Zhang, H. Transcriptomics and metabolomics analyses provide insights into the difference in toxicity of benzo[*a*]pyrene and 6-chlorobenzo[*a*]pyrene to human hepatic cells. *Science of The Total Environment* **2022**, *812*, 152242.

(145) Sakakibara, H.; Ohura, T.; Kido, T.; Yamanaka, N.; Tanimura, N.; Shimoi, K.; Guruge, K. S. Organ-specific distribution of 7-chlorinated benz[*a*]anthracene and regulation of selected cytochrome P450 genes in rats. *J Toxicol Sci* **2013**, *38* (1), 137-143. DOI: 10.2131/jts.38.137

(146) Su, C. H.; Chen, S. P.; Chen, L. Y.; Yang, J. J.; Lee, Y. C.; Lee, S. S.; Chen, H. H.; Ng, Y. Y.; Kuan, Y. H. 3-Bromofluoranthene-induced cardiotoxicity of zebrafish and apoptosis in the vascular endothelial cells via intrinsic and extrinsic caspase-dependent pathways. *Ecotoxicol Environ Saf* **2021**, *228*, 112962. DOI: 10.1016/j.ecoenv.2021.112962

(147) Park, J.; Kim, Y.; Jeon, H. J.; Kim, K.; Kim, C.; Lee, S.; Son, J.; Lee, S. E. Acute and developmental toxic effects of mono-halogenated and halomethyl naphthalenes on

zebrafish (*Danio Rerio*) embryos: Cardiac malformation after 2-bromomethyl naphthalene exposure. *Environ Pollut* **2022**, *297*, 118786. DOI: 10.1016/j.envpol.2021.118786

(148) Putz, M. V.; Putz, A.-M. Dft chemical reactivity driven by biological activity: Applications for the toxicological fate of chlorinated PAHs. *Applications of Density Functional Theory to Biological and Bioinorganic Chemistry* **2013**, 181-231.

Chapter 2: Methodology

2.1 Sample Preparation

Sample preparation is a process whereby a substance or compounds of interest (analytes) are separated and isolated from other complex components of the sample (matrix). Sample preparation can increase the selectivity of method and sensitivity of the assay by removal of interferents and enrichment of analytes; it can also convert the analyte into a more suitable form for detection and separation when necessary. Finally, it may provide a matrix-independent method that is rugged and reproducible.[1]

Extraction is one of the most common sample preparation methods, in which analytes are separated from the sample matrix and partitioned to the extracting solvent. Ideally, the solvent of choice is selective to dissolve the analytes but not interferents. Solvents can be organic liquids, supercritical liquids, or liquids bonded to a support material. The selectivity of extraction can be altered and achieved by optimizing multiple factors, such as solvent, pH, temperature, and pressure.[1]

Liquid-liquid extraction (LLE) and solid-liquid extraction (SLE) are commonly used in analytical and environmental chemistry. Liquid-liquid extraction often involves two immiscible liquids, usually an aqueous liquid and an organic solvent. However, typically large volumes of solvent and multiple extraction steps are needed to achieve exhaustive extractions, which leads to dilution of analytes and consequently extra solvent evaporation step and clean-up steps. The nature of conventional LLE makes it become both time-consuming and labor-intensive.[1,2]

When samples are not readily able to dissolve directly in solvents and chemical digestion

cannot be employed, it is not uncommon that SLE is used for sample preparation. In order to exhaustively extract hydrophobic organic analytes, Soxhlet extraction was developed in 1879, in which large volumes of solvents are refluxing boiled and recycled through the sample for long periods of time.[3]

2.2 Accelerated Solvent Extraction (ASE)

Accelerated solvent extraction (ASE) is a novel extraction method, which is accepted by the US EPA for extraction of solid matrices.[4] Unlike conventional SLE and Soxhlet extraction, ASE, also known as pressurized liquid extraction (PLE), employs a closed system and stainless-steel extraction cells, which allows the use of organic solvents at high temperatures typically greater than their boiling points.[1] It was first reported and introduced by Ezzell *et al*. and Ritcher *et al*.[5,6]

A schematic of an ASE system is shown in Figure 2.1. During the extraction, solvent is pumped into heated extraction cells filled with solid or semisolid sample (50 – 200 °C) under high pressure (500 – 3000 psi) for a prescribed period. Extracts are delivered to a collection vial for further clean-up or analysis. Advantages of ASE relative to convention extraction techniques are: i) semi-automation, ii) enhanced extraction efficiency and, iii) reduced volume of extracting solvent.

The elevated temperature can increase the solubility and diffusion rates of thermally stable analyte in solvents;[7-9] it can also enhance the disruption of interactions between analytes and sample matrix, and decrease the viscosity of solvents.[7,10] Temperatures above boiling points of solvents can be employed under high pressure conditions; high pressure can ensure faster contact of solvents and analytes.[6]

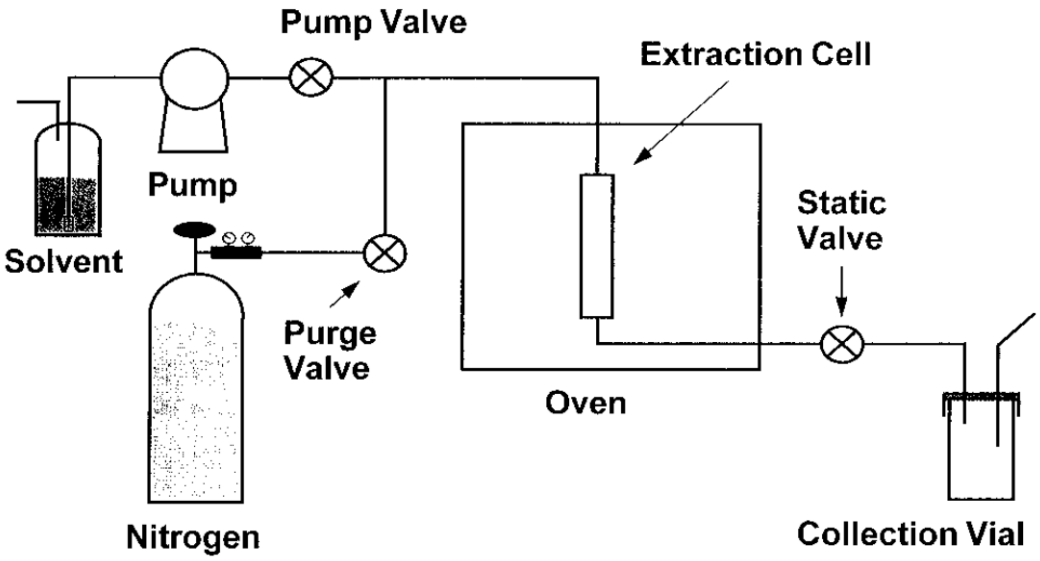

Figure 2.1: *Schematic of accelerated solvent extraction (ASE).*[6]

Analysis of soil, sediments, biota samples and other solid matrices for PAHs requires the extraction of analytes from matrix. The development of ASE extraction methods for PAHs can be traced back to the 1990s.[6,11]

For my study, there are two ASE methods used for extraction. The difference between the two ASE methods is shown in Figure 2.2. Biota samples (*ca.* 5.0 g, wet weight) are extracted as in the traditional ASE method with no addition of copper powder, 5% deactivated alumina, and silica gel. For soil or sediment samples (*ca.* 0.5 – 2.0 g), a one-step ASE method that I designed is employed.

In an ASE cell for the one-step ASE method, a glass fiber filter is first placed at the bottom of the 34 mL ASE cell. A mixture of 1 g copper, 4.5 g 5% deactivated alumina, and 5 g silica gel is placed into the cell. Samples are weighed, mixed with diatomaceous earth (DE) dispersant (1.5 g), and added to the ASE cells. The samples are then spiked with a recovery internal standard (RIS, account for the loss of analyte during sample preparation). Finally, Ottawa sand is used to fill the

dead volume of the cell to approximately 0.5 cm below the top of the cell.

The ASE extraction conditions used are as follows: system pressure 1500 psi, oven temperature 120 °C with a heat up time of 6 min., static time of 15 min. with 1 static cycle, dichloromethane (DCM) as the extraction solvent, 60% flush volume and a nitrogen purge for 80 seconds at 150 psi. After extraction, the extract was treated with sodium sulphate to remove moisture, and subsequently transferred to a 250 mL flat bottom flask.

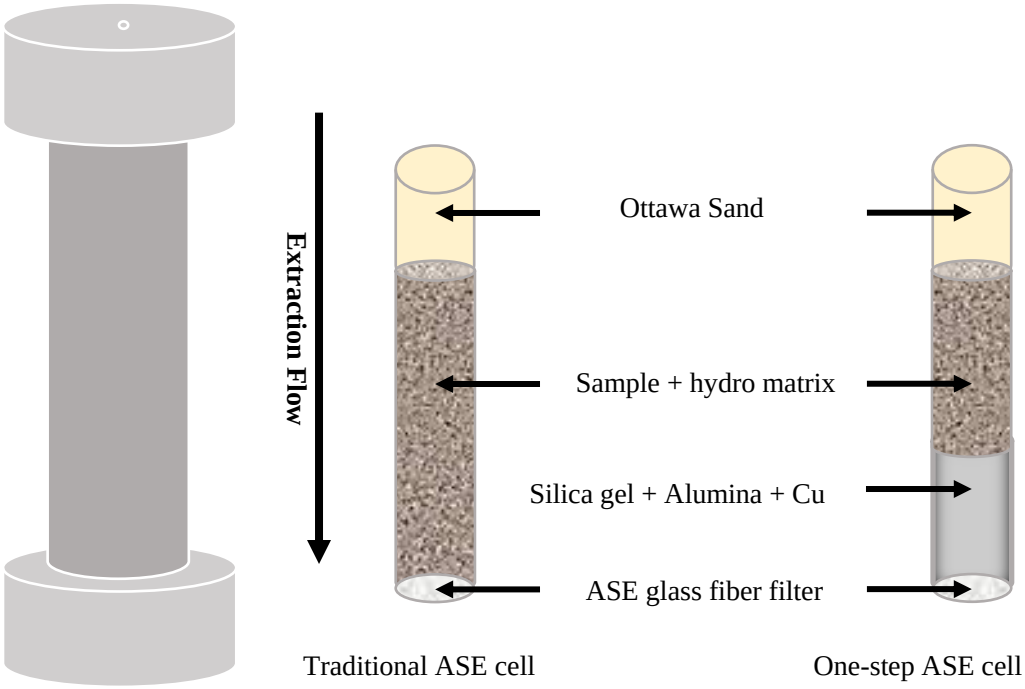

Figure 2.2: *ASE cell filled with sample for traditional (middle) and one-step ASE method (right).*

In soil and sediments, elemental sulfur, usually in octahedral sulfur form (S_8), are usually found.[12] Retention parameters of S_8 are similar to PAHs and polychlorinated biphenyls (PCBs), which could be co-extracted in solvent extraction.[13-15] However, sulfur can interrupt and impact the chromatographic quality of analysis by masking chromatographic peaks of PACs.[16] Various methods have been applied to remove sulfur, such as sodium sulfite,[17,18] mercury,[17] copper powder or granules,[19-21] silver ions,[17] gel permeation chromatography,[17] saponification (with ethanolic

potassium hydroxide).[17]

For my study, activated copper powder was employed to remove sulfur in my one-step ASE method. The chemical equation of desulfurization is shown in Equation 2.1. The activated copper powder is first prepared by adding 5% nitric acid (v/v) to copper powder (*ca.* 5 – 10 g) in a flat bottom flask (FBF) with a glass stopper. The FBF is then swirled thoroughly and occasionally for about 30 minutes. Then, the mixture is allowed to settle, and the 5% nitric acid is decanted. Copper powder is rinsed thoroughly with water (three times). Copper is then rinsed with acetone (three times), and dried by rotary evaporation and stored in a vial with screw cap before use in one-step ASE method.

$$Cu + S \rightarrow CuS \qquad \textit{Equation 2.1}$$

2.3 Microbead Beating Extraction

Currently for extraction of PACs from avian eggs, common approaches, for instance traditional liquid-liquid extraction (LLE), Soxhlet extraction methods[22-24] and the QuEChERS method,[25] are employed.

Microbead beating tissue homogenization has been used extensively in biomedical research to lysis or grind soft and hard biological material, such as brain,[26] tumor,[27] and skin tissue.[28] Sample tubes that are packed with microbeads (ceramic or stainless steel) and tissue material to be homogenized are agitated in 3-dimensions. Thus, fluidic homogenates are formed by multidirectional forceful motion for additional processing workflows.

In my study, five (5.0 ± 0.01) g of the in-house egg reference material is weighed into 15 mL high-density polypropylene microbead extraction tubes (MBETs, Bertin Technologies, Montingy-

le-Bretonneux, France) contained 2.5 g of ceramic microbeads. Eight (8) mL of dichloromethane (DCM) were then added to the MBETs. Samples are then extracted using a Precellys Evolution Homogenizer (Bertin Technologies, Montigny-le-Bretonneux, France) at 6500 rpm with 3 cycles for 20 s and 120 s between the cycles (total time of 5 min). The MBETs are then centrifuged at 5000 revolutions per minute (rpm) at 10°C for 10 min. The extract (bottom layer) is transferred to a 60 mL glass collection vial. The MBETs are then rinsed with 2 × 8 mL DCM, vortexed for 1 min and centrifuged again at 5000 rpm at 10 °C for 10 min. The rinses are combined with the initial extract. After extraction, the extract is treated with sodium sulphate to remove moisture, and subsequently transferred to a 60 mL glass collection vial with 3 washes of hexane (~2 mL each). The sample volume is then reduced to approximately 10 mL using a gentle stream of ultra-high purity (UHP) nitrogen. 500 µL of extracts are transferred to pre-weighed aluminium boats for lipid determination. Extracts (9.5 mL) are then reduced to 2.5 mL and transferred to a test tube. The glass tube is rinsed with 2 × 1 mL DCM, and rinses are then combined with the extract. The volume of extract is then adjusted to 5 mL using DCM.

2.4 Gel Permeation Chromatography (GPC)

Gel permeation chromatography (GPC), also known as size exclusion chromatography, contains a stationary phase (porous particles or beads) and a mobile phase (solvent). As shown in Figure 2.3, molecules are separated based on the molecular size and molecular hydrodynamic volume; bigger molecules (marked in red) are excluded by smaller sized pores and eluted faster, while smaller molecules (marked in blue) can pass through pores and are eluted slower due to the longer distance traveled.[29,30]

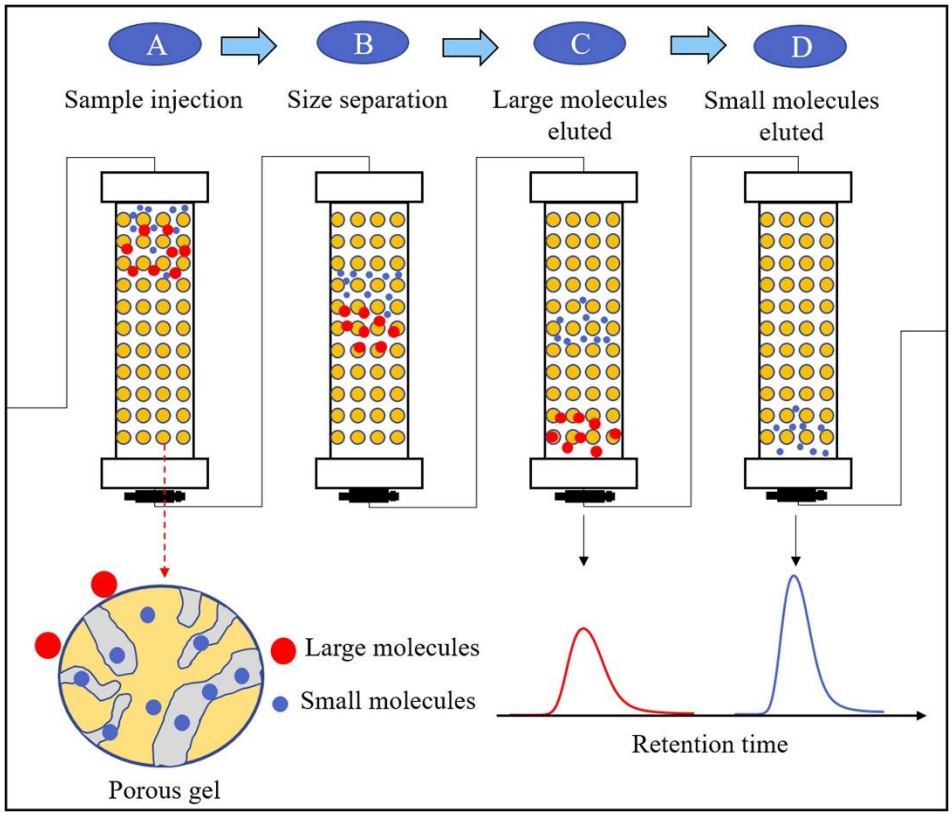

Figure 2.3: *Schematic diagram of the separation principle of gel permeation chromatography (GPC) test.*[29]

In the analyses of PACs, GPC is widely used to remove co-extracted biogenic molecules and other sample matrices, such as lipids, fats, and pigments, which can interfere or mask GC/MS analyses of biological tissues for organic contaminants.[31-34]

In my study, extracts of biological samples (5 mL) are applied to the head of a GPC column (J_2-scientific AccuPrepMPSTM, Columbia, MO, USA) packed with 60 g of S-X3 Biobeads to remove excess lipids and protein. The following parameters are employed by the fully-automated system; injection volume: 5 mL; pressure: 8 psi; flow rate: 5 mL/min; mobile phase: hexane:DCM (1:1, v/v). The first 120 mL of effluent contained lipids and other biogenic material is discarded.

The fraction containing analytes eluted from 125 – 250 mL and is collected in a 250 mL round bottom flask; the extract is then reduced in volume to 200 µL via Genevac Rocket Evaporator (Ipswich, Suffolk, UK) and nitrogen evaporation. An instrument performance internal standard (IPIS, d_{10}-anthracene, account for the instrument fluctuation and small difference in the volume of final extract) is then added to the sample. Amber GC vials are used to store the samples under refrigeration at 4 °C until they are analyzed.

2.5 Dispersive Solid Phase Extraction (dSPE)

Dispersive solid phase extraction (dSPE) was first reported by Anastassiades *et al*. as a clean-up method for pesticide extraction of food samples.[35] As an iteration of SPE, sorbent material (stationary phase) in dSPE is dispersed directly into extracts or sample matrix. In dSPE, faster mass transfer can be achieved due to closed contact between sorbent and analytes, which allows an increase in the contact surface area and thus quickens the establishment of extraction equilibrium.[36,37] In addition, the dSPE approach, also known as QuEChERS (quick, easy, cheap, effective, rugged and safe), has been commercially successful as it can be sold in ready-to-use containers, such as centrifuge tubes. To remove the matrix from the liquid phase, it is important to add a low amount of solid into the liquid phase. The retention of analytes in the liquid phase after clean-up leads to an improvement in the selectively of the method.[38]

For the determination of PACs, USEPA issued an official dSPE based method for water and sediment.[39,40] Development of various sorbent materials for dSPE method of PACs have been widely reported, for example carbon nanotubes,[41] graphene and derivatives,[42-44], metal-organic frameworks,[45-49], polymer materials,[50-53] ionic liquids,[54-57] silica-based sorbents,[58-63] and

miscellaneous materials.[64-75] Dispersive SPE has also been employed to extract PACs from other matrices, such as water,[41-43] leaves,[76] fish embryos,[77] seafood,[78] and Chinese herbal medicines.[79]

For my study, the 1 mL extracts of biological samples are transferred to a 125 mL round bottom flask with successive washes of 70:30% (DCM: hexane, 2 x 10 mL each) containing silica gel (4 g), 0.5 g sodium sulfate, and 5% deactivated alumina (1 g). The flasks are allowed to sit for 30 min with periodic swirling every 10 min. Extracts are then transferred to a 60 mL ASE tube, reduced to 200 µL by Genevac under nitrogen, fortified with the IPIS (100 pg μL^{-1}) and stored at 4 ºC in amber vials.

2.6 Silica Gel / Alumina Chromatography

It is widely acknowledged that adsorption column chromatography can be used to separate complex mixtures, such as petroleum and bitumen samples, into fractions, which contain various class of compounds.[80-82] The US EPA has adopted adsorption chromatography for the cleanup of environmental samples for the separation and detection of PAHs in their standard methods.[83-85] Adsorption column chromatography has also been employed for fractionating PACs in multiple environmental matrices, such as, dust,[86] soil and sediment,[87-89] water and wastewater,[88,90] biota and food,[91-94] oils and tars.[95,96]

Polar micro-powdered synthetic absorbents, for example silica gel and alumina, are usually employed as the stationary phase for adsorption column chromatography, as they have a nano-porous structure made of silicon dioxide and aluminum oxide.[97]

Because both silica gel and alumina are polar, the polar components of sample extracts can be retained and separated by the stationary phase. Besides, there is a subtle difference between silica

gel and alumina; silica gel is weakly acidic and thus silica preferentially retains basic compounds; alumina is slightly basic and retains acidic components.[98,99]

Various solvents and their mixtures with different polarity can be used as the mobile phase in adsorption column chromatography, for example, cyclohexane, hexane, toluene, methanol and, DCM.[80-84] For PACs, solvent mixtures such as DCM and hexane, can be used to elute the aromatic fraction of an extract off the column.[100]

The choice of stationary phase has been investigated for PACs. In general, alumina is less popular than silica gel in adsorption column chromatography due in part to the fact that alumina column chromatography is less reproducible and can decompose sensitive compounds.[97,101] In 1965, Sawicki *et al.* reported using alumina column chromatography to fractionate heterocyclic PACs, specifically nitrogen containing PACs, from air particulate matter.[102] It is not uncommon that alumina is pretreated prior to use, for example low-temperature heating, which can dehydrate aluminum hydroxide (a mixture of γ-alumina and alumina monohydrate). Freshly prepared γ-alumina is very active but tends to be affected and deactivated by adsorbing moisture.[101,102] Rosen and Middleton developed an adsorption column chromatography with silica gel to separate aliphatic, aromatic and polar fractions from petroleum refinery wastes by isooctane, benzene, and mixture of chloroform and methanol (1:1, v/v).[90]

Better selectivity and reproducibility can be achieved by activated silica gel as shown in comparative studies. The presented silanol groups (Si–O–H) on the surface of silica gel can interact with water molecules to form a hydration film around silica particles, which can affect and suppress the interaction of silica surface and PAC molecules.[97]

For my study, silica gel (*ca.* 11 g) is made into a slurry with about 20 mL of DCM, which is then transferred to a Supelco® chromatographic plugged column (300 mm × 10 mm) containing

DCM. After the silica gel settled in the column, 5 % deactivated alumina (*ca.* 1 g) is added to the column followed by sodium sulfate (*ca.* 0.5 g). Then, DCM is drained to the head of the column bed and 25 mL of hexane is added for solvent exchange. The extract (1 mL) off the GPC column is then added to the head of the column bed and first eluted with 25 mL of hexane, containing the aliphatic fraction which is discarded for my study. Then, the fraction containing PACs, which is eluted with 25 mL of DCM:hexane (50:50, v/v), is collected and evaporated to a final volume of 5 mL. The instrument performance internal standard (IPIS) is then spiked into the final extract prior to analysis on the gas chromatograph.

2.7 Gas Chromatography (GC)

Invented in 1952, GC was first reported by James and Martin for the separation of volatile fatty acids, with a stationary phase using silicone-stearic acid supported on diatomaceous earth and a mobile phase of nitrogen gas.[103] However, the concept of GC was introduced by Martin and Synge in 1941, in which they proposed that using a vapour as mobile phase should allow the separation of volatile substances.[104] Since then, GC has become one of the most important and most applied analytical techniques and has played an essential role in modern chemistry.

In GC, sample extracts are injected through an injection port, in which analytes are volatilized to gaseous state by high temperature. The mobile phase, usually an inert gas such as helium, argon, or nitrogen, is constantly fed to the GC, where analytes are separated by partitioning between the mobile- and stationary phases. The stationary phase can be a microscopic layer of liquid on inert solid support held in a capillary column. Depended on the properties of analytes, GC ovens can be run isothermally or with a ramped program; the former can be used in separation of analytes with

similar retention time, while the ramped temperature program allows for reduced analysis times.[105] At the end of the column, analytes enter the detector through a heated transfer-line. Multiple detectors can be used for detection, such as electron capture detector (ECD),[106-108] flame ionization detector (FID),[109-111] nitrogen phosphorus detector (NPD),[112-114] thermal conductivity detector (TCD),[115,116] flame photometric detector (FPD),[117,118] photoionization detector (PID),[119-121] and mass spectrometer (MS).[32,122,123] Because MS is used as the detector in my study, it will be discussed in detail in section 2.9.

The separation of analytes is determined by their p-chem properties (*e.g.*, boiling point, polarity, vapor pressure), the polarity of the stationary phase, column temperature, carrier gas flow rate and column length. The retention of analytes is also affected by these factors; analytes, which have more similar polarity to that of the stationary phase, are partitioned longer in the stationary phase and eluted later. Of all the parameters, it is boiling point of analytes that governs retention and elution; electron-rich functional groups and shape of analytes (*e.g.*, chirality) can also affect the partitioning by selective interactions.[124]

Helium is the most popular carrier gas because it is safe, inert and can reach the desired purity.[125] Hydrogen can also provide similar chromatography performance with shorter time as it offers higher optimum linear velocity for GC.[125,126] Considering the decreased availability and high cost of helium, hydrogen has been studied as a replacement of helium as the carrier gas.[127,128]

Gas chromatography has been widely employed in analyses of separation for complex mixtures, such as plant extracts,[129] essential oil,[130] tobacco smoke,[131] blood,[132], and petroleum.[130-132] Gas chromatography has also been extensively applied in the determination and quantification of PACs from various matrices, for instance biota,[133,134] air,[135-138] water,[88,139-141] foods,[142-144] soil and sediment.[87,88,145]

For the analysis of PACs in my study, an Agilent 7890B gas chromatograph (Agilent Technologies, Wilmington, DE, USA) is used. Analytes of interests are injected on an Agilent J&W DB-5ms Ultra Inert column of 30 m length, 0.25 mm internal diameter and 0.25 μm film thickness. Helium is used as the carrier gas at a constant flow rate of 1.2 mL/min. The injection volume of sample extract used is 1 μL via a PAL RSI 85 auto sampler in splitless mode at 250 ºC. The GC oven temperature is held at 60 °C for 1 min then raised to 210°C at 35 °C/min, further ramped up to 260 °C at 2 °C/min, 300 °C at 10 °C/min and held for 5 min, and finally to 325 °C at 50 °C/min and held for 5.5 min. Both transfer line and source temperature are set at 320 °C and UHP nitrogen is used as the collision gas at 60 psi.

2.8 Two-dimensional Gas Chromatography (2D GC / GC × GC)

Two-dimensional gas chromatography (2D GC) was first proposed by Giddings in 1984.[146] In brief, the best result of 2D separation can be obtained by two independent separation mechanisms. For example, in two-dimensional thin layer chromatography (2D TLC), analytes are first separated in one direction with one solvent and then separated by another solvent in another direction that is perpendicular to the primary direction.[147] In order to perform 2D GC, the separation mechanism of two columns must be different to be qualified as perpendicular.[148,149]

The resolution of 2D separation (R_{2D}) is shown in Equation 2.2, where R_1 and R_2 are resolutions of primary and secondary dimensions.[146] For GC, the resolution (R) can be calculated as shown in Equation 2.3, where t_r represents retention time and W represents the width of chromatographic peaks.[105]

$$R_{2D} = \sqrt{(R_1^2 + R_2^2)} \qquad \textit{Equation 2.2}$$

$$R = \frac{t_{r,2} - t_{r,1}}{0.5(W_2 + W_1)} = \frac{\Delta t_r}{W_2 + W_1} \qquad \textit{Equation 2.3}$$

Conventionally, the heart-cutting approach was employed for 2D GC, in which only a portion of material from the first column is introduced to the second column. This technique can only improve the resolution of analytes in the selected region. In modern 2D GC, also known as comprehensive 2D GC, two capillary columns with two different stationary phases (orthogonal phases) and often with different column lengths are employed for the 2D separation. As shown in Figure 2.4, 2D GC typically includes the following parts: (a) injector; (b) primary column; (c) column connectors; (d) GC × GC interface; (e) secondary column; (f) detector; (g) optional division for secondary oven. At the thermal modulator, all effluent from the primary column is injected into the secondary column by periodical pulsing with heating and a cooling process.[148] In 1991, 2D GC was first reported by Liu and Phillips, in which an on-column thermal modulator was used to analyze hydrocarbon mixture.[150]

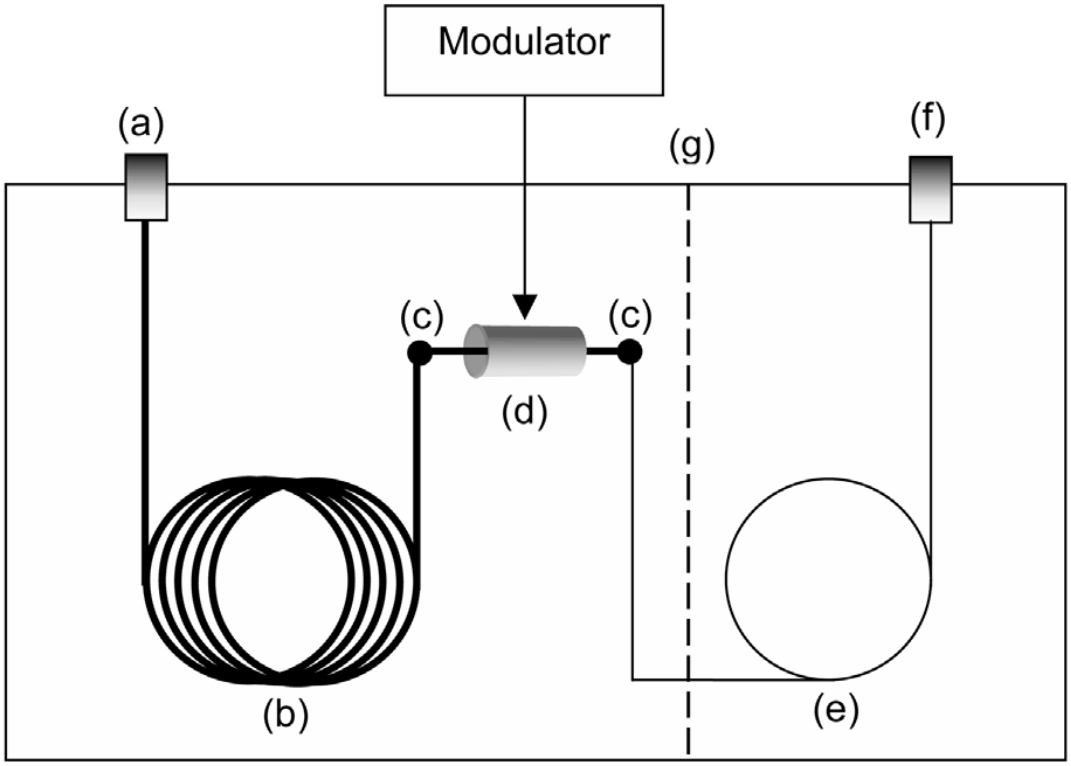

Figure 2.4: *Block diagram of a GC × GC system.*[148]

Reprinted with permission from Gorecki, T.; Harynuk, J.; Panic, O. The evolution of comprehensive two-dimensional gas chromatography (GC × GC). *J Sep Sci* **2004**, *27* (5-6), 359-379. Copyright ©2004 John Wiley and Sons.

There are two main types of modulation, namely thermal modulation and valve-based modulation. The cryogen-based thermal modulator, which is used in my study, is a type of thermal modulator; peaks from the primary column are trapped and cryogenically refocused by rapid cooling by liquid nitrogen, and then injected to the secondary column.

For my study, a 7890A gas chromatograph (Agilent Technologies, Wilmington, DE, USA) is employed, which is fitted with a split/splitless injector, GC x GC thermal modulator (operated at -80 ºC) and a secondary oven. The column configurations consist of an Rxi -5Sil MS (60 m × 0.25 mm × 0.25 μm) (Restek, Bellefonte, PA, USA) followed by an Rxi-17Sil MS (2 m × 0.25 mm × 0.25 μm) (Restek, Bellefonte, PA, USA). The GC columns are connected using an SGE micro

union connector. Standards and samples (2 μL) are injected at 250 ºC in the splitless mode. The sample is analyzed in both one dimensional and 2D modes. In one dimensional mode, the oven is programmed from 80 ºC (held for 1 min), heated to 210 ºC at 35 ºC/min, further to 260 ºC at 3◦C/min and finally to 315 ºC at 10 ºC/min (held for 5 min). The temperature ramp in 2D mode is programmed from 55ºC (held for 2 min) heated to 110ºC at 10ºC/min, further to 210ºC at 3 ºC/min and finally to 310 ºC at 8 ºC/min (held for 15 min). The modulation period is 2 s (hot pulse, 0.7 s; cool time, 0.3 s). Other parameters used in both modes are the MS transfer line temperature set to 300 ºC and helium (carrier gas) is supplied at 1.4 mL/min.

2.9 Mass Spectrometry (MS)

Mass spectrometry (MS) is a useful and powerful technique in modern analytical chemistry and used to determine the mass and structure of compounds.[105] In 1897, Sir Joseph J. Thomson, 1906 Nobel Laureate in Physics,[151] discovered unknown negatively charged particles (electrons) when studying the cathode rays and determined the charge to mass ratio (*e/m*) of electrons.[152] Thomson also found the isotope of stable elements in 1913.[153,154] MS was created by Francis W. Aston in 1919, who also identified isotopes of 212 naturally occurring isotopes and won the Nobel Prize for chemistry in 1922.[105,155] In recent decades, there have been significant advances in the field and MS is now perhaps the most common method found in the modern-day analytical laboratory.

The most important parts in a typical mass spectrometer include the ionization source, the mass analyzer and detector, which are shown in Figure 2.5. Generally, analytes are volatilized into their gaseous state inside the ionization source; after they are ionized, analytes are accelerated by

an electrical field and transferred to a mass analyzer, where analyte particles and fragments are separated by their mass-to-charge ratio (*m/z* ratio); then, the analytes and fragments are detected by a detector, and corresponding signals are sent and integrated on computer systems as peaks on the mass spectrum.[105]

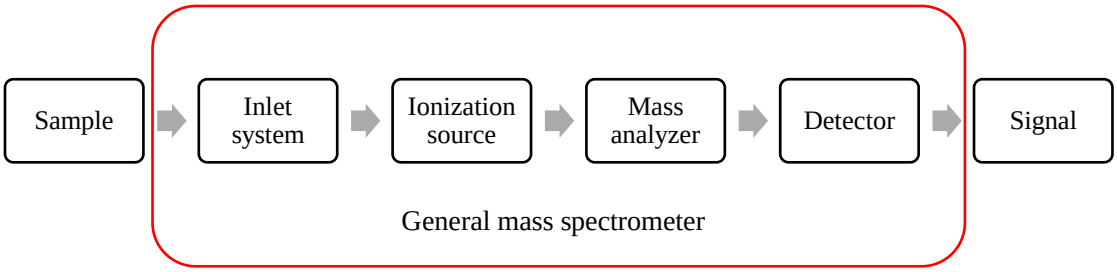

Figure 2.5: *Block diagram of a general mass spectrometer.*

It is not uncommon that the MS is coupled with various instrumentation, such as gas chromatography (GC), high-performance liquid chromatography (HPLC), and inductively coupled plasma (ICP), which could be useful for separating target analytes from complex matrices. Mass spectrometry is one of the most widely adapted techniques for identification and quantification for organic compounds; its use has been reported in different subdisciplines of scientific research, such as agriculture,[156] food science,[157] pharmacology,[158] toxicology,[159] medicinal science,[160] and environmental science.[136]

2.10 Ionization Techniques

Neutral analyte molecules are injected into the MS and first ionized in the ionization source; multiple techniques have been adapted based on properties of analytes and the requirement of detection. Depending on the ionization mode, the ionization process can produce either positive or negative ions. There are various ionization techniques, including electron ionization (EI), chemical

ionization (CI), electrospray ionization (ESI), matrix-assisted laser desorption/ionization (MALDI), fast atom bombardment (FAB), desorption electrospray ionization (DESI), direct analysis in real time (DART), atmospheric-pressure chemical ionization (APCI), inductively coupled plasma (ICP), field desorption. In my study, EI is the only ionization technique that has been employed and will be discussed in detail.

Electron ionization is also known as electron impact ionization, was first reported by Arthur J. Dempster in 1918 but then the beam of electrons directly ionized solid surfaces.[161] In 1929, Walker Bleakney used focused monoenergetic electrons to ionize gaseous atoms.[162] EI is a common technique for MS, and it can be used for small, volatile, thermally stable compounds.

In the EI ionization source, volatilized analyte molecules (M) are bombarded with a stream of electrons with a spiral trajectory (usually 70 eV kinetic energy) that are emitted from a heated tungsten or rhenium filament and accelerated by an electric field; then, analyte molecules will lose of an electron and form the molecular ion ($M^{+\cdot}$) as the kinetic energy of the electron is higher than ionization energy of the molecule. Ionization efficiency of EI is about 0.01% to 0.1%.[105,163] The process of ionization is shown in Equation 2.4.

$$M + e^- \rightarrow M^{+\cdot} + 2e^- \qquad \textit{Equation 2.4}$$

Because the ionization energy for typical organic compounds is about 10 to 20 eV, excess energy from 70 eV will be stored in organic molecules as internal energy; since internal energy of organic molecules outpaces vibrational or bond energy, they will fragment in the ionization source, which is the reason why EI is regarded as a hard ionization technique. By lowering the kinetic energy of the electron in the EI ionization source, it can make the ionization process softer and retain more intact molecular ions.

2.11 Mass Analyzers

Ions that are produced in the ionization source are introduced into a mass analyzer, where they are separated according to their *m/z* ratio. There are several common types of mass analyzers, including sectors, quadrupoles, triple quadrupoles (QqQ), ion traps, Fourier transform ion cyclotron resonance (FTICR) and time-of-flight (TOF). In my study, both QqQ and TOF mass analyzers were employed and thus are described below.

The performance of a mass analyzer can be assessed based on five main characteristics, *viz.,* the mass range limit, the analysis speed (scan speed), the transmission, the mass accuracy and the resolution.

The mass range determines the range of *m/z* can be measured by a mass analyzer. The scan speed is the rate of the mass analyzer that can measure over the mass range. The transmission can be evaluated by the ratio of the number of ions reaching the detector and the number of the ions entering the mass analyzer. The accuracy of the *m/z* value provided by the analyzer can be used to assess the measurement error of instrument The mass measurement error, also known as the mass accuracy, can be further determined as the difference between the theoretical *m/z* and the measured *m/z* by the spectrometer as shown in Equation 2.5. Since mass accuracy can be either positive or negative, ± 5 parts per million (ppm) is an accepted limit for compound identification and/or assigning correct formulae with high level of confidence.[164]

$$mass\ accuracy = \frac{{}^{m}/_{z_{measured}} - {}^{m}/_{z_{theoretical}}}{{}^{m}/_{z_{theoretical}}} \times 10^{6} \qquad \textit{Equation 2.5}$$

Resolution (R) is used to measure the ability of a mass analyzer to distinguish peaks for two ions with a small m/z difference in the mass spectrum. R can be calculated by Equation 2.6, where M is *m/z* value of (the second) peak and ΔM is the difference of *m/z* between the two ions. The

greater the R value, the more capable a mass analyzer to distinguish ions with small *m/z* difference.

$$R = \frac{M}{\Delta M} \qquad \textit{Equation 2.6}$$

For my study, an Agilent 7000C QqQMS (Agilent Technologies, Wilmington, DE, USA) and TOF analyzer (Pegasus 4D HRT, LECO, St Joseph, MI, USA) were employed. The detailed EI fragmentation patterns and measured experimental masses are shown in Table I.1.

2.11.1 Triple Quadrupole Mass Spectrometer (QqQMS)

In the 1970s, Christie G. Enke and Richard Yost published an experiment on a triple quadrupole mass spectrometer (QqQMS), with the instrument of J.D. Morrison.[165] Although it is named as triple quadrupole, only the first and third quadrupoles (Q_1, Q_3) are used as real quadrupoles, which are opposing parallel hyperbolic metal rods; the second "quadrupole" (q_2), the collision cell, uses a hexapole in practice.[166,167] The schematic of QqQ is shown in Figure 2.6. QqQMS allows transmission of a small mass region (generally < 1 amu, atomic mass unit) of selected *m/z* values for certain ions or a range of *m/z* values by applying a combination of constantly varying voltages through the axis of the rods.

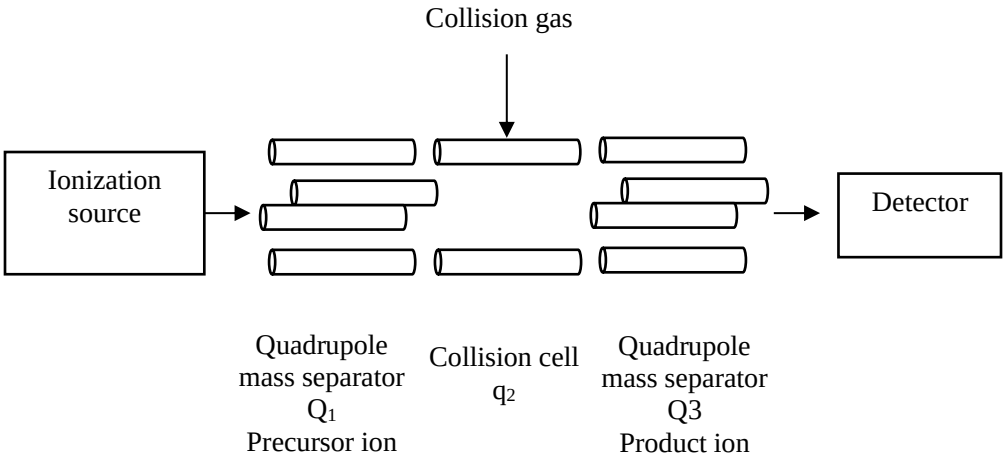

Figure 2.6: *Triple quadrupole mass spectrometer.*

Quadrupole MS is known to have high sensitivity, good dynamic range, slower scan rates than TOFMS.[168] Besides, it also has a relatively short distance between the ion source and the detector due to efficient focusing features. Ions are resolved by quadrupole MS on their *m/z* rather than the momentum or kinetic energy of ions like TOF analyzers.[169]

The QqQMS can be operated in full scan mode, by which all ions are scanned. It can also be operated in single ion monitoring (SIM) mode and multiple reaction monitoring (MRM) mode. In MRM mode of QqQMS, ions in Q_1 are filtered for known *m/z* for precursor ions and then enter q_2 will be bombarded with collision gas, like N_2, where ions fragment into smaller pieces under collision induced dissociation (CID). All ion fragments enter Q_3 are filtered for certain *m/z* for product ions. Since MRM detects specific mass transitions for certain precursor ion to product ion, this makes QqQMS more selective than quadrupole MS in identifying and quantifying analytes.

2.11.2 Time-of-Flight Mass Spectrometer (TOFMS)

Time-of-flight MS is a technique that separates ions based on their *m/z* ratio as a function of their flight time. Pulsed ions are accelerated by an electric field (potential is V); ions enter the TOF tube under low pressure with specific length d; if there is a reflector, the direction of ion path is reflected and then detected by detector. A reflector or ion mirror, first proposed by Mamyrin,[170] is a series of evenly spaced electrodes with applied linear electric field and can reduce the KE distribution of ions. The reflector is in the field-free region opposite to the ion source, while the detector is located on the side of the ion source to capture the reflected ions.

The principle of *m/z* separation is shown in Equation 2.7; *m/z* is related to the square of flight time, t^2. Other than by using a reflector, resolution of TOFMS can also be enhanced by applying delayed extraction grid and orthogonal ion injection.[171-173]

$$\frac{m}{z} = \frac{2eVt^2}{d^2} \qquad \textit{Equation 2.7}$$

With delayed extraction mode, there is a time lag that is applied between ion formation and extraction; then, ions are extracted by a voltage pulse. In continuous extraction mode, voltage is applied for immediate extraction.

Time-of-flight MS is a high-resolution MS with resolving power ranging from 1 000 to 25 000 and mass accuracy down to about 0.001 ppm; its mass range is up to 10^6 *m/z* units, but in reality, it might be limited by the time of flight of heavy ions.[105] Sensitivity of detection for time signal current image is affected and hindered by internal and thermal noise. Due to the high transmission efficiency, TOFMS has good sensitivity.

The resolution of TOFMS is proportional to the flight path and time, which indicates that increasing the length of TOF tube can increase resolution.[174] However, increased TOF tube length can result in increasing loss of ions by scattering after collision with gas molecules in TOF tube and thus decreasing the performance of the TOF analyzer.

For my study, the TOF instrument (Pegasus 4D HRT, LECO, St Joseph, MI, USA) used is made to have a novel flight path named the folded flight path (FFP™) technology. Similar to the reflector TOF, FFP™ increases flight path for ions and hence increased resolving power. Ions that are generated in the ion source can be refocused by passing through a parallel set of electrostatic mirrors. FFP™ also mitigates the dispersion based on energy, which improve transmission efficiency of the ions and improves sensitivity. The FFP™ technology in combination with the KADAS, a patented ion statistics-based, acquisition system on LECO instrument permits mass accuracy better than 1ppm, with acquisition rates of 200 spectra/second in the two-dimensional mode.[175]

2.12 References

(1) Smith, R. M. Before the injection—modern methods of sample preparation for separation techniques. *Journal of Chromatography A* **2003**, *1000* (1-2), 3-27.

(2) Ramos, L. Critical overview of selected contemporary sample preparation techniques. *J Chromatogr A* **2012**, *1221*, 84-98. DOI: 10.1016/j.chroma.2011.11.011

(3) Soxhlet, F. v. Die gewichtsanalytische bestimmung des milchfettes. *Dingler's Polytechnisches Journal* **1879**, *232* (461-465).

(4) U.S. EPA. Method 3545a (SW-846): Pressurized fluid extraction (PFE). 2007.

(5) Ezzell, J. L.; Richter, B. E.; Felix, W. D.; Black, S. R.; Meikle, J. E. A comparison of accelerated solvent extraction with conventional solvent extraction of organophosphorus pesticides and herbicides. *LC GC* **1995**, *13* (5), 390-398.

(6) Richter, B. E.; Jones, B. A.; Ezzell, J. L.; Porter, N. L.; Avdalovic, N.; Pohl, C. Accelerated solvent extraction: A technique for sample preparation. *Analytical chemistry* **1996**, *68* (6), 1033-1039.

(7) Perry, R. H.; Green, D. W.; Southard, M. Z. Perry's chemical engineers' handbook. 6 ed.; McGraw-Hill 1984.

(8) Pitzer, K. S.; Brewer, L. Thermodynamics. 2 ed.; McGraw-Hill, 1961.

(9) Sekine, T.; Hasegawa, Y. *Solvent extraction chemistry*; Marcel Dekker, 1977.

(10) Pimentel, G. C.; McClellan, A. L. *The hydrogen bond*; WH freeman, 1960.

(11) Schantz, M. M.; Nichols, J. J.; Wise, S. A. Evaluation of pressurized fluid extraction for the extraction of environmental matrix reference materials. *Analytical Chemistry* **1997**, *69* (20), 4210-4219.

(12) Mechlińska, A.; Wolska, L.; Namieśnik, J.; Wolska, L. Removal of sulfur from a solvent extract. *TrAC Trends in Analytical Chemistry* **2012**, *31*, 129-133. DOI: 10.1016/j.trac.2011.06.022

(13) Billon, G.; Ouddane, B.; Boughriet, A. Chemical speciation of sulfur compounds in surface sediments from three bays (Fresnaye, Seine and Authie) in northern France, and identification of some factors controlling their generation. *Talanta* **2001**, *53* (5), 971-981. DOI: 10.1016/s0039-9140(00)00586-5

(14) Rogowska, J.; Sychowska, J.; Cieszynska-Semenowicz, M.; Wolska, L. Elemental sulfur in sediments: Analytical problems. *Environ Sci Pollut Res Int* **2016**, *23* (24), 24871-24879. DOI: 10.1007/s11356-016-7739-1

(15) Schubert, P.; Fernández-Escobar, I.; Rosenberg, E.; Bayona, J.-M. Evaluation of desulfurization procedures for the elimination of sulfur interferences in the organotin analysis of sediments. *Journal of Chromatography A* **1998**, *810* (1-2), 245-251. DOI: 10.1016/s0021-9673(98)00197-6

(16) Muir, D.; Sverko, E. Analytical methods for pcbs and organochlorine pesticides in environmental monitoring and surveillance: A critical appraisal. *Anal Bioanal Chem* **2006**, *386* (4), 769-789. DOI: 10.1007/s00216-006-0765-y

(17) Smedes, F.; De Boer, J. Determination of chlorobiphenyls in sediments—analytical methods. *TrAC Trends in Analytical Chemistry* **1997**, *16* (9), 503-517.

(18) Japenga, J.; Wagenaar, W.; Smedes, F.; Salomons, W. A new, rapid clean-up procedure for the simultaneous determination of different groups of organic micropollutants in sediments; application in two european estuarine sediment studies. *Environmental Technology* **1987**,

8 (1-12), 9-20.
(19) Goerlitz, D. F.; Law, L. M. Note on removal of sulfur interferences from sediment extracts for pesticide analysis. *Bull Environ Contam Toxicol* **1971**, *6* (1), 9-10. DOI: 10.1007/BF01559064
(20) Covaci, A.; Gheorghe, A.; Redekker, E. S.; Schepens, P. Distribution of organochlorine and organobromine pollutants in two sediment cores from the Scheldt Estuary (Belgium). *Organohalogen compounds* **2002**, *57*, 329-332.
(21) Pardos, M.; Benninghoff, C.; Thomas, R. L.; Khim‐Heang, S. Confirmation of elemental sulfur toxicity in the microtox® assay during organic extracts assessment of freshwater sediments. *Environmental Toxicology and Chemistry: An International Journal* **1999**, *18* (2), 188-193.
(22) Hebert, C. E.; Weseloh, D. V.; Macmillan, S.; Campbell, D.; Nordstrom, W. Metals and polycyclic aromatic hydrocarbons in colonial waterbird eggs from Lake Athabasca and the Peace-Athabasca Delta, Canada. *Environ Toxicol Chem* **2011**, *30* (5), 1178-1183. DOI: 10.1002/etc.489
(23) Pereira, M. G.; Walker, L. A.; Wright, J.; Best, J.; Shore, R. F. Concentrations of polycyclic aromatic hydrocarbons (PAHs) in the eggs of predatory birds in Britain. *Environ Sci Technol* **2009**, *43* (23), 9010-9015. DOI: 10.1021/es901805e).
(24) Power, A.; White, P.; McHugh, B.; Berrow, S.; McKeown, A.; Crowley, D.; Newton, S.; McGovern, E.; Murphy, S.; O'Connor, I. Polycyclic aromatic hydrocarbons (PAHs) in seabird eggs in Ireland. *Mar Pollut Bull* **2021**, *170*, 112636. DOI: 10.1016/j.marpolbul.2021.112636
(25) Patil, R.; Chatterjee, N. S.; Kamble, N.; Nerpagar, A.; Langade, N.; Kandaswamy, C.; Presley, S. I. D.; Banerjee, K. Multiresidue analysis of pesticides, polyaromatic hydrocarbons and polychlorinated biphenyls in poultry meat and chicken eggs by GC-MS/MS: Method development and validation. *J Environ Sci Health B* **2022**, *57* (4), 263-283. DOI: 10.1080/03601234.2022.2047390
(26) Homogenization of 6 mouse brains in a 15ml precellys® tube using the precellys® evolution. *BioTechniques* **2018**, *65* (4), 231-231. DOI: 10.2144/btn-2018-2007
(27) Dubacq, S. Performing efficient sample preparation with hard tumor tissue: Precellys® bead-beating homogenizer solution. *Nature Methods* **2016**, *13* (4), i-iii. DOI: 10.1038/nmeth.f.394
(28) Ayella, A.; Verollet, R. Comparison of protein and DNA profiles of mice skin tissue and colon cancer, cells extracted with the precellys® 24 vs. Conventional methods. *The FASEB Journal* **2008**, *22* (S2), 251-251. DOI: 10.1096/fasebj.22.2_supplement.251
(29) Ma, J.; Sun, G.; Sun, D.; Yu, F.; Hu, M.; Lu, T. Application of gel permeation chromatography technology in asphalt materials: A review. *Construction and Building Materials* **2021**, *278*, 122386. DOI: 10.1016/j.conbuildmat.2021.122386
(30) Williams, T. Gel permeation chromatography: A review. *Journal of Materials Science* **1970**, *5* (9), 811-820. DOI: 10.1007/bf00562169
(31) Fromberg, A.; Hojgard, A.; Duedahl-Olesen, L. Analysis of polycyclic aromatic hydrocarbons in vegetable oils combining gel permeation chromatography with solid-phase extraction clean-up. *Food Addit Contam* **2007**, *24* (7), 758-767. DOI: 10.1080/02652030601150505
(32) Hassan, J.; Farahani, A. GC–MS determination of PAHs in fish samples following salting-out-assisted solvent extraction-gel permeation chromatography. *Chromatographia* **2011**, *74* (5-6), 477-482. DOI: 10.1007/s10337-011-2093-4

(33) Lian, W.; Ren, F.; Tang, L.; Dong, D. Analysis of polycyclic aromatic hydrocarbons in cigarette samples using gel permeation chromatography clean-up by gas chromatography–tandem mass spectrometry. *Microchemical Journal* **2016**, *129*, 194-199. DOI: 10.1016/j.microc.2016.06.021
(34) Luo, Y. B.; Chen, X. J.; Zhang, H. F.; Jiang, X. Y.; Li, X.; Li, X. Y.; Zhu, F. P.; Pang, Y. Q.; Hou, H. W. Simultaneous determination of polycyclic aromatic hydrocarbons and tobacco-specific n-nitrosamines in mainstream cigarette smoke using in-pipette-tip solid-phase extraction and on-line gel permeation chromatography-gas chromatography-tandem mass spectrometry. *J Chromatogr A* **2016**, *1460*, 16-23. DOI: 10.1016/j.chroma.2016.07.018
(35) Anastassiades, M.; Lehotay, S. J.; Štajnbaher, D.; Schenck, F. J. Fast and easy multiresidue method employing acetonitrile extraction/partitioning and "dispersive solid-phase extraction" for the determination of pesticide residues in produce. *Journal of AOAC INTERNATIONAL* **2003**, *86* (2), 412-431. DOI: 10.1093/jaoac/86.2.412
(36) Sajid, M.; Nazal, M. K.; Ihsanullah, I. Novel materials for dispersive (micro) solid-phase extraction of polycyclic aromatic hydrocarbons in environmental water samples: A review. *Anal Chim Acta* **2021**, *1141*, 246-262. DOI: 10.1016/j.aca.2020.07.064
(37) Scigalski, P.; Kosobucki, P. Recent materials developed for dispersive solid phase extraction. *Molecules* **2020**, *25* (21), 4869. DOI: 10.3390/molecules25214869
(38) Chisvert, A.; Cárdenas, S.; Lucena, R. Dispersive micro-solid phase extraction. *TrAC Trends in Analytical Chemistry* **2019**, *112*, 226-233. DOI: 10.1016/j.trac.2018.12.005
(39) US EPA. Method 8272—parent and alkyl polycyclic aromatics in sediment pore water by solid-phase microextraction and gas chromatography/mass spectrometry in selected ion monitoring mode. US EPA Washington, DC, USA: 2007.
(40) Lashgari, M.; Singh, V.; Pawliszyn, J. A critical review on regulatory sample preparation methods: Validating solid-phase microextraction techniques. *TrAC Trends in Analytical Chemistry* **2019**, *119*, 115618.
(41) Yazdanpanah, M.; Nojavan, S. Micro-solid phase extraction of some polycyclic aromatic hydrocarbons from environmental water samples using magnetic β-cyclodextrin-carbon nano-tube composite as a sorbent. *Journal of Chromatography A* **2019**, *1585*, 34-45.
(42) Pang, L.; Zhang, W.; Zhang, W.; Chen, P.; Yu, J.; Zhu, G.-T.; Zhu, S. Magnetic graphene solid-phase extraction in the determination of polycyclic aromatic hydrocarbons in water. *RSC advances* **2017**, *7* (85), 53720-53727.
(43) Mateos, R.; Vera-López, S.; Saz, M.; Díez-Pascual, A. M.; San Andrés, M. P. Graphene/sepiolite mixtures as dispersive solid-phase extraction sorbents for the anaysis of polycyclic aromatic hydrocarbons in wastewater using surfactant aqueous solutions for desorption. *Journal of Chromatography A* **2019**, *1596*, 30-40.
(44) Han, Q.; Wang, Z.; Xia, J.; Chen, S.; Zhang, X.; Ding, M. Facile and tunable fabrication of Fe_3O_4/graphene oxide nanocomposites and their application in the magnetic solid-phase extraction of polycyclic aromatic hydrocarbons from environmental water samples. *Talanta* **2012**, *101*, 388-395.
(45) Huo, S. H.; Yan, X. P. Facile magnetization of metal-organic framework MIL-101 for magnetic solid-phase extraction of polycyclic aromatic hydrocarbons in environmental water samples. *Analyst* **2012**, *137* (15), 3445-3451. DOI: 10.1039/c2an35429b
(46) Zhang, S.; Yao, W.; Ying, J.; Zhao, H. Polydopamine-reinforced magnetization of zeolitic imidazolate framework ZIF-7 for magnetic solid-phase extraction of polycyclic aromatic hydrocarbons from the air-water environment. *J Chromatogr A* **2016**, *1452*, 18-26. DOI:

10.1016/j.chroma.2016.05.039
(47) Xia, L.; Liu, L.; Dou, Y.; Guo, L.; Li, G.; Sun, Z.; You, J. A stable mesoporous metal‐organic framework as highly efficient sorbent of dispersive micro solid‐phase extraction for the determination of polycyclic aromatic hydrocarbons by HPLC. *Journal of separation science* **2018**, *41* (23), 4331-4339.
(48) Amiri, A.; Ghaemi, F.; Maleki, B. Hybrid nanocomposites prepared from a metal-organic framework of type MOF-199 (Cu) and graphene or fullerene as sorbents for dispersive solid phase extraction of polycyclic aromatic hydrocarbons. *Microchimica Acta* **2019**, *186*, 1-8.
(49) Rocio-Bautista, P.; Pino, V.; Ayala, J. H.; Pasan, J.; Ruiz-Perez, C.; Afonso, A. M. A magnetic-based dispersive micro-solid-phase extraction method using the metal-organic framework HKUST-1 and ultra-high-performance liquid chromatography with fluorescence detection for determining polycyclic aromatic hydrocarbons in waters and fruit tea infusions. *J Chromatogr A* **2016**, *1436*, 42-50. DOI: 10.1016/j.chroma.2016.01.067
(50) Villar-Navarro, M.; Martin-Valero, M. J.; Fernandez-Torres, R. M.; Callejon-Mochon, M.; Bello-Lopez, M. A. Easy, fast and environmental friendly method for the simultaneous extraction of the 16 EPA PAHs using magnetic molecular imprinted polymers (mag-MIPs). *J Chromatogr B Analyt Technol Biomed Life Sci* **2017**, *1044-1045*, 63-69. DOI: 10.1016/j.jchromb.2016.12.009
(51) Mehdinia, A.; Haddad, H.; Mozaffari, S. Polyimide‐coated magnetic nanoparticles as a sorbent in the solid‐phase extraction of polycyclic aromatic hydrocarbons in seawater samples. *Journal of Separation Science* **2016**, *39* (17), 3418-3427.
(52) Reyes-Gallardo, E. M.; Lucena, R.; Cardenas, S.; Valcarcel, M. Magnetic nanoparticles-nylon 6 composite for the dispersive micro solid phase extraction of selected polycyclic aromatic hydrocarbons from water samples. *J Chromatogr A* **2014**, *1345*, 43-49. DOI: 10.1016/j.chroma.2014.04.033
(53) Amiri, A.; Baghayeri, M.; Kashmari, M. Magnetic nanoparticles modified with polyfuran for the extraction of polycyclic aromatic hydrocarbons prior to their determination by gas chromatography. *Microchimica Acta* **2016**, *183*, 149-156.
(54) Galan-Cano, F.; Alcudia-Leon Mdel, C.; Lucena, R.; Cardenas, S.; Valcarcel, M. Ionic liquid coated magnetic nanoparticles for the gas chromatography/mass spectrometric determination of polycyclic aromatic hydrocarbons in waters. *J Chromatogr A* **2013**, *1300*, 134-140. DOI: 10.1016/j.chroma.2013.03.026
(55) Zhang, Q.; Yang, F.; Tang, F.; Zeng, K.; Wu, K.; Cai, Q.; Yao, S. Ionic liquid-coated Fe_3O_4 magnetic nanoparticles as an adsorbent of mixed hemimicelles solid-phase extraction for preconcentration of polycyclic aromatic hydrocarbons in environmental samples. *Analyst* **2010**, *135* (9), 2426-2433.
(56) Shahriman, M. S.; Ramachandran, M. R.; Zain, N. N. M.; Mohamad, S.; Manan, N. S. A.; Yaman, S. M. Polyaniline-dicationic ionic liquid coated with magnetic nanoparticles composite for magnetic solid phase extraction of polycyclic aromatic hydrocarbons in environmental samples. *Talanta* **2018**, *178*, 211-221. DOI: 10.1016/j.talanta.2017.09.023
(57) Liu, X.; Lu, X.; Huang, Y.; Liu, C.; Zhao, S. Fe_3O_4@ ionic liquid@ methyl orange nanoparticles as a novel nano-adsorbent for magnetic solid-phase extraction of polycyclic aromatic hydrocarbons in environmental water samples. *Talanta* **2014**, *119*, 341-347.
(58) Liu, Y.; Li, H.; Lin, J. M. Magnetic solid-phase extraction based on octadecyl functionalization of monodisperse magnetic ferrite microspheres for the determination of

polycyclic aromatic hydrocarbons in aqueous samples coupled with gas chromatography-mass spectrometry. *Talanta* **2009**, *77* (3), 1037-1042. DOI: 10.1016/j.talanta.2008.08.013
(59) Nascimento, M. M.; Olimpio da Rocha, G.; Bittencourt de Andrade, J. Simple and effective dispersive micro-solid phase extraction procedure for simultaneous determination of polycyclic aromatic compounds in fresh and marine waters. *Talanta* **2019**, *204*, 776-791. DOI: 10.1016/j.talanta.2019.06.061
(60) Galán-Cano, F.; Lucena, R.; Cárdenas, S.; Valcárcel, M. Direct coupling of dispersive micro-solid phase extraction and thermal desorption for sensitive gas chromatographic analysis. *Analytical Methods* **2011**, *3* (4), 991-995.
(61) Cai, Y.; Yan, Z.-H.; Wang, N.-Y.; Cai, Q.-Y.; Yao, S.-Z. Preparation of naphthyl functionalized magnetic nanoparticles for extraction of polycyclic aromatic hydrocarbons from river waters. *RSC Advances* **2015**, *5* (69), 56189-56197.
(62) Rozi, S. K. M.; Nodeh, H. R.; Kamboh, M. A.; Manan, N. S. A.; Mohamad, S. Novel palm fatty acid functionalized magnetite nanoparticles for magnetic solid-phase extraction of trace polycyclic aromatic hydrocarbons from environmental samples. *J Oleo Sci* **2017**, *66* (7), 771-784. DOI: 10.5650/jos.ess17016
(63) Cai, Y.; Yan, Z.; NguyenVan, M.; Wang, L.; Cai, Q. Magnetic solid phase extraction and gas chromatography-mass spectrometrical analysis of sixteen polycyclic aromatic hydrocarbons. *J Chromatogr A* **2015**, *1406*, 40-47. DOI: 10.1016/j.chroma.2015.06.024
(64) Xue, S. W.; Tang, M. Q.; Xu, L.; Shi, Z. G. Magnetic nanoparticles with hydrophobicity and hydrophilicity for solid-phase extraction of polycyclic aromatic hydrocarbons from environmental water samples. *J Chromatogr A* **2015**, *1411*, 9-16. DOI: 10.1016/j.chroma.2015.07.104
(65) Al-Rashdi, A. Double-functionalized magnetic nanoparticles for preconcentration and determination of polycyclic aromatic hydrocarbons in water samples. *Analytical Chemistry Research* **2016**, *10*, 9-17.
(66) Cai, Y.; Yan, Z.; Yang, M.; Huang, X.; Min, W.; Wang, L.; Cai, Q. Polydopamine decorated 3d nickel foam for extraction of sixteen polycyclic aromatic hydrocarbons. *J Chromatogr A* **2016**, *1478*, 2-9. DOI: 10.1016/j.chroma.2016.11.030
(67) Nuhu, A. A.; Basheer, C.; Shaikh, A. A.; Al-Arfaj, A. R. Determination of polycyclic aromatic hydrocarbons in water using nanoporous material prepared from waste avian egg shell. *Journal of Nanomaterials* **2012**, *2012*, 102-102.
(68) Ballesteros-Gomez, A.; Rubio, S. Hemimicelles of alkyl carboxylates chemisorbed onto magnetic nanoparticles: Study and application to the extraction of carcinogenic polycyclic aromatic hydrocarbons in environmental water samples. *Anal Chem* **2009**, *81* (21), 9012-9020. DOI: 10.1021/ac9016264
(69) Ding, J.; Gao, Q.; Luo, D.; Shi, Z. G.; Feng, Y. Q. N-octadecylphosphonic acid grafted mesoporous magnetic nanoparticle: Preparation, characterization, and application in magnetic solid-phase extraction. *J Chromatogr A* **2010**, *1217* (47), 7351-7358. DOI: 10.1016/j.chroma.2010.09.074
(70) Xi, J.; Zhang, J.; Zhao, H. Novel uniform Fe_3O_4 hollow spheres for magnetic solid-phase extraction of polycyclic aromatic hydrocarbons. *Anal Sci* **2017**, *33* (9), 999-1005. DOI: 10.2116/analsci.33.999
(71) Cai, Y.; Yan, Z.; Wang, L.; NguyenVan, M.; Cai, Q. Magnetic solid phase extraction and static headspace gas chromatography-mass spectrometry method for the analysis of polycyclic aromatic hydrocarbons. *J Chromatogr A* **2016**, *1429*, 97-106. DOI:

10.1016/j.chroma.2015.12.032
(72) Ciric, S.; Mitic, V.; Jovanovic, S.; Ilic, M.; Nikolic, J.; Stojanovic, G.; Stankov Jovanovic, V. Dispersive micro-solid phase extraction of 16 priority polycyclic aromatic hydrocarbons from water by using thermally treated clinoptilolite, and their quantification by gc-ms. *Mikrochim Acta* **2018**, *185* (12), 556. DOI: 10.1007/s00604-018-3091-0
(73) Benedetti, B.; Di Carro, M.; Magi, E. Multivariate optimization of an extraction procedure based on magnetic molecular imprinted polymer for the determination of polycyclic aromatic hydrocarbons in sea water. *Microchemical Journal* **2019**, *145*, 1199-1206. DOI: 10.1016/j.microc.2018.12.048
(74) de Barros Caetano, V. C. L.; da Costa Cunha, G.; Oliveira, R. V. M.; da Rosa Alexandre, M.; Romão, L. P. C. Magnetic hybrid support for ultrasound-assisted magnetic solid-phase extraction of polycyclic aromatic hydrocarbons from produced water. *Microchemical Journal* **2019**, *146*, 1195-1203. DOI: 10.1016/j.microc.2019.02.055
(75) Abboud, A. S.; Sanagi, M. M.; Ibrahim, W. A. W.; Keyon, A. S. A.; Aboul-Enein, H. Y. Calcium alginate-caged multiwalled carbon nanotubes dispersive microsolid phase extraction combined with gas chromatography-flame ionization detection for the determination of polycyclic aromatic hydrocarbons in water samples. *J Chromatogr Sci* **2018**, *56* (2), 177-186. DOI: 10.1093/chromsci/bmx095
(76) Xiao, J.; Chen, C.; Li, Y.; Fan, J.; Yan, Z.; Cai, Y. Determination of polycyclic aromatic hydrocarbons (PAHs) in indigowoad leaves with preconcentration by cholesterol-decorated nickel foam (NF) dispersive solid-phase extraction (dSPE) and determination by gas chromatography–mass spectrometry (GC-MS). *Analytical Letters* **2022**, *56* (9), 1465-1477. DOI: 10.1080/00032719.2022.2134886
(77) Sorensen, L.; Silva, M. S.; Booth, A. M.; Meier, S. Optimization and comparison of miniaturized extraction techniques for PAHs from crude oil exposed atlantic cod and haddock eggs. *Anal Bioanal Chem* **2016**, *408* (4), 1023-1032. DOI: 10.1007/s00216-015-9225-x
(78) Fernando, H.; Ju, H.; Kakumanu, R.; Bhopale, K. K.; Croisant, S.; Elferink, C.; Kaphalia, B. S.; Ansari, G. A. S. Distribution of petrogenic polycyclic aromatic hydrocarbons (PAHs) in seafood following Deepwater Horizon oil spill. *Mar Pollut Bull* **2019**, *145*, 200-207. DOI: 10.1016/j.marpolbul.2019.05.015
(79) Li, Y.; Yan, Z.; Fan, J.; Yao, X.; Cai, Y. Preparation of COF-coated nickel foam adsorbents for dispersive solid-phase extraction of 16 polycyclic aromatic hydrocarbons from Chinese herbal medicines. *Talanta* **2023**, *265*, 124916. DOI: 10.1016/j.talanta.2023.124916
(80) Wallace, D.; Henry, D.; Pongar, K.; Zimmerman, D. Evaluation of some open column chromatographic methods for separation of bitumen components. *Fuel* **1987**, *66* (1), 44-50. DOI: 10.1016/0016-2361(87)90210-9
(81) Selucky, M.; Chu, Y.; Ruo, T.; Strausz, O. Chemical composition of Athabasca bitumen. *Fuel* **1977**, *56* (4), 369-381. DOI: 10.1016/0016-2361(77)90061-8
(82) Snyder, L. R. Routine compound class separation and analysis of heavy petroleum fractions by adsorpton chromatography. *Analytical Chemistry* **2002**, *37* (6), 713-717. DOI: 10.1021/ac60225a022
(83) US EPA. Method 3630C silica gel cleanup. **1996**.
(84) US EPA. Method 610: Polynuclear aromatic hydrocarbons. US Environmental Protection Agency (EPA) Washington, DC, USA: 1984.
(85) US EPA. Compendium method to 13A—determination of polycyclic aromatic hydrocarbons

(PAHs) in ambient air using gas chromatography/mass spectrometry (GC/MS). United States Environmental Protection Agency Washington, DC: 1999.
(86) Iwegbue, C. M. A.; Iteku-Atata, E.-O. C.; Odali, E. W.; Egobueze, F. E.; Tesi, G. O.; Nwajei, G. E.; Martincigh, B. S. Distribution, sources and health risks of polycyclic aromatic hydrocarbons (PAHs) in household dusts from rural, semi-urban and urban areas in the Niger Delta, Nigeria. *Exposure and Health* **2018**, *11* (3), 209-225. DOI: 10.1007/s12403-018-0276-z
(87) Iwegbue, C. M. A.; Tesi, G. O.; Overah, L. C.; Emoyan, O. O.; Nwajei, G. E.; Martincigh, B. S. Effects of flooding on the sources, spatiotemporal characteristics and human health risks of polycyclic aromatic hydrocarbons in floodplain soils of the lower parts of the River Niger, Nigeria. *Polycyclic Aromatic Compounds* **2017**, *40* (2), 228-244. DOI: 10.1080/10406638.2017.1403329
(88) Li, M.; Shi, M.; Hu, T.; Liu, W.; Mao, Y.; Yu, Y.; Yu, H.; Xu, A.; Yang, W.; Xing, X.; Zhang, J.; Qi, S. Geochemical characteristics and behaviors of polycyclic aromatic hydrocarbons (PAHs) in soil, water, and sediment near a typical nonferrous smelter. *Journal of Soils and Sediments* **2023**, *23* (5), 2258-2272. DOI: 10.1007/s11368-023-03476-0
(89) Ahad, J. M. E.; Jautzy, J. J.; Cumming, B. F.; Das, B.; Laird, K. R.; Sanei, H. Sources of polycyclic aromatic hydrocarbons (PAHs) to northwestern Saskatchewan Lakes east of the Athabasca Oil Sands. *Organic Geochemistry* **2015**, *80*, 35-45. DOI: 10.1016/j.orggeochem.2015.01.001
(90) Rosen, A. A.; Middleton, F. M. Identification of petroleum refinery wastes in surface water. *Analytical Chemistry* **2002**, *27* (5), 790-794. DOI: 10.1021/ac60101a027
(91) Gharbi, I.; Moret, S.; Chaari, O.; Issaoui, M.; Conte, L. S.; Lucci, P.; Hammami, M. Evaluation of hydrocarbon contaminants in olives and virgin olive oils from Tunisia. *Food Control* **2017**, *75*, 160-166. DOI: 10.1016/j.foodcont.2016.12.003
(92) Fasano, E.; Yebra-Pimentel, I.; Martínez-Carballo, E.; Simal-Gándara, J. Profiling, distribution and levels of carcinogenic polycyclic aromatic hydrocarbons in traditional smoked plant and animal foods. *Food Control* **2016**, *59*, 581-590. DOI: 10.1016/j.foodcont.2015.06.036
(93) Dost, K.; İdeli, C. Determination of polycyclic aromatic hydrocarbons in edible oils and barbecued food by HPLC/UV–Vis detection. *Food Chemistry* **2012**, *133* (1), 193-199. DOI: 10.1016/j.foodchem.2012.01.001
(94) Khan, S.; Cao, Q. Human health risk due to consumption of vegetables contaminated with carcinogenic polycyclic aromatic hydrocarbons. *Journal of Soils and Sediments* **2011**, *12* (2), 178-184. DOI: 10.1007/s11368-011-0427-3
(95) Callén, M.; Hall, S.; Mastral, A. M.; Garcia, T.; Ross, A.; Bartle, K. D. PAH presence in oils and tars from coal–tyre coprocessing. *Fuel Processing Technology* **2000**, *62* (1), 53-63. DOI: 10.1016/s0378-3820(99)00062-4
(96) Konan, N. G. F. D. S.; Li, M.; Shi, S.; Liu, X.; Tang, Y.; Kojo, A.; Toyin, A. Simple column chromatography separation procedure for polycyclic aromatic hydrocarbons: Controlling factor(s). *Arabian Journal of Geosciences* **2022**, *15* (15). DOI: 10.1007/s12517-022-10625-1
(97) Ncube, S.; Madikizela, L.; Cukrowska, E.; Chimuka, L. Recent advances in the adsorbents for isolation of polycyclic aromatic hydrocarbons (PAHs) from environmental sample solutions. *TrAC Trends in Analytical Chemistry* **2018**, *99*, 101-116. DOI: 10.1016/j.trac.2017.12.007

(98) Dugger, D. L.; Stanton, J. H.; Irby, B. N.; McConnell, B. L.; Cummings, W. W.; Maatman, R. W. The exchange of twenty metal ions with the weakly acidic silanol group of silica gel. *The Journal of Physical Chemistry* **2002**, *68* (4), 757-760. DOI: 10.1021/j100786a007
(99) Lee, B. I.; Rives, J. P. Dispersion of alumina powders in nonaqueous media. *Colloids and Surfaces* **1991**, *56*, 25-43. DOI: 10.1016/0166-6622(91)80112-2
(100) Idowu, I.; Francisco, O.; Thomas, P. J.; Johnson, W.; Marvin, C.; Stetefeld, J.; Tomy, G. T. Validation of a simultaneous method for determining polycyclic aromatic compounds and alkylated isomers in biota. *Rapid Commun Mass Spectrom* **2018**, *32* (3), 277-287. DOI: 10.1002/rcm.8035
(101) Perreault, H. Development of chromatographic and mass spectrometric techniques for the analysis of complex mixtures containing aromatic compounds. Dalhousie University, Halifax, Nova Scotia, 1992.
(102) Sawicki, E.; Meeker, J. E.; Morgan, M. J. The quantitative composition of air pollution source effluents in terms of aza heterocyclic compounds and polynuclear aromatic hydrocarbons. *Air Water Pollut* **1965**, *9*, 291-298.
(103) James, A. T.; Martin, A. J. Gas-liquid partition chromatography; the separation and micro-estimation of volatile fatty acids from formic acid to dodecanoic acid. *Biochem J* **1952**, *50* (5), 679-690. DOI: 10.1042/bj0500679
(104) Martin, A. J.; Synge, R. L. A new form of chromatogram employing two liquid phases: A theory of chromatography. 2. Application to the micro-determination of the higher monoamino-acids in proteins. *Biochem J* **1941**, *35* (12), 1358-1368. DOI: 10.1042/bj0351358
(105) Harris, D. C. *Quantitative chemical analysis*; W. H. Freeman and Company, 2010.
(106) Güzel, B.; Canlı, O. An environmental friendly and stable analytical method for the determination of indicator polychlorinated biphenyls (PCBs) in solid and waste oil samples by gas chromatography-electron capture detector (GC-ECD). *Microchemical Journal* **2022**, *178*, 107325. DOI: 10.1016/j.microc.2022.107325
(107) Albadri, A. E. A. E.; Elbashir, A. A.; Ahmed, H. E.-o.; Mihaina, I. A. M.; Aboul-Enein, H. Y. A gas chromatographic method with electron-capture detector (GC-ECD) for simultaneous determination of fenpropathrin, λ-cyhalothrin, and deltamethrin residues in tomato and its applications to kinetic studies after field treatment. *Food Anal. Methods* **2012**, *5* (6), 1296-1302. DOI: 10.1007/s12161-012-9382-x
(108) Hariri, M.; Ghani, K.; Damiri, S. Purification of 2,4,6-trinitrotoluene by digestion with sodium sulfite and determination of its impurities by gas chromatography–electron capture detector (GC-ECD). *Journal of the Iranian Chemical Society* **2019**, *16* (11), 2401-2408. DOI: 10.1007/s13738-019-01709-z
(109) Tankiewicz, M.; Morrison, C.; Biziuk, M. Application and optimization of headspace solid-phase microextraction (HS-SPME) coupled with gas chromatography–flame-ionization detector (GC–FID) to determine products of the petroleum industry in aqueous samples. *Microchemical Journal* **2013**, *108*, 117-123. DOI: 10.1016/j.microc.2012.10.010
(110) Lee, S.-H.; Mukherjee, S.; Brewer, B.; Ryan, R.; Yu, H.; Gangoda, M. A laboratory experiment to measure Henry's Law constants of volatile organic compounds with a bubble column and a gas chromatography flame ionization detector (GC-FID). *Journal of Chemical Education* **2012**, *90* (4), 495-499. DOI: 10.1021/ed200303x
(111) Silva, L. I.; Rocha-Santos, T. A.; Duarte, A. C. Comparison of a gas chromatography-optical fibre (GC-OF) detector with a gas chromatography-flame ionization detector (GC-FID) for

determination of alcoholic compounds in industrial atmospheres. *Talanta* **2008**, *76* (2), 395-399. DOI: 10.1016/j.talanta.2008.03.012
(112) Kim, S. H.; Hwang, J.-H.; Lee, K.-G. Analysis of acrylamide using gas chromatography-nitrogen phosphorus detector (GC-NPD). *Food Science and Biotechnology* **2011**, *20* (3), 835-839. DOI: 10.1007/s10068-011-0116-4
(113) Greiner-Sosanko, E.; Giannoutsos, S.; Lower, D. R.; Virji, M. A.; Krasowski, M. D. Drug monitoring: Simultaneous analysis of lamotrigine, oxcarbazepine, 10-hydroxycarbazepine, and zonisamide by HPLC-UV and a rapid gc method using a nitrogen-phosphorus detector for levetiracetam. *J Chromatogr Sci* **2007**, *45* (9), 616-622. DOI: 10.1093/chromsci/45.9.616
(114) Wang, P.; Rashid, M.; Liu, J.; Hu, M.; Zhong, G. Identification of multi-insecticide residues using GC-NPD and the degradation kinetics of chlorpyrifos in sweet corn and soils. *Food Chem* **2016**, *212*, 420-426. DOI: 10.1016/j.foodchem.2016.05.008
(115) Araujo, M. N.; Vargas, S. R.; Soares, L. A.; Trindade, L. F.; Fuess, L. T.; Adorno, M. A. T. Rapid method for determination of biogas composition by gas chromatography coupled to a thermal conductivity detector (GC-TCD). *International Journal of Environmental Analytical Chemistry* **2023**, *ahead-of-print* (ahead-of-print), 1-18. DOI: 10.1080/03067319.2023.2210055
(116) Budiman, H.; Zuas, O. Validation of analytical method for determination of high level carbon dioxide (CO_2) in nitrogen gas (N_2) matrix using gas chromatography thermal conductivity detector. *Periódico Tchê Química* **2015**, *12* (24), 7-16. DOI: 10.52571/PTQ.v12.n24.2015.7_P_24_pgs_7_16.pdf
(117) Zhu, J.; Xiao, Z. Characterization of odor-active volatiles in hawthorn puree using thermal desorption system coupled to gas chromatography-mass spectrometry-olfactometry and gc-flame photometric detector. *J Agric Food Chem* **2018**, *66* (46), 12296-12305. DOI: 10.1021/acs.jafc.8b04636
(118) Ahmadi, F.; Assadi, Y.; Hosseini, S. M.; Rezaee, M. Determination of organophosphorus pesticides in water samples by single drop microextraction and gas chromatography-flame photometric detector. *J Chromatogr A* **2006**, *1101* (1-2), 307-312. DOI: 10.1016/j.chroma.2005.11.017
(119) Akbar, M.; Shakeel, H.; Agah, M. GC-on-chip: Integrated column and photoionization detector. *Lab Chip* **2015**, *15* (7), 1748-1758. DOI: 10.1039/c4lc01461h
(120) Peng, F. M.; Xie, P. H.; Shi, Y. G.; Wang, J. D.; Liu, W. Q.; Li, H. Y. Photoionization detector for portable rapid gc. *Chromatographia* **2007**, *65* (5-6), 331-336. DOI: 10.1365/s10337-006-0169-3
(121) Zhang, W.-Q.; Li, H.; Zhang, Y.-J.; Bi, F.; Meng, L.-S.; Zhang, X.-M.; Mao, J.-Y.; Cheng, N.-L.; Fang, B.; Yang, Y.; Chen, C.; Guo, K.-X.; Zhan, G.-E.; Sha, J.; Wang, X.-Z. Fast determination of monocyclic aromatic hydrocarbons in ambient air using a portable gas chromatography–photoionization detector. *Chromatographia* **2017**, *80* (8), 1233-1247. DOI: 10.1007/s10337-017-3331-1
(122) Bass, S. W.; Flynn, L. R.; Meierer, R. E. *Headspace screening/capillary column GC/MS analysis for volatile organics : Validation studies and applications*; ASTM International, 1991. DOI: 10.1520/STP25471S.
(123) Gomez-Ruiz, J. A.; Cordeiro, F.; Lopez, P.; Wenzl, T. Optimisation and validation of programmed temperature vaporization (PTV) injection in solvent vent mode for the analysis of the 15+1 eu-priority PAHs by GC-MS. *Talanta* **2009**, *80* (2), 643-650. DOI:

10.1016/j.talanta.2009.07.041
(124) Bartle, K. D.; Myers, P. History of gas chromatography. *TrAC Trends in Analytical Chemistry* **2002**, *21* (9-10), 547-557. DOI: 10.1016/s0165-9936(02)00806-3
(125) Galletta, M.; Zoccali, M.; Jones, N.; Mondello, L.; Tranchida, P. Q. Flow-modulated comprehensive two-dimensional gas chromatography combined with time-of-flight mass spectrometry: Use of hydrogen as a more sustainable alternative to helium. *Anal Bioanal Chem* **2022**, *414* (21), 6371-6378. DOI: 10.1007/s00216-022-04086-4
(126) Rood, D. Gas chromatography problem solving and troubleshooting. *J Chromatogr Sci* **2002**, *40* (5), 304-305. DOI: 10.1093/chromsci/40.1.57
(127) Munoz-Guerra, J. A.; Prado, P.; Garcia-Tenorio, S. V. Use of hydrogen as a carrier gas for the analysis of steroids with anabolic activity by gas chromatography-mass spectrometry. *J Chromatogr A* **2011**, *1218* (41), 7365-7370. DOI: 10.1016/j.chroma.2011.08.009
(128) Nnaji, C. N.; Williams, K. C.; Bishop, J. M.; Verbeck, G. F. Hydrogen as a GC/MS carrier and buffer gas for use in forensic laboratories. *Sci Justice* **2015**, *55* (3), 162-167. DOI: 10.1016/j.scijus.2015.01.003
(129) Vieira, I. J.; Medeiros, W. L.; Monnerat, C. S.; Souza, J. J.; Mathias, L.; Braz-Filho, R.; Pinto, A. C.; Sousa, P. M.; Rezende, C. M.; Epifanio Rde, A. Two fast screening methods (GC-MS and TLC-ChEI assay) for rapid evaluation of potential anticholinesterasic indole alkaloids in complex mixtures. *An Acad Bras Cienc* **2008**, *80* (3), 419-426. DOI: 10.1590/s0001-37652008000300003
(130) Kubeczka, K. H. Vortrennung ätherischer Öle und ähnlich komplexer stoffgemische für die GC-analyse durch modifizierte trockensäulen-chromatographie. *Chromatographia* **1973**, *6* (2), 106-108. DOI: 10.1007/bf02270547
(131) Elder, J. F.; Gordon, B. M.; Uhrig, M. S. Complex mixture analysis by capillary-to-capillary column heartcutting GC/MS. *Journal of Chromatographic Science* **1986**, *24* (1), 26-33. DOI: 10.1093/chromsci/24.1.26
(132) Morinaga, M.; Hara, K.; Kageura, M.; Hieda, Y.; Takamoto, M.; Kashimura, S. A simple, rapid and simultaneous analysis of complex volatile hydrocarbon mixtures in blood using gas chromatography/mass spectrometry with a wide-bore capillary column. *Z Rechtsmed* **1990**, *103* (8), 567-572. DOI: 10.1007/BF01261420
(133) Zeng, Z.; Gao, Y.; Cui, J.; Lin, M.; Tang, J.; Wang, F.; Yang, Y.; Yu, Y.; Ma, S. Liquid-liquid extraction combined with online cleanup for the simultaneous determination of PAHs by GC-MS/MS and their hydroxylated metabolites by LC-MS/MS in human fingernails. *J Chromatogr B Analyt Technol Biomed Life Sci* **2022**, *1188*, 123057. DOI: 10.1016/j.jchromb.2021.123057
(134) Angioni, A.; Cau, A.; Secci, M.; Addis, P. GC-ITMS analysis of PAH contamination levels in the marine sea urchin paracentrotus lividus in Sardinia. *Mar Pollut Bull* **2014**, *82* (1-2), 201-207. DOI: 10.1016/j.marpolbul.2014.02.031
(135) Jin, R.; Liu, G.; Jiang, X.; Liang, Y.; Fiedler, H.; Yang, L.; Zhu, Q.; Xu, Y.; Gao, L.; Su, G.; Xiao, K.; Zheng, M. Profiles, sources and potential exposures of parent, chlorinated and brominated polycyclic aromatic hydrocarbons in haze associated atmosphere. *Sci Total Environ* **2017**, *593-594*, 390-398. DOI: 10.1016/j.scitotenv.2017.03.134
(136) Jin, R.; Liu, G.; Zheng, M.; Jiang, X.; Zhao, Y.; Yang, L.; Wu, X.; Xu, Y. Secondary copper smelters as sources of chlorinated and brominated polycyclic aromatic hydrocarbons. *Environ Sci Technol* **2017**, *51* (14), 7945-7953. DOI: 10.1021/acs.est.7b02031
(137) Jin, R.; Bandowe, B. A. M.; Zheng, M.; Liu, G.; Nezikova, B.; Prokes, R.; Cupr, P.; Klanova,

J.; Lammel, G. Atmospheric deposition of chlorinated and brominated polycyclic aromatic hydrocarbons in central Europe analyzed by GC-MS/MS. *Environ Sci Pollut Res Int* **2021**, *28* (43), 61360-61368. DOI: 10.1007/s11356-021-15038-3

(138) Lin, B.; Yang, Y.; Yang, L.; Liu, G.; Li, C.; Xu, J.; Hou, S.; Zheng, M. Congener profiles and process distributions of polychlorinated biphenyls, polychlorinated naphthalenes and chlorinated polycyclic aromatic hydrocarbons from secondary copper smelting. *J Hazard Mater* **2022**, *423* (Pt B), 127125. DOI: 10.1016/j.jhazmat.2021.127125

(139) Liu, Q.; Xu, X.; Lin, L.; Yang, G.; Wang, D. Occurrence, health risk assessment and regional impact of parent, halogenated and oxygenated polycyclic aromatic hydrocarbons in tap water. *J Hazard Mater* **2021**, *413*, 125360. DOI: 10.1016/j.jhazmat.2021.125360

(140) Liu, Q.; Xu, X.; Lin, L.; Wang, D. Occurrence, distribution and ecological risk assessment of polycyclic aromatic hydrocarbons and their derivatives in the effluents of wastewater treatment plants. *Sci Total Environ* **2021**, *789*, 147911. DOI: 10.1016/j.scitotenv.2021.147911

(141) Rajasekhar, B.; Nambi, I. M.; Govindarajan, S. K. Investigating the degradation of nC12 to nC23 alkanes and PAHs in petroleum-contaminated water by electrochemical advanced oxidation process using an inexpensive Ti/Sb-SnO2/PbO2 anode. *Chemical Engineering Journal* **2021**, *404*. DOI: 10.1016/j.cej.2020.125268

(142) Masuda, M.; Wang, Q.; Tokumura, M.; Miyake, Y.; Amagai, T. Simultaneous determination of polycyclic aromatic hydrocarbons and their chlorinated derivatives in grilled foods. *Ecotoxicol Environ Saf* **2019**, *178* (April), 188-194. DOI: 10.1016/j.ecoenv.2019.04.046

(143) Yan, K.; Wu, S.; Gong, G.; Xin, L.; Ge, Y. Simultaneous determination of typical chlorinated, oxygenated, and european union priority polycyclic aromatic hydrocarbons in milk samples and milk powders. *J Agric Food Chem* **2021**, *69* (13), 3923-3931. DOI: 10.1021/acs.jafc.1c00283

(144) Li, W.; Wu, S. Halogenated polycyclic aromatic hydrocarbons and their parent compounds in ready-to-eat seafood rich in salt: Method validation, profiles, correlation, and exposure risks. *Food Control* **2022**, *136*. DOI: 10.1016/j.foodcont.2022.108864

(145) Du, Y.; Xu, X.; Liu, Q.; Lin, L.; Bai, L.; Wang, D. Contribution of atmospheric deposition to halogenated polycyclic aromatic hydrocarbons in surface sediments: A validation study. *Sci Total Environ* **2022**, *815*, 152889. DOI: 10.1016/j.scitotenv.2021.152889

(146) Giddings, J. C. Two-dimensional separations: Concept and promise. *Anal Chem* **1984**, *56* (12), 1258A-1260A, 1262A, 1264A passim. DOI: 10.1021/ac00276a003

(147) Consden, R.; Gordon, A. H.; Martin, A. J. Qualitative analysis of proteins: A partition chromatographic method using paper. *Biochem J* **1944**, *38* (3), 224-232. DOI: 10.1042/bj0380224

(148) Gorecki, T.; Harynuk, J.; Panic, O. The evolution of comprehensive two-dimensional gas chromatography (GC × GC). *J Sep Sci* **2004**, *27* (5-6), 359-379. DOI: 10.1002/jssc.200301650

(149) Marriott, P.; Shellie, R. Principles and applications of comprehensive two-dimensional gas chromatography. *TrAC Trends in Analytical Chemistry* **2002**, *21* (9-10), 573-583. DOI: 10.1016/s0165-9936(02)00814-2

(150) Liu, Z.; Phillips, J. B. Comprehensive two-dimensional gas chromatography using an on-column thermal modulator interface. *Journal of Chromatographic Science* **1991**, *29* (6), 227-231. DOI: 10.1093/chromsci/29.6.227

(151) Thomas, J. M. Jj thomson: Winner of the Nobel Prize for Physics 1906. *Angewandte Chemie*

International Edition **2006**, *45* (41), 6797-6800.
(152) Thomson, J. J. Xl. Cathode rays. *The London, Edinburgh, and Dublin Philosophical Magazine and Journal of Science* **1897**, *44* (269), 293-316.
(153) Thomson, J. J. Xix. Further experiments on positive rays. *The London, Edinburgh, and Dublin Philosophical Magazine and Journal of Science* **2009**, *24* (140), 209-253. DOI: 10.1080/14786440808637325
(154) Thomson, J. J. Lxxxiii. Rays of positive electricity. *The London, Edinburgh, and Dublin Philosophical Magazine and Journal of Science* **2009**, *20* (118), 752-767. DOI: 10.1080/14786441008636962
(155) Aston, F. W. Lxxiv. A positive ray spectrograph. *The London, Edinburgh, and Dublin Philosophical Magazine and Journal of Science* **2009**, *38* (228), 707-714. DOI: 10.1080/14786441208636004
(156) Pattanapo, W. Effect of electrolyzed reducing water with various concentration on carbendazim fungicide removal in cherry tomato. *International Journal of GEOMATE* **2020**, *18* (68), 54-59. DOI: 10.21660/2020.68.5618
(157) Kowalska, J.; Kowalska, H.; Cieslak, B.; Majewska, E.; Ciecierska, M.; Derewiaka, D.; Lenart, A. Influence of sucrose substitutes and agglomeration on volatile compounds in powdered cocoa beverages. *J Food Sci Technol* **2020**, *57* (1), 350-363. DOI: 10.1007/s13197-019-04067-z
(158) Zhang, H.; Zhao, J.; Chen, B.; Ma, Y.; Li, Z.; Shou, X.; Wen, L.; Yuan, Y.; Gao, H.; Ruan, J.; Li, H.; Lu, S.; Gong, Y.; Wang, J.; Wen, H. Pharmacokinetics and tissue distribution study of liposomal albendazole in naturally Echinococcus granulosus infected sheep by a validated UPLC-Q-TOF-MS method. *J Chromatogr B Analyt Technol Biomed Life Sci* **2020**, *1141*, 122016. DOI: 10.1016/j.jchromb.2020.122016
(159) Cambien, G.; Venisse, N.; Migeot, V.; Rabouan, S.; Belmouaz, M.; Binson, G.; Albouy-Llaty, M.; Ayraud-Thevenot, S.; Dupuis, A. Simultaneous determination of bisphenol a and its chlorinated derivatives in human plasma: Development, validation and application of a uhplc-ms/ms method. *Chemosphere* **2020**, *242*, 125236. DOI: 10.1016/j.chemosphere.2019.125236
(160) Zu, Y.; Yan, M.; Fu, Y.; Liu, W.; Zhang, L.; Gu, C.; Efferth, T. Determination and quantification of astragalosides in Radix Astragali and its medicinal products using LC-MS. *J Sep Sci* **2009**, *32* (4), 517-525. DOI: 10.1002/jssc.200800499
(161) Dempster, A. J. A new method of positive ray analysis. *Physical Review* **1918**, *11* (4), 316-325. DOI: 10.1103/PhysRev.11.316
(162) Bleakney, W. A new method of positive ray analysis and its application to the measurement of ionization potentials in mercury vapor. *Physical Review* **1929**, *34* (1), 157-160. DOI: 10.1103/PhysRev.34.157
(163) Hoffmann, E. d.; Stroobant, V. *Mass spectrometry : Principles and applications*; J. Wiley, 2007.
(164) Brenton, A. G.; Godfrey, A. R. Accurate mass measurement: Terminology and treatment of data. *J Am Soc Mass Spectrom* **2010**, *21* (11), 1821-1835. DOI: 10.1016/j.jasms.2010.06.006
(165) Yost, R. A.; Enke, C. G. Selected ion fragmentation with a tandem quadrupole mass spectrometer. *Journal of the American Chemical Society* **2002**, *100* (7), 2274-2275. DOI: 10.1021/ja00475a072
(166) Bouyssiere, B.; Baco, F.; Savary, L.; Lobinski, R. Speciation analysis for mercury in gas

condensates by capillary gas chromatography with inductively coupled plasma mass spectrometric detection. *J Chromatogr A* **2002**, *976* (1-2), 431-439. DOI: 10.1016/s0021-9673(02)01151-2

(167) Tranchida, P. Q.; Aloisi, I.; Giocastro, B.; Zoccali, M.; Mondello, L. Comprehensive two-dimensional gas chromatography-mass spectrometry using milder electron ionization conditions: A preliminary evaluation. *J Chromatogr A* **2019**, *1589*, 134-140. DOI: 10.1016/j.chroma.2019.01.006

(168) Yip, L. Y.; Chan, E. C. Y. Gas chromatography/mass spectrometry-based metabonomics. In *Proteomic and metabolomic approaches to biomarker discovery*, Elsevier, 2013; pp 133-147.

(169) Miller, P. E.; Denton, M. B. The quadrupole mass filter: Basic operating concepts. *Journal of Chemical Education* **1986**, *63* (7), 617. DOI: 10.1021/ed063p617

(170) Mamyrin, B.; Karataev, V.; Shmikk, D.; Zagulin, V. The mass-reflectron, a new nonmagnetic time-of-flight mass spectrometer with high resolution. *Zh. Eksp. Teor. Fiz* **1973**, *64* (1), 82-89.

(171) Krutchinsky, A.; Loboda, A.; Spicer, V.; Dworschak, R.; Ens, W.; Standing, K. Orthogonal injection of matrix ‐ assisted laser desorption/ionization ions into a time ‐ of ‐ flight spectrometer through a collisional damping interface. *Rapid Communications in Mass Spectrometry* **1998**, *12* (9), 508-518.

(172) Chernushevich, I. V.; Ens, W.; Standing, K. G. Peer reviewed: Orthogonal-injection TOFMS for analyzing biomolecules. *Anal Chem* **1999**, *71* (13), 452A-461A. DOI: 10.1021/ac990524l

(173) Cotter, R. J. Peer reviewed: The new time-of-flight mass spectrometry. *Anal Chem* **1999**, *71* (13), 445A-451A. DOI: 10.1021/ac9904617

(174) De Hoffmann, E.; Stroobant, V. *Mass spectrometry: Principles and applications*; John Wiley & Sons, 2007.

(175) Willis, P.; Jaloszynski, J.; Artaev, V. Improving duty cycle in the folded flight path high-resolution time-of-flight mass spectrometer. *International Journal of Mass Spectrometry* **2021**, *459*, 116467. DOI: 10.1016/j.ijms.2020.116467

Chapter 3: Identification of Halogenated Polycyclic Aromatic Hydrocarbons in Biological Samples from Alberta Oil-Sands Region

A version of this chapter has already been published as Zhe Xia, Ifeoluwa Idowu, Chris Marvin, Philippe J. Thomas, Wesley Johnson, Olga Francisco, Jörg Stetefeld, Bernard Crimmins, Mark Fry and Gregg T. Tomy. Identification of halogenated polycyclic aromatic hydrocarbons in biological samples from Alberta Oil-Sands Region. *Chemosphere*, **2019**, *215*, 206–213.

3.1 Abstract

Halogenated polycyclic aromatic hydrocarbons (HPAHs) were identified in biological samples from the Alberta Oil-Sands Region (AOSR) using gas chromatography coupled with high-resolution time-of-flight mass spectrometry (GC-HRTOF-MS) at a resolving power of 25,000. Knowledge of the electron ionization (EI) fragmentation behavior of individual HPAH isomers, achieved by injecting authentic standards in full-scan MS mode, was paramount in identifying a suite of HPAHs in samples from the AOSR. Confirmation of compounds in biological samples was based on the measured mass accuracy (± 3 ppm) of 2 characteristic ions prominent in the EI mass spectra of each compound. Numerous compounds were detected in the high-resolution total ion chromatogram in liver extracts of 4 biological species from the AOSR: river otter (*Lontra Canadensis*), northern pike (*Esox lucius*), lake whitefish (*Coregonus clupeaformis*) and snails (*Gastropod sp.*) many of which remain unidentified. Careful examination of the high-resolution accurate mass data suggests that dichloro-anthracene/phenanthrene, bromo-anthracene/phenanthrene and dibromo-fluorene were present in the biological samples. Lipid corrected concentrations of dichloro-PAHs were estimated to be 16.3 ± 11.4 (*n = 4*) and 5.5 (*n = 1*) ng/g in lake whitefish and river otter, respectively. Concentrations of mono-bromo-PAHs were an order of magnitude greater in snails (170.5 ng/g) than in northern pike (12.5 ng/g) while concentrations of dibromo-PAHs were 4 times greater in snails than in northern pike. The detection of these compounds in biota implies that these compounds are bioaccumulative. The liver-based biomagnification factor of the dichloro-PAH congener in the river otter/lake whitefish feeding relationship is much smaller than 1 implying that this compound does not biomagnify.

3.2 Introduction

Polycyclic aromatic compounds (PACs) are a complex class of compounds derived from incomplete combustion or diagenesis of plant matter forming petroleum oils. The most common PACs are the polycyclic aromatic hydrocarbons (PAHs) of which 16 have been identified as priority pollutants by the United States Environmental Protection Agency (US EPA) and are also listed under Schedule 1 of the Canadian Environmental Protection Act.[1] However, there are other important PACs that to date have received less attention. These include halogenated polycyclic aromatic hydrocarbons (HPAHs), non-halogenated alkylated PAHs and heterocyclic aromatic compounds that contain S-, O- and N- atoms.[1,2] Like some of the other PACs, HPAHs are likely to be persistent in the environment.[2] In addition, the toxicity of some HPAHs have been found to be similar to dibenzo-p-dioxins and dibenzofurans (PCDD/Fs).[3-5]

HPAHs have been detected and quantified in environmental matrices and abiotic samples, such as waste incinerators, electronic wastes, atmosphere, and sediment.[3,5-8] HPAHs have also been detected in biological organisms, such as seafood from south China, fish from the Great Lakes and blue mussels (*Mytilus edulis*) from Massachusetts, USA.[6,9,10] In general, however, there is a paucity of reports of HPAHs in biological organisms and is due, in part, to the lack of validated analytical methods for the identification and quantification of these compounds and a lack of analytical standards for HPAHs.[11]

The Canadian oil sands are naturally occurring mixtures of crude bitumen (thick, heavy crude oil), sand, clay, ultrafine mineral solids and water that are rich in PACs. PACs are released naturally from oil sands deposits (*i.e.,* due to erosion of the bitumen deposits) and from bitumen extraction and upgrading (*i.e.,* oil sands activity).[12-14] However, several studies have clearly shown that

increases in the concentrations of C_1–C_4 alkylated PAHs and dibenzothiophenes (prominent components of Athabasca Oil Sands Region (AOSR) bitumen[15]) in the atmosphere, water, soil and sediments, plants, wildlife and fish in the AOSR are a result of proximity to oil sands activity.[12-14,16-21] Historically, the AOSR was covered by sea water millions of years ago, which would have contained high concentrations of chloride and bromide ions.[22,23] During the oil sand formation, the elevated pressures and temperatures of diagenesis and catagenesis combined with the catalytic activation of halides formed with abundant crustal elements Al and Fe (AlX_3 and FeX_3, X = Cl or Br) likely created an environment conducive to organohalogen formation. HPAH formation has also been associated with the presence of halogen ions and strong sunlight.[24] Based on these two potential formation pathways, it is hypothesized that HPAHs resulting from non-combustion sources are present in biological organisms from the AOSR. Having higher log K_{ow} than their parent compounds, HPAHs have a higher propensity to partition into lipids, and as a result, bioaccumulate and biomagnify in biological organisms.[15,25] This could in turn induce toxicity due to the activation of the AhR pathway.[26]

Our study hypothesis, therefore, is that HPAHs are present in biological samples from the AOSR. Before testing these hypotheses, it was first necessary to establish an analytical method for identification and quantification of these compounds. We chose to use gas chromatography coupled with high resolution time-of-flight mass spectrometry (GC/HRTOF-MS). The mass spectral behavior of 16 authentic standards under electron ionization conditions was first elucidated. Our GC/HRTOF-MS system operating at a resolving power (RP) of 25,000 acquired HR mass spectra and the exact masses of two prominent ions characteristic to each HPAHs were then used to identify HPAHs in biological samples from AOSR. The results from this study provide preliminary identification of a new class of compounds in the AOSR that to date have gone

undetected.

3.3 Materials and Methods

3.3.1 Chemicals

All high-purity (Optima grade) organic solvents, silica gel (923 grade, 100–200 mesh), alumina (60–325 mesh), Ottawa sand and anhydrous sodium sulfate, diatomaceous earth (DE) were purchased from Fisher Chemicals (Ottawa, Canada). The analytical standards and their acronyms are provided in Table II.1.

3.3.2 Sample Preparation

All samples were collected in 2014 and 2015 from the sites shown in Figure 3.1. Individual liver samples of otters ($n = 6$), northern pike ($n = 4$) and lake whitefish ($n = 4$) were extracted separately. Snails collected in the field were placed in clean water for 24 hours to depurate the contents of their guts. Shells were then removed and tissues were pooled and homogenized. A sub-sample of this pooled material was then extracted ($n = 3$). Analyte extractions were performed using an accelerated solvent extractor (ASE, Thermo Scientific, Waltham, MA). Approximately 1.5 grams of each sample (wet weight) was weighed and mixed with DE and transferred to an ASE extraction cell size and spiked with the recovery internal standard (RIS, 100 ng) prior to extraction. Details of the extraction and clean-up steps can be found in Idowu *et al*.[27]

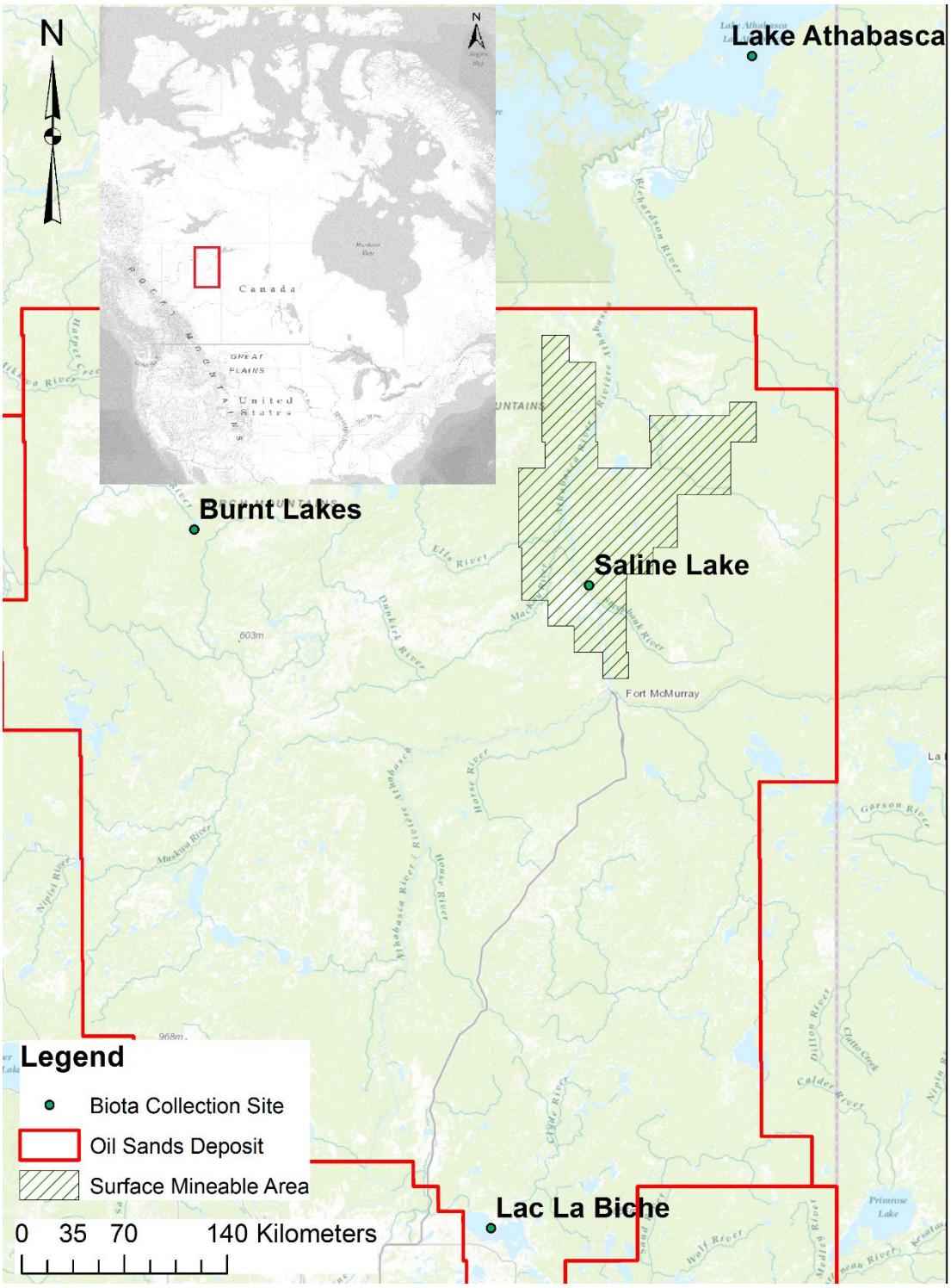

Figure 3.1: *Sample Collection Sites in the AOSR.*

3.3.3 GC-HRTOF-MS Analysis

Analysis of the HPAH extracts and standard solutions were performed in one-dimensional mode using a 7890A GC (Agilent Technologies, Wilmington, DE, USA) fitted with a split/splitless injector, coupled with a Pegasus HRT 4D (LECO, St Joseph, MI, USA) operated in electron ionization (EI) mode and calibrated with perfluorotributylamine, PFTBA, as the mass calibrant. The stationary phase was Rxi-5Sil MS (60 m × 0.25 mm × 0.25 μm) (Restek, Bellefonte, PA, USA). Standards and samples (2 μL) were injected at 250ºC in splitless mode. The GC program started at 80ºC (held for 1 min), heated to 210ºC at 35 ºC/min, further to 260ºC at 3ºC/min and finally to 315ºC at 10ºC/min (held for 5 min). The MS transfer line temperature was at 300ºC, ion source temperature at 250ºC and He as carrier gas at a flow-rate of 1.4 mL/min. The HRTOF-MS was operated at a mass range of *m/z* 50 – 500 with an acquisition rate of 13 spectra/second at 70 eV. The RP (full width half height) of the system was typically greater than 25 000 based on the peak width of the lock-masses *m/z* 218.9851 (C_4F_9) and 263.9871 ($C_5F_{10}N$) of PFTBA. During the chromatographic run, PFTBA was continuously bled into the ion source to serve as an internal standard for instrument mass calibration. Run to run repeatability, measured as the mean mass accuracy (Δm) of all sample and standard injections were smaller than ± 0.5 ppm for both lock-masses.

3.3.4 GC/MS/MS Analysis

Instrumental details of the analysis and quantitation of PAHs and alkyl-PAHs (APAHs) are given in Idowu *et al*.[27]

3.3.5 Method Verification

Our laboratory is ISO-17025 certified and a fully validated PAH method in accordance with the Eurachem Guide has been previously reported.[27,28] Based on the Eurachem guidelines, a comprehensive assessment of all the performance characteristics for HPAHs is unnecessary.[28] Instead, a truncated validation, *i.e.*, verification, is warranted which must demonstrate that HPAHs behave similar to the PAHs during the sample extraction and clean-up steps. To test this, a mixture of PAHs and HPAHs [3 levels: high (500 ng), medium (200 ng), low (50 ng); (*n = 5*)] was fortified in an ASE cell containing C_{18}-silica gel (used as a surrogate for biological tissue) and taken through the entire protocol. In this manner, recoveries of PAHs and HPAHs can be compared directly. Limits of detection (LODs) for individual HPAHs were achieved by fortifying ASE cells (*n = 10*) containing C_{18}-silica gel with each HPAHs (50 pg) and LODs calculated as 3× the standard deviation of the 10 replicate measurements. Calibration standards in hexane (1, 5, 10, 25, 50 and 100 pg/µL) were injected in triplicates to generate our calibration curves for each HPAH.

3.4 Results and Discussion

There were no statistical differences (Student *t*-test, $p < 0.05$) in the average recoveries of HPAHs relative to PAHs at any of the fortification levels. The repeatability of the method for HPAHs (and PAHs) expressed as the average % RSD of all 16 HPAHs and (16 PAHs) were 5.4 (6.7), 7.5 (4.9) and 13.9 (6.4), for the high, medium, and low spikes, respectively. Limits of detection ranged from 1.8 – 5.9 pg. The linear dynamic range of the method ranged from 1 – 100 pg (for all analytes). Based on the Eurachem Guide, the performance characteristics experimental results imply that the ISO-17025 PAHs validated method established in our laboratory is suitable

for use with the HPAHs.[28]

3.4.1 EI Mass Spectra

Reference EI mass spectra were generated using individual standards commercially available. As examples, the full-scan EI mass spectra of 1,5-dichloroanthrancene, 3-bromophenanthrene and 2,7-dibromofluorene are shown in Figure 3.2. The mono- and di-Cl containing PAHs EI mass spectra was dominated by the molecular ion ($M^{+\bullet}$) (*see* Table II.2). For the mono-Cl isomers, with the exception of 1-Cl-Pyr, the second most dominant ion arose from a neutral loss of HCl from the $M^{+\bullet}$ ion; the second most abundant peak for 1-Cl-Pyr arose from the loss of a Cl radical from the $M^{+\bullet}$. A neutral loss of 2 Cl atoms was the second most dominant ion observed in the EI mass spectra of the 2 di-Cl isomers. The appearance of the EI full-scan mass spectra for the mono-Br ions was more complicated than the Cl-containing PAHs. In 3 cases (3-Br-Phe, 9-Br-Phe and 1-Br-Pyr), the $M^{+\bullet}$ ions dominated the EI mass spectra of the mono-Br isomers. For 7-Br-BaA, the base peak arose from a neutral loss of HBr from the $M^{+\bullet}$ while for the 5-Br-Ana and 2-Br-Fle the base peak arose from a free radical loss of Br from the $M^{+\bullet}$. Losses of 2 Br atoms from the parent ion (9,10-Br_2-Ant and 9,10-Br_2-Phe) or concomitant loss of $Br^{\bullet}$ and HBr (2,7-Br_2-Phe) were the dominant ions in the EI mass spectra of the di-Br PAHs.

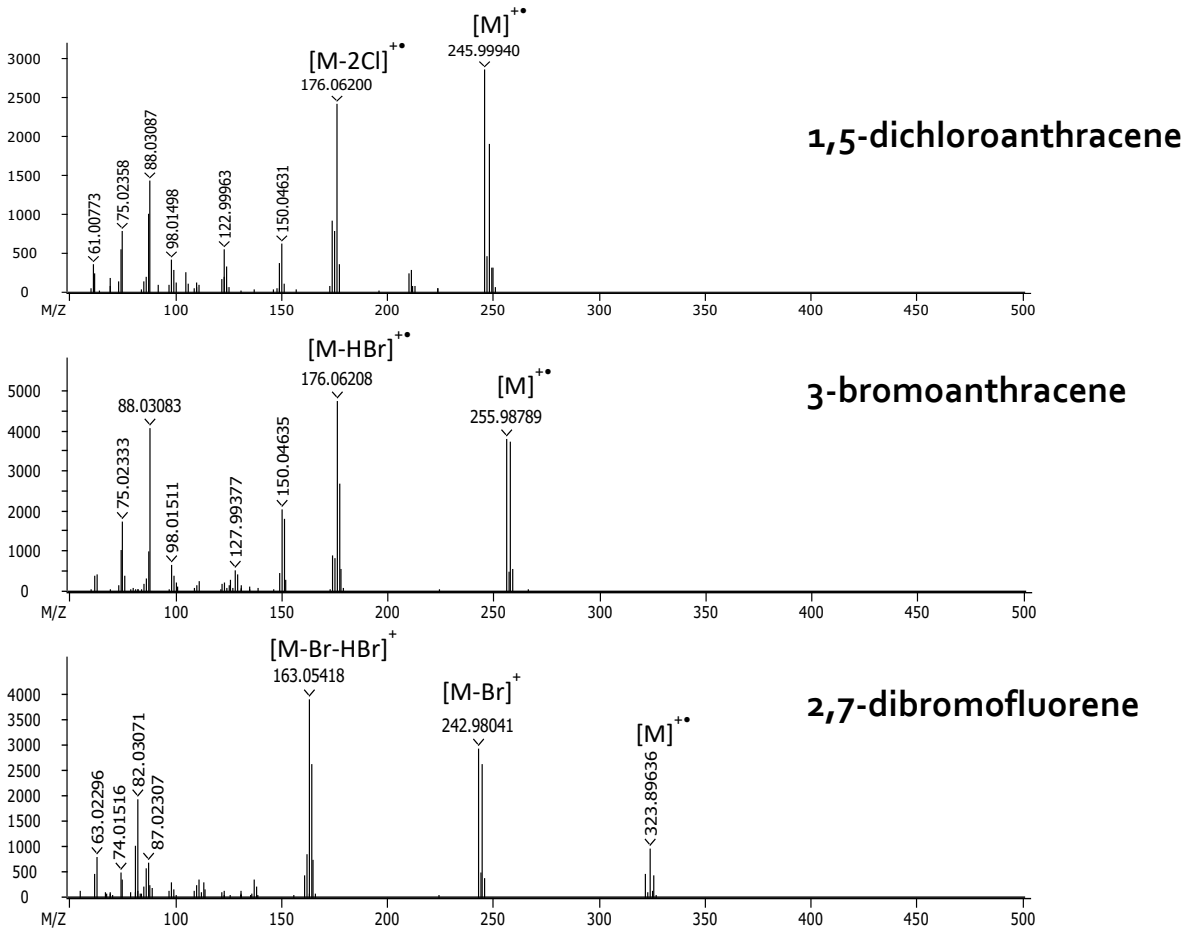

Figure 3.2: *EI full-scan (m/z 50–500 amu) high resolution mass spectra of 1,5-dichloroanthracene (top panel), 3-bromoanthracene (middle panel) and 2,7-dibromofluorene (bottom panel).*

3.4.2 Instrument Verification

As a check of the performance of the GC/HRTOF-MS, the mean experimental Δm was first measured on the 16 HPAHs. Based on our knowledge of the EI fragmentation behavior of the HPAHs, the theoretical exact *m/z* values for the abundant ions characteristic to each HPAH were compared with the experimentally measured mean *m/z* values (5 replicate injections of the standard solution) determined by our system at a RP of 25 000 (*see* Table II.2). The repeatability of replicate measurements of the analytical standards ranged from 0.1 to 0.6 mmu and in all cases the mean

mass accuracy was lower than ±3.5 ppm. Taken together, these results imply that the GC/HRTOF-MS is well-suited for identifying these compounds in environmental samples.

3.4.3 Compound Identification Workflow

The next step was to use the verified method to determine if any HPAHs could be identified in any of the 4 biota samples. To do so, total ion chromatograms were first deconvoluted by constructing exact mass extracted ion chromatograms (XICs) for the 2 prominent ions characteristic of each of the 16 HPAHs. The ions selected that are shown in Table II.2 were determined from repeated injections ($n = 5$) of the standard solution. Figure 3.3 shows examples of the ion chromatograms at exact mass for HPAHs tentatively identified in our samples. The next step in the identification workflow was to compare the experimentally measured mass for the 2 prominent ions in the samples to the theoretically expected *m/z* value. Measured Δm of ±5 ppm was considered reliable for analyte identification. An attempt was made to compare the experimentally measured abundances of ions in the isotopic cluster of identified compounds in samples to their theoretical values. However, because of the small mass of material analyzed and the small concentrations of analytes we were unable to do so. Another consideration in the identification of HPAHs in our samples was to ensure that t_r of identified compounds eluted closely to the analytical standard of similar halogenated content and PAH backbone. A similar approach was also adopted by Millow *et al.* and Manzano *et al*.[29,30] Concentrations of HPAHs in the samples were then estimated by comparing the electronically integrated area of peaks in the exact mass XIC in the samples to the area of HPAH external standard of similar halogenated type and content and with a similar PAH backbone. The mass labeled PAH internal standard used to assess recoveries was > 80% and no recovery correction was applied to the data. We ensured that reported

concentrations were greater than MDL values. With the limited number of isomers currently available it is not possible to discern the substitution positions of the halogen atoms on the HPAHs identified in our samples.

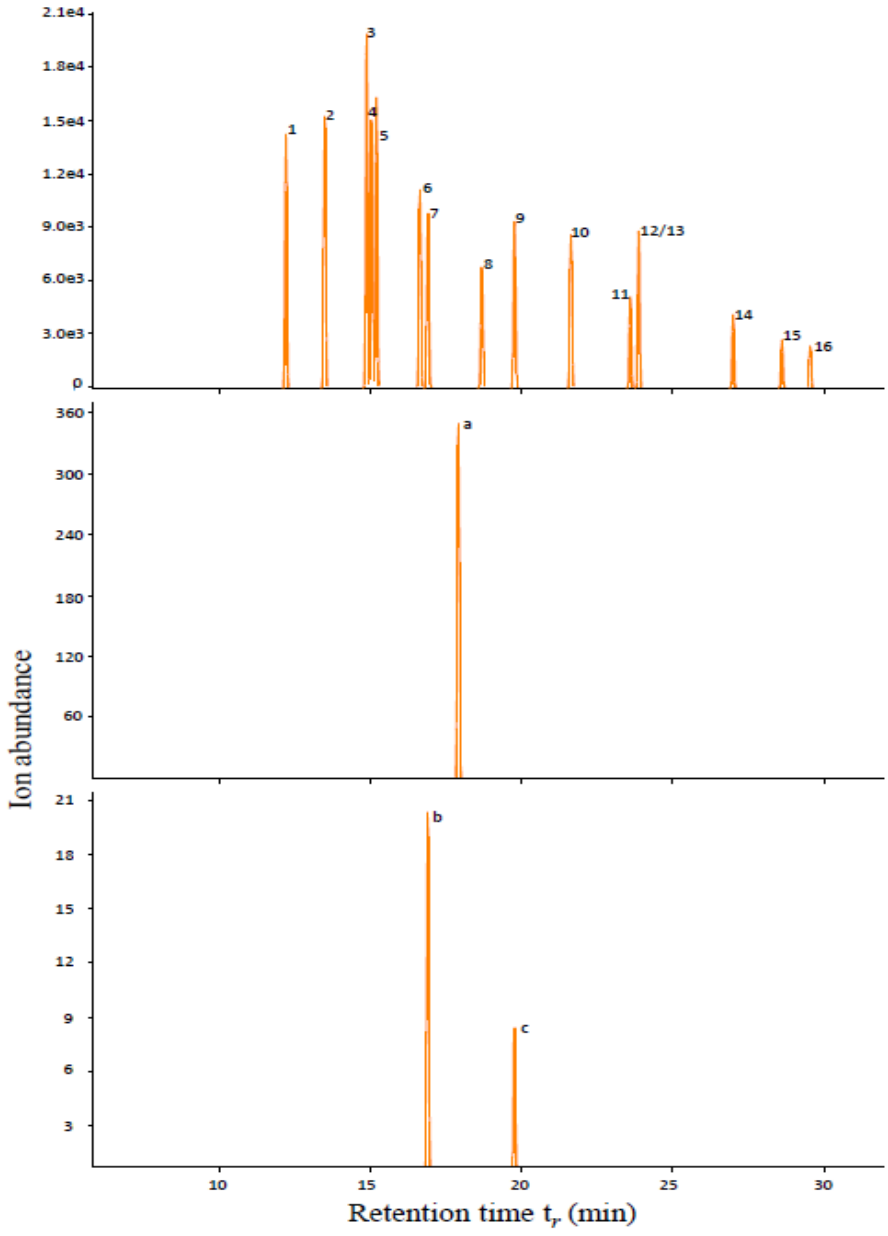

Figure 3.3: *Ion chromatograms of 16 HPAHs standard mixture (top panel), a lake whitefish extract (middle panel; a=Cl_2-Ant/Phe) and a snail extract (bottom panel; b=Br-Ant/Phe, c=Br_2-Fle). Elution order and t_r of HPAHs standards can be found in Table II.2.*

3.4.4 Identification of HPAHs

Figure 3.3 shows the elution profile of 3 HPAHs tentatively identified in our samples. For lake whitefish, a dichloro-anthracene/phenanthrene [$C_{14}H_8Cl_2$, (Cl_2-Ant/Phe)] was identified in all 4 samples. The t_r time of this compound was ~18.0 min which was in good agreement with the elution of the 1,5-Cl_2-Ant standard. Because there are 15 and 25 possible constitutional isomers for a Cl_2-Ant and Cl_2-Phe, respectively, it is perhaps not surprising that the t_r of the compound in the samples was not the same as that of the 1,5-Cl_2-Ant standard.[31] The mean experimental measured masses ($n = 4$) of the 2 characteristic ions in the EI mass spectra of this compound were 245.9995±0.0007 and 176.0619±0.0003 amu. Compared with the theoretically expected mass, this corresponds to respective Δm's of -1.10±1.50 and -0.94±0.84 ppm. Using the response factor of 1,5-Cl_2-Ant, mean concentrations of Cl_2-Ant were estimated to be 16.3 ± 11.4 ng/g, lipid weight (lw, gravimetrically measured, *see* Table 3.1).

Dichloro-anthracene/phenanthrene was also identified and detected in one river otter sample. The peak in the chromatogram that we ascribe to be Cl_2-Ant/Phe had an experimental measured mass of 245.9993 $[M]^{+\bullet}$ and 176.0625 $[M\text{-}2Cl]^{+\bullet}$ with Δm's of -1.75 and 2.73 ppm, respectively. The concentration of this compound in the river otter sample was ~3× smaller than those measured in lake whitefish.

Two brominated compounds were also tentatively identified in our samples (*see* Figure 3.3). A monobrominated anthracene/phenanthrene [$C_{14}H_9Br$, (Br-Ant/Phe)] and a dibrominated fluorene [$C_{13}H_8Br_2$, (Br_2-Fle)] were detected in northern pike and in a snail sample (*see* Table 3.1). The t_r of the Br-Ant/Phe was the same as that of the 9-Br-Phe, however, there are 3 and 5 constitutional isomers of mono-Br Ant and Phe, respectively.[31] But with an identical t_r it is

reasonable to suggest that the compound in our sample is 9-Br-Phe. The experimentally measured masses of the 2 characteristic ions of Br-Ant/Phe in the northern pike samples were 255.9876/176.0621 which correspond to a Δm of -2.38/0.51 ppm, respectively. Similarly for the snail sample, Δm values of 0.82 and 1.99 ppm were obtained for the 2 prominent ions characteristic to this compound. The lipid-normalized concentrations of Br-Ant/Phe were estimated to be 12.5 and 170.5 ng/g in northern pike and snail, respectively, and were based on the response factor of 9-Br-Phe.

Table 3.1: *Concentrations of 3 HPAHs and APAHs in biota samples from AOSR.*

Species	HPAH Detected	Measured Mass	Mass Accuracy (Δm, ppm)	Concentrations (ng/g, lw)[a]	
				HPAHs	APAHs[b]
Lake Whitefish	Cl_2-Ant/Phe	245.9995 176.0619	-1.0976 -0.9372	16.3 ± 11.4[c]	7.3 ± 3.9[c,d]
Snails	Br-Ant/Phe	255.9884 176.0624	0.8203 1.9879	170.5	529.9[e]
	Br_2-Fle	323.8959 163.0541	-2.4390 -0.6746	111.4	316.5[f]
River Otter	Cl_2-Ant/Phe	245.9993 176.0625	-1.7480 2.7263	5.5	--
Northern Pike	Br-Ant/Phe	255.9876 176.0621	-2.3829 0.5111	12.5	--
	Br_2-Fle	323.8971 163.0547	1.1114 2.7598	26.7	3.2[g]

[a] *lw= lipid weight*
[b] *alkylated PAHs*
[c] *mean ± SD measured in 4 samples*
[d] *C_2-Phe*
[e] *sum of 2-, 3- and 4/9-methyl-Phe*
[f] *C_2-Fle*

The experimentally measured *m/z*-values for the 2 prominent ions of Br_2-Fle in the northern pike and snail samples were 323.8971/163.0567 and 323.8959/163.0541, respectively. The t_r of this compound in our samples agreed well with that of 2,7-Br_2-Fle (19.8 min) but because there are 25 theoretical constitutional isomers for a Br_2-Fle we cannot say with certainty that the compound in our samples is in fact 2,7-Br_2-Fle.[31] Measured Δm's were all within ± 3 ppm of the expected *m/z* value. Based on the response factor of 2,7-Br_2-Fle, the concentration of Br_2-Fle in northern pike and snail were estimated to be 26.7 and 111.4 ng/g, lipid weight, respectively.

It is instructive to compare concentrations of HPAHs to those of PAHs and APAHs measured in our samples. There are, however, two caveats that must be acknowledged before doing so. First, the total body burden of HPAHs in organisms from this study is probably underestimated because there are likely other HPAHs that remain undetected. Due to the small sample mass available for our study and limited availability of analytical standards, detection of additional HPAHs (and other HPACs) is challenging. Second, the external standard approach to quantitation whereby the response factor of one standard is used to quantify an analyte in our sample likely introduces a bias in our measurements. To make comparisons more meaningful concentrations of HPAHs were compared to those of APAHs with the same alkyl-substitution content. For example, the concentrations of Br_2-Fle were compared with C_2-Fle and Br-Ant/Phe were compared with C_1-Ant/Phe. Concentrations of PAHs were generally below detection limits in the samples.

In lake whitefish, the mean concentrations of Cl_2-Ant/Phe were ~2× greater than C_2-Ant/Phe. Conversely, the concentrations of APAHs in snails were ~3× greater than HPAHs. In northern pike, Br_2-Fle concentrations were 8× greater than that of C_2-Fle. Similar comparisons for Cl_2-Ant/Phe in river otter and Br-Ant/Phe in northern pike could not be made as APAHs of similar alkyl-content were below detection limits in the samples.

The biomagnification factor (BMF) indicates the extent of enrichment or trophic dilution of a compound between a particular predator/prey feeding relationship and intuitively provides insight into the relative susceptibility of compounds to biotransformation/clearance in biota.[32] Using the river otter/lake whitefish feeding relationship, the liver-based BMF for the dichloro-PAH congener was 0.34. Although the elution times of the dichloro-PAHs congener in both the river otter and lake whitefish were identical, it is possible that the substitution position of the chlorine atoms could be different. We further acknowledge that this BMF represents a single predator/prey feeding relationship, but it does suggest that this HPAH congener is susceptible to metabolism and/or clearance in aquatic organisms.

Interestingly, the concentrations of HPAHs and APAHs were greatest in snails relative to the other pelagic organisms. Snails are benthic feeders and accumulation of HPAHs (and APAHs) would be from their direct interaction with sediments. This suggests that the concentrations of HPAHs in sediments are elevated and/or the metabolic activity of snails are low for these compounds. Further work on characterizing concentrations of HPAHs in sediments is clearly warranted.

Analogous to PAHs and APAHs, organism exposure to HPAHs in the AOSR is likely to be from continuous chronic inputs of these compounds into the receiving environment. By definition, bioaccumulation of environmental contaminants in biological organisms occurs when the rate of chemical uptake and storage exceeds the rate of clearance (*i.e.*, depuration and/or metabolism). It is quite plausible then that clearance of HPAHs is rapid, but that continuous exposure leads to detection in organism. While our study was not designed to specifically address this issue it is clear that further work is needed to understand the toxicokinetics of these compounds in biological organisms.

3.5 Conclusions

To date non-halogenated PACs have been the focus of monitoring activities in the AOSR, but the presence of the halogenated PAHs suggests these compounds may be important to AOSR impacted environments. Challenges to developing a better understanding of the burden of these PAH analogues are prompted by the lack of authentic standards available limiting our ability to identify a comprehensive suite of these compounds in our samples and could have in part contributed to an underestimation of the body burdens detected. More commercially available standards will facilitate a more thorough understanding of the EI fragmentation of these compounds that in turn will help to identify more of these compounds in samples. Admittedly, our study was limited to a small number of HPAHs. It is conceivable that other HPAHs and even halogenated derivatives of APAHs may also be environmentally relevant compounds in the AOSR considering that the APAHs are detected in greater concentrations than their parent PAHs. Using larger sample mass, work is ongoing to identify more of these novel compounds in samples from the AOSR and to assess the efficacy by which these compounds are biotransformed in biological organisms. The identification of 3 HPAHs in samples from the AOSR is the first report of these compounds from this region and the uncertainty in the formation pathway; photochemical vs. diagenesis requires more research to ensure halogenation is not a byproduct of oil sands energy development. Finally, examination of HPAHs concentrations in conjunction with stable isotope analysis will help understand the trophodynamics of these compounds in food webs from the AOSR.

3.6 Acknowledgements

We wish to thank the Alberta Trappers Association and Treaty 8 Nations (including Fort McKay First Nations, Athabasca Chipewyan First Nations and Mikisew Cree First Nation) for providing the samples in this work and for helping with field logistics. This work was supported by a Natural Sciences and Engineering Research Council of Canada (NSERC) Discovery and Engage Grants to GTT. JS and GTT were supported by a NSERC-Strategic Partnership Grant (ID: STPGP: 479210-2015). Funding was also provided by Environment and Climate Change Canada, and the Canada-Alberta Oil Sands Monitoring Program to CM and PJT.

3.7 References

(1) Achten, C.; Andersson, J. T. Overview of polycyclic aromatic compounds (PAC). *Polycycl Aromat Compd* **2015**, *35* (2-4), 177-186. DOI: 10.1080/10406638.2014.994071

(2) Lee, K.; Boufadel, M.; Chen, B.; Foght, J.; Hodson, P.; Swanson, S.; Venosa, A. *Expert panel report on the behaviour and environmental impacts of crude oil released into aqueous environments*; Royal Society of Canada, 2005.

(3) Ma, J.; Horii, Y.; Cheng, J.; Wang, W.; Wu, Q.; Ohura, T.; Kannan, K. Chlorinated and parent polycyclic aromatic hydrocarbons in environmental samples from an electronic waste recycling facility and a chemical industrial complex in China. *Environ Sci Technol* **2009**, *43* (3), 643-649. DOI: 10.1021/es802878w

(4) Ohura, T.; Morita, M.; Makino, M.; Amagai, T.; Shimoi, K. Aryl hydrocarbon receptor-mediated effects of chlorinated polycyclic aromatic hydrocarbons. *Chem Res Toxicol* **2007**, *20* (9), 1237-1241. DOI: 10.1021/tx700148b

(5) Ohura, T.; Sawada, K.; Amagai, T.; Shinomiya, M. Discovery of novel halogenated polycyclic aromatic hydrocarbons in urban particulate matters: Occurrence, photostability, and ahr activity. *Environ Sci Technol* **2009**, *43* (7), 2269-2275. DOI: 10.1021/es803633d

(6) Horii, Y.; Ohura, T.; Yamashita, N.; Kannan, K. Chlorinated polycyclic aromatic hydrocarbons in sediments from industrial areas in Japan and the united states. *Arch Environ Contam Toxicol* **2009**, *57* (4), 651-660. DOI: 10.1007/s00244-009-9372-1

(7) Horii, Y.; Ok, G.; Ohura, T.; Kannanct, K. Occurrence and profiles of chlorinated and brominated polycyclic aromatic hydrocarbons in waste incinerators. *Environ Sci Technol* **2008**, *42* (6), 1904-1909. DOI: 10.1021/es703001f

(8) Jin, R.; Liu, G.; Jiang, X.; Liang, Y.; Fiedler, H.; Yang, L.; Zhu, Q.; Xu, Y.; Gao, L.; Su, G.; Xiao, K.; Zheng, M. Profiles, sources and potential exposures of parent, chlorinated and brominated polycyclic aromatic hydrocarbons in haze associated atmosphere. *Sci Total Environ* **2017**, *593-594*, 390-398. DOI: 10.1016/j.scitotenv.2017.03.134

(9) Kannan, K.; Yamashita, N.; Imagawa, T.; Decoen, W.; Khim, J. S.; Day, R. M.; Summer, C. L.; Giesy, J. P. Polychlorinated naphthalenes and polychlorinated biphenyls in fishes from Michigan waters including the Great Lakes. *Environmental Science & Technology* **2000**, *34* (4), 566-572. DOI: 10.1021/es990966e

(10) Ni, H. G.; Guo, J. Y. Parent and halogenated polycyclic aromatic hydrocarbons in seafood from south China and implications for human exposure. *J Agric Food Chem* **2013**, *61* (8), 2013-2018. DOI: 10.1021/jf304836q

(11) Ieda, T.; Ochiai, N.; Miyawaki, T.; Ohura, T.; Horii, Y. Environmental analysis of chlorinated and brominated polycyclic aromatic hydrocarbons by comprehensive two-dimensional gas chromatography coupled to high-resolution time-of-flight mass spectrometry. *J Chromatogr A* **2011**, *1218* (21), 3224-3232. DOI: 10.1016/j.chroma.2011.01.013

(12) Kelly, E. N.; Schindler, D. W.; Hodson, P. V.; Short, J. W.; Radmanovich, R.; Nielsen, C. C. Oil sands development contributes elements toxic at low concentrations to the Athabasca River and its tributaries. *Proc Natl Acad Sci U S A* **2010**, *107* (37), 16178-16183. DOI: 10.1073/pnas.1008754107

(13) Kurek, J.; Kirk, J. L.; Muir, D. C.; Wang, X.; Evans, M. S.; Smol, J. P. Legacy of a half century of Athabasca Oil Sands development recorded by lake ecosystems. *Proc Natl Acad Sci U S A* **2013**, *110* (5), 1761-1766. DOI: 10.1073/pnas.1217675110

(14) Thienpont, J. R.; Desjardins, C. M.; Kimpe, L. E.; Korosi, J. B.; Kokelj, S. V.; Palmer, M. J.; Muir, D. C. G.; Kirk, J. L.; Smol, J. P.; Blais, J. M. Comparative histories of polycyclic aromatic compound accumulation in lake sediments near petroleum operations in western Canada. *Environ Pollut* **2017**, *231* (Pt 1), 13-21. DOI: 10.1016/j.envpol.2017.07.064
(15) Strausz, O. P.; Lown, E. M. *The chemistry of Alberta Oil Sands, bitumens and heavy oils*; Alberta Energy Research Institute Calgary, AB, 2003.
(16) Boutin, C.; Carpenter, D. J. Assessment of wetland/upland vegetation communities and evaluation of soil-plant contamination by polycyclic aromatic hydrocarbons and trace metals in regions near oil sands mining in Alberta. *Sci Total Environ* **2017**, *576*, 829-839. DOI: 10.1016/j.scitotenv.2016.10.062
(17) Fernie, K. J.; Marteinson, S. C.; Chen, D.; Eng, A.; Harner, T.; Smits, J. E. G.; Soos, C. Elevated exposure, uptake and accumulation of polycyclic aromatic hydrocarbons by nestling tree swallows (Tachycineta Bicolor) through multiple exposure routes in active mining-related areas of the Athabasca Oil Sands Region. *Sci Total Environ* **2018**, *624*, 250-261. DOI: 10.1016/j.scitotenv.2017.12.123
(18) Lundin, J. I.; Riffell, J. A.; Wasser, S. K. Polycyclic aromatic hydrocarbons in caribou, moose, and wolf scat samples from three areas of the Alberta Oil Sands. *Environ Pollut* **2015**, *206*, 527-534. DOI: 10.1016/j.envpol.2015.07.035
(19) Ohiozebau, E.; Tendler, B.; Hill, A.; Codling, G.; Kelly, E.; Giesy, J. P.; Jones, P. D. Products of biotransformation of polycyclic aromatic hydrocarbons in fishes of the Athabasca/Slave river system, Canada. *Environ Geochem Health* **2016**, *38* (2), 577-591. DOI: 10.1007/s10653-015-9744-6
(20) Parrott, J. L.; Marentette, J. R.; Hewitt, L. M.; McMaster, M. E.; Gillis, P. L.; Norwood, W. P.; Kirk, J. L.; Peru, K. M.; Headley, J. V.; Wang, Z.; Yang, C.; Frank, R. A. Meltwater from snow contaminated by oil sands emissions is toxic to larval fish, but not spring river water. *Sci Total Environ* **2018**, *625*, 264-274. DOI: 10.1016/j.scitotenv.2017.12.284
(21) Ohiozebau, E.; Tendler, B.; Codling, G.; Kelly, E.; Giesy, J. P.; Jones, P. D. Potential health risks posed by polycyclic aromatic hydrocarbons in muscle tissues of fishes from the Athabasca and Slave Rivers, Canada. *Environ Geochem Health* **2017**, *39* (1), 139-160. DOI: 10.1007/s10653-016-9815-3
(22) Musial, G.; Reynaud, J.-Y.; Gingras, M. K.; Féniès, H.; Labourdette, R.; Parize, O. Subsurface and outcrop characterization of large tidally influenced point bars of the Cretaceous Mcmurray Formation (Alberta, Canada). *Sedimentary Geology* **2012**, *279*, 156-172. DOI: 10.1016/j.sedgeo.2011.04.020
(23) Ranger, M. J.; Pemberton, S. G. Marine influence on the mcmurray formation in the primrose area, Alberta. **1988**.
(24) Sankoda, K.; Nomiyama, K.; Yonehara, T.; Kuribayashi, T.; Shinohara, R. Evidence for in situ production of chlorinated polycyclic aromatic hydrocarbons on tidal flats: Environmental monitoring and laboratory scale experiment. *Chemosphere* **2012**, *88* (5), 542-547. DOI: 10.1016/j.chemosphere.2012.03.017
(25) Sun, J. L.; Zeng, H.; Ni, H. G. Halogenated polycyclic aromatic hydrocarbons in the environment. *Chemosphere* **2013**, *90* (6), 1751-1759. DOI: 10.1016/j.chemosphere.2012.10.094
(26) Horii, Y.; Khim, J. S.; Higley, E. B.; Giesy, J. P.; Ohura, T.; Kannan, K. Relative potencies of individual chlorinated and brominated polycyclic aromatic hydrocarbons for induction

of aryl hydrocarbon receptor-mediated responses. *Environ Sci Technol* **2009**, *43* (6), 2159-2165. DOI: 10.1021/es8030402
(27) Idowu, I.; Francisco, O.; Thomas, P. J.; Johnson, W.; Marvin, C.; Stetefeld, J.; Tomy, G. T. Validation of a simultaneous method for determining polycyclic aromatic compounds and alkylated isomers in biota. *Rapid Commun Mass Spectrom* **2018**, *32* (3), 277-287. DOI: 10.1002/rcm.8035
(28) Magnusson, B.; Örnemark, U. *The fitness for purpose of analytical methods – a laboratory guide to method validation and related topics (2nd ed)*; 2014.
(29) Manzano, C. A.; Marvin, C.; Muir, D.; Harner, T.; Martin, J.; Zhang, Y. Heterocyclic aromatics in petroleum coke, snow, lake sediments, and air samples from the Athabasca oil sands region. *Environ Sci Technol* **2017**, *51* (10), 5445-5453. DOI: 10.1021/acs.est.7b01345
(30) Millow, C. J.; Mackintosh, S. A.; Lewison, R. L.; Dodder, N. G.; Hoh, E. Identifying bioaccumulative halogenated organic compounds using a nontargeted analytical approach: Seabirds as sentinels. *PLOS ONE* **2015**, *10* (5), e0127205. DOI: 10.1371/journal.pone.0127205
(31) Johnson, W.; Idowu, I.; Francisco, O.; Marvin, C.; Thomas, P. J.; Stetefeld, J.; Tomy, G. T. Enumeration of the constitutional isomers of environmentally relevant substituted polycyclic aromatic compounds. *Chemosphere* **2018**, *202*, 9-16. DOI: 10.1016/j.chemosphere.2018.03.035
(32) Leeuwen, C. J. v.; Vermeire, T. G. *Risk assessment of chemicals: An introduction*; Springer Nature, 2007.

Chapter 4: New Approaches to Reduce Sample Processing Times for the Determination of Polycyclic Aromatic Compounds in Environmental Samples

A version of this chapter has already been published as Zhe Xia, Ifeoluwa Idowu, Evan Kerr, Nicole Klaassen, Harsimran Assi, Harley Bray, Chris Marvin, Philippe J. Thomas, Jörg Stetefeld and Gregg T. Tomy. New approaches to reduce sample processing times for the determination of polycyclic aromatic compounds in environmental samples. *Chemosphere*, **2021**, *274*, 129738.

4.1 Abstract

This study validates two approaches to streamlining the processing of sediment and biota for a suite of polycyclic aromatic compounds (PACs) with a wide range of chemical properties, including polycyclic aromatic hydrocarbons (PAHs) and alkyl-PAHs (APAHs), and a new class of environmental contaminants, halogenated-PACs (HPAHs). One method is based on one-step *in situ* extraction/cleanup using accelerated solvent extraction (ASE) in which a mixture of copper, deactivated alumina and silica gel were added directly to the ASE cell along with sample; the second technique is based on dispersive solid phase extraction (dSPE) using alumina/silica for cleanup of biota samples to augment conventional ASE extraction combined with gel permeation chromatography. Validation protocols were performed in accordance with the ISO/IEC 17025 guidelines, whereby method performance characteristics, *i.e.*, accuracy, precision, linearity, limits of detection and ruggedness, were evaluated. Accuracies generally ranged from 70 – 120% for the *in situ* ASE method and 70 – 100% for the dSPE technique. Limits of detection/quantitation for the 45 target analytes for *in situ* ASE and dSPE methods were determined to be < 2.5/8 pg μL^{-1}, and < 20/60 pg μL^{-1}, respectively. Intra and inter-day repeatability for both methods were < 25% except for 1 APAH which had an inter-day precision of 35% using the dSPE method. Neither method was affected by any of the purposeful changes attempted which implies that both methods are robust. Results of our validation studies showed excellent data quality for both methods in addition to achieving a reduction in sample processing times.

4.2 Introduction

Polycyclic aromatic compounds (PACs) can be found naturally in compounds such as bitumen or generated through the incomplete combustion of organic materials, but predominantly they are introduced into the environment by anthropogenic means.[1,2] The burning of coal, wood and oil or accidental crude oil spills, for example, can release these PACs into the environment.[3] These compounds have been detected globally in many environmental compartments including biota, soil, water and in the air.[3-7] Polycyclic aromatic compounds can have deleterious impacts on biological systems because many of these compounds have the ability to bioaccumulate and have exhibited a multitude of toxic, carcinogenic and/or mutagenic effects.[3,6] Environmental monitoring of PACs is paramount to understanding their spatial and temporal distribution and global sampling/monitoring campaigns have already been established by many countries.[4,6,8-11] Naturally, concomitant with these large global monitoring networks for PACs is the need to identify and quantify PACs in a timely and cost-effective manner.

Currently, a common method for extracting PACs from a given matrix uses pressurised liquid extraction (PLE), often referred to as accelerated solvent extraction (ASE).[4,12,13] By utilizing elevated temperature and pressure for a prescribed amount of time with appropriately selected organic solvents, it is possible to efficiently disrupt target analyte-matrix interaction(s) and exhaustively extract the desired analyte from various environmental matrices.[14] Following ASE extraction, it is typical to further purify sample extracts using column chromatography to remove any co-extracted interferences. The overarching principle of this step is to exploit differences in the chemical interactions of the target analyte(s) with the solvent(s) and adsorbent used in the column chromatography step. Like any other analytical protocol, the introduction of an additional

step in a sample workflow can lead to potential losses of analyte and an increase in overall sample processing times.

In an effort to minimize sample preparation cost and time, the QuEChERS method for analysis of PACs in environmental samples was introduced.[15-20] The technique, developed by Anastassiades *et al.*, was designed to provide a rapid and inexpensive method for analysis of pesticide residues in fruits and vegetables.[21] Because QuEChERS exploits partition properties of target analyte(s) with their matrix and extraction solvents, the technique has been used in processing of samples for PAC analysis. Specifically, QuEChERS has been used to analyze polycyclic aromatic hydrocarbons (PAHs) in biotic and abiotic samples.[16-20]

While the QuEChERS method does have advantages relative to other methods for PAH analysis, there are some inherent disadvantages to the method, especially when there is a large range in chemical properties of target analytes. Selecting the appropriate QuEChERS solvent(s) to efficiently and exhaustively extract the PACs examined in this study, which have a large disparity in octanol-water partition coefficients (K_{OW}), is challenging.[22] Furthermore, others have shown that there is often a requirement for a low sample weight to final extract ratio that can lead to varying degrees of matrix effects depending on the type of tissue material processed.[23-25] In addition, it has been shown that there is a potential for PAH contamination stemming from the choice of dispersant used and QuEChERS tubes.[15,26]

Due to the apparent deficiencies of QuEChERS for some analytes, we have considered alternative techniques for combining extraction and cleanup of environmental samples while retaining the performance characteristic of classical methods. Idowu *et al.* (2018) presented a validated method for the analysis of PAHs and alkylated PAHs in environmental samples based

on ASE extraction and gas chromatography mass spectrometry for detection and quantitation.[12] This work was conducted in an ISO accredited laboratory and under strict adherence with the Eurachem Guide on method validation. Recently, three studies reported on a layered, *in situ* one-step ASE extraction and clean-up of PACs from sediment samples with no additional sample processing steps.[27-29] With the elevated pressures employed in ASE and the forceful nature of solvent introduction into the ASE cell it is likely that the carefully prepared layers would be disturbed and that mixing of the layers would arise. Based on this we hypothesize that an *in situ* non-layered ASE approach with judicious selection of extraction solvent(s) would enable exhaustive extraction of a broad suite of PACs. In keeping with our intention of streamlining sample workflows for PAC analyses, we also validated a simple alumina/silica dispersive solid phase extraction (dSPE) clean-up step *post*-ASE and gel permeation chromatography (GPC) for biota extracts to replace the time-consuming column chromatography step.

4.3 Materials and Methods

4.3.1 Chemicals

Organic solvents and diatomaceous earth (DE) dispersant were purchased from Fisher Scientific (Ottawa, ON). Silica gel, alumina, anhydrous sodium sulphate and Ottawa Sand were also from Fisher Chemicals. Details of target analytes and their suppliers can be found in the Table III.1. The suite of labelled internal standards used for recovery internal standard (RIS) were d_8-naphthalene, d_8-acenaphthylene, d_{10}-acenaphthene, d_{10}-fluorene, d_{10}-phenanthrene, d_{10}-pyrene, d_{12}-benz(*a*)anthracene, d_{12}-chrysene, d_{12}-benzo(*b*)fluoranthene, d_{12}-benzo(*k*)fluoranthene, d_{12}-benzo(*a*)pyrene, d_{12}-indeno(1,2,3-*c*,*d*)pyrene, d_{14}-dibenz(*a*,*h*)anthracene, and d_{14}-

benzo(*g,h,i*)perylene. Labelled anthracene (d_{10}-anthracene) was used as the instrument performance internal standard (IPIS).

4.3.2 Samples

Two samples were used to evaluate the performance characteristics of the one-step extraction method. As part of our ISO accreditation status, our laboratory participates in biannual performance testing (PT) exercises that are coordinated by the Canadian Association for Laboratory Accreditation (CALA, Ontario, ON). A soil sample from CALA containing PACs (CALA C18-1) was used for the study. In addition, the Standard Reference Material (SRM) 1944 [National Institute of Standards and Technology (NIST), Gaithersburg, MD], which is a sample of New York/New Jersey Waterway sediment and contains certified concentrations of many PACs, was also used. Validation of the dSPE technique using silica gel/alumina was performed on a lake trout (*Salvelinus namaycush*) purchased from a local retail store (Winnipeg, MB). The fillet was homogenized using a Knife Mill Grindomix Retsch GM 200 (Newton, PA, USA) and stored in a -80 º C freezer.

4.3.3 Abbreviations

For the HPAHs, the abbreviations used here begin with the substitution positions of the halogen(s), following by the substituted halogen atom and parent PAHs structure. The abbreviation of PAHs is listed below: Naphthalene (Nap), acenaphthylene (Acy), acenaphthene (Ace), fluorene (Flu), phenanthrene (Phe), anthracene (Ant), fluoranthene (Flu), pyrene (Pyr), benzo[*a*]anthracene (BaA), chrysene (Chr), benzo[*b*]fluoranthene (BbF), benzo[*k*]fluoranthene (BkF), benzo[*a*]pyrene (BaP), benzo[*g,h,i*]perylene (BghiP), indeno[1,2,3-*c,d*]pyrene (Ind), and dibenz[*a,h*]anthracene (DahA). The full list of abbreviations can be found in the Table III.1.

4.3.4 One-step Layerless Extraction Protocol for Sediment

Both CALA C18-1 and SRM1944 were extracted using a Dionex ASE 350 (ThermoFisher, Mississauga, ON). A glass fiber filter was first placed at the bottom of the 34 mL ASE cell. A mixture of 1 g reduced copper, 4.5 g 5% deactivated alumina, and 5 g silica gel was placed into the cell. Sub-samples of well mixed CALA C18-1 (0.5 g) were weighed, mixed with DE dispersant (1.5 g, baked at 600°C for 6 hours), and added to the ASE cells. The samples were then spiked with a recovery internal standard. Finally, Ottawa sand (baked at 600°C for 6 hours) was used to fill the dead volume of the cell to approximately 0.5 cm below the top of the cell. The ASE extraction conditions were as follows: system pressure 1500 psi, oven temperature 120 °C with a heat up time of 6 min., static time of 15 min. with 1 static cycle, DCM as the extraction solvent, 60% flush volume and a nitrogen purge for 80 seconds at 150 psi. After extraction, the extract was treated with sodium sulphate to remove moisture, and subsequently transferred to a 250 mL round bottom flask. The sample volume was reduced to approximately 2 mL, transferred to a glass test tube with 3 washes of hexane (~5 mL each) and reduced to 5 mL using a gentle stream of ultra-high purity (UHP) nitrogen. An instrument performance internal standard (IPIS, d_{10}-anthracene) was added to in the sample. Amber GC vials were used to store the samples under refrigeration at 4 °C until they were analyzed.

4.3.5 Method Validation of One-step Layerless Extraction

The Eurachem Guide – The Fitness for the Purpose of Analytical Methods, stipulates performance characteristics for method validation, namely detection limits, working range, accuracy/recovery, precision, and ruggedness.[30]

4.3.5.1 Detection Limits

LOD/LOQs for our one-step extraction study were calculated by extracting *ca.* 1.5 g DE dispersant (*n = 10*) fortified with 10 µL of 5 ng μL^{-1} PAHs and HPAHs. The standard deviations (s_0) of signals for each target analyte were calculated and an adjusted standard deviation (s_0') as defined in the Eurachem Guide was calculated by the ratio $s_0' = s_0/\sqrt{n}$. Finally, target analyte specific LODs were determined as 3* s_0', and LOQs as 10* s_0'.[30] LODs and LOQs are reported on a mass volume basis.

4.3.5.2 Working Range

For HPAHs, a six-point external calibration curve (10, 200, 400, 600, 800, 1000 pg μL^{-1}) was constructed with a 100 pg μL^{-1} IPIS added to each point to account for small systematic errors that may arise from fluctuations in injection volumes and ionization efficiencies between injections. Calibration standards were run randomly in triplicate with a solvent blank injection. The signal response of each analyte was normalised to the IPIS and plotted as a function of concentration. Linearity was evaluated by the magnitude of R^2 (correlation coefficient) and the level of significance (*i.e.*, *p*-value) for each of the analytes. Residual plots were also examined to ensure that data was randomly distributed about zero. For PAHs and APAHs, working range could be found in Idowu *et al*.[12]

4.3.5.3 Accuracy

For our one-step extraction validation study, CALA C18-1 (*n = 6*) were extracted and analysed for PAHs; for HPAHs, DE dispersant (*n = 10*) was fortified with the 100 pg μL^{-1} HPAH mixture.

4.3.5.4 Precision

Repeatability on our one step extraction was calculated by extracting and quantifying PAH from CALA C18-1 in replicate over a 24 h period (intraday, $n = 6$) and over 3 consecutive days (interday, $n = 3$); for HPAHs, precision was determined by replicate extraction of fortified DE dispersant over a 24 h period (intraday, $n = 6$) and over 3 consecutive days (interday, $n = 3$).

4.3.5.5 Ruggedness

The ruggedness of our one-step extraction protocol was determined by purposefully changing the extraction temperature (115 °C and 125 °C, $n = 3$ in each case) or the flush volume of solvent used in extraction (55% and 65%, $n = 3$ in each case). Student *t*-tests ($t = 0.05$, two-tail) were run to determine if any of the changes resulted in statistically significant differences.

4.3.5.6 Extraction and dSPE Protocol for Biota

A glass fiber filter was placed in the bottom of the 34 mL ASE cell. A 5.0 g sub-sample of homogenized lake trout sample was weighed and mixed with DE Dispersant (3.5 g). The mixture was transferred to 34 mL ASE cells. Ottawa sand was used to fill the dead volume of the cell to approximately 0.5 cm below the top of the cell. The ASE condition was the same as for the one-step extraction study. Following extraction, samples were reduced in volume to 5 mL using a Genevac SP scientific evaporation system (Warminster, PA, USA). Sodium sulfate (3 g) was added to remove water and the extracts further reduced to 2.6 mL under a gentle stream of UHP nitrogen and then brought to 5.2 mL with hexane. 200 µL of extracts were transferred to pre-weighed aluminium boats for lipid determination. Extracts (5.0 mL) were then applied to the head of a GPC column (J_2-scientific AccuPrepMPSTM, Columbia, Missouri, USA) packed with 60 g

of S-X3 Biobeads to remove excess lipids and protein. The following parameters were employed by the fully-automated system; injection volume: 5 mL; pressure: 8 psi; flow rate: 5 mL/min; mobile phase: hexane:DCM (1:1, v/v). The first 120 mL of effluent contained lipids and other biogenic material and was discarded. The fraction containing analytes eluted from 125 – 250 mLs and was collected in a 250 mL round bottom flask; the extract was reduced in volume to 1 mL via Genevac solvent evaporator and nitrogen evaporation. The 1 mL extract was transferred to a 125 mL round bottom flask with successive washes of 70:30% (DCM: hexane, 2 x 10 mL each) containing silica gel (4 g), 0.5 g sodium sulfate and 5% deactivated alumina (1 g). The flasks were allowed to sit for 30 min with periodic swirling every 10 min. The extract was then transferred to a 60 mL ASE tube and reduced to 200 μL by Genevac under nitrogen. Extracts were then fortified with the IPIS (100 pg μL^{-1}) and stored at 4 ºC in amber vials.

4.3.6 Method Validation for dSPE

4.3.6.1 Detection Limits

For the dSPE study, LOD/LOQs were determined by replicate extractions of lake trout samples (*n = 8*) fortified with a 10 μL of 1 ng μL^{-1} PAC mixture. The standard deviations (s_0) of signals for each target analyte were calculated and an adjusted standard deviation (s_0'), as defined in the Eurachem Guide, was calculated uisng the ratio $s_0' = s_0/\sqrt{n}$. Analyte specific LODs were determined as 3* s_0', and LOQs as 10* s_0'.[30] LODs and LOQs are reported on a mass to volume basis.

4.3.6.2 Accuracy

For validation of the dSPE, lake trout samples (5.0 g, *n = 8*) were used for accuracy tests, which were fortified with 10 µL of 0.2, 2 or 10 ng μL^{-1} PAC mixture, respectively.

4.3.6.3 Precision

For dSPE study, precision was determined by replicate extractions of lake trout samples (*n = 8*) fortified with mixture of 10 µL of 2 ng μL^{-1} PACs.

4.3.6.4 Ruggedness

For dSPE study, ruggedness was determined by changing either the mass of silica gel (3.5 g and 4.5 g, *n = 3* in each case), mass of deactivated alumina (0.8 g and 1.2 g, *n = 3* in each case), composition of the solvent systems (65:35%, DCM:hexane, *n = 3*) and the rinse cycle on the dSPE (1 cycled, *n = 3*). Student *t*-tests (*t* = 0.05, two-tail) were run to determine if any of the changes result in statistically significant differences.

4.3.7 GC-MS/MS Conditions

An Agilent 7890 GC coupled with a triple quadrupole Agilent 7000C mass spectrometer fitted with electron ionization (EI) source was used for the MS/MS acquisition. An Agilent J&W DB-5ms ultra inert column (30 m × 0.25 mm × 0.25 µm) and helium was used as the carrier gas at a constant flow rate of 1.2 mL/min. 1 µL of sample was injected with a PAL RSI 85 auto sampler at 250 °C in splitless mode. The oven temperature for PAHs and APAHs was held at 60 °C for 1 min then raised to 120 °C at 35 °C/min, further ramped up to 220 °C at 14 °C/min, to 260 °C at 3 °C/min and held for 5 min, 300 °C at 10 °C/min, and finally to 310 °C at 50 °C/min. For HPAHs, the oven

temperature for PAHs was held at 60 °C for 1 min then raised to 210°C at 35 °C/min, further ramped up to 260 °C at 2 °C/min, 300 °C at 10 °C/min and held for 5 min, and finally to 325 °C at 50 °C/min and held for 5.5 min. Both transfer line and source temperature were set at 320 °C and UHP nitrogen was used as the collision gas at 60 psi. The quantification and confirmation ions and the multiple reaction monitoring (MRM) ion transitions for PAHs and APAHs can be found in Idowu *et al.*[12] Similar details for HPAHs MRM ion transitions that were developed for this study can be found in Table III.2.

4.4 Results and Discussion

The performance characteristics for the one-step extraction and dSPE techniques are shown in Table 4.1 and Table 4.2, respectively. For the one-step extraction, LODs/LOQs for PAHs were all ≤ 1.9/6.3 pg μL^{-1} and ranged from 0.5/1.5 pg μL^{-1} (benzo[*b*]fluoranthene) to 1.9/6.3 pg μL^{-1} (benzo[*a*]pyrene). The LODs/LOQs for HPAHs were all below 10 pg μL^{-1} and ranged from 0.3/1.0 pg μL^{-1} (9-Cl-Phe) to 2.3/7.8 pg μL^{-1} (2-Br-Ant). We also examined LODs for all our target analytes using the layered approach described in our previous study and compared them to the LODs using the layerless approach.[29] Interestingly, LODs for all PAHs using our layerless extraction approach were consistently smaller (Student *t*-test, $p < 0.05$) than LODs using the layered method (data not shown). For HPAHs, while LODs obtained from the layerless method were smaller than the layered approach (data not shown), only for 10 of the 22 analytes were those differences statistically significant (Student *t*-test, $p < 0.05$). Visual inspection of *post*-ASE extracts obtained from the layerless approach were found to be clearer than those from the layered

method. Whether clearer extracts are related to the smaller LOD values obtained from the layerless approach remains unclear.

The dSPE method exhibited comparable LODs/LOQs for PAHs, which ranged from 1.5/5.0 pg μL^{-1} (benzo[*g,h,i*]perylene) to 6.1/20.4 pg μL^{-1} (fluorene). However, HPAHs, however, had overall higher LODs/LOQs, ranging from 5.6/18.7 pg μL^{-1} (4-Br-Pyr) to 15.6/52.1 pg μL^{-1} (2,7-Br_2-Phe). For APAHs, LODs/LOQs ranged from 3.3/11.0 pg μL^{-1} (1-methylfluorene) to 15.3/51.0 pg μL^{-1} (1,2,5,6-tetramethylnaphthalene).

Calibration standards for HPAHs were run randomly in triplicate with blanks injected between each concentration level to prevent carry-over in each subsequent set of injections. Slopes of the calibration curve for HPAHs had correlation coefficients (R^2) that were all > 0.99 with *p*-values < 0.05. Residual plots demonstrated data normality scattered around zero (data not shown). Taken together, the linear regression models obtained for each analyte were considered acceptable.[31]

The accuracy of our one-step method for PAHs was determined by comparing the reported values in the CALA C18-1 and SRM-1944 samples to our measured values. Table 4.3 shows the results of these comparisons. While there are no universally accepted criteria for assessing method bias, our laboratory uses a systematic error (*i.e.*, bias) threshold of ± 30% as being acceptable. This is consistent with the criterion used for acceptable limits for NIST interlaboratory analytical comparison studies.[32] In generally, our measured values are all between 70–120% except for naphthalene (123.2%) and fluorene (57.1%). For HPAHs, our method accuracy was assessed by fortifying the DE with known amounts of HPAHs and comparing the measured values to fortified amounts. With the exception of 2,3,9,10-Br_4-Ant, the accuracies for all HPAHs were all between

70-120%. The high bias observed for 2,3,9,10-Br_4-Ant could be attributed to its very low response factor on GC-MS/MS which can sometimes make detection and quantification problematic.

For our dSPE study, there were no statistically significant differences in recoveries for PAHs at the low and medium spiking level (Student *t*-test, $p > 0.05$) while for a few exceptions for recoveries of PAHs at the high dose were higher (Student *t*-test, $p < 0.05$) compared to the low and medium treatment levels. At the low and medium spiking levels, PAH recoveries ranged from 68.2 to 116.4%. At the high dose, average recoveries for all 16 PAHs were 106% and 3 PAHs (acenaphthene, anthracene and fluorene) showed biases that were slightly greater than 30% (*see* Table 4.2). For HPAHs, all recoveries were determined to be in the range 60–120%, except 2,3,9,10-Br_4-Ant at the spiking level of 10 pg μL^{-1} (58.9%) and 500 pg μL^{-1} (56.6%). The low recovery of 2,3,9,10-Br_4-Ant could be due in part to the quantification difficulty resulted from low response factor. The recoveries for APAHs at all spiking levels were generally higher than those of the HPAHs except for a few compounds were within the ± 30% desirable range.

We compared the recoveries of PAHs in fish from our study to a similar study done using the QuEChERS method.[16] Double-tailed, paired student *t*-tests were run for all PAHs at the respective spiking levels. For all of PAHs, all *p*-values had been found to be greater than 0.05, which implied that there is no statistical difference between the performance of the two methods. As mentioned earlier, there are no reports on the use of the QuEChERS for other PACs likely because of the difficulty in selecting a solvent system that would effectively be able to extract PACs that span a large range of K_{OW} values like those examined in this study.

In our one-step extraction study, precision (relative standard deviation, RSD) for PAHs were calculated using 6 separate extractions over 24 h (intraday), and over 3 consecutive days (interday).

They ranged from 5.7% to 16.6% (acenaphthylene and anthracene, intraday) and 6.7% to 25.4% (fluorene and benzo[*b*]fluoranthene, interday). The precision was lower than 20% for all HPAHs; intraday precision ranged from 3.4% to 19.0% (5,6-Br_2-Ana and 1,5,9,10-Cl_4-Ant); interday precision ranged 7.3% to 18.8% (9-Br-Phe and 7,12-Cl_2-BaA). The repeatability for PAHs and HPAHs in our dSPE study were all lower than 20%. For APAHs, the repeatability values were all lower than 20%, except 2,6-dimethylnaphthalene at the spiking level of 10 pg μL^{-1} (34.3%).

The ruggedness of our methods was tested by making small, purposeful changes to our adopted procedure. We selected what we felt to be steps that would have the potential to impact the quality of our analytical data the most. Despite the changes made, neither method showed any deterioration to the quality of the data and implies that both methods are robust.

It is acknowledged that soil and/or sediment rich in elemental sulphur can compromise the quality of analytical data.[33,34] This is especially true if sulphur is not efficiently removed from sample extracts prior to GC-injection. In our study we did not observe any of pernicious effects of sulphur implying that our 1 g of reduced copper in our ASE cell was effective at removing the sulphur present in our two test materials. Greater amounts of copper can be used to clean-up samples with higher sulphur content.

4.5 Conclusions

The overarching goal of developing new approaches to chemical analyses is to reduce sample processing times without compromising the quality of analytical data. Using ISO-accepted guidelines for generating data quality in analytical chemistry, we validated two approaches to

streamlining the processing of biota and abiotic samples for a wide range of PACs. For sediments, the one step *in situ* ASE extraction and clean-up method negated the need for further sample processing, with the exception of volume reduction. Similarly, for biota samples, lipid-free extracts were purified using the dSPE approach with alumina/silica as the dispersant. This approach eliminated the need for column chromatography and, unlike some dispersants used in the QuEChERS method, did not contain detectable amounts of any of our target analytes. The quality of the data generated in our validation procedures implied both methods can reduce resource requirements, compared to more classical methods, in addition to providing superior quality performance objectives.

4.6 Acknowledgements

This work was supported by a Natural Sciences and Engineering Research Council – Discovery Grant to GTT (Grant #: RGPIN-05354-2019).

Table 4.1: *Method validation parameters for the analysis of PAHs and HPAHs GC-EI-MS/MS for one-step extraction study in sediment and soil.*

Compounds	Working Range (pg µL^{-1})	Accuracy (%)	Precision (RSD %)		LOD (pg µL^{-1})	LOQ (pg µL^{-1})
			Intraday	Interday		
PAHs						
Acenapthene	10–1000[12]	71.6	11.3	7.0	0.6	1.8
Acenapthylene		69.8	5.7	14.5	0.6	1.9
Anthracene		90.1	16.6	14.8	1.9	6.3
Benz[*a*]anthracene		76.1	10.8	11.9	1.0	3.5
Benzo[*a*]pyrene		85.5	16.4	17.9	1.6	5.3
Benzo[*b*]fluoranthene		78.7	10.4	25.4	0.5	1.5
Benzo[*g,h,i*]perylene		95.5	16.2	16.6	1.7	5.8
Benzo[*k*]fluoranthene		88.4	12.6	11.9	1.2	3.9
Chrysene		88.9	13.0	13.2	0.9	2.8
Dibenzo[*a,h*]anthracene		80.2	12.6	10.6	1.5	5.1
Fluoranthene		77.5	13.2	12.7	0.8	2.5
Fluorene		57.1	9.9	6.7	0.6	1.9
Indeno[1,2,3-*c,d*]pyrene		82.8	12.2	14.8	1.9	6.2
Naphthalene		123.2	9.7	18.0	0.9	3.1
Phenanthrene		80.6	12.3	10.3	1.2	4.1
Pyrene		71.2	13.1	15.0	0.8	2.8
HPAHs						
1,2-Br_2-Any	10–500	90.1	4.3	10.7	0.6	2.1
1,4-Cl_2-Ant		92.9	4.7	12.5	0.8	2.5
1,5,9,10-Cl_4-Ant		100.6	19.0	14.7	0.9	2.9
1,5-Cl_2-Ant		89.9	10.0	9.9	0.7	2.4
1,6-Br_2-Pyr		97.1	16.9	18.2	1.5	5.0
1,8-Br_2-Ant		94.4	7.5	18.5	0.9	2.9
1-Br-Ant		77.7	8.1	14.8	1.5	5.1
1-Cl-Ant		74.7	7.1	13.0	1.7	5.7

1-Cl-Pyr	82.4	9.6	14.0	2.1	7.1
2,3,9,10-Br_4-Ant	156.0	13.4	13.1	1.7	5.8
2,7-Br_2-Fle	90.8	7.0	11.8	1.3	4.4
2,7-Br_2-Phe	83.3	14.6	18.0	1.0	3.5
2.7-Cl_2-Fle	99.6	5.6	15.8	0.8	2.8
2-Br-Ant	76.8	16.7	10.6	2.3	7.8
2-Br-Fle	89.0	4.6	9.5	0.8	2.7
2-Cl-Fle	87.8	6.5	7.7	0.5	1.6
3-Br-Flu	101.1	6.2	17.4	0.8	2.8
3-Br-Phe	85.2	3.9	9.7	0.6	2.0
4-Br-BaA	78.9	11.7	17.3	1.8	6.1
4-Br-Pyr	82.1	7.9	16.8	1.8	6.1
5,6-Br_2-Ana	87.7	3.4	14.2	0.6	1.9
5-Br-Ana	83.1	9.2	9.9	0.9	3.0
7,12-Cl_2-BaA	81.0	11.1	18.8	0.9	3.1
7-Cl-BaA	87.4	11.8	18.5	1.9	6.5
9,10-Br_2-Phe	85.7	17.8	14.6	0.6	2.1
9-Br-1,5-Cl_2-Ant	84.5	8.4	17.9	0.7	2.4
9-Br-Phe	91.9	5.9	7.3	0.4	1.3
9-Cl-Ant	73.0	12.6	13.9	1.7	5.6
9-Cl-Phe	88.8	4.8	7.9	0.3	1.0

Table 4.2: *Method validation parameters for the analysis of PAHs, HPAHs and APAHs using GC-EI-MS/MS for dSPE study in biota.*

Compounds	Working Range (pg µL^{-1})	Linearity[a]	Spiking Level						LOD (pg µL^{-1})	LOQ (pg µL^{-1})
			10 pg µL^{-1}		100 pg µL^{-1}		500 pg µL^{-1}			
			Accuracy (%)	Precision (RSD %)	Accuracy (%)	Precision (RSD %)	Accuracy (%)	Precision (RSD %)		
PAHs										
Acenapthene	10–1000[12]		85.0	4.8	93.4	11.7	130.9	2.2	2.1	7.1
Acenapthylene			82.9	5.5	90.3	10.7	125.7	1.5	2.4	8.1
Anthracene			82.5	11.6	90.6	7.0	133.4	4.0	5.1	16.9
Benz[*a*]anthracene			71.7	5.8	75.5	12.1	99.5	2.4	2.2	7.4
Benzo[*a*]pyrene			68.7	10.1	73.5	10.2	85.1	5.4	3.7	12.3
Benzo[*b*]fluoranthene			68.2	5.9	74.1	12.1	95.4	3.9	2.2	7.2
Benzo[*g,h,i*]perylene			75.7	3.8	81.3	10.8	89.8	2.2	1.5	5.0
Benzo[*k*]fluoranthene			79.0	7.8	82.2	12.9	115.2	2.9	3.3	10.9
Chrysene			87.9	5.2	89.9	9.9	120.9	1.2	2.4	8.1
Dibenzo[*a,h*]anthracene			77.3	6.1	79.4	10.1	96.4	4.1	2.5	8.3
Fluoranthene			85.6	5.1	88.8	10.7	101.4	4.1	2.3	7.8
Fluorene			101.7	11.3	107.4	14.0	130.4	1.4	6.1	20.4
Indeno[1,2,3-*c,d*]pyrene			70.8	4.1	77.6	11.7	95.0	3.8	1.6	5.2
Naphthalene			116.6	5.4	75.2	8.1	75.4	9.7	3.3	11.1
Phenanthrene			76.5	6.5	83.0	12.3	114.0	2.3	2.6	8.8
Pyrene			81.5	4.7	83.5	9.8	102.2	2.4	2.0	6.7
HPAHs										
1,2-Br_2-Any	10–1000	0.997	72.9	12.0	92.7	10.0	64.1	8.2	8.9	29.6
1,4-Cl_2-Ant		0.996	95.8	12.8	106.2	10.4	77.6	7.0	12.4	41.4
1,5,9,10-Cl_4-Ant		0.993	83.6	15.1	97.1	11.9	67.7	10.1	12.8	42.6
1,5-Cl_2-Ant		0.998	111.0	13.6	115.2	10.0	91.0	7.5	15.3	51.1
1,6-Br_2-Pyr		0.995	95.2	15.7	113.0	12.6	89.8	9.0	15.2	50.6
1,8-Br_2-Ant		0.994	97.1	13.8	115.0	11.2	85.6	8.6	13.6	45.4
1-Br-Ant		0.997	79.9	16.8	90.0	9.7	72.3	7.5	13.7	45.5
1-Cl-Ant		0.997	93.7	13.2	109.6	9.7	81.6	6.3	12.6	42.0

2,3,9,10-Br_4-Ant		0.992	58.9	10.6	83.1	15.0	56.6	18.8	6.3	21.1
2,7-Br_2-Fle		0.996	84.7	15.6	103.9	10.6	79.5	7.4	13.5	44.9
2,7-Br_2-Phe		0.996	98.0	15.7	112.9	10.6	89.6	7.2	15.6	52.1
2.7-Cl_2-Fle		0.997	81.5	15.8	93.8	10.3	71.9	6.5	13.1	43.5
2-Br-Ant		0.995	75.3	15.6	92.2	11.5	71.7	7.2	11.9	39.7
2-Br-Fle		0.997	79.7	15.9	92.8	9.8	72.8	6.2	12.9	42.9
2-Cl-Ant		0.996	84.5	15.5	100.3	11.4	76.1	6.3	13.3	44.4
2-Cl-Fle		0.997	72.0	14.9	86.4	9.4	63.8	4.8	10.9	36.4
3-Br-Flu		0.996	92.3	16.1	106.4	10.3	84.2	8.0	15.1	50.3
3-Br-Phe		0.997	84.3	15.1	96.7	10.1	74.2	6.5	12.9	43.1
4-Br-BaA		0.993	105.5	12.7	117.1	14.6	104.4	7.9	13.6	45.4
4-Br-Pyr		0.996	92.5	6.0	101.9	10.3	79.6	8.0	5.6	18.7
5,6-Br_2-Ana		0.992	60.6	17.1	84.6	13.2	71.1	8.4	10.6	35.2
5-Br-Ana		0.997	91.4	13.3	100.0	9.9	78.6	5.8	12.3	41.1
9,10-Br_2-Ant		0.997	88.9	9.5	106.2	9.7	78.5	9.2	8.6	28.5
9-Br-Phe		0.997	80.9	15.2	93.6	9.6	70.8	6.7	12.5	41.6
9-Cl-Fle		0.996	87.9	16.2	89.4	19.0	109.0	9.2	14.5	48.2
APAHs										
2-Methylnaphthalene	2–500[12]		88.4	16.3	63.8	19.8	117.1	12.6	7.6	25.4
1-Methylnaphthalene			79.5	18.4	63.1	16.7	113.6	10.8	7.7	25.8
2,6-dimethylnaphthalene			158.0	12.3	66.5	11.9	102.5	15.5	10.3	34.5
1,6-dimethylnaphthalene			137.5	19.0	82.4	14.6	114.8	9.8	13.8	46.2
2,3,5-trimethylnaphthalene			71.7	20.0	67.9	13.1	110.0	5.5	7.6	25.4
1,2,5,6-tetramethylnaphthalene			84.2	34.3	53.0	6.2	121.2	3.5	15.3	51.0
1,4,6,7-tetramethylnaphthalene			155.2	18.2	82.2	13.7	121.3	3.6	15.0	49.9
3-Methylphenanthrene			137.3	19.4	75.9	13.0	126.7	3.8	14.1	47.1
2-Methylphenanthrene			110.4	12.9	69.8	16.1	120.8	4.3	7.6	25.2
9-Methylphenanthrene			123.1	17.4	71.6	15.3	119.8	3.9	11.4	37.9
1-Methylphenanthrene			120.7	17.1	67.2	15.1	118.4	3.5	10.9	36.5

3,6-Dimethylphenanthrene	114.6	17.4	69.2	14.5	107.6	4.5	10.6	35.3
1,3-Dimethylphenanthrene	99.3	16.3	66.5	15.4	107.3	3.8	8.6	28.6
1,7-Dimethylphenanthrene	110.5	15.2	66.2	12.9	107.8	4.5	8.9	29.7
1,8-Dimethylphenanthrene	117.3	15.7	67.5	13.2	110.7	4.9	9.8	32.5
1,2,6-trimethylphenanthrene	125.0	16.5	74.4	14.1	130.6	3.5	10.9	36.4
Retene	132.4	16.3	71.6	11.4	119.5	3.8	11.5	38.2
1,2,6,9-tetramethylphenanthrene	133.7	14.3	72.5	14.0	124.0	5.8	10.2	33.9
2-methylchrysene	137.0	17.8	85.2	14.9	138.2	13.0	12.9	43.0
1,3,6-trimethylchrysene	131.1	18.6	84.6	12.8	131.7	9.0	12.9	43.0
1-methylfluorene	72.1	8.6	78.0	8.6	82.8	4.0	3.3	11.0
1,7-dimethylfluorene	84.7	9.3	90.9	6.6	79.6	3.7	4.2	13.9
4-methylpyrene	71.9	11.6	72.2	7.4	84.0	5.5	4.4	14.8
1-methylpyrene	64.1	11.6	63.6	6.2	79.3	3.9	3.9	13.2
4,5-dimethylpyrene	72.1	15.2	74.3	13.6	81.4	10.9	5.8	19.4
7-methylbenzo[*a*]pyrene	73.1	12.6	89.8	13.1	75.8	14.6	10.2	34.0

[a]*Linearity for PAHs and APAHs reported in Idowu et al.*[12]

Table 4.3: *Certified and measured concentrations (mean ± standard deviation, mg/kg) of PAHs in CALA C18-1 and SRM 1944 samples by GC-EI-MS/MS.*

Compound	**Certified/ Reference Concentrations (mg/kg)**		**Measured Concentrations (mg/kg)**	
	SRM 1944	**CALA C18-1**	**SRM 1944[1] (%)[c]**	**CALA C18-1[1] (%)[c]**
Acenaphthene	0.39 ± 0.03[b]	0.323 ± 0.02[a]	0.22 ± 0.03 (56.4%)	0.231 ± 0.036 (71.5%)
Acenaphthylene	n/a	0.554 ± 0.05[a]	0.80 ± 0.14 (n/a)	0.387 ± 0.031 (69.8%)
Anthracene	1.13 ± 0.07[b]	0.577 ± 0.03[a]	1.14 ± 0.06 (100.8%)	0.520 ± 0.096 (90.1%)
Benz[*a*]anthracene	4.72 ± 0.11[a]	1.89 ± 0.08[a]	3.15 ± 0.19 (66.7%)	1.439 ± 0.204 (76.1%)
Benzo[*a*]pyrene	4.30 ± 0.13[a]	1.77 ± 0.08[a]	2.83 ± 0.25 (65.8%)	1.513 ± 0.290 (85.5%)
Benzo[*b*]fluoranthene	3.87 ± 0.42[a]	2.07 ± 0.2[a]	2.97 ± 0.18 (76.7%)	1.628 ± 0.216 (78.6%)
Benzo[*g,h,i*]perylene	2.84 ± 0.10[a]	1.51 ± 0.07[a]	2.42 ± 0.16 (85.2%)	1.443 ± 0.245 (95.6%)

Benzo[*k*]fluoranthene	2.30 ± 0.20[a]	1.15 ± 0.08[a]	1.98 ± 0.19 (86.1%)	1.016 ± 0.145 (88.3%)
Chrysene	4.86 ± 0.10[a]	2.03 ± 0.09[a]	4.93 ± 0.29 (101.3%)	1.806 ± 0.264 (89.0%)
Dibenzo[*a*,*h*]anthracene	0.424 ± 0.069[a]	0.357 ± 0.02[a]	0.63 ± 0.04 (148.6%)	0.286 ± 0.045 (80.1%)
Fluoranthene	8.92 ± 0.32[a]	5.05 ± 0.2[a]	7.22 ± 0.44 (80.9%)	3.915 ± 0.668 (77.5%)
Fluorene	0.48 ± 0.04[b]	0.494 ± 0.04[a]	0.25 ± 0.03 (52.1%)	0.282 ± 0.049 (57.1%)
Indeno[1,2,3-*c*,*d*]pyrene	2.78 ± 0.10[a]	1.56 ± 0.08[a]	1.91 ± 0.10 (68.7%)	1.292 ± 0.190 (82.8%)
Naphthalene	1.28 ± 0.04[b]	4.67 ± 0.3[a]	0.88 ± 0.11 (68.8%)	5.754 ± 0.453 (123.2%)
Phenanthrene	5.27 ± 0.22[a]	4.12 ± 0.2[a]	4.59 ± 0.35 (87.1%)	3.321 ± 0.507 (80.6%)
Pyrene	9.70 ± 0.42[a]	3.61 ± 0.1[a]	6.59 ± 0.39 (67.9%)	2.570 ± 0.474 (71.1%)

[1]*Six replicate measurements;*
[a] *Certified Mass Fractions;*
[b] *Reference Mass Fraction;*
[c] *% bias.*

4.7 References

(1) Abdel-Shafy, H. I.; Mansour, M. S. M. A review on polycyclic aromatic hydrocarbons: Source, environmental impact, effect on human health and remediation. *Egyptian Journal of Petroleum* **2016**, *25* (1), 107-123. DOI: 10.1016/j.ejpe.2015.03.011

(2) Sörensen, A.; Wichert, B. Asphalt and bitumen. In *Ullmann's encyclopedia of industrial chemistry*, 2009.

(3) Sun, J. L.; Zeng, H.; Ni, H. G. Halogenated polycyclic aromatic hydrocarbons in the environment. *Chemosphere* **2013**, *90* (6), 1751-1759. DOI: 10.1016/j.chemosphere.2012.10.094

(4) Ahad, J. M. E.; Macdonald, R. W.; Parrott, J. L.; Yang, Z.; Zhang, Y.; Siddique, T.; Kuznetsova, A.; Rauert, C.; Galarneau, E.; Studabaker, W. B.; Evans, M.; McMaster, M. E.; Shang, D. Polycyclic aromatic compounds (PACs) in the Canadian environment: A review of sampling techniques, strategies and instrumentation. *Environ Pollut* **2020**, *266* (Pt 2), 114988. DOI: 10.1016/j.envpol.2020.114988

(5) Jin, R.; Bu, D.; Liu, G.; Zheng, M.; Lammel, G.; Fu, J.; Yang, L.; Li, C.; Habib, A.; Yang, Y.; Liu, X. New classes of organic pollutants in the remote continental environment - chlorinated and brominated polycyclic aromatic hydrocarbons on the Tibetan Plateau. *Environ Int* **2020**, *137* (February), 105574. DOI: 10.1016/j.envint.2020.105574

(6) Wallace, S. J.; de Solla, S. R.; Head, J. A.; Hodson, P. V.; Parrott, J. L.; Thomas, P. J.; Berthiaume, A.; Langlois, V. S. Polycyclic aromatic compounds (PACs) in the Canadian environment: Exposure and effects on wildlife. *Environ Pollut* **2020**, *265* (Pt B), 114863. DOI: 10.1016/j.envpol.2020.114863

(7) Youngblood, W. W.; Blumer, M. Polycyclic aromatic hydrocarbons in the environment: Homologous series in soils and recent marine sediments. *Geochimica et Cosmochimica Acta* **1975**, *39* (9), 1303-1314. DOI: 10.1016/0016-7037(75)90137-4

(8) Droppo, I. G.; di Cenzo, P.; Power, J.; Jaskot, C.; Chambers, P. A.; Alexander, A. C.; Kirk, J.; Muir, D. Temporal and spatial trends in riverine suspended sediment and associated polycyclic aromatic compounds (PAC) within the Athabasca oil sands region. *Sci Total Environ* **2018**, *626*, 1382-1393. DOI: 10.1016/j.scitotenv.2018.01.105

(9) Jin, R.; Zheng, M.; Lammel, G.; Bandowe, B. A. M.; Liu, G. Chlorinated and brominated polycyclic aromatic hydrocarbons: Sources, formation mechanisms, and occurrence in the environment. *Progress in Energy and Combustion Science* **2020**, *76*, 100803. DOI: 10.1016/j.pecs.2019.100803

(10) Landis, M. S.; Studabaker, W. B.; Patrick Pancras, J.; Graney, J. R.; Puckett, K.; White, E. M.; Edgerton, E. S. Source apportionment of an epiphytic lichen biomonitor to elucidate the sources and spatial distribution of polycyclic aromatic hydrocarbons in the Athabasca Oil Sands Region, Alberta, Canada. *Sci Total Environ* **2019**, *654*, 1241-1257. DOI: 10.1016/j.scitotenv.2018.11.131

(11) Musa Bandowe, B. A.; Wei, C.; Han, Y.; Cao, J.; Zhan, C.; Wilcke, W. Polycyclic aromatic compounds (PAHs, oxygenated PAHs, nitrated PAHs and azaarenes) in soils from China and their relationship with geographic location, land use and soil carbon fractions. *Sci Total Environ* **2019**, *690*, 1268-1276. DOI: 10.1016/j.scitotenv.2019.07.022

(12) Idowu, I.; Francisco, O.; Thomas, P. J.; Johnson, W.; Marvin, C.; Stetefeld, J.; Tomy, G. T. Validation of a simultaneous method for determining polycyclic aromatic compounds and

alkylated isomers in biota. *Rapid Commun Mass Spectrom* **2018**, *32* (3), 277-287. DOI: 10.1002/rcm.8035
(13) US EPA. Method 3545a pressurized fluid extraction (PFE). *Method 3545A* **2007**.
(14) Richter, B. E.; Jones, B. A.; Ezzell, J. L.; Porter, N. L.; Avdalovic, N.; Pohl, C. Accelerated solvent extraction: A technique for sample preparation. *Analytical chemistry* **1996**, *68* (6), 1033-1039.
(15) Johnson, Y. S. Determination of polycyclic aromatic hydrocarbons in edible seafood by quechers-based extraction and gas chromatography-tandem mass spectrometry. *J Food Sci* **2012**, *77* (7), T131-137. DOI: 10.1111/j.1750-3841.2012.02758.x
(16) Khorshid, M.; Souaya, E. R.; Hamzawy, A. H.; Mohammed, M. N. Quechers method followed by solid phase extraction method for gas chromatographic-mass spectrometric determination of polycyclic aromatic hydrocarbons in fish. *Int J Anal Chem* **2015**, *2015*, 352610. DOI: 10.1155/2015/352610
(17) Miossec, C.; Lanceleur, L.; Monperrus, M. Adaptation and validation of quechers method for the simultaneous analysis of priority and emerging pollutants in sediments by gas chromatography—mass spectrometry. *International Journal of Environmental Analytical Chemistry* **2018**, *98* (8), 695-708. DOI: 10.1080/03067319.2018.1496245
(18) Slámová, T.; Sadowska-Rociek, A.; Fraňková, A.; Surma, M.; Banout, J. Application of QuEChERS-EMR-Lipid-DLLME method for the determination of polycyclic aromatic hydrocarbons in smoked food of animal origin. *Journal of Food Composition and Analysis* **2020**, *87*. DOI: 10.1016/j.jfca.2020.103420
(19) Sun, Y.; Wu, S. Analysis of PAHs in oily systems using modified quechers with EMR-lipid clean-up followed by GC-QQQ-MS. *Food Control* **2020**, *109*. DOI: 10.1016/j.foodcont.2019.106950
(20) Wang, D.; Ma, J.; Li, H.; Zhang, X. Concentration and potential ecological risk of PAHs in different layers of soil in the petroleum-contaminated areas of the Loess Plateau, China. *Int J Environ Res Public Health* **2018**, *15* (8). DOI: 10.3390/ijerph15081785
(21) Anastassiades, M.; Lehotay, S. J.; Štajnbaher, D.; Schenck, F. J. Fast and easy multiresidue method employing acetonitrile extraction/partitioning and "dispersive solid-phase extraction" for the determination of pesticide residues in produce. *Journal of AOAC INTERNATIONAL* **2003**, *86* (2), 412-431. DOI: 10.1093/jaoac/86.2.412
(22) Forsberg, N. D.; Wilson, G. R.; Anderson, K. A. Determination of parent and substituted polycyclic aromatic hydrocarbons in high-fat salmon using a modified quechers extraction, dispersive SPE and GC-MS. *J Agric Food Chem* **2011**, *59* (15), 8108-8116. DOI: 10.1021/jf201745a
(23) Bordin, D. C.; Alves, M. N.; de Campos, E. G.; De Martinis, B. S. Disposable pipette tips extraction: Fundamentals, applications and state of the art. *J Sep Sci* **2016**, *39* (6), 1168-1172. DOI: 10.1002/jssc.201500932
(24) Paya, P.; Anastassiades, M.; Mack, D.; Sigalova, I.; Tasdelen, B.; Oliva, J.; Barba, A. Analysis of pesticide residues using the quick easy cheap effective rugged and safe (QuEChERS) pesticide multiresidue method in combination with gas and liquid chromatography and tandem mass spectrometric detection. *Anal Bioanal Chem* **2007**, *389* (6), 1697-1714. DOI: 10.1007/s00216-007-1610-7
(25) Prestes, O. D.; Friggi, C. A.; Adaime, M. B.; Zanella, R. Quechers: Um método moderno de preparo de amostra para determinação multirresíduo de pesticidas em alimentos por

métodos cromatográficos acoplados à espectrometria de massas. *Química Nova* **2009**, *32* (6), 1620-1634. DOI: 10.1590/s0100-40422009000600046
(26) Forsberg, N. D.; Wilson, G. R.; Anderson, K. A. Addition to determination of parent and substituted polcyclic aromatic hydrocarbons in high-fat salmon using a modified QuEChERS extraction, dispersive spe and gc–ms. *Journal of Agricultural and Food Chemistry* **2011**, *59* (19), 10773-10773. DOI: 10.1021/jf203649c
(27) Choi, M.; Kim, Y. J.; Lee, I. S.; Choi, H. G. Development of a one-step integrated pressurized liquid extraction and cleanup method for determining polycyclic aromatic hydrocarbons in marine sediments. *J Chromatogr A* **2014**, *1340*, 8-14. DOI: 10.1016/j.chroma.2014.03.015
(28) Titaley, I. A.; Eriksson, U.; Larsson, M. Rapid extraction method of polycyclic aromatic compounds in soil using basic silica selective pressurized liquid extraction. *J Chromatogr A* **2020**, *1618*, 460896. DOI: 10.1016/j.chroma.2020.460896
(29) Xia, Z.; Klaassen, N.; Idowu, I.; Tomy, G. *A more cost-efficient extraction method for polycyclic aromatic hydrocarbons (PAH) in sediments and soils using accelerated solvent extraction (ASE)*.
(30) Magnusson, B.; Örnemark, U. *The fitness for purpose of analytical methods – a laboratory guide to method validation and related topics (2nd ed)*; 2014.
(31) Weitzel, M. L. J.; Johnson, W. M. *Application of ISO/IEC 17025 technical requirements in industrial laboratories: Method validation*; Friesen Press, 2013.
(32) Schantz, M. M.; Kucklick, J. R. *Interlaboratory analytical comparison study to support DEEPWATER HORIZON natural resource damage assessment : Description and results for marine sediment QA10SED01, NISTIR 7793*; National Institute of Standards and Technology, Gaithersburg, MD, 2011.
(33) Blumer, M. Removal of elemental sulfur from hydrocarbon fractions. *Analytical Chemistry* **2002**, *29* (7), 1039-1041. DOI: 10.1021/ac60127a014
(34) Jin, M.; Xia, Y.; Lei, T.; Ma, S.; Qiu, J.; Zhang, R. Note on sulfur removal with metallic copper. *Geochemistry International* **2007**, *45* (6), 620-623. DOI: 10.1134/s0016702907060109

Chapter 5: Microbead Beating Extraction of Avian Eggs for Polycyclic Aromatic Compounds

A version of this chapter has already been published as Zhe Xia, Ifeoluwa Idowu, Thor Halldorson, Amica-Mariae Lucas, Claire Stein, Manpreet Kaur, Thane Tomy, Chris Marvin, Philippe J. Thomas, Craig E. Hebert, Reyd A. Smith, Frederic Dwyer-Samuel, Jennifer F. Provencher and Gregg T. Tomy. Microbead beating extraction of avian eggs for polycyclic aromatic compounds. *Chemosphere*, **2023**, *335*, 139059.

5.1 Abstract

Due to their relatively high trophic position and importance as a food source for many communities in the circumpolar north, seabird eggs are an important matrix for monitoring contaminant levels. In fact, many countries, including Canada, have established long-term seabird egg contaminant monitoring programs, with oil related compounds a contaminant of emerging concern for seabirds in several regions. Current approaches to measuring many contaminant burdens in seabird eggs are time-consuming and often require large volumes of solvent. Here we propose an alternative approach, based on the principle of microbead beating tissue extraction using custom designed stainless-steel extraction tubes and lids, to measure a suite of 75 polycyclic aromatic compounds (polycyclic aromatic hydrocarbons (PAHs), alkyl-PAHs, halogenated-PAHs and some heterocyclic compounds) comprising a wide-range of chemical properties. Our method was conducted in strict accordance with ISO/IEC 17025 guidelines for method validation. Accuracies for our analytes generally ranged from 70 – 120%, and intra and inter-day repeatability for most analytes were < 30%. Limits of detection/quantitation for the 75 target analytes were < 0.2/0.6 ng g^{-1}. The level of contamination in our method blanks was significantly smaller in our stainless-steel tubes/lids relative to commercially available high-density polypropylene plastic alternatives. Overall, our method meets our data quality objectives and results in a notable reduction in sample processing times relative to current approaches.

5.2 Introduction

Tracking and monitoring of environmental pollutants can be carried out to study their fate and effects on biota. Long-term monitoring of contaminants can be used to understand how contaminants are transported through and between ecosystems.[1] For example, tracking of mercury in biota across the Arctic has allowed researchers to understand that mercury produced at temperate latitudes can travel great distances via atmospheric and ocean currents which are then be deposited in the Arctic.[2] In addition to studies focused on fate, contaminants monitoring can also be aimed at understanding the biological effects of contaminants. The impacts of contaminants can include a range of effects from biochemical to behavioural. Contaminants have been found to alter methylation of DNA in vertebrates, including alligators and fish.[3,4] Increased concentrations have also been found to be associated with changes in the endocrine systems of polar bears.[5] In common loons and fish, behavioural changes have been found to be associated with elevated levels of contaminants.[6,7] Understanding environmental levels of chemicals and their biological effects are critical to informing policies and regulatory actions under international conventions and agreements aimed at mitigating and reducing any impacts.

Several long-term monitoring programs in Canada use bird eggs as a relatively non-invasive and easy matrix to collect. These long-term monitoring programs have been used to inform how environmental contaminant concentrations change in relation to policy actions.[8-10] For example, in the Laurentian Great Lakes region legacy contaminants have been tracked for almost 50 years using herring gull (*Larus argentatus*) eggs.[11] Additionally, longitudinal studies of contaminants in eggs can be paired with environmental data in order to understand the transport of contaminants to biota.[12] A recent study examining mercury concentrations in avian eggs from northern Alberta highlighted the important role of riverine transport in regulating mercury and exposure in gulls

and terns breeding downstream.[13] In the Arctic, studies using seabird eggs across several oceanographic zones have demonstrated how the source of contaminants in the Arctic can differ by region and trends need to be considered within this context.[14]

Some studies utilise wild eggs in lab-based studies to examine the impacts of defined doses of contaminants. For example, Braune *et al.* exposed seabird eggs collected from colonies in northern Canada to understand the concentrations of Hg that lead to embryonic malformations and egg non-viability.[15] Other studies focus on using eggs from domesticated species to understand individual level impacts. One study used chickens to examine toxicity when birds ingest mercury through their food at different exposure lengths.[16] Similarly, studies using domestic birds have tracked pollutants to better understand how contaminant burdens can vary across tissues and be used as proxies for each other.[17] An important component of relating these studies to impacts on the environment is being able to relate the experimental concentrations associated with deleterious effects to environmentally relevant exposures in wildlife.

Evaluating contaminant impacts is complicated by the fact that organisms are exposed to mixtures of chemicals that may have antagonistic, additive or synergistic effects. Furthermore, cumulative effects of contaminant exposure and environmental stressors are increasingly recognized as significant threat to some species experiencing sub-lethal exposures to anthropogenic contaminants. Of note is that environmental contaminants are not a stressor that act alone. For example, polar bears are at threat from both contaminants and climate change as warming temperatures are altering contaminant cycling in the Arctic and resultant exposure to chemicals in polar bear.[18] Additionally, avian studies have shown that exposure to multiple stressors including contaminants and extreme temperatures, can influence avian reproductive behavior, with potential implications on population stability especially when combined with

predation pressures.[19-21] Pathogens and contaminants can also interact, with recent work examining avian influenza in relation to mercury burdens in waterfowl found that higher mercury burdens were most associated with higher levels of infection of avian influenza.[22]

Polycyclic aromatic compounds [PACs, defined here as the sum of polycyclic aromatic hydrocarbons (PAHs), halo- and alkyl-PAHs] are a well-known and complex class of contaminants that have been detected in many biological matrices, such fish, snail, otter, and avian eggs.[23-28] Current approaches for extraction of PACs from avian eggs involves the use of traditional liquid-liquid extraction (LLE), Soxhlet extraction methods[23-25] and the QuEChERS method.[29] A major shortcoming of LLE can be the limited mass-transfer of the analyte from the matrix to the extraction solvent especially when aggressive agitation methods are not employed. Extractions based on Soxhlet are lengthy and typically employ large solvent volumes, which can potentially introduce contamination from impurities in the solvent. In our previous study, we highlighted a few concerns with the QuEChERS approach for PAHs analysis.[30] For instance, QuEChERS is not suitable for analytes that cover a wide range of chemical properties, it is limited in sample size, and there are issues with PAH contamination from dispersant.[31-36]

Microbead beating tissue homogenization has been used extensively in biomedical research to lysis or grind soft and hard biological material. The principle of operation is that tubes packed with microbeads (ceramic or stainless steel) and tissue material to be homogenized are agitated in 3-dimensions. This multidirectional forceful motion results in homogenates that are fluidic in nature lending them to additional processing workflows. Based on this, we hypothesize that PACs can be efficiently extracted from avian eggs using microbead beating tissue homogenization with concomitant solvent extraction. Here we present the results of a controlled study to test our

hypothesis and examine the quality of our method performance objectives as well as present some of the potential pitfalls to be avoided.

5.3 Materials and Methods

5.3.1 Chemicals

Organic solvents and anhydrous sodium sulphate were purchased from Fisher Scientific (Ottawa, ON). The suite of labelled internal standards used for recovery internal standard (RIS) were d_8-naphthalene, d_8-acenaphthylene, d_{10}-acenaphthene, d_{10}-fluorene, d_{10}-phenanthrene, d_{10}-pyrene, d_{12}-benz(*a*)anthracene, d_{12}-chrysene, d_{12}-benzo(*b*)fluoranthene, d_{12}-benzo(*k*)fluoranthene, d_{12}-benzo(*a*)pyrene, d_{12}-indeno(1,2,3-*c,d*)pyrene, d_{14}-dibenz(*a,h*)anthracene, and d_{14}-benzo(*g,h,i*)perylene. Labelled anthracene (d_{10}-anthracene) was used as the instrument performance internal standard (IPIS). For the HPAHs, the abbreviations used here begin with the substitution positions of the halogen(s), followed by the substituted halogen atom and parent PAHs structure. The abbreviation of PAHs is listed below: naphthalene (Nap), acenaphthylene (Acy), acenaphthene (Ace), fluorene (Flu), phenanthrene (Phe), anthracene (Ant), fluoranthene (Flu), pyrene (Pyr), benzo[*a*]anthracene (BaA), chrysene (Chr), benzo[*b*]fluoranthene (BbF), benzo[*k*]fluoranthene (BkF), benzo[*a*]pyrene (BaP), benzo[*g,h,i*]perylene (BghiP), indeno[1,2,3-*c,d*]pyrene (Ind), and dibenz[*a,h*]anthracene (DahA). The full list of abbreviations, details of target analytes and their suppliers can be found in Xia *et al*.[30]

5.3.2 Preparation of Reference Egg Samples

To our knowledge, a standard reference material of avian eggs with known PAC concentrations is not commercially available. In light of this, we created our own in-house reference material. Chicken egg samples were purchased from a local retail store (Winnipeg, MB, Canada) and used to evaluate the performance characteristics of our method. Eggs were cracked, combined (*n = 10*) and homogenized using a Polytron extraction unit (Kinematica, Switzerland) in a stainless steel 50 mL tube. Homogenates were aliquoted into separate tubes and spiked with known amounts of PACs to give final concentrations of 10, 100 and 500 pg μL^{-1} which equates to wet weight concentrations of 0.4, 4 and 20 ng/g, respectively. Non-spiked homogenates were used as our method blanks. These homogenates were used as our in-house reference material and subsequently stored at -80^{o} C. To assess the utility of our method for the analysis of wild bird eggs, we analysed eggs collected in Nunatsiavut from wild common eiders (*Somateria mollissima*), black guillemots (*Cepphus grylle*), and great black-backed gulls (*Larus marinus*) were used to verify lipid extraction efficiencies. These birds were collected under scientific permits from the Nunatsiavut Government as part of an ongoing collaborative research program on the effects of oil pollution in the environment.

5.3.3 Microbead Extraction

Five (5.0 +/- 0.01 g) of the in-house egg reference material was weighed into 15 mL high-density polypropylene microbead extraction tubes (MBETs, Bertin Technologies, Montingy-le-Bretonneux, France) that contained either 0, 0.5, 1.0 or 2.5 g of ceramic microbeads. Eight (8) mL of dichloromethane (DCM) were added to the MBETs. Samples were extracted using a Precellys Evolution Homogenizer (Bertin Technologies, Montigny-le-Bretonneux, France) at 6500 rpm

with 3 cycles for 20 s and 120 s between the cycles (total time of 5 min). The MBETs were then centrifuged at 5000 rpm at 10°C for 10 min. The extract (bottom layer) was transferred to a 60 mL glass collection vial. The MBETs were rinsed with 2 × 8 mL DCM, vortexed for 1 min and centrifuged again at 5000 rpm at 10 °C for 10 min. The rinses were combined with the initial extract. After extraction, the extract was treated with sodium sulphate to remove moisture, and subsequently transferred to a 60 mL glass collection vial with 3 washes of hexane (~2 mL each). The sample volume was reduced to approximately 10 mL using a gentle stream of ultra-high purity (UHP) nitrogen. 500 µL of extracts were transferred to pre-weighed aluminium boats for lipid determination. Extracts (9.5 mL) were then reduced to 2.5 mL and transferred to a test tube. The glass tube was rinsed with 2 × 1 mL DCM, and rinses were combined with the extract. The volume of extract was adjusted to 5 mL using DCM.

5.3.4 Automated Gel Permeation Chromatography

Extracts (5 mL) were applied to the head of a GPC column (J_2-scientific AccuPrep, Columbia, MO, USA) packed with 60 g of S-X3 Biobeads to remove excess lipids and protein. The following parameters were employed by the fully-automated system; injection volume: 5 mL; pressure: 8 psi; flow rate: 5 mL/min; mobile phase: hexane:DCM (1:1, v/v). The first 120 mL of effluent contained lipids and other biogenic material and was discarded. The fraction containing analytes eluted from 125 – 250 mL and was collected in a 250 mL round bottom flask; the extract was reduced in volume to 200 µL via a Genevac Rocket Evaporator (Ipswich, Suffolk, UK) and nitrogen evaporation. The IPIS, d_{10}-anthracene, was then added to the sample. Amber GC vials were used to store the samples under refrigeration at 4 °C until they were analyzed.

5.3.5 GC-MS/MS Conditions

An Agilent 7890 GC coupled with a triple quadrupole Agilent 7000C mass spectrometer fitted with an electron ionization (EI) source was used for the MS/MS acquisition. Agilent J&W HP-5ms ultra inert column (30 m × 0.25 mm × 0.25 µm) and helium was used as the carrier gas at a constant flow rate of 1.2 mL/min. 1 µL of sample was injected with a PAL RSI 85 auto sampler at 250 °C in splitless mode. The oven temperature for PAHs and APAHs was held at 60 °C for 1 min then raised to 120 °C at 35 °C/min, further ramped up to 220 °C at 14 °C/min, to 260 °C at 3 °C/min and held for 5 min, 300 °C at 10 °C/min, and finally to 310 °C at 50 °C/min. For HPAHs, the oven temperature for PAHs was held at 60 °C for 1 min then raised to 210°C at 35 °C/min, further ramped up to 260 °C at 2 °C/min, 300 °C at 10 °C/min and held for 5 min, and finally to 325 °C at 50 °C/min and held for 5.5 min. Both transfer line and source temperature were set at 320 °C and UHP nitrogen was used as the collision gas at 60 psi. The quantification and confirmation ions and the multiple reaction monitoring (MRM) ion transitions for PACs can be found in Idowu *et al.* and Xia *et al.*[30,37]

5.3.6 Quality Data Objectives

The Eurachem Guide – The Fitness for the Purpose of Analytical Methods, explicitly states that performance characteristics for method validation, should include an assessment of detection limits, accuracy/recovery, precision, and ruggedness.[38]

5.3.6.1 Detection Limits

LOD/LOQs for our microbead extraction study were calculated by extracting our chicken egg reference sample ($n = 8$) fortified with 10 pg μL^{-1} PAHs, APAHs and HPAHs. The standard

deviations (s_0) of signals for each target analyte were calculated and an adjusted standard deviation (s_0') as defined in the Eurachem Guide was calculated by the ratio $s_0' = s_0/\sqrt{n}$. Finally, target analyte specific LODs were determined as 3* s_0', and LOQs as 10* s_0'.[1] LODs and LOQs are reported on a mass-to-mass wet weight basis.[38]

5.3.6.2 Accuracy

For our microbead extraction validation study, our chicken egg reference sample (*n = 8*) used for accuracy tests, which were fortified with 10, 100 or 500 pg μL^{-1} PACs mixture.

5.3.6.3 Precision

Repeatability on our microbead extraction was calculated by extracting chicken egg reference samples fortified with 100 pg μL^{-1} PACs mixture and quantifying PACs in replicate over a 24 h period (intraday, *n = 8*) and over 3 consecutive days (interday, *n = 4*).

5.3.6.4 Ruggedness

The extraction efficiencies of microbead extraction were also compared to accelerated solvent extraction (ASE) method by chicken egg reference samples (*n = 6*) fortified with 100 pg μL^{-1} PACs mixture and LLE method by chicken egg reference samples (*n = 3*) fortified with 10 pg μL^{-1} PAHs. To test interference from high-density polypropylene plastic, 15 mL MBETs (*n = 3*) were pre-soaked in DCM for 24 h and sonicated 20 min prior to use. Un-soaked MBETs (*n = 3*) were used as blank. To test the efficiency of extracting lipid, egg of common eider (*n = 12*), black guillemot (*n = 12*), great black-backed gull (*n = 12*) and chicken (*n = 3*) were extracted by DCM and hexane using 2.5 g of microbeads, respectively and lipid content (% lipid, w/w) were

determined gravimetrically according to Idowu *et al*.[37] Student *t*-tests ($t = 0.05$, two-tail) were run to determine if any of the changes results in statistically significant differences.

5.4 Results and Discussion

Because PACs are non-polar and partition into lipids, we chose to test the extraction efficiencies of lipids from different taxa of bird eggs using hexane and DCM using 1.0 g of microbeads. In general, smaller amounts of lipids were recovered in hexane compared to DCM (*see* Figure 5.1). Unsurprisingly, we also found that PACs were extracted more efficiently from egg tissue using DCM than hexane (data not shown). Our validation method therefore was based on DCM as the extracting solvent.

It is acknowledged that plastic could be a source of contamination of PACs.[39-41] Because our extraction method was tested using strongly non-polar organic solvents under vigorous agitation with microbeads, which likely leads to some level of heat formation, it is important to first test if the bead-beating approach results in any background contamination from the high-density plastic MBETs themselves. To test this, we added different masses of microbeads (0, 0.5, 1.0 and 2.5 g) into each tube, along with 8 mL of our extraction solvent and used our protocol as described in Section 5.3.3. Our study showed that increasing the mass of microbeads results in elevated amounts of some PACs measured in our method blanks *i.e.*, DCM (*see* Figure IV.1). Additionally, the greater mass of microbeads also leads to greater deterioration of the lids of the tubes (*see* Figure IV.2). To reduce the leaching of PACs from the MBETs and lids, we pre-soaked both tubes/lids in DCM for 24 h and performed the extraction in an identical manner to tubes/lids that were unsoaked. Expectedly, PAC concentrations in our method blanks from tubes/lids that were pre-soaked were lower than tubes/lids that were unsoaked (*see* Table 5.1). Specifically, there were statistically

significant differences in concentrations for 6 PAHs and 7 APAHs in method blanks from pre-soaked and unsoaked tubes/lids (Student *t*-test, $p > 0.05$, *see* Table 5.1). In addition, concentrations of 7 APAHs in our method blanks all exceeded 100 pg μL^{-1} irrespective if tubes/lids were pre-soaked. Taken together, while pre-soaking tubes/lids greatly reduces the level of contamination of PACs in our method blanks, background levels of many PACs remained unacceptably high.

To circumvent leaching of PACs from our extraction tubes, we customed machined stainless-steel tubes/lids using the 15 mL commercially available high-density plastic tubes as a template (*see* Figure 5.2). To create a seal, Teflon inserts were placed inside the lids prior to fitting over the stainless-steel tube. Using the extraction protocol described in Section 5.3.3, we first determined if there were differences in lipid extraction efficiencies using 2.5 or 1.0 g of microbeads in our stainless-steel tubes. Because there was no statistical difference between either of the weights tested on lipid extraction efficiencies, we chose to perform all future tests using 1.0 g of ceramic microbeads in our stainless-steel tubes. PACs contamination in our method blanks from our stainless-steel tube/lids ($n = 6$) was assessed as described above. Background concentrations of all our PACs studied were significantly smaller using the stainless-steel tubes/lids relative to the high-density plastic tubes, with PACs concentrations lower than the smallest spiking concentration used in our study (10 pg μL^{-1}) (*see* Table 5.2). Furthermore, background concentrations of all PAHs except naphthalene were all smaller than their respective LODs. Recall that background concentrations of 7 APAHs from our high-density plastic MBETs were greater than 100 pg μL^{-1} (see Table 5.1); using our stainless-steel tubes these concentrations were all smaller than their lowest respective calibration standard (*i.e.*, 2 pg μL^{-1}). Because we were satisfied with the small amount of PAC contamination arising from our stainless-steel tubes, we chose to perform our

validation studies using them and 1.0 g of ceramic microbeads. The following sections describe the data quality objectives of our method.

The overall performance characteristics of the extraction protocol are shown in Table 5.3. LODs/LOQs for PAHs were all ≤ 4 ng g^{-1} and ranged from 0.019/0.062 ng g^{-1} (acenaphthylene) to 0.108/0.361 ng g^{-1} (naphthalene). The LODs/LOQs for HPAHs were all below 0.4 ng g^{-1} and ranged from 0.017/0.057 ng g^{-1} (1-Cl-Ant) to 0.109/0.363 ng g^{-1} (9,10-Br_2-Ant). For APAHs, LODs/LOQs ranged from 0.025/0.084 ng g^{-1} (2-methylphenanthrene) to 0.190/0.634 ng g^{-1} (2-methylnaphthalene).

The accuracy of our extraction method for PACs was determined by comparing the measured values to the fortified amounts from our in-house chicken egg reference material which were created by fortifying eggs with known amounts of PACs at three levels (10, 100 and 500 pg μL^{-1}). For PAHs in the 10 pg μL^{-1} spiking level, our measured values are all between the criteria set by Association of Official Agricultural Chemists (AOAC), 60–115%,[42] except for acenaphthene (119.3%), benz[*a*]anthracene (118.6%), chrysene (120.7%), fluoranthene (123.4%) and naphthalene (115.8%). In the other two spiking levels, our results are also in good agreement with AOAC criteria. For HPAHs, recoveries ranged from 31.8% to 81.2%, 52.4% to 132.2% and 47.4% to 121.7% for 10, 100 and 500 pg μL^{-1} spiking level respectively; only about half of our measured results fall into the range of recovery set by AOAC, due in part to the fact there was no recovery correction to track the loss in the extraction procedure. The results for APAHs have a low bias especially for higher spiking levels, which could be a result of the lack of authentic recovery internal standards that are used to track the losses during the extraction process.

The precision (relative standard deviation, RSD) for PAHs of our method were calculated using 8 separate extractions over 24 h (intraday), and 12 separate extractions over 3 consecutive

days (interday) on our chicken egg reference sample. They ranged from 3.8% to 14.1% (acenaphthene and phenanthrene, intraday) and 3.9% to 28.2% (acenaphthene and fluoranthene, interday); only the interday repeatability for 3 PAHs fell outside the AOAC expected precision criteria (15%),[42] namely benzo[*g,h,i*]perylene (17.5%), fluoranthene (28.2%), and pyrene (21.8%). For all HPAHs, all the intraday precisions were lower than 20%, ranging from 9.1% to 17.3% (2-Cl-Fle and 5,6-Br_2-Ana, intraday) and 9.2% to 18.8% (2,7-Br_2-Fle and 5,6-Br_2-Ana, interday). For APAHs, most measured intraday repeatability was lower than 30%, except 4,5-dimethylpyrene (38.1%), 7-methylbenzo[*a*]pyrene (33.4%) and 7,10-dimethylbenzo[*a*]pyrene (39.3%). The major drawback of the validation data appears to be the repeatability for APAHs, especially for alkylated fluorene, alkylated dibenzothiophene, alkylated pyrene, and alkylated benzo[*a*]pyrene, which were injected in a separated injection without MRM transitions of authentic deuterated PAH surrogates. Deuterated PAH surrogates were used to estimate recovery, which was then applied to correct the loss of the closest eluting APAHs in extraction process.[37] The lack of authentic internal standards and inability to use an isotope dilution method to accurately quantify APAHs likely contributed, in part, to the poorer precision in our data for these compounds.

Patil *et al*. reported recoveries of nine PAHs from eggs which ranged from 69% (acenapthalene) to 112% (dibenz[*a,h*]anthracene) using a modified QuEChERs.[29] Chung *et al.* used ultrasound-assisted solvent for the extraction of 35 PACs in black-tailed gull eggs.[43] Although no assessment of blank contamination was presented, the overall performance characteristics of the method was similar to those reported here. Additionally, while both methods used similar volumes of extracting solvent, the extraction processing times for an individual sample using the Chung *et al.* method was *ca*. 70 min while for our method it was *ca*. 25 min.

Finally, the performance of our microbead method was compared to that of ASE using parameters described in a previous study.[37] Overall, there were negligible differences in accuracy and repeatability between the methods (data not shown). However, the microbead method described here uses considerably less solvent than the ASE (16 mL vs 50 mL) and can simultaneously process 6 samples in *ca*. 25 min while the ASE takes *ca*. 40 min per sample.

5.5 Conclusions

Despite every effort to pre-clean commercially available high-density plastic tubes, there were still leaching issues which are especially problematic if measuring PACs at the sub-ppm levels. Also, because the microbead beating approach is aggressive, deterioration of the plastic caps and cap-liners does occur and likely contributes to PACs in method blanks. Soaking tubes and caps in DCM for 24 h did mitigate contamination issues but background levels of many PACs, specifically the APAHs, were greater than 100 pg μL^{-1}. Our custom machined stainless-steel tubes/lids greatly reduced background contamination in our method blanks and enabled us to validate an extraction method for processing avian egg samples for a wide range of PACs even at the sub-ppm level. Because of its principle of operation, the microbead beating extraction led to high mass transfer of our analytes from fortified matrix into our extraction solvent resulting in high analyte recoveries. That our system is fully automated also makes it highly repeatable and less labour-intensive than traditional methods leading to faster sample processing times. We anticipate that our method will be amenable to other tissue-types where small sample weights need to be processed.

Table 5.1: *Mean background concentrations (pg μL^{-1}, n = 6) and % RSD of selected PACs arising from pre-soaked and unsoaked high-density MBETs.*

Compounds	Pre-soaked MBETs		Unsoaked MBETs		Decreasing ratio
	Concentration (pg μL^{-1})	% RSD	Concentration (pg μL^{-1})	% RSD	
Benz[*a*]anthracene	0.154	17.8	0.805	30.4	5.23
Benzo[*g,h,i*]perylene	0.0932	56.5	1.12	27.5	12.1
Chrysene	0.294	17.4	1.84	31.0	6.27
Fluoranthene	1.57	40.8	6.86	16.6	4.36
Naphthalene	9.04	16.7	12.4	2.76	1.37
Pyrene	5.41	31.8	39.6	25.3	7.32
2-Methylnaphthalene	216	29.6	379	15.8	1.75
1-Methylnaphthalene	234	33.4	400	10.1	1.70
C_2-Dimethylnaphthalene[a]	221	36.1	414	9.45	1.88
C_3-Trimethylnaphthalene[a]	199	28.0	447	11.6	2.24
3-Methylphenanthrene	163	48.1	398	29.3	2.45
2-Methylphenanthrene	178	57.0	463	27.7	2.60
C_2-Dimethylphenanthrene[a]	117	44.4	891	29.9	7.62

[a] *The entire cluster of isomers were integrated and quantified.*

Table 5.2: *Mean background concentrations (pg μL^{-1}, n = 6) and % RSD of PAHs arising from stainless-steel metal tubes.*

Compounds	**Concentration (pg μL^{-1})**	**% RSD**
Acenaphthene	<LOD	19.17
Acenaphthylene	<LOD	38.29
Anthracene	<LOD	66.15
Benz[*a*]anthracene	<LOD	18.54
Benzo[*a*]pyrene	<LOD	14.98
Benzo[*b*]fluoranthene	<LOD	9.29
Benzo[*g,h,i*]perylene	<LOD	50.85
Benzo[*k*]fluoranthene	<LOD	29.08
Chrysene	<LOD	28.78
Dibenzo[*a,h*]anthracene	<LOD	58.78
Fluoranthene	<LOD	37.23
Fluorene	<LOD	21.06
Indeno[1,2,3-*c,d*]pyrene	<LOD	54.53
Naphthalene	4.52	9.19
Phenanthrene	<LOD	12.34
Pyrene	<LOD	34.35

Table 5.3: *Method performance characteristics of our method for the analysis of PACs in chicken eggs using microbead beating extraction and GC-EI-MS/MS detection and quantitation.*

Compounds	Working Range (pg µL^{-1})	Spiking Level						Interday Precision (RSD %)	LOD (ng g^{-1})	LOQ (ng g^{-1})
		10 pg µL^{-1}		100 pg µL^{-1}		500 pg µL^{-1}				
		Accuracy (%)	Precision (RSD %)	Accuracy (%)	Precision (RSD %)	Accuracy (%)	Precision (RSD %)			
PAHs										
Acenapthene	10 – 1000[37]	119.3	13.9	111.8	3.8	113.4	2.4	3.9	0.070	0.234
Acenapthylene		105.3	4.2	110.4	4.4	103.6	3.5	5.1	0.019	0.062
Anthracene		84.6	11.4	70.6	11.4	53.4	5.2	14.5	0.041	0.137
Benz[*a*]anthracene		118.6	9.7	106.1	4.9	108.4	2.6	4.3	0.049	0.163
Benzo[*a*]pyrene		81.0	12.2	73.1	7.9	70.7	3.2	6.6	0.042	0.140
Benzo[*b*]fluoranthene		99.6	12.5	75.6	7.4	72.1	4.0	7.6	0.053	0.176
Benzo[*g,h,i*]perylene		86.8	6.4	77.7	6.0	82.5	1.4	17.5	0.024	0.079
Benzo[*k*]fluoranthene		115.0	19.7	89.4	13.0	91.3	7.5	11.3	0.096	0.321
Chrysene		120.7	14.9	131.6	4.0	130.5	7.1	6.2	0.076	0.255
Dibenzo[*a,h*]anthracene		69.5	14.6	82.2	12.4	81.8	10.2	13.1	0.043	0.143
Fluoranthene		123.4	15.2	106.9	6.1	112.5	6.2	28.2	0.080	0.266
Fluorene		70.1	10.8	126.4	7.0	118.6	5.1	11.2	0.032	0.107
Indeno[1,2,3-*c,d*]pyrene		74.7	14.8	79.3	4.0	85.3	2.9	10.0	0.047	0.156
Naphthalene		115.8	22.0	83.8	13.3	91.9	3.3	11.4	0.108	0.361
Phenanthrene		90.5	23.6	95.1	5.6	96.4	2.4	11.2	0.091	0.303
Pyrene		98.2	15.1	91.1	14.1	112.1	7.3	21.8	0.063	0.210
HPAHs										
1,2-Br_2-Any		37.4	19.5	65.5	11.7	61.5	4.3	13.7	0.031	0.103

1,4-Cl_2-Ant	10 – 1000[30]	67.6	14.6	124.5	9.6	111.4	6.1	10.4	0.042	0.140
1,5,9,10-Cl_4-Ant		46.2	25.4	87.0	16.9	80.7	10.1	18.0	0.050	0.166
1,6-Br_2-Pyr		39.8	21.5	132.2	13.4	121.7	5.3	14.7	0.036	0.121
1-Br-Ant		66.2	12.9	103.9	10.2	92.7	6.0	10.9	0.036	0.120
1-Cl-Ant		31.8	12.6	52.4	9.4	47.4	5.9	10.0	0.017	0.057
2,7-Br_2-Fle		49.1	19.8	112.7	9.9	100.7	6.0	9.2	0.041	0.138
2,7-Br_2-Phe		51.4	21.8	112.2	10.7	102.0	4.9	10.4	0.047	0.158
2,7-Cl_2-Fle		66.2	16.2	105.6	9.6	95.5	5.9	9.8	0.045	0.152
2-Br-Fle		46.4	10.6	88.7	10.3	77.5	6.3	9.3	0.021	0.070
2-Cl-Fle		63.7	7.6	86.2	9.1	78.3	5.8	10.8	0.021	0.069
3-Br-Flu		75.4	25.4	120.8	10.4	107.2	5.5	11.1	0.081	0.271
3-Br-Phe		65.7	11.1	105.4	9.8	94.7	5.7	11.9	0.031	0.103
4-Br-BaA		61.8	22.1	124.3	11.2	114.8	4.7	13.5	0.058	0.194
4-Br-Pyr		81.2	21.7	117.3	10.9	103.7	5.0	11.5	0.075	0.250
5,6-Br_2-Ana		33.8	33.1	61.5	17.3	55.7	9.1	20.7	0.047	0.158
5-Br-Ana		57.8	11.6	89.1	9.5	80.6	5.3	10.8	0.028	0.095
9,10-Br_2-Ant		59.6	43.1	112.1	11.0	101.8	4.9	10.4	0.109	0.363
9-Cl-Fle		51.3	12.4	60.3	11.6	59.7	7.3	18.1	0.027	0.090
APAHs										
2-Methylnaphthalene	2 – 500[37]	111.0	40.4	38.0	15.0	39.8	10.2	13.1	0.190	0.634
1-Methylnaphthalene		109.5	19.6	38.2	10.0	37.2	9.2	7.5	0.091	0.303
2,6-Dimethylnaphthalene		51.0	26.3	49.4	5.5	51.9	8.8	11.1	0.057	0.190
1,6-Dimethylnaphthalene		73.9	14.2	68.5	5.5	64.0	8.4	10.8	0.045	0.148
2,3,5-Trimethylnaphthalene		75.1	24.5	76.1	6.5	92.2	7.9	15.4	0.078	0.260
1,2,5,6-Tetramethylnaphthalene		70.8	21.3	69.1	6.7	75.3	10.7	29.8	0.064	0.213
1,4,6,7-Tetramethylnaphthalene		62.3	19.6	58.6	6.4	63.9	10.4	29.1	0.052	0.173
3-Methylphenanthrene		22.3	27.7	59.7	10.7	63.6	11.9	25.6	0.026	0.088
2-Methylphenanthrene		29.0	20.5	53.5	10.9	56.7	11.6	24.6	0.025	0.084

9-Methylphenanthrene	36.7	43.4	62.1	10.7	70.2	12.3	22.9	0.068	0.225
1-Methylphenanthrene	29.1	40.8	53.2	10.6	57.4	11.5	26.2	0.050	0.168
3,6-Dimethylphenanthrene	41.8	44.8	56.1	9.0	68.1	14.7	20.7	0.080	0.265
2,6-Dimethylphenanthrene	47.8	45.1	58.0	9.6	62.7	14.6	15.9	0.091	0.305
1,3-Dimethylphenanthrene	27.5	26.5	59.0	11.2	70.1	12.8	14.2	0.031	0.103
1,7-Dimethylphenanthrene	63.4	33.4	67.8	8.8	78.0	13.4	14.1	0.090	0.299
1,8-Dimethylphenanthrene	62.8	23.3	62.8	9.3	71.5	11.5	18.2	0.062	0.207
1,2,6-Trimethylphenanthrene	76.9	21.5	64.4	6.3	74.7	8.4	14.1	0.070	0.233
Retene	63.6	27.0	62.2	7.3	78.0	9.8	18.4	0.073	0.243
1,2,6,9-Tetramethylphenanthrene	74.5	13.8	125.3	9.9	157.3	10.7	24.4	0.044	0.145
Benzo[*b*]naphtho[1,2-*d*]thiophene	103.2	16.4	91.1	6.7	85.6	6.4	10.1	0.072	0.239
Benzo[*b*]naphtho[2,3-*d*]thiophene	146.0	14.2	118.3	9.7	95.0	10.1	12.7	0.088	0.292
2-Methylchrysene	51.8	17.0	64.3	17.7	46.8	9.1	19.3	0.037	0.124
7,12-Dimethylbenz[*a*]anthracene	47.2	14.3	61.8	8.0	58.6	8.0	12.6	0.029	0.095
1,3,6-Trimethylchrysene	36.8	39.0	70.0	8.2	67.9	10.3	4.7	0.061	0.203
1-Methylfluorene	48.5	38.3	96.9	25.5	53.8	18.7	37.5	0.079	0.263
Dibenzothiophene	36.0	46.1	57.6	23.1	37.1	16.2	23.5	0.070	0.234
9-n-Propylfluorene	106.6	23.0	71.7	24.0	44.6	15.5	27.8	0.104	0.347
1,7-Dimethylfluorene	100.2	22.1	75.2	27.0	49.7	16.9	40.6	0.094	0.313
4-Methyldibenzothiophene	53.9	51.1	80.8	24.5	50.8	16.2	24.0	0.117	0.389
9-n-Butylfluorene	106.1	17.7	83.7	23.9	51.7	15.5	26.5	0.080	0.266
2,8-Dimethyldibenzothiophene	76.5	16.9	60.0	26.8	38.0	14.8	22.8	0.055	0.183
2,4,7-Trimethyldibenzothiophene	95.5	40.3	87.7	28.3	54.5	14.6	31.4	0.163	0.544
4-n-Butyldibenzothiophene	78.8	49.0	96.9	27.6	60.3	14.2	28.1	0.164	0.546

4-Methylpyrene	145.7	12.6	104.8	27.4	65.1	16.0	28.6	0.078	0.259
1-Methylpyrene	48.1	70.1	92.6	30.0	56.8	16.7	31.5	0.143	0.477
1-n-Propylpyrene	113.8	26.3	101.6	27.1	63.2	13.2	27.4	0.127	0.423
4,5-Dimethylpyrene	142.6	30.2	88.9	38.1	37.0	11.6	50.7	0.183	0.609
1-n-Butylpyrene	163.4	27.1	111.1	27.8	66.4	11.6	27.2	0.188	0.625
7-Methylbenzo[*a*]pyrene	106.4	14.0	52.8	33.4	43.5	16.9	48.2	0.063	0.211
7,10-Dimethylbenzo[*a*]pyrene	120.1	35.8	64.0	39.3	36.3	22.3	44.3	0.182	0.608

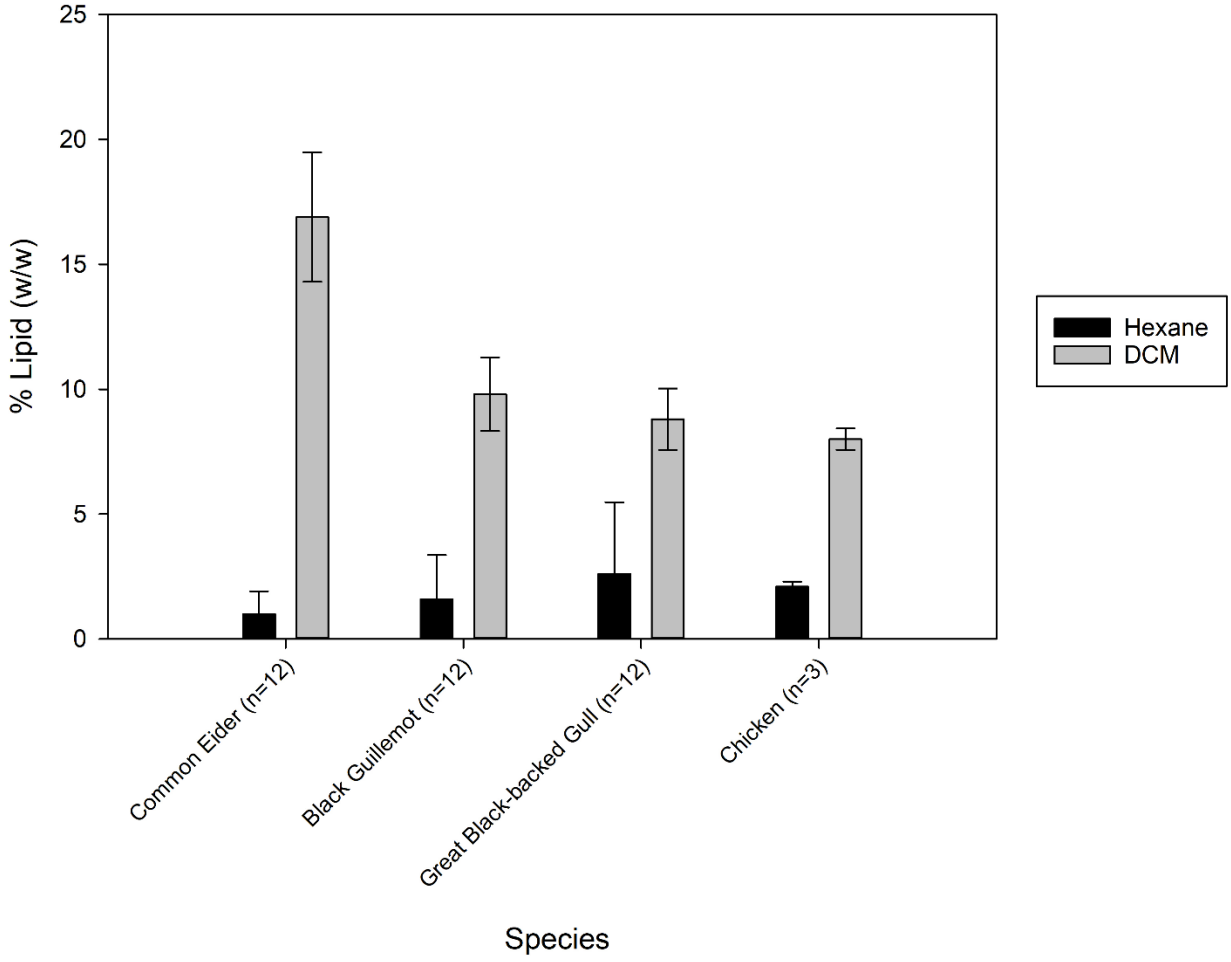

Figure 5.1: *Mean lipid percent ± standard deviation (n = 12) recovered from egg samples of various avian species extracted using hexane and DCM.*

Figure 5.2: *Custom designed 15 mL stainless-steel tube with lid fitted with a Teflon septum.*

5.6 References

(1) Marvin, C. H.; Berthiaume, A.; Burniston, D. A.; Chibwe, L.; Dove, A.; Evans, M.; Hewitt, L. M.; Hodson, P. V.; Muir, D. C. G.; Parrott, J.; Thomas, P. J.; Tomy, G. T. Polycyclic aromatic compounds in the Canadian environment: Aquatic and terrestrial environments. *Environ Pollut* **2021**, *285*, 117442. DOI: 10.1016/j.envpol.2021.117442

(2) Douglas, T. A.; Loseto, L. L.; Macdonald, R. W.; Outridge, P.; Dommergue, A.; Poulain, A.; Amyot, M.; Barkay, T.; Berg, T.; Chételat, J.; Constant, P.; Evans, M.; Ferrari, C.; Gantner, N.; Johnson, M. S.; Kirk, J.; Kroer, N.; Larose, C.; Lean, D.; Nielsen, T. G.; Poissant, L.; Rognerud, S.; Skov, H.; Sørensen, S.; Wang, F.; Wilson, S.; Zdanowicz, C. M. The fate of mercury in arctic terrestrial and aquatic ecosystems, a review. *Environ. Chem.* **2012**, *9* (4), 321. DOI: 10.1071/en11140

(3) Cediel Ulloa, A.; Gliga, A.; Love, T. M.; Pineda, D.; Mruzek, D. W.; Watson, G. E.; Davidson, P. W.; Shamlaye, C. F.; Strain, J. J.; Myers, G. J.; van Wijngaarden, E.; Ruegg, J.; Broberg, K. Prenatal methylmercury exposure and DNA methylation in seven-year-old children in the seychelles child development study. *Environ Int* **2021**, *147*, 106321. DOI: 10.1016/j.envint.2020.106321

(4) Nilsen, F. M.; Parrott, B. B.; Bowden, J. A.; Kassim, B. L.; Somerville, S. E.; Bryan, T. A.; Bryan, C. E.; Lange, T. R.; Delaney, J. P.; Brunell, A. M.; Long, S. E.; Guillette, L. J., Jr. Global DNA methylation loss associated with mercury contamination and aging in the American alligator (Alligator mississippiensis). *Sci Total Environ* **2016**, *545-546*, 389-397. DOI: 10.1016/j.scitotenv.2015.12.059

(5) Pedersen, K. E.; Letcher, R. J.; Sonne, C.; Dietz, R.; Styrishave, B. Per- and polyfluoroalkyl substances (PFASs) - new endocrine disruptors in polar bears (Ursus maritimus)? *Environ Int* **2016**, *96*, 180-189. DOI: 10.1016/j.envint.2016.07.015

(6) Evers, D. C.; Savoy, L. J.; DeSorbo, C. R.; Yates, D. E.; Hanson, W.; Taylor, K. M.; Siegel, L. S.; Cooley, J. H., Jr.; Bank, M. S.; Major, A.; Munney, K.; Mower, B. F.; Vogel, H. S.; Schoch, N.; Pokras, M.; Goodale, M. W.; Fair, J. Adverse effects from environmental mercury loads on breeding common loons. *Ecotoxicology* **2008**, *17* (2), 69-81. DOI: 10.1007/s10646-007-0168-7

(7) Swaddle, J. P.; Diehl, T. R.; Taylor, C. E.; Fanaee, A. S.; Benson, J. L.; Huckstep, N. R.; Cristol, D. A. Exposure to dietary mercury alters cognition and behavior of zebra finches. *Curr Zool* **2017**, *63* (2), 213-219. DOI: 10.1093/cz/zox007

(8) Burgess, N. M.; Bond, A. L.; Hebert, C. E.; Neugebauer, E.; Champoux, L. Mercury trends in herring gull (Larus argentatus) eggs from Atlantic Canada, 1972-2008: Temporal change or dietary shift? *Environ Pollut* **2013**, *172*, 216-222. DOI: 10.1016/j.envpol.2012.09.001

(9) Elliott, J. E.; Noble, D. G.; Norstrom, R. J.; Whitehead, P. E. Organochlorine contaminants in seabird eggs from the pacific coast of Canada, 1971-1986. *Environ Monit Assess* **1989**, *12* (1), 67-82. DOI: 10.1007/BF00396737

(10) Elliott, J. E.; Noble, D. G.; Norstrom, R. J.; Whitehea, P. E.; Simon, M.; Pearce, P. A.; Peakall, D. B. Patterns and trends of organic contaminants in Canadian seabirds, 1968-1990. In *Persistent pollutants in the marine environment.*, Ch Walker, D. R. L. Ed.; Pergamon Press, 1992; pp 181-194.

(11) Solla, S. R. d.; Weseloh, D. V. C.; Hughes, K. D.; Moore, D. J. Forty-year decline of organic contaminants in eggs of herring gulls (Larus argentatus) from the Great Lakes, 1974 to 2013. *cowa* **2016**, *39* (sp1), 166-179. DOI: 10.1675/063.039.sp117

(12) Lucia, M.; Verboven, N.; Strom, H.; Miljeteig, C.; Gavrilo, M. V.; Braune, B. M.; Boertmann, D.; Gabrielsen, G. W. Circumpolar contamination in eggs of the high-Arctic ivory gull Pagophila Eburnea. *Environ Toxicol Chem* **2015**, *34* (7), 1552-1561. DOI: 10.1002/etc.2935

(13) Hebert, C. E. The river runs through it: The Athabasca River delivers mercury to aquatic birds breeding far downstream. *PLOS ONE* **2019**, *14* (4), e0206192. DOI: 10.1371/journal.pone.0206192 (acccessed 2022/08/24/01:21:30).

(14) Dietz, R.; Letcher, R. J.; Desforges, J.-P.; Eulaers, I.; Sonne, C.; Wilson, S.; Andersen-Ranberg, E.; Basu, N.; Barst, B. D.; Bustnes, J. O.; Bytingsvik, J.; Ciesielski, T. M.; Drevnick, P. E.; Gabrielsen, G. W.; Haarr, A.; Hylland, K.; Jenssen, B. M.; Levin, M.; McKinney, M. A.; Nørregaard, R. D.; Pedersen, K. E.; Provencher, J.; Styrishave, B.; Tartu, S.; Aars, J.; Ackerman, J. T.; Rosing-Asvid, A.; Barrett, R.; Bignert, A.; Born, E. W.; Branigan, M.; Braune, B.; Bryan, C. E.; Dam, M.; Eagles-Smith, C. A.; Evans, M.; Evans, T. J.; Fisk, A. T.; Gamberg, M.; Gustavson, K.; Hartman, C. A.; Helander, B.; Herzog, M. P.; Hoekstra, P. F.; Houde, M.; Hoydal, K.; Jackson, A. K.; Kucklick, J.; Lie, E.; Loseto, L.; Mallory, M. L.; Miljeteig, C.; Mosbech, A.; Muir, D. C. G.; Nielsen, S. T.; Peacock, E.; Pedro, S.; Peterson, S. H.; Polder, A.; Rigét, F. F.; Roach, P.; Saunes, H.; Sinding, M.-H. S.; Skaare, J. U.; Søndergaard, J.; Stenson, G.; Stern, G.; Treu, G.; Schuur, S. S.; Víkingsson, G. Current state of knowledge on biological effects from contaminants on Arctic wildlife and fish. *Science of The Total Environment* **2019**, *696*, 133792. DOI: 10.1016/j.scitotenv.2019.133792

(15) Braune, B. M.; Scheuhammer, A. M.; Crump, D.; Jones, S.; Porter, E.; Bond, D. Toxicity of methylmercury injected into eggs of thick-billed murres and Arctic terns. *Ecotoxicology* **2012**, *21* (8), 2143-2152. DOI: 10.1007/s10646-012-0967-3

(16) Zhou, C.; Xu, P.; Huang, C.; Liu, G.; Chen, S.; Hu, G.; Li, G.; Liu, P.; Guo, X. Effects of subchronic exposure of mercuric chloride on intestinal histology and microbiota in the cecum of chicken. *Ecotoxicol Environ Saf* **2020**, *188*, 109920. DOI: 10.1016/j.ecoenv.2019.109920

(17) Kim, J. H.; Lee, H. K.; Park, G. H.; Choi, H. S.; Ji, S. Y.; Kil, D. Y. Determination of the toxic level of dietary mercury and prediction of mercury intake and tissue mercury concentrations in broiler chickens using feather mercury concentrations. *Journal of Applied Poultry Research* **2019**, *28* (4), 1240-1247. DOI: 10.3382/japr/pfz090

(18) Borga, K.; McKinney, M. A.; Routti, H.; Fernie, K. J.; Giebichenstein, J.; Hallanger, I.; Muir, D. C. G. The influence of global climate change on accumulation and toxicity of persistent organic pollutants and chemicals of emerging concern in Arctic food webs. *Environ Sci Process Impacts* **2022**, *24* (10), 1544-1576. DOI: 10.1039/d1em00469g

(19) Bårdsen, B.-J.; Hanssen, S. A.; Bustnes, J. O. Multiple stressors: Modeling the effect of pollution, climate, and predation on viability of a sub-arctic marine bird. *Ecosphere* **2018**, *9* (7), e02342. DOI: 10.1002/ecs2.2342

(20) Hallinger, K. K.; Cristol, D. A. The role of weather in mediating the effect of mercury exposure on reproductive success in tree swallows. *Ecotoxicology* **2011**, *20* (6), 1368-1377. DOI: 10.1007/s10646-011-0694-1

(21) Smith, R. A.; Albonaimi, S. S.; Hennin, H. L.; Gilchrist, H. G.; Fort, J.; Parkinson, K. J. L.; Provencher, J. F.; Love, O. P. Exposure to cumulative stressors affects the laying phenology and incubation behaviour of an arctic-breeding marine bird. *Sci Total Environ* **2022**, *807* (Pt 2), 150882. DOI: 10.1016/j.scitotenv.2021.150882

(22) Teitelbaum, C. S.; Ackerman, J. T.; Hill, M. A.; Satter, J. M.; Casazza, M. L.; De La Cruz, S. E. W.; Boyce, W. M.; Buck, E. J.; Eadie, J. M.; Herzog, M. P.; Matchett, E. L.; Overton, C. T.; Peterson, S. H.; Plancarte, M.; Ramey, A. M.; Sullivan, J. D.; Prosser, D. J. Avian influenza antibody prevalence increases with mercury contamination in wild waterfowl. *Proc Biol Sci* **2022**, *289* (1982), 20221312. DOI: 10.1098/rspb.2022.1312

(23) Hebert, C. E.; Weseloh, D. V.; Macmillan, S.; Campbell, D.; Nordstrom, W. Metals and polycyclic aromatic hydrocarbons in colonial waterbird eggs from Lake Athabasca and the Peace-Athabasca Delta, Canada. *Environ Toxicol Chem* **2011**, *30* (5), 1178-1183. DOI: 10.1002/etc.489 (acccessed 2022/08/24/02:09:50).

(24) Pereira, M. G.; Walker, L. A.; Wright, J.; Best, J.; Shore, R. F. Concentrations of polycyclic aromatic hydrocarbons (PAHs) in the eggs of predatory birds in Britain. *Environ Sci Technol* **2009**, *43* (23), 9010-9015. DOI: 10.1021/es901805e (acccessed 2022/08/24/02:07:51).

(25) Power, A.; White, P.; McHugh, B.; Berrow, S.; McKeown, A.; Crowley, D.; Newton, S.; McGovern, E.; Murphy, S.; O'Connor, I. Polycyclic aromatic hydrocarbons (PAHs) in seabird eggs in Ireland. *Mar Pollut Bull* **2021**, *170*, 112636. DOI: 10.1016/j.marpolbul.2021.112636

(26) Tan, J.; Lu, X.; Fu, L.; Yang, G.; Chen, J. Quantification of cl-PAHs and their parent compounds in fish by improved ase method and stable isotope dilution GC-MS. *Ecotoxicol Environ Saf* **2019**, *186* (October), 109775. DOI: 10.1016/j.ecoenv.2019.109775

(27) Wickrama-Arachchige, A. U.; Hirabayashi, T.; Imai, Y.; Guruge, K. S.; Dharmaratne, T. S.; Ohura, T. Accumulation of halogenated polycyclic aromatic hydrocarbons by different tuna species, determined by high-resolution gas chromatography Orbitrap mass spectrometry. *Environ Pollut* **2020**, *256*, 113487. DOI: 10.1016/j.envpol.2019.113487

(28) Xia, Z.; Idowu, I.; Marvin, C.; Thomas, P. J.; Johnson, W.; Francisco, O.; Stetefeld, J.; Crimmins, B.; Fry, M.; Tomy, G. T. Identification of halogenated polycyclic aromatic hydrocarbons in biological samples from Alberta Oil-Sands Region. *Chemosphere* **2019**, *215*, 206-213. DOI: 10.1016/j.chemosphere.2018.10.050

(29) Patil, R.; Chatterjee, N. S.; Kamble, N.; Nerpagar, A.; Langade, N.; Kandaswamy, C.; Presley, S. I. D.; Banerjee, K. Multiresidue analysis of pesticides, polyaromatic hydrocarbons and polychlorinated biphenyls in poultry meat and chicken eggs by GC-MS/MS: Method development and validation. *J Environ Sci Health B* **2022**, *57* (4), 263-283. DOI: 10.1080/03601234.2022.2047390

(30) Xia, Z.; Idowu, I.; Kerr, E.; Klaassen, N.; Assi, H.; Bray, H.; Marvin, C.; Thomas, P. J.; Stetefeld, J.; Tomy, G. T. New approaches to reduce sample processing times for the determination of polycyclic aromatic compounds in environmental samples. *Chemosphere* **2021**, *274*, 129738. DOI: 10.1016/j.chemosphere.2021.129738

(31) Bordin, D. C.; Alves, M. N.; de Campos, E. G.; De Martinis, B. S. Disposable pipette tips extraction: Fundamentals, applications and state of the art. *J Sep Sci* **2016**, *39* (6), 1168-1172. DOI: 10.1002/jssc.201500932

(32) Forsberg, N. D.; Wilson, G. R.; Anderson, K. A. Addition to determination of parent and substituted polcyclic aromatic hydrocarbons in high-fat salmon using a modified quechers

extraction, dispersive SPE and GC–MS. *Journal of Agricultural and Food Chemistry* **2011**, *59* (19), 10773-10773. DOI: 10.1021/jf203649c

(33) Forsberg, N. D.; Wilson, G. R.; Anderson, K. A. Determination of parent and substituted polycyclic aromatic hydrocarbons in high-fat salmon using a modified QuEChERS extraction, dispersive SPE and GC-MS. *J Agric Food Chem* **2011**, *59* (15), 8108-8116. DOI: 10.1021/jf201745a

(34) Johnson, Y. S. Determination of polycyclic aromatic hydrocarbons in edible seafood by quechers-based extraction and gas chromatography-tandem mass spectrometry. *J Food Sci* **2012**, *77* (7), T131-137. DOI: 10.1111/j.1750-3841.2012.02758.x

(35) Paya, P.; Anastassiades, M.; Mack, D.; Sigalova, I.; Tasdelen, B.; Oliva, J.; Barba, A. Analysis of pesticide residues using the quick easy cheap effective rugged and safe (QuEChERS) pesticide multiresidue method in combination with gas and liquid chromatography and tandem mass spectrometric detection. *Anal Bioanal Chem* **2007**, *389* (6), 1697-1714. DOI: 10.1007/s00216-007-1610-7

(36) Prestes, O. D.; Friggi, C. A.; Adaime, M. B.; Zanella, R. Quechers: Um método moderno de preparo de amostra para determinação multirresíduo de pesticidas em alimentos por métodos cromatográficos acoplados à espectrometria de massas. *Química Nova* **2009**, *32* (6), 1620-1634. DOI: 10.1590/s0100-40422009000600046

(37) Idowu, I.; Francisco, O.; Thomas, P. J.; Johnson, W.; Marvin, C.; Stetefeld, J.; Tomy, G. T. Validation of a simultaneous method for determining polycyclic aromatic compounds and alkylated isomers in biota. *Rapid Commun Mass Spectrom* **2018**, *32* (3), 277-287. DOI: 10.1002/rcm.8035

(38) Magnusson, B.; Örnemark, U. *The fitness for purpose of analytical methods – a laboratory guide to method validation and related topics (2nd ed)*; 2014.

(39) Conesa, J. A.; Nuñez, S. S.; Ortuño, N.; Moltó, J. PAH and POP presence in plastic waste and recyclates: State of the art. *Energies* **2021**, *14* (12), 3451. DOI: 10.3390/en14123451 (acccessed 2022/08/25/03:35:48).

(40) Fisner, M.; Taniguchi, S.; Majer, A. P.; Bicego, M. C.; Turra, A. Concentration and composition of polycyclic aromatic hydrocarbons (PAHs) in plastic pellets: Implications for small-scale diagnostic and environmental monitoring. *Mar Pollut Bull* **2013**, *76* (1-2), 349-354. DOI: 10.1016/j.marpolbul.2013.09.045 (acccessed 2022/08/25/03:36:24).

(41) Li, C. T.; Zhuang, H. K.; Hsieh, L. T.; Lee, W. J.; Tsao, M. C. PAH emission from the incineration of three plastic wastes. *Environ Int* **2001**, *27* (1), 61-67. DOI: 10.1016/s0160-4120(01)00056-3 (acccessed 2022/08/25/03:35:54).

(42) Aoac international. In *Guidelines for standard method performance requirements (spmrs). Aoac official methods of analysis*, Vol. Appendix F; 2016; pp 1-18.

(43) Chung, D.; Lee, J. H.; Lee, S. Y.; Oh, Y. S.; Shin, H. S. Quantitative analysis and contamination profiles of PCBs, OCPs, and PAHs in black-tailed gull eggs in the republic of korea. *Environ Monit Assess* **2022**, *194* (4), 268. DOI: 10.1007/s10661-022-09905-w

Chapter 6: Comparison of Different Approaches to Quantify Substituted Polycyclic Aromatic Compounds

A version of this chapter has already been published as Ifeoluwa G. Idowu, Zhe Xia, Courtney D. Sandau, Michelle Misselwitz, Chris Marvin, Philippe Thomas, Gregg T. Tomy. Comparison of different approaches to quantify substituted polycyclic aromatic compounds. *Journal of Chromatography A*, **2021**, *1651*, 462317.

6.1 Abstract

Unlike native polycyclic aromatic hydrocarbons (PAHs), quantitation of substituted polycyclic aromatic compounds (PACs) has been a challenge in the environmental industry. The challenge can be attributed in part to the large number of theoretically possible isomers and the lack of authentic standards for quantitation. In addition, the lack of a unified approach to the quantitation of these compounds has led to poor interlaboratory accuracy. Because these compounds are often used for toxicology studies or to delineate sources and fingerprinting, it is vital that a standardized approach to quantifying them is established. This study evaluated different quantitation approaches to quantifying both 16 individual PACs and 32 groups/clusters of substituted PACs in three standard reference materials (SRM 1944 - New York / New Jersey waterway sediments, SRM 1597 - a coal tar sample and SRM 2779 - Gulf of Mexico crude oil). The methods employed include: (1) external calibration taking into account recovery correction factor for each analyte, (2) an average relative response factor (ARRF) of PACs obtained with a recovery correction, (3) ARRF of PACs obtained using uncorrected peak areas (*i.e.*, no recovery correction), (4) ARRF of PACs calculated by normalization to deuterated PAHs and (5) ARRF of native PAHs to quantify substituted PACs. The evaluation of concentrations of individually substituted PACs from the different quantitative approaches compared to the certified/reference values showed that methods 1, 2 and 3 performed best. The average percentage of compounds that fell within our acceptable limit (± 30%) using methods 1, 2 and 3 for SRM-1944, -1597 and -2779 was 87, 75 and 100%, respectively. and resulted in concentrations within the acceptable limit (± 30%) for most of the compounds evaluated. Using native PAHs to quantify their substituted analogs resulted in data of the poorest quality. Irrespective of the approach used, there were significant systematic errors in measurements on clusters/groups PACs most notably C_1- and C_2- benzanthracenes/chrysenes/triphenylenes, and C_2-

and C_3-dibenzothiophenes being consistently greater than 100% of the stated value. Commercial availability of more substituted PAC will mitigate the biases associated with the quantitation of PAC clusters/groups.

6.2 Introduction

Polycyclic aromatic compounds (PACs) are ubiquitous environmental pollutants commonly found in crude oil/petroleum, creosote, sediments, coal tar, and also associated waste.[1-4] Due to their ubiquity, toxicity and their usefulness in source identification considerable attention has been given to them by the scientific community.[1,5-9] Polycyclic aromatic compounds have a similar backbone structure to polycyclic aromatic hydrocarbons (PAHs), however, substitutions can occur on one or more of the rings (*e.g.*, alkyl, halogens) or within the ring itself (*e.g.*, N-, S-, O-atoms).[10-13] Substitutions on the ring(s) leads to a large number of theoretically possible compounds which leads to the inherent complexity associated with this class of compounds.[14]

Evaluation of the environmental toxicity, risk, and source apportionment of PACs is often hampered by challenges associated with their identification and quantification.[2,12,15,16] Two of the main challenges that the analytical chemist face in measuring the occurrence and concentrations of these compounds are the lack of commercially available authentic standards and the large number of theoretically possible isomers.[12,14] Wilton listed other factors that can lead to poor data quality: (i) the use of a single ion detection method (*i.e.*, single/selective ion monitoring technique – SIM), (ii) difficulty in identifying and integrating the correct homolog peaks and (iii) interference of compounds that elute within the same retention time window.[16-18] Numerous studies have examined approaches to accurately quantify these compounds.[18-21]

Naturally, the lack of a standardized/unified approach to quantifying PACs has led to variability in environmental measurements among laboratories. For example, in an interlaboratory comparison study to support work related to the Deepwater Horizon natural resource damage assessment, there was a higher variability in the data for alkyl-PAC measurements compared to PAHs among participating laboratories.[17,20,22] Also, the common practice to employ single/selective ion monitoring for PACs, especially the C_2 to C_4 homologues, provides lower degree of confidence in identification of retention time window and estimated concentrations.[16,17,20] Adopting a unified approach to quantifying PACs will minimize inter-laboratory variability and lead to more reliable measurements which is vital to environmental risk assessments and forensics studies.

While several studies have largely examined data acquisition challenges to address interlaboratory variability, there is a paucity of information on comparing different PAC quantitative approaches and the subsequent impact on data quality. This study attempted to address this critical data-gap and examined a variety of different quantitative approaches to determining PACs in environmental samples. Three standard reference materials (SRMs) were selected for our study: SRM 1944 – New York / New Jersey waterway sediments, SRM 1597 – a coal tar sample and SRM 2779 which is a Gulf of Mexico crude oil. The accuracy and precision of each method was compared to the certified or reference values as reported in the certificate of analysis for each SRM. It should be noted that the reference values provided for SRMs are from multiple laboratories, likely using a variety of quantification methods. Therefore, these reference values represent the average of the methods employed by the laboratories that provided results for the SRM characterizations. Our studies were performed in an ISO/IEC 17025:2017 accredited laboratory and adhered to the strict guidelines for obtaining meaningful data quality objectives.

6.3 Materials and Methods

6.3.1 Materials

Organic solvents used for this study were purchased from Fisher Chemicals (Ottawa, Ontario, Canada). Various analytical standards were employed including sixteen (16) unsubstituted PAHs as a native mix, deuterium mass labeled d_{10}-anthracene, d_8-naphthalene, d_8-acenaphthylene, d_{10}-acenaphthene, d_{10}-fluorene, d10-phenanthrene, d_{10}-pyrene, d_{12}-benz(*a*)anthracene, d_{12}-chrysene, d_{12}-benzo(*b*)fluoranthene, d_{12}-benzo(*k*)fluoranthene, d_{12}-benzo(*a*)pyrene, d_{12}-indeno(1,2,3-*c,d*)pyrene, d_{14}-dibenzo(*a,h*)anthracene, d_{14}-benzo(*g,h,i*)perylene and twenty-eight (28) individual substituted PACs as presented in Table V.1. All standards were > 98% purity and purchased from AccuStandard Inc., New Haven, Connecticut, USA and Chiron Chemicals, Trondheim, Norway. An isotope dilution internal standard (IDIS) consisted of 15 of the 16 deuterated PAHs while the 16th compound, d_{10}-anthracene, was used as the instrument performance internal standard (IPIS). The standard reference materials, SRM 1944 – New York / New Jersey waterway sediments, SRM 1597 – a coal tar sample and SRM 2779 Gulf of Mexico crude oil were purchased from the National Institute of Science and Technology (NIST Gaithersburg, Maryland, USA). Silica gel (923 grade, 100–200 mesh), alumina (60–325 mesh), and anhydrous sodium sulfate were all purchased from Fisher Chemical. Diatomaceous earth (DE) dispersant was purchased from Fisher Scientific (Ottawa, ON).

6.3.2 Sample Processing

6.3.2.1 New York / New Jersey Waterway Sediments (SRM 1944)

An accurately weighed amount of SRM 1944 (0.5 g, *n = 7*) was mixed with DE dispersant and packed into a 10 mL ASE cell. The cell was then packed with Ottawa Sand to fill the dead volume and subsequently spiked with IDIS (50 ng μL^{-1}, 10 µL) Procedural blanks (*n = 6*) were prepared by only using DE dispersant and Ottawa Sand. Dichloromethane (DCM) was used as the extracting solvent; details of the ASE parameters can be found in Idowu *et al.*[24] The extract obtained from the ASE was then transferred to a round bottom flask and carefully reduced to 2 mL. Reduced copper (~ 0.5 g) was added to the extract to remove any elemental sulfur that might be present. The extract was solvent exchanged into hexane and then carefully reduced to 1 mL under a gentle stream of UHP nitrogen. Extracts were then added to the head of an adsorption chromatography column that contained 11 g silica gel in the bottom, followed by 1 g of deactivated alumina, and topped with 1 g of Na_2SO_4 as the stationary phase. The elution of compounds was performed with solvents of increasing polarity starting with 25 mL of hexane for non-polar/saturate fraction and afterward, 25 mL DCM: Hexane (1:1, v/v) to elute the PAC fraction. The PAC fractions were then evaporated to 1 mL, fortified IPIS (50 ng μL^{-1}, 10 µL), and transferred to GC vials.

6.3.2.2 Gulf of Mexico Oil (SRM 2779)

An accurately weighed amount of SRM 2779 (0.01 g, *n = 5*) was spiked with IDIS (10 ng μL^{-1}, 10 µL) and cleaned up using a silica/alumina open column chromatography as described previously. The PAC fraction was then evaporated to1 mL, spiked with IPIS (10 ng μL^{-1}, 10 µL), and transferred to GC vials.

6.3.2.3 Coal Tar (SRM 1597a)

An accurately weighed amount of SRM 1579a (0.01 g, *n* = *3*) was dissolved in DCM, sonicated for 20 min and then diluted to 1 mL in a volumetric flask with hexane. This solution was also spiked with IDIS (10 ng μL^{-1}, 10 µL) and IPIS (10 ng μL^{-1}, 10 µL) before sample injection.

6.3.3 Gas Chromatography Tandem Mass Spectrometry

An Agilent 7890a GC coupled with a triple quadrupole mass spectrometer fitted with electron ionization (EI) source was used for the MS/MS acquisition. Chromatographic separation was achieved using an Agilent J&W HP-5ms ultra inert column (30 m × 0.25 mm × 0.25 µm), with helium as the carrier gas at a constant flow rate of 1.2 mL/min. 1 µL of sample was injected with a PAL RSI 85 auto sampler at 250 °C in splitless mode for all SRM analyzed. The oven temperature was held at 60 °C for 1 min then raised to 120 °C at 35 °C/min, further ramped up to 220 °C at 14 °C/min, 260 °C at 5 °C/min and held for 5 min, to 300 °C at 10 °C/min and finally to 310 °C at 50 °C/min. Both transfer line and source temperature were set at 320 °C. Multiple reaction monitoring (MRM) precursor → product ion transitions were used for all analytes. To improve detection limits, pseudo-MRM transitions were used for indeno(1,2,3-*c,d*)pyrene, dibenz(*a,h*)anthracene and benzo(*g,h,i*)perylene; details of MRM transitions are as described in Idowu *et al*.[23]

6.3.4 Description of Approaches to Quantification

6.3.4.1 Quantitation of PAHs in SRMs

The concentration of PAHs in the SRM materials were determined using the isotope dilution method of quantitation. The first step in this approach is to determine the relative response factors

(RRFs) of native PAHs relative to their deuterated analogs over a prescribed concentration range of the native species. Standard solutions of 16 native PAHs at nominal concentrations of 10, 200, 400, 600, 800, and 1000 pg μL^{-1} (*n = 3* or more in each case) were individually spiked with their d-PAH surrogates (100 pg μL^{-1}). To account for small systematic errors that may arise from fluctuations in injection volumes and ionization efficiencies between injections, a known amount of IPIS (100 pg μL^{-1}) is added to all the standard solutions (and samples) which is then used to calculate the percentage recovery and to normalize the response of the target analytes. The relative response factor (RRF) of native PAH at each concentration level is determined and then an average RRF (ARRF) is calculated across the entire concentration range. The ARRF is then used to calculate the concentration of native PAHs in the sample.

6.3.4.2 Quantitation of PACs in SRMs

Five different approaches were used to quantify substituted PACs. Differences in each of the methods lie primarily in the way the response of the PAC group/cluster is related to an external standard. In instances where multiple isomers exist for a substituted PAC, the approach to quantitation requires prudent selection of an authentic substituted PAC as the external standard. Admittedly, this can be somewhat subjective and often based on commercial availability of solutions. In addition, where multiple isomers exist for a PAC grouping, the accepted approach is to sum the electronically integrated areas of all the isomers in the MRM ion channel from samples and compare it to the area of judiciously selected external standard(s) as shown in Table V.2.[24,25] By way of an example, 3-methyl-phenanthrene is used to quantify the C_1 phenanthrenes/anthracenes group. The implicit assumption with this approach is that the response factors of all substituted PACs in the group are similar to 3-methyl-phenanthrene. Others have

shown that it is unrealistic to assume this is the case but in the absence of chromatographic separation of all isomers in a PAC group and lack of availability of all isomers this is the most reasonable and best approach.[24,26] In those instances where no representative standard for a particular substituted PAC group existed, we selected the structurally closest isomer available to serve as its external standard. For example, 4,5-dimethyl-pyrene was used to quantify all the isomers in the C_3 pyrene group. As stated above, the total electronically integrated area of the ion signals of an MRM characteristic of a specific PAC group was compared to the response of the external standard selected for quantitation. We will begin by describing our approach to quantifying individual and group PAC isomers using external standardization.

Differences in the choice and approach to treating the electronically integrated areas and how they are compared to the external standards will now be described.

6.3.4.2.1 Method 1

In this approach, a calibration curve is generated using standard solutions containing individual substituted PACs at nominal concentrations of 2, 100, 200, 300, 400 and 500 pg/µL each spiked with IPIS (100 pg μL^{-1}). Corrected peak areas (*i.e.*, area of native species corrected to area of IPIS) and nominal concentrations are plotted to give slopes and intercepts for each individual substituted PAC standard. These slopes and intercepts are then used with the corrected peak areas of sample extracts to obtain the concentrations of an individual substituted PAC.

Potential loss of analyte during sample processing is the last consideration that we account for when quantifying analytes using the external standardization method. Here a solution containing mass labeled PAHs are spiked into the sample prior to processing and any losses observed are used to correct for losses of the target PAC. We selected the mass labeled PAH eluting closest to the

PAC isomer or group to correct for potential losses. It could be argued that the unsubstituted mass labeled PAH could also be used to quantify its substituted analog, however, we chose to use the mass labeled compound eluting closest to the substituted group of interest.[23] Table V.4 shows the closest eluting mass labeled PAHs for each group of substituted PACs grouping.

The next four methods rely on different approaches using the relative response factors to quantify PACs. The first step is to calculate response factors of individually substituted PACs over a concentration range (2, 100, 200, 300, 400, 500 pg μL^{-1}) relative to an internal standard (IS) and then uses an ARRF over the entire calibration range to calculate the concentration of PACs in samples.

The RRF for PACs is obtained using Equation 6.1.

$$RRF_{Substitued\ PAC} = \frac{\left(\frac{Area_{substituted\ PACs}}{Area_{Internal\ standard}}\right)}{\left(\frac{Conc_{Substituted\ PACs}}{Conc_{Internal\ standard}}\right)} \qquad \textit{Equation 6.1}$$

Using the ARRF, concentrations (mass per volume) of both individual and group substituted PACs in each sample extract can be determined using Equation 6.2.

$$Extract\ Conc_{substituted\ PAC}\ \left(\frac{pg}{\mu L}\right) = \left[\frac{\left(\frac{Area_{Substituted\ PAC}}{Area_{IS}}\right)}{ARRF_{Substituted\ PAC}}\right] \times\ Conc_{IS} \qquad \textit{Equation 6.2}$$

Finally, concentrations of individual and group substituted PACs (on weight-to-weight basis) can be determined using the equation by Equation 6.3.

$$Substituted\ PAC\ \left(\frac{ng}{g}\right) = \frac{\left(\frac{Extract\ Conc_{\ Substituted\ PAC}\ \times\ V_{Extract}\ (\mu L)}{Sample\ mass\ (g)}\right)}{(1000\ pg/ng)} \qquad \textit{Equation 6.3}$$

The choice of *internal standard* used in the equations above led to four (4) different quantitation approaches each described below in detail.

6.3.4.2.2 Method 2

This method normalizes the response of the selected substituted PAC standard to the IPIS (d_{10}-anthracene) which is added to samples prior to GC-injection. The ARRF is determined over a range of concentrations (2, 100, 200, 300, 400, 500 pg μL^{-1}) that is used to calculate the concentration of PACs in samples. Naturally, the response of targeted PACs in our samples are also normalized to the IPIS. Hence, d_{10}-anthracene is substituted as the internal standard in Equation 6.1 and Equation 6.2.

The peak area of the substituted PACs in our sample extracts are further corrected for losses as described in the external calibration approach using the closest eluting d-PAH. We refer to this as the corrected area in the equation below. The ARRF listed in Table V.4 were used to calculate the extract concentrations in each of the samples using Equation 6.3.

6.3.4.2.3 Method 3

This method is similar to that reported in Yang *et al*.[21] The ARRF is obtained using the same approach as *Method 1* (Equation 6.1). The difference between *Method 3* and *Method 2* is that no correction is applied to the data for loss of analyte during sample processing *i.e.*, d-PAHs are not used to correct the areas of the target analytes in the samples.

The ARRF in Table V.5 were used to calculate the extract concentrations in each of the samples following Equation 6.2.

6.3.4.2.4 Method 4

This quantitation approach is a modification to the method described in Yang *et al*.[21] In *methods 2 and 3*, the peak area is normalized to an internal standard in the form of an IPIS to

calculate the ARRF in Equation 6.1. Here, the area of mass labeled standards that are analogs of native PAHs are used to normalize the electronically integrated area of the group of substituted PACs (Equation 6.1). For example, d_{10}-phenanthrene was used to obtain the ARRF for all substituted phenanthrenes/anthracenes. The ARRF (Table V.5) was used in calculating the extract concentrations. The concentrations (mass per volume) of substituted PACs in each sample extract was determined using Equation 6.2 where the area of the internal standard used is the mass labelled PAH.

6.3.4.2.5 Method 5

For this method, the ARRF obtained for native unsubstituted PAHs (Table V.3) was used to quantify the substituted PAC isomers. Similar to *Methods 3 and 4*, the concentration of substituted PAHs was then determined using Equation 6.2 and Equation 6.3. In Equation 6.2, ARRF of native PAH is used instead of the ARRF of substituted PACs.

6.4 Results and Discussion

Although the primary focus of our study is to examine different quantitative approaches to substituted PACs, we felt it noteworthy to begin our discussions by looking at the quality of the data obtained for PAHs in the 3 SRMs. Table V.6 and Figure V.1 shows the results obtained for the 16 US-EPA PAHs [arithmetic mean ± standard deviation (SD)] in the 3 SRM materials. For comparison, the certified/reference values (arithmetic mean ± SD) are also presented along with calculated % bias. Values are either designated as a reference or certified mass fraction values reported on a dry-mass basis. The certified mass fraction is a weighted mean of mass fraction from

two or more analytical methods while the reference values are based on unweighted means of the result from two or more analytical methods. Our ISO accredited laboratory has set a systematic error (*i.e.*, bias) threshold of ± 30% as being acceptable, this matches the criterion used for acceptable limits for NIST interlaboratory analytical comparison studies.[22]

With a few exceptions, our measured PAH values are in close agreement to the stated certified values in all 3 SRMs. For SRM 1944, there was a general negative bias in the data with only 5 PAHs showing positive biases. Measured values for two PAHs were outside the desired range with dibenz(*a,h*)anthracene showing the largest bias of +86%. Measured PAH values in SRM 1597a were all within ± 30% of the certified values. Similar to SRM 1944, we also observed a high systematic error for dibenz(*a,h*)anthracene (> +200%) in SRM 2779. The bias observed for dibenz(*a,h*)anthracene in SRM 1597a was small (~ +17%), but greater than 80% in SRMs 1944 and 2779, which strongly suggests there is an interference in these two SRMs that were augmenting the signal of dibenz(a,h)anthracene in our MRM ion channel. There were four other compounds measured in SRM 2779 in which the observed bias was greater than 30% (see Table V.6, Figure V.1).

The next sections will discuss the quality of the data obtained for PACs using the 5 quantitative approaches described in the Experimental Section.

6.4.1 Method 1

With a few exceptions, our measured PAC concentrations for individual isomers are in very good agreement with the reference/certified values listed in the 3 SRMs (*see* Table V.7, Figure 6.1 and Figure 6.2). For SRM 1944 in which there are eleven reported reference concentrations, the range in the bias for our measured values was +18% to -38%. SRM 1597a has reported values for

sixteen individual substituted PACs of which twelve are listed as reference values. For the 4 certified values, only 9- and 4-methyl-phenanthrene had a bias that was outside of an acceptable range of ± 30%. Five of the twelve substituted PACs measured in SRM 1597a with reported reference values fell outside this acceptable range. In addition to reported values of ten individual substituted PACs, SRM 2779, also lists reference values for 22 groups of substituted PACs. All the measured values for the 10 individually substituted PACs were within the acceptable range of ± 30%.

Conversely, there was a large spread in the bias for the measured concentrations for the PAC groups with a range of -78% (C_4-benzanthracenes/chrysenes/triphenylenes) to > 259% (C_2-benzanthracenes/chrysenes/triphenylenes) and only 3 PAC groups (C_4-dibenzothiophenes, C_3-benzanthracenes/chrysenes/triphenylenes and C_4-naphthalenes) had measured values that were within the acceptable range of ± 30% (*see* Figure 6.2). Six PAC clusters/groups, C_3-naphthalenes, C_1- and C_2- benzanthracenes/chrysenes/triphenylenes, C_2- and C_3- dibenzothiophenes and C_1-fluoranthenes/pyrenes had biases that were over 100%.

6.4.2 Method 2

This approach resulted in measured values that were in strong agreement with the 11 reference values for individual substituted compounds in SRM 1944 with a range in bias of -29% to +0.6% (Figure 6.1 and Figure 6.2, Table V.8). For SRM 1597a, there are 16 values reported on individually substituted PACs, 12 of which are reference values. The range in bias of our measured values to the reported values is -17% to 88%. Four compounds, 2-methylnaphthalene, 9/4-methylphenanthrene, 2,6-dimethylnaphthalene and dibenzothiophene all had measured values that were greater than 30% of the reported values. All measured values for individually substituted

PACs in SRM 2779 were within ± 30% of the reported certified or reference values. That none of the measured values for 2-methylnaphthalene, 9/4-methylphenanthrene, 2,6-dimethylnaphthalene and dibenzothiophene exceeded 30% in SRM 2779 strongly suggests the presence of chemical interferences in SRM 1597a that are augmenting their respective MRM ion signals.

When *Method 2* was used to quantify PAC groups/clusters the agreement between measured values and reference values was poor. In fact, for the 22 reference values reported for SRM 2779 only three PAC groups fall within the desired ± 30% range. A very high positive bias was reported for C_2-benz(*a*)anthracenes/chrysenes/triphenylenes as seen in Figure 6.2 similar to C_1-group. It should be noted that the average of peak areas obtained from C_1-chrysene *i.e.*, 5-methylchrysene and C_3-chrsyene *i.e.*, 1,3,6-trimethylchrysene was used to quantify C_2-group. We did not observe the same bias with C_4-group where a C_3-chrysene *i.e.*, 1,3,6-trimethylchrysene was used to quantify the group semi-quantitatively.

6.4.3 Method 3

This approach resulted in measured values that were in good agreement with the 11 reference values for individual substituted compounds in SRM 1944 with 9 compounds falling within the ± 30% value (Figure 6.1 and Figure 6.2, Table V.9). For SRM 1597a, only 3 measured values were greater than 30% of the reported value. Similar to *Method 2*, 9/4-methylphenanthrene, 2,6-dimethylnaphthalene and dibenzothiophene had measured values that were greater than 30% of the reported values when quantified using *Method 3*. All measured values for individually substituted PACs in SRM 2779 were within ± 30% of the reported certified or reference values. Much like *Method 2*, there was an overall poor agreement between reference values for PAC groups and measured values determined using *Method 3* with only 6 of the 22 measured values

within the desired ± 30% range (*see* Figure 6.2). In fact, much like *Method 2*, the C_1 and C_2-benzanthracenes/chrysenes/triplenylenes and C_2- and C_3- dibenzothiophenes showed biases greater than 100% when quantified using *Method 3*.

6.4.4 Method 4

When this method was used to quantify individually substituted compounds in SRM 1994, 7 compounds were within the ± 30% value (*see* Figure 6.1, Table V.10). Five compounds, 4-methylpyrene, 1-methylpyrene, 2,6-dimethylnaphthalene, 4-methyldibenzothiophene, benzo[*b*]naphtho[1,2-*d*]thiophene and benzo[*b*]naphtho[2,3-*d*]thiophene, all had measured values that were outside the acceptable range of ± 30% of the reported values in the SRM 1597a material. Similar to *Methods 1–3*, there was overall poor agreement between reference values for PAC groups and measured values determined using *Method 4* with only 5 of the 22 measured values within the desired ± 30% range (*see* Figure 6.2).

6.4.5 Method 5

The ARRF of the 16 US-EPA native PAHs in all the 3 SRMs evaluated in this study is as shown in Table V.3. Reported and measured concentrations of PACs on a weight/weight basis using *Method 5* are presented in Table V.11. For SRM 1944 and 2779, none of the 11 individually substituted PACs measured using *Method 5* fell within ± 30% (*see* Figure 6.1). Similarly, there was also a high bias on the individually substituted compounds measured in SRM 1597a and only 2 compounds (1- and 2-methylnaphthalene) had biases that were within ± 30%. *Method 5* was also extremely poor at measuring concentrations of individual substituted PACs in SRM 2779 and only 3 PAC groups/clusters (C_4-naphthalenes, C_1-dibenzothiophenes and C_3-dibenzothiophenes) had

measured values that were within ± 30% (*see* Figure 6.2). Surprisingly, only the C_3-napthalene cluster/group showed a bias of greater than 100%.

6.4.6 Comparison of Different Approaches to Quantify PACs

It is instructive to compare the 5 quantitative approaches and to provide insights into the quality of data. It is worth reiterating that SRM 1944 was ASE extracted and further cleaned up using adsorption chromatography, while SRM 2779 required only adsorption chromatography and SRM 1597a was simply diluted in solvent prior to analyses.

We investigated the calculated average absolute bias of the 5 methods for the 11 substituted PACs in SRM 1944. There was no statistical difference (Student *t*-test, $p < 0.05$) between the average absolute bias of *Methods 1 and 2* (13.7 ± 11.5% and 11.6 ± 8.8%, respectively) and the average absolute bias for both methods were significantly smaller than all the other methods. While the average absolute bias for *Methods 3 and 4* were significantly greater than *Methods 1 and 2* ($p < 0.05$) the difference between the two methods (*i.e.*, 3 and 4) was not statistically dissimilar ($p > 0.05$). With an average absolute bias of 73.6 ± 38.9%, *Method 5* was significantly greater ($p < 0.05$) than the other 4 methods. Clearly, quantitation based on *Methods 1 and 2* results in analytical data of the highest quality for the 11 PACs in SRM 1944. What is unique to *Methods 1 and 2*, relative to the other methods, is the use of an IS to explicitly correct for analyte losses during sample processing. *Methods 3 and 5* do not use an IS to account for recovery, and *Method 4* uses a mass labeled PAH to perform the dual role of an IPIS and a RIS. Where considerable sample processing is required, we recommend that an IS be added to samples prior to handling to account for losses during workup.

In general, the quality of the analytical data, based on our calculated average absolute biases

for substituted PACs in SRM 1597a and 2779 using the 5 methods showed similar trends. *Methods 1, 2 and 3* all showed statistically similar average absolute biases in both SRMs, while *Method 4* had in average absolute biases slightly greater than *Methods 1, 2 and 3* and *Method 5* always resulted in data of the poorest quality. With little sample processing required for both SRM 1597a and 2779, it is not surprising that method 3, which does not make use of an IS to account for analyte losses during workup, resulted in average absolute biases that were similar to *Methods 1 and 2*. Similar to our observations with SRM 1944, *Method 5*, which relies on unsubstituted PAHs to quantify its substituted PAC analog, had average absolute biases significantly greater ($p < 0.05$) than all the other methods. This strongly suggests that the response factors of unsubstituted PAHs are dissimilar to their substituted analogs and that quantitation based on this approach will lead to data that is unreliable.

Regardless of the method used, the quality of the data for cluster/group PACs in SRM 2779 were in poor agreement with the reference values. The average absolute biases were greater than 60% in all cases and there were no statistical differences in the average absolute biases amongst the quantitative methods ($p < 0.05$). Overwhelmingly, quantitation of the C_1 and C_2-benzanthracenes/chrysenes/triplenylenes, and C_2- and C_3- dibenzothiophenes consistently had the greatest systematic errors irrespective of the quantitative method. It is clear that the use of an individually substituted PAC to quantify a cluster/group of PACs in which there can be hundreds of constitutional isomers generates data that does not meet the minimum quality performance objectives. Differences in the ion fragmentation behavior of substituted PACs is likely the major driver in the large observed systematic errors.[24] The commercial availability of more individually substituted PACs should lead to improvements in data quality. As more isomers become available, we can expect less bias in the average response factors between standards and samples ultimately

leading to more accurate measurements

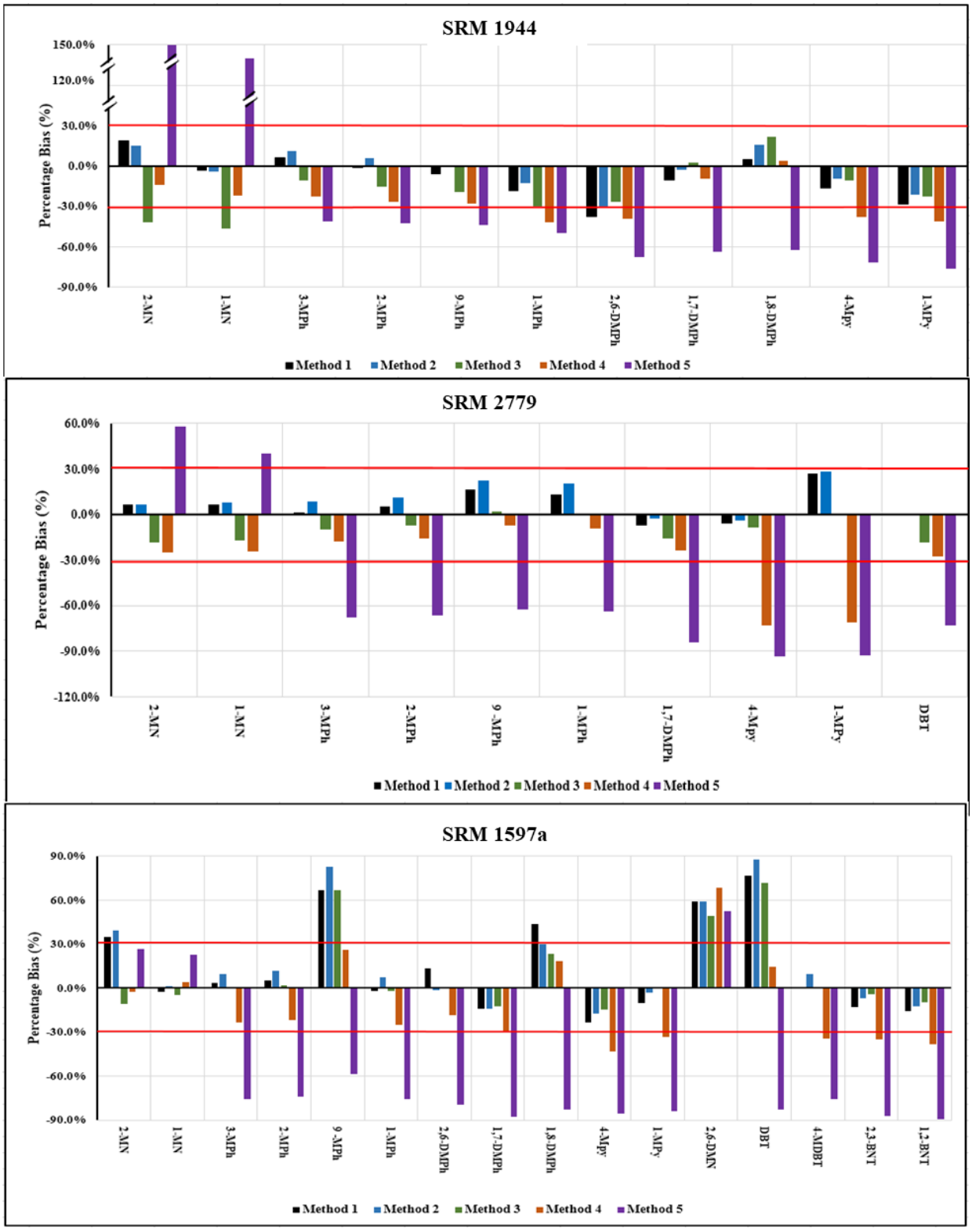

Figure 6.1: *Comparison of the percent bias of a suite of individually substituted PACs in SRM 1944 (top panel), SRM 2779 (middle panel) and SRM 1597a (bottom panel) using the five quantitative approaches described in our text.*

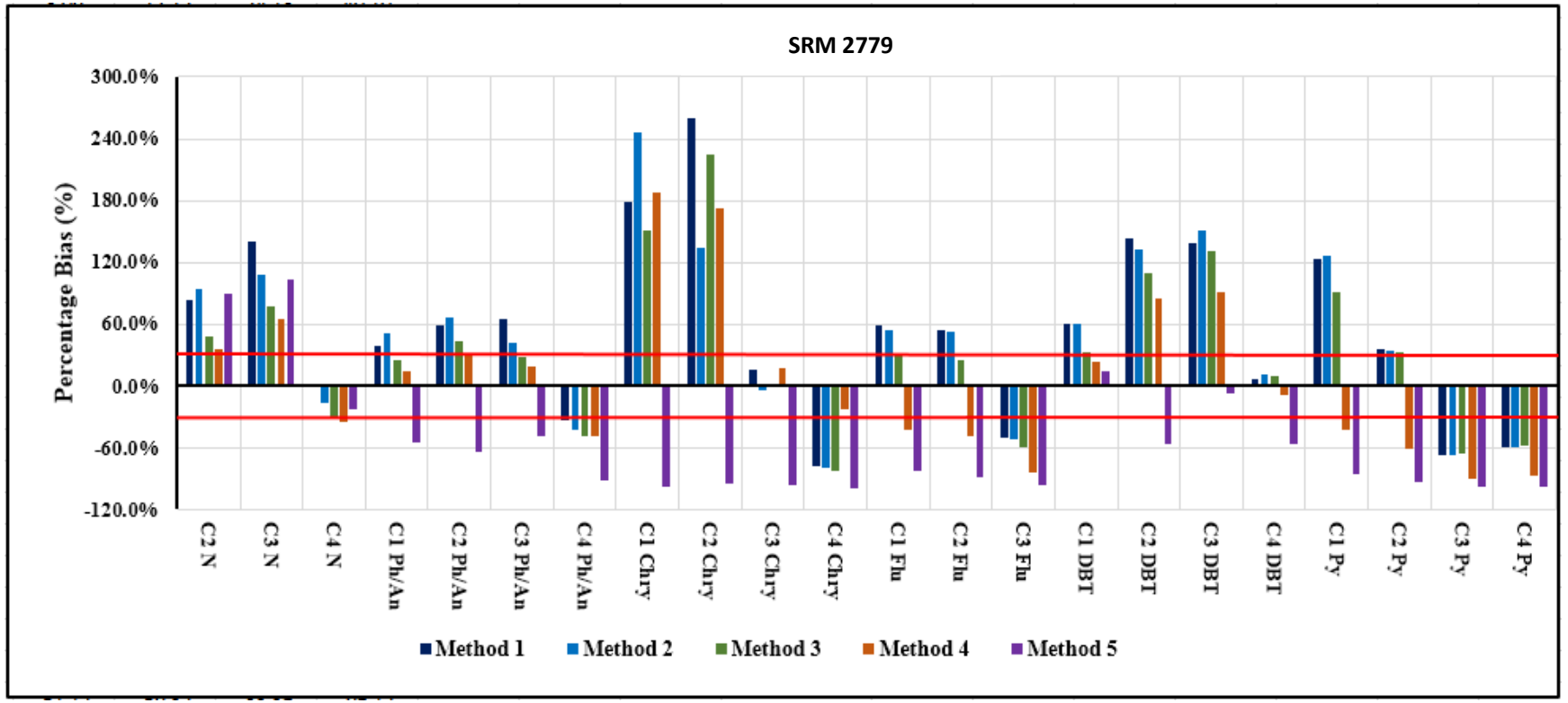

Figure 6.2: *Comparison of the percent bias of a suite of group substituted PACs in SRM 2779 using the five quantitative approaches described in our text.*

6.5 Conclusions

The overarching goal of our study was to examine the quality of analytical data when different approaches are used to quantify PACs in environmental media. Five different quantitative approaches were tested with mixed results in terms of variability amongst the methods. Perhaps not surprising, there was poor agreement among the data for quantitation of PAC clusters compared to using the methods to quantify individual PACs. In fact, using parent PAHs to quantify their substituted PAH analogs resulted in data of the poorest quality. Naturally, bias in measurement data will lead to systemic errors in source delineation and environmental forensic studies. With the advent of more commercially available substituted PACs it is likely a good time for laboratories coordinating interlaboratory exercises to revisit certifying more of these compounds in SRMs and, if feasible, to make recommendations on a standardized approach to quantify PACs in environmental media.

6.6 Acknowledgements

This work was supported by a Natural Sciences and Engineering Research Council – Discovery Grant to GTT (Grant #: RGPIN-05354-2019).

6.7 References

(1) Abdel-Shafy, H. I.; Mansour, M. S. M. A review on polycyclic aromatic hydrocarbons: Source, environmental impact, effect on human health and remediation. *Egyptian Journal of Petroleum* **2016**, *25* (1), 107-123. DOI: 10.1016/j.ejpe.2015.03.011

(2) Neff, J. M.; Stout, S. A.; Gunster, D. G. Ecological risk assessment of polycyclic aromatic hydrocarbons in sediments: Identifying sources and ecological hazard. *Integr Environ Assess Manag* **2005**, *1* (1), 22-33. DOI: 10.1897/ieam_2004a-016.1

(3) Hodson, P. V.; Wallace, S. J.; de Solla, S. R.; Head, S. J.; Hepditch, S. L. J.; Parrott, J. L.; Thomas, P. J.; Berthiaume, A.; Langlois, V. S. Polycyclic aromatic compounds (PACs) in the Canadian environment: The challenges of ecological risk assessments. *Environ Pollut* **2020**, *266* (Pt 2), 115165. DOI: 10.1016/j.envpol.2020.115165

(4) Tsibart, A. S.; Gennadiev, A. N. Polycyclic aromatic hydrocarbons in soils: Sources, behavior, and indication significance (a review). *Eurasian Soil Sci.* **2013**, *46* (7), 728-741, Review. DOI: 10.1134/s1064229313070090

(5) Honda, M.; Suzuki, N. Toxicities of polycyclic aromatic hydrocarbons for aquatic animals. *Int J Environ Res Public Health* **2020**, *17* (4), 1363. DOI: 10.3390/ijerph17041363

(6) Lawal, A. T. Polycyclic aromatic hydrocarbons. A review. *Cogent Environmental Science* **2017**, *3* (1), 1339841.

(7) Rengarajan, T.; Rajendran, P.; Nandakumar, N.; Lokeshkumar, B.; Rajendran, P.; Nishigaki, I. Exposure to polycyclic aromatic hydrocarbons with special focus on cancer. *Asian Pacific Journal of Tropical Biomedicine* **2015**, *5* (3), 182-189.

(8) Manzetti, S. Polycyclic aromatic hydrocarbons in the environment: Environmental fate and transformation. *Polycyclic Aromatic Compounds* **2013**, *33* (4), 311-330.

(9) Tobiszewski, M.; Namiesnik, J. Pah diagnostic ratios for the identification of pollution emission sources. *Environ Pollut* **2012**, *162*, 110-119. DOI: 10.1016/j.envpol.2011.10.025

(10) Ahad, J. M.; Macdonald, R.; Parrot, J.; Yang, Z.; Zhang, Y.; Siddique, T.; Kuznetsova, A.; Rauert, C.; Galarneau, E.; Studabaker, W. Polycyclic aromatic compounds (PACs) in the Canadian environment: A review of sampling techniques, strategies and instrumentation. *Environmental Pollution* **2020**, 114988.

(11) Achten, C.; Andersson, J. T. Overview of polycyclic aromatic compounds (PAC). *Polycycl Aromat Compd* **2015**, *35* (2-4), 177-186. DOI: 10.1080/10406638.2014.994071

(12) Andersson, J. T.; Achten, C. Time to say goodbye to the 16 epa PAHs? Toward an up-to-date use of PACs for environmental purposes. *Polycycl Aromat Compd* **2015**, *35* (2-4), 330-354. DOI: 10.1080/10406638.2014.991042

(13) Idowu, O.; Semple, K. T.; Ramadass, K.; O'Connor, W.; Hansbro, P.; Thavamani, P. Beyond the obvious: Environmental health implications of polar polycyclic aromatic hydrocarbons. *Environ Int* **2019**, *123*, 543-557, Review. DOI: 10.1016/j.envint.2018.12.051

(14) Johnson, W.; Idowu, I.; Francisco, O.; Marvin, C.; Thomas, P. J.; Stetefeld, J.; Tomy, G. T. Enumeration of the constitutional isomers of environmentally relevant substituted polycyclic aromatic compounds. *Chemosphere* **2018**, *202*, 9-16. DOI: 10.1016/j.chemosphere.2018.03.035

(15) Long, A. S.; Lemieux, C. L.; Gagne, R.; Lambert, I. B.; White, P. A. Genetic toxicity of complex mixtures of polycyclic aromatic hydrocarbons: Evaluating dose-additivity in a

transgenic mouse model. *Environ Sci Technol* **2017**, *51* (14), 8138-8148. DOI: 10.1021/acs.est.7b00985

(16) Wilton, N. M.; Wise, S. A.; Robbat, A., Jr. Errors in alkylated polycyclic aromatic hydrocarbon and sulfur heterocycle concentrations caused by currently employed standardized methods. *Anal Chim Acta* **2017**, *977*, 20-27, Article. DOI: 10.1016/j.aca.2017.04.017

(17) Antle, P. M.; Zeigler, C. D.; Wilton, N. M.; Robbat, J. A. A more accurate analysis of alkylated PAH and PASH and its implications in environmental forensics. *International Journal of Environmental Analytical Chemistry* **2013**, *94* (4), 332-347. DOI: 10.1080/03067319.2013.840886

(18) Zeigler, C.; Macnamara, K.; Wang, Z.; Robbat, A., Jr. Total alkylated polycyclic aromatic hydrocarbon characterization and quantitative comparison of selected ion monitoring versus full scan gas chromatography/mass spectrometry based on spectral deconvolution. *J Chromatogr A* **2008**, *1205* (1-2), 109-116. DOI: 10.1016/j.chroma.2008.07.086

(19) Zeigler, C.; Wilton, N.; Robbat, A., Jr. Toward the accurate analysis of C1-C4 polycyclic aromatic sulfur heterocycles. *Anal Chem* **2012**, *84* (5), 2245-2252. DOI: 10.1021/ac202845x

(20) Zhao, Y.; Hong, B.; Fan, Y.; Wen, M.; Han, X. Accurate analysis of polycyclic aromatic hydrocarbons (PAHs) and alkylated PAHs homologs in crude oil for improving the gas chromatography/mass spectrometry performance. *Ecotoxicol Environ Saf* **2014**, *100*, 242-250. DOI: 10.1016/j.ecoenv.2013.10.018

(21) Yang, C.; Zhang, G.; Wang, Z.; Yang, Z.; Hollebone, B.; Landriault, M.; Shah, K.; Brown, C. E. Development of a methodology for accurate quantitation of alkylated polycyclic aromatic hydrocarbons in petroleum and oil contaminated environmental samples. *Anal. Methods* **2014**, *6* (19), 7760-7771. DOI: 10.1039/c4ay01393j

(22) Schantz, M. M.; Kucklick, J. R. *Interlaboratory analytical comparison study to support Deepwater Horizon natural resource damage assessment: Description and results for marine sediment QA10SED01, NISTIR 7793*; National Institute of Standards and Technology, Gaithersburg, MD, 2011.

(23) Idowu, I.; Francisco, O.; Thomas, P. J.; Johnson, W.; Marvin, C.; Stetefeld, J.; Tomy, G. T. Validation of a simultaneous method for determining polycyclic aromatic compounds and alkylated isomers in biota. *Rapid Commun Mass Spectrom* **2018**, *32* (3), 277-287. DOI: 10.1002/rcm.8035

(24) John, G. F.; Yin, F.; Mulabagal, V.; Hayworth, J. S.; Clement, T. P. Development and application of an analytical method using gas chromatography/triple quadrupole mass spectrometry for characterizing alkylated chrysenes in crude oil samples. *Rapid Commun Mass Spectrom* **2014**, *28* (8), 948-956. DOI: 10.1002/rcm.6868

(25) Sorensen, L.; Meier, S.; Mjos, S. A. Application of gas chromatography/tandem mass spectrometry to determine a wide range of petrogenic alkylated polycyclic aromatic hydrocarbons in biotic samples. *Rapid Commun Mass Spectrom* **2016**, *30* (18), 2052-2058. DOI: 10.1002/rcm.7688

(26) Idowu, I.; Johnson, W.; Francisco, O.; Obal, T.; Marvin, C.; Thomas, P. J.; Sandau, C. D.; Stetefeld, J.; Tomy, G. T. Comprehensive two-dimensional gas chromatography high-resolution mass spectrometry for the analysis of substituted and unsubstituted polycyclic aromatic compounds in environmental samples. *J Chromatogr A* **2018**, *1579*, 106-114. DOI: 10.1016/j.chroma.2018.10.030

Chapter 7: Alkylation of Benz[*a*]anthracene Affects Toxicity to Early–Life Stage Zebrafish and *In Vitro* Aryl Hydrocarbon Receptor 2 Transactivation in a Position-Dependent Manner

A version of this chapter has already been published as Justin Dubiel, Derek Green, Yamin Raza, Hunter M Johnson, Zhe Xia, Gregg T Tomy, Alice Hontela, Jon A Doering, Steve Wiseman. Alkylation of Benz[*a*]anthracene Affects Toxicity to Early‐Life Stage Zebrafish and *In Vitro* Aryl Hydrocarbon Receptor 2 Transactivation in a Position‐Dependent Manner. *Environmental Toxicology and Chemistry*, **2022**, *41*, 1993-2002.

7.1 Abstract

Polycyclic aromatic hydrocarbons (PAHs) are complex organic chemicals with different chemical structure, which can affect fish health and result in adverse effects by activating the aryl hydrocarbon receptor 2 (AhR2). Apart from the environmental ubiquity of PAHs, certain environment matrices can contain more alkyl PAHs. However, there is limited understanding of how alkylation affects *in vivo* fish toxicity of PAHs and the relationship between alkylation and *in vitro* activation of AhR2. Thus, this study is aimed at determinating the toxicity of benz[*a*]anthracene and three alkylated homologs with different alkylation positions to zebrafish (*Danio rerio*) at early life stages and assessment of potency for these four analytes activating zebrafish AhR2 by a standardized *in vitro* AhR transactivation assay. There was a dose-dependent correlation between the exposure of embryos to each of the PAHs and their adverse health effects, such as mortality and malformations characteristic of AhR2 activation. The *in vivo* toxicities and *in vitro* AhR2 activation potencies for alkyl-benz[*a*]anthracenes are dependent on alkylation position and different from parent benz[*a*]anthracene. Nevertheless, there is a lack of statistically significant linear connection between the responses in these assays. Considering the results, further exploration of alkylation-resulted fish toxicity of PAHs and the importance of alkylated PAHs in ecological risk assessments is warranted.

7.2 Introduction

The chemical structure of polycyclic aromatic hydrocarbons (PAHs), a class of environmentally ubiquitous organic compounds, contains two or more fused aromatic rings.[1] PAHs can be emitted to the environment by natural or human activities, which includes oil spills, petroleum refining, and incomplete combustion of organic materials, for instance fossil fuels and wood.[1] Recently, human activities have contributed to the increase of PAH emissions, which has been detected with the highest concentrations vicinal to populated urban and industrial areas.[2,3] These hydrophobic PAHs can be persistent and accumulated under specific conditions, which are detected from sediments.[4] In early development stage, fish embryos can possibly be exposed to PAHs as it is commonly buried and deposited on or next to the sediment surface containing PAHs, which can result in many adverse biological effects.[5,6]

Some four-to-six-ring PAHs can bind to and dysregulate signaling of the aryl hydrocarbon receptor (AhR) with, which can be toxic to fish and some vertebrates.[5] The AhR can be activated by ligand and act as a transcription factor, which is involved in the regulation of thousands of genes across various physiological pathways.[7,8] In fishes, there are a number of isoforms of the AhR, while a dose-dependent early-life stage mortality and developmental malformations in fish embryos is connected to the dysregulation of the AhR2 isoform, including pericardial and yolk sac edemas, spinal and cranial deformities, cardiovascular deformities, anemia, and reductions in growth.[6,9-11] Besides, PAHs can also harm the organism without AhR activation.[12,13] Therefore, PAHs are been ranked as the ninth most hazardous substance group by the US Agency for Toxic Substances and Disease Registry (ATSDR), and 16 PAHs are listed as "priority pollutants" by US Environmental Protection Agency (USEPA).[14]

To date, 16 USEPA priority PAHs, representing unsubstituted parent compounds, have been

the focus of the majority of studies. Nonetheless, compared to unsubstituted parent compounds, alkyl PAHs are more enriched in the environment affected by fossil fuel development and extraction. For instance, USEPA priority PAHs account on average less than 15% of total PAHs in coal.[15] Likewise, total PAHs in oil contains about 5% – 15% priority PAHs and 85% – 95% alkyl PAHs.[16] In deposits of bitumen from Athabasca oil sands region, Alberta, Canada, the major form of PAHs are alkyl PAHs, where increasing amount and concentration of alkyl PAHs have been observed in sediment and tributaries of the lower Athabasca River because of bitumen mining.[17,18] In the Norwegian Arctic, there is a 10- to 30-fold increase in PAH concentrations with major composition of alkyl PAHs.[19] Like PAHs, alkyl PAHs can be toxic to fish.[20,21] Thus, alkyl PAHs can possibly pose ecological risks to fishes due to potential toxicity and environmental abundance.

Alkyl PAHs, like four-to-six-ring PAHs, show higher toxicity, which is depended on positioning of alkylation, than parent compounds by binding to the AhR in *in vitro* cell-based AhR activation assays.[22] Alkylated chrysene homologs transactivate AhR2 of Atlantic cod (*Gadus morhua*) in a standard i*n vitro* AhR transactivation assay while the parent chrysene, a US-EPA priority PAH, does not activate the AhR2.[23] In zebrafish (*Danio rerio*) and Japanese medaka (*Oryzias latipes*), developmental toxicity of benzo[*a*]pyrene and benz[*a*]anthracene (BAA) depends on the position of the alkyl group.[20,21] As shown by these studies, although the AhR can be *in vitro* activated by alkylated PAHs with higher potencies comparing with parent PAHs and resulting in affecting developmental toxicity, little is known about the relationship between early-life stage toxicities, activation of the AhR, and alkyl positioning among PAHs. This study is aimed at better understanding of this relationship as a preliminary step. Particularly, the early-life stage mortality and sensitivity to activation of AhR2 for a US-EPA priority four-ring PAH, BAA, and three alkylated BAA with different alkylation positions is investigated. BAA and alkyl BAA have

been detected from environmental matrices related to fossil fuel development or combustion.[19,24] Zebrafish embryos, a common laboratory model species, were exposed to BAA, 4-methylbenz[*a*]anthracene (4-MBAA), 8-methylbenz[*a*]anthracene (8-MBAA), or 7,12-dimethylbenz[*a*]anthracene (7,12-DMBAA), in a standard in vitro AhR transactivation assay, with the potency of each PAH determined by activation of the AhR2.[23,25,26]

7.3 Material and Methods

7.3.1 Chemicals and Reagents

Analytical-grade BAA, 4-MBAA, 8-MBAA, 7,12-DMBAA were procured from Millipore-Sigma (St Louis, MO, USA). For each chemical, there were four dosing solutions prepared in full-strength dimethylsulfoxide (DMSO; > 99.9% pure), started from maximum solubility. The nominal concentrations of solution are shown in Table 7.1, which is obtained by three-fold serial dilution and ranged from 0.15 – 24 mg/mL. Optima grade organic solvents silica gel, sodium sulfate, and alumina were acquired from Fisher Scientific (Ottawa, ON, CA) and used in quantifying PAHs. The details of analytical standards have been listed previously.[27]

7.3.2 Embryo Experimentation

7.3.3 Collection of Embryos

Zebrafish embryos (Tupfel long fin strain) were acquired from a breeding culture in the Aquatic Research Facility in the Alberta Water and Environmental Science Building at the University of Lethbridge, Canada. Vertical flow-through racks (Tecniplast) were used for housing the breeding colony with the supply of dechlorinated City of Lethbridge municipal water. The

light:dark photoperiod was kept as 16:8-h. *Artemia* (Brine Shrimp Direct) was used to feed zebrafish twice per day. One hour after the onset of light, clutches of embryos from individual breeding fish groups were collected for the use in microinjections. All experimental protocols were in accordance with the University of Lethbridge Animal Welfare Protocol 1714.

7.3.3.1 Microinjection Experimental Design

Previously, waterborne exposure was widely employed in studies of the toxicity of PAHs to early life stages of fishes.[6,9,11,28] However, microinjection was chosen to be employed in this study. The chorion is bypassed by the microinjection, which allows entire dose to be administered at once. Consequently, embryos can be frozen immediately for the quantification of chemicals, and thus the adsorption, distribution, metabolism, and excretion (ADME) of PAHs can be minimized. However, since the ADME processes can be active throughout the exposure period, analysis of embryos from waterborne exposure would only snapshot the dose of PAHs. An IM-400 Electric Microinjector (Narishige Group) was employed for microinjection, which was calibrated to deliver the desired solution volume beforehand for each treatment by established methods.[29] Room temperature of approximately 25 °C was maintained for microinjection. Prior to completion of gastrulation (6 h postfertilization [hpf]), *ca.* 1.5 nL of solution was administrated directly into the yolk. Each treatment group is comprised of 50 injected embryos in triplicates. Embryos without microinjection were used as the negative control treatment. Embryos injected with full-strength DMSO were used as process control treatment. Both control treatments and the four PAH treatment groups were included in experimental replicate, for which embryos were collected from independent breeding events on different days. In addition, each treatment had 200 mg embryos (about 400 embryos) and was frozen immediately at −80 °C for PAHs quantification to determine

exposure dose.

7.3.3.2 Embryo Rearing and Assessment

Plastic Petri dishes containing dechlorinated City of Lethbridge municipal tap water at 25 ± 1 °C was used for the embryo incubation for a duration of 24h. Dead or nonviable embryos were discarded after 24h, and individual embryos were transferred to independent wells of a 24-well plate (Eppendorf Canada) containing 2 mL of dechlorinated City of Lethbridge municipal water, which was maintained at 25 ± 1 °C until 15 days postfertilization (dpf). As determined by a preliminary method development study, study duration was set to 15 days for complete utilization of the yolk sac and exposure to the full dose (data not shown). Embryos were evaluated every day during the experiment using a Motic K Series stereomicroscope (Motic Instruments) or a Zeiss Discovery.V12 stereo microscope (Carl Zeiss) for quantifying the occurrences of pericardial edema, yolk sac edema, spinal deformity, or mortality, in accordance with the guidelines of the Organisation for Economic Cooperation and Development.[30] It was recorded for the percentage of embryos exhibiting one or more malformations. Images were captured using a Zeiss Axiocam 105 Colour camera (Carl Zeiss) and ZEN lite imaging software (Carl Zeiss). Starting at 5 dpf, 50% water renewal was carried out daily when all embryos had hatched.

7.3.3.3 Real-time Polymerase Chain Reaction

To assess the *in vivo* activation of the AhR in early life stages of zebrafish, abundance of transcripts of cytochrome P450 1A (*cyp1a*) was quantified, which is a common biomarker for AhR activation. As described previously in section 7.3.3.1 (Microinjection Experimental Design), DMSO or each PAH at the median lethal dose (LD_{50}) was injected to embryos, which is reared until 5 dpf at 25 ± 1 °C. Total RNA was extracted from 10-15 larvae per replicate using TRIzol™

reagent (ThermoFisher Scientific), and RNA concentrations were determined using a NanoDrop One spectrophotometer (ThermoFisher Scientific). By using a QuantiNova™ reverse-transcription kit, complementary DNA (cDNA) was synthesized from 1–2 µg of total RNA, which includes a step for genomic DNA removal (Qiagen). A 35 µL reaction mixture was prepared for each cDNA and primer combination, including 3.5 µL of cDNA, 1.75 µL of primer pair (10 pM final concentration), 17.5 µL of SensiFAST™ SYBR® No-ROX Kit (Meridian Bioscience), and 12.25 µL of nuclease-free water for the quantitative polymerase chain reaction (qPCR). The qPCR protocol included initial denaturation at 95 °C for 2 min, followed by 40 cycles of denaturation at 95 °C for 5 s, and annealing at 60 °C for 10 s. A melt curve analysis was performed to ensure the amplification of a single PCR product, and a no-template control was included to verify the absence of contamination. All qPCRs were carried out in triplicate using 10-µL volumes in 96-well plates in a CFX96 Touch Real-Time PCR Detection System (Bio-Rad). The primer sequences used were as follows: for *cyp1a*, F: GCATTACGATACGTTCGATAAGGAC, R: GCTCCGAATAGGTCATTGACGAT; for *18s*, F: CCACTCCCGAGATCCAACTA, R: CAAATTACCCATTCCCGACA. The transcript abundance of *18s* was used to normalize the transcript abundance of *cyp1a*. Changes in *cyp1a* transcript expression in embryos exposed to PAHs were determined relative to the DMSO control using the efficiency-corrected method developed by Pfaffl.[31] Efficiencies of the *cyp1a* and *18s* reactions were determined to be 102% and 95%, respectively.[32,33]

7.3.4 In Vitro AhR Transactivation Assay

As described in previously methods[34] with aforementioned modifications,[35] maintenance of COS-7 cells, transfection of constructs, and the luciferase reporter gene assay were performed in 96-well plates. In brief, COS-7 cells were transfected with 8 ng of zebrafish AhR2,[36] 1.55 ng of white sturgeon AhR nuclear translocator 2,[35] 20 ng of the rat CYP1A reporter construct,[37,38] and 0.75 ng of the Renilla luciferase vector (Promega) per well. Ten (10) serial concentrations of BAA, 4-MBAA, 8-MBAA, or 7,12-DMBAA, with nominal concentrations 0.00003 – 100 nM, were exposed to transfected cells. Full-strength DMSO from the same stock as described previously (*see* section 7.3.1) was used to prepare dosing solutions. SpectraMax i3x plate reader (Molecular Devices) was used to measure luciferase. Triplicated measurements were performed for each chemical, with four separate wells per replicate. All procedures were conducted in the SynBridge core facility at the University of Lethbridge.

7.3.5 Quantification of PAHs

Extraction and analysis were conducted at the Centre for Oil and Gas Research and Development, University of Manitoba, an accredited laboratory under the International Organization for Standardization (ISO-17025:2017). Fifteen (15) mass-labeled PAH recovery internal standards (100 ng, 16 USEPA PAHs except anthracene) were added to 200 mg of embryos in a 2 mL vial prior to extraction. Then, spiked embryos were transferred to 2 mL Precellys reinforced tube with 2 × 1.5 mL dichloromethane rinse, in which three 1.4 mm zirconium oxide beads were added. A Precellys Evolution Homogenizer (Bertin Technologies) was used for extraction at 6500 rpm for 6 cycles, with intervals of 20 and 10 s between cycles. After extraction, the supernatant from each extract was transferred with 2 × 2 mL dichloromethane into a 30 mL

test tube containing silica gel (2 g), 0.5 g sodium sulfate, and 5% deactivated alumina (0.5 g) for dispersive solid-phase extraction cleanup.[39] Then, 3 mL hexane was aliquoted to the test tube, which is settled for 30 min with occasional vortex at every 10 min. Final volume of 1 mL for extract was obtained by transferring cleaned-up extract to a prerinsed tube using 5 mL of dichloromethane:hexane (70:30) and reduced under a gentle stream of ultra-high-purity nitrogen. Instrument performance internal standard, d_{10}-anthracene (100 ng), was spiked to the extraction prior to injection of gas chromatography/tandem mass spectrometer (GC/MS/MS). After vortexing, samples were stored in 2 mL amber glass autosampler vials at 4 °C. The same extraction protocol of embryo was applied to laboratory blanks ($n = 4$). The detailed method of GC/MS/MS detection and quantification has been previously described.[27]

Contamination and performance characteristics such as detection limits, accuracy/recovery, and precision were evaluated by a method validation.[40] Target analytes ($n = 9$, 100 pg μL^{-1}) were spiked to 1.5 mL dichloromethane, which was extracted as described above. Analysis of procedural blanks ($n = 3$) suggests that there is no contamination of target analytes. Method performance characteristics are given in Table VI.1.

7.3.6 Data Analysis

A four-parameter logistic model was adopted for response curves by GraphPad Prism 9 software. Lethal doses causing 20% mortality and LD_{50} were determined for early life stages of zebrafish. Besides, concentrations causing effects of 20% (EC_{20}), 50% (EC_{50}), and 80% (EC_{80}) in COS-7 cells transfected with zebrafish AhR2 were calculated. Because the lowest treatment concentration was statistically significant ($p \leq 0.05$) from the DMSO control treatment, it was defined as the effect concentration threshold for activation of zebrafish AhR2. One-way analysis

of variance followed by Dunnett's post hoc test was employed to determine the significant differences between treatments and controls ($p \leq 0.05$) by IBM SPSS Statistics 20 software. The Shapiro-Wilk test was used to determine normality, Levene's test was used to test homogeneity of variance of each data set, and logarithmic transformation was employed if necessary. Relative potencies (RePs) for *in vivo* data were computed based on nanomoles per gram of egg by Equation 7.1.

$$ReP = \frac{\mathrm{LD_{50}\ Compound\ of\ Interest}}{\mathrm{LD_{50}\ Reference\ Compound}} \qquad \textit{Equation 7.1}$$

In Equation 7.1, the compounds being compared to the reference compound (*i.e.*, BAA or 2,3,7,8-tetrachlorodibenzo-p-dioxin [TCDD]) was defined as compound of interest. Equation 7.2 was used to determine the relative potencies for *in vitro* data from this study, where EC_{XX} represents the average of EC_{20}, EC_{50}, and EC_{80} values.

$$ReP = \frac{\mathrm{EC_{xx}\ Compound\ of\ Interest}}{\mathrm{EC_{xx}\ Reference\ Compound}} \qquad \textit{Equation 7.2}$$

7.4 Results and Discussion

Zebrafish embryos were exposed to BAA and alkylated BAA in serial doses to assess mortality and occurrences of AhR-mediated malformations, as an initial step for unraveling the relationship between alkylation and AhR-mediated toxicity of PAHs to early life stages of fishes. A dose-dependent relationship for mortality had been discovered for embryos exposed to BAA, the parent compound (Figure 7.1A and Table 7.1), with an LD_{50} of 26,129 ng/g-egg (Table 7.1). Previously, zebrafish were determined to be one of the least sensitive fish species to the prototypical AhR

agonist, TCDD, with an LD50 of 2.61 ng/g-egg.[10,41] Like BAA, similar dose-dependent relationship in mortality between embryos to three alkylated homologs, 4-MBAA, 8-MBAA, and 7,12-DMBAA, had been observed (Figure 7.1B–D and Table 7.1), while 8-MBAA was the most potent and 4-MBAA was the least potent (Table 7.2). Compared to BAA, the relative potencies of 4-MBAA, 7,12-DMBAA, and 8-MBAA were 1.1, 1.4, and 5.6, respectively (Table 7.3). The potency factor for various PAHs, including BAA, 8-MBAA, and 7,12-DMBAA, was determined based on ethoxyresorufin-O-deethylase (EROD) activity or AhR binding affinity in various vertebrate assay systems in previous work.[22] Relative to BAA, the relative potencies for 8-MBAA and 7,12-DMBAA were 8.0 and 2.0, respectively (Table 7.3). Compared to the *in vivo* relative potencies determined in the current study for early–life stage mortality in zebrafish, both of the relative potencies are within a two-fold range (Table 7.3). Thus, the results of the current study suggest that alkylation of BAA either increased or had no effect on potency in inducing early-life stage mortality in zebrafish, which agrees with results from previous *in vitro* assays.

Along with early-life stage mortality, the percentage of embryos displaying one or more malformations were raised, such as yolk sac edema, pericardial edema, and spinal curvatures, when exposed to both BAA and the three alkylated homologs (Table 7.1). Representative images of malformations can be found in Figure VI.1. These observed malformations agree with the effects commonly related to the activation of the AhR in vertebrates, including fishes.[10,42,43] The elevation in CYP1A is a prototypical hallmark for activation of the AhR.[44] The increase of *cyp1a* transcript and protein abundance as well as EROD activity across various fish and bird species results from the binding of four-to-six-ring PAHs, including BAA, to AhR.[8,45-49] In avian species, sensitivity to early–life stage mortality among species can be predicted by response of *cyp1a* in hepatocytes exposed to PAHs.[50] The present study with zebrafish has shown a similar effect that exposure to

both BAA and each alkylated homolog led to an increase in *cyp1a* transcript abundance (Figure 7.2). Embryo mortality can result from exposure to BAA and the three alkylated homologs likely due to, at least in part, AhR-mediated processes, which is supported by occurrence of characteristic AhR-mediated malformations and the greater abundance of *cyp1a* messenger RNA in early–life stage zebrafish.

The sensitivity of different fish species to early–life stage mortality resulting from exposure to dioxin-like compounds (DLCs), another class of agonists of the AhR, can vary more than 200-fold.[41,51] These differences in interspecies sensitivity can be attributed to the variations in the AhR2 protein structure, which alters its sensitivity to DLC activation.[25,52-54] A predictive mechanism-based biological model, known as a quantitative adverse outcome pathway (qAOP), is generated based on this understanding of interspecies sensitivity, which is able to predict dose-response curves for DLC-induced early-life stage mortality in any fish species by utilizing data from a standardized *in vitro* AhR transactivation assay involving COS-7 cells transfected with the relevant AhR isoform from the species of interest.[52,55] While some variation in sensitivity from different bird species has been demonstrated,[47,50] the extent of such variation among fish species is not well investigated, and it remains unknown if AhR2 activation sensitivity can be used to predict early-life stage mortality for fishes. As an initial attempt to explore this knowledge gap, this study utilized the standardized *in vitro* AhR transactivation assay to determine the sensitivity of zebrafish AhR2 activation by BAA and the three alkylated homologs. Similar to DLCs, BAA, 4-MBAA, 8-MBAA, and 7,12-DMBAA activated zebrafish AhR2 in a concentration-dependent manner (Table 7.3). The order of potency was 8-MBAA > 4-MBAA > BAA > 7,12-DMBAA (Table 7.4). Compared to BAA, the relative potencies for zebrafish AhR2 activation by 4-MBAA, 8-MBAA, and 7,12-DMBAA were 5.1, 18.4, and 0.6, respectively (Table 7.3). These relative sensitivities for

AhR2 activation differed by 4.6-fold, 3.3-fold, and 2.3-fold from their corresponding early-life stage mortality sensitivities and were close to previous *in vitro* assay results (Table 7.3).[22] Nonetheless, a statistically significant linear relationship between early-life stage mortality sensitivity and AhR2 activation sensitivity among the four investigated PAHs in zebrafish was not observed ($p = 0.27$, $p = 0.86$; Figure 7.4). In addition, as agonists, these PAHs were more potent than TCDD of the zebrafish AhR2 in the standardized *in vitro* AhR transactivation assay, although PAHs were significantly less potent at resulting early-life stage mortality (Table VI.2).[10,56] Therefore, these PAHs do not fit to the previously reported relationship between AhR2 activation sensitivity and early-life stage mortality sensitivity within the qAOP (Figure 7.4).[52,55]

There might be some factors that contribute to the lack of agreement in relationships between early-life mortality and *in vitro* AhR2 activation for DLCs and PAHs. Metabolism is a possible factor. Previously, DLCs have been shown to be bioaccumulate in tissues,[57] and cause persistent EROD activity.[58,59] In contrast, PAHs are not long-term accumulative or increasing in tissue concentrations,[60] likely due to the existence of robust enzyme systems for vertebrates, which convert PAHs into epoxides or hydroxylated derivatives during phase I biotransformation and excreted by cells in phase II metabolism.[61] Although the exact extent of phase I and phase II metabolism in early life stages of zebrafish remains uncertain, it is suggested that functional biotransformation pathways are present in zebrafish embryos and larvae by current evidence.[62] Previous studies have detected EROD activity as early as 2.5 hpf,[63] while the abundance of *cyp1a* transcript have elevated in larvae at 120 hpf in present study (Figure 7.2). It is suggested that biotransformation might contribute to the decrease availability of PAHs to interact with the AhR in zebrafish early-life stages over time, hence preventing the sustained AhR activation seen with TCDD and other DLCs. On the other hand, there is limited capacity for COS-7 cells transfected

with AhRs to exhibit phase I biotransformation, and thus continuous AhR activation can be induced by PAHs, suggesting that PAHs could bind to the receptor with a higher affinity comparing to TCDD.[64,65] However, despite the disparity between early-life stage mortality sensitivity and AhR2 activation sensitivity in the standardized *in vitro* AhR transactivation assay between DLCs and PAHs and the data gap of a statistically significant linear relationship in the current research, a small data set for the development of a robust linear relationship is presented by this study. Future inclusion of additional data might lead to a significant predictive relationship for PAHs between these assays different from DLCs (Figure 7.4).

The present study was aimed to better characterize the relationship between alkylation and the *in vivo* toxicity of BAA to early life stages of zebrafish, a model fish species, and to investigate the correlation between this sensitivity and the potency for activation of the AhR2 in the standardized *in vitro* AhR transactivation assay. These findings agree with previous results from a variety of cell- or embryo-based assays, suggesting that alkylation could increase the AhR-mediated toxicity of PAHs.[20-23] Only the 16 US-EPA priority PAHs were used for ecological risk assessments, which was estimated to underestimate the risk by as much as 40- to 70-fold.[66] This uncertainty in the ecological risk assessment of PAHs, along with the confirmation that alkylation can amplify the *in vivo* toxicity of PAHs to fish, suggests the pressing need to evaluate the toxicity of other parent PAHs and their alkylated homologs, as well as other patterns of alkylation and substitutions involving multicarbon side chains. However, it is not practical to conduct early-life stage toxicity tests involving numerous PAHs and alkylation patterns in both zebrafish and native species of regulatory concern due to the limited time, cost, and challenges associated with certain species, such as their rarity, conservation status, and incompatibility with laboratory cultivation. Thus, determination whether a predictive relationship exists between PAH sensitivity to AhR2

activation in the standardized *in vitro* assay and early-life stage mortality should be focused by ongoing research. However, it is important to note that this predictive relationship could only be applicable to PAHs that act as AhR agonists and not to those causing toxicity entirely independent of the AhR.[12,13] Investigation of potential models accounting for PAHs inducing AhR-independent toxicities needs to be explored by further research. Given the ample amount of alkyl PAHs in the environment and the uncertainty surrounding their toxicity compared to parent compounds, it could be essential to develop of predictive tools, for instance the qAOP, for more pragmatic assessments of these toxicities across various fish species, thus facilitating more objective ecological risk evaluations for this class of chemical.

7.5 Acknowledgment

S. Wiseman was supported by a Tier II Canada Research Chair in Aquatic and Mechanistic Toxicology, an infrastructure grant from the Canadian Foundation for Innovation (project 35224), the Government of Alberta Research Capacity Program, and a Discovery Grant from the Natural Science and Engineering Research Council of Canada (NSERC; RGPIN-2017-04474). J. Dubiel was supported by an Alberta Conservation Authority Grant in Biodiversity (030-00-90-140/990). D. Green was supported by an NSERC Postgraduate Scholarship (PGSD3-504735-2017). Y. Raza was supported by an NSERC Undergraduate Student Research Award. G. T. Tomy was supported by an NSERC Discovery Grant (RGPIN-2019-05354). A. Hontela was supported by an NSERC Discovery Grant (RGPIN-2014-06541). J. A. Doering was supported by an NSERC Postdoctoral Fellowship (PDF-546121-2020). The authors acknowledge support from E. Stock and the staff in the Aquatic Research Facility at the University of Lethbridge. Thanks to M. Denison (University

of California, Davis, CA, USA) for donation of rat CYP1A1 reporter construct and to R. Tanguay (Oregon State University, Corvallis, Or, USA) for donation of zebrafish AhR2 expression construct.

Table 7.1: *Measurement parameters of zebrafish used in early–life stage toxicity testing and cumulative effects observed.*

Treatment	Dosing Solution Concentration (ng/nL)	Nominal Embryo Concentration (ng/g-egg)	Measured Embryo Concentration (ng/g-egg)	Malformations (%) [a]	Total Mortality (%)
Negative Control	N/A[b]	0.00	0.00	3 (3)	8 (6)
Process control	0.00	0.00	0.00	4 (4)	16 (10)
BAA	0.89	2222	842	5 (2)	28 (12)
	2.67	6666	3556	14 (6)	31 (5)
	8.00	20000	9849	11 (2)	41 (2)
	24.00	60000	33896	11 (1)	62 (8)
4-MBAA	0.19	463	401	15 (19)	18 (2)
	0.56	1388	890	11 (7)	27 (5)
	1.67	4166	3416	8 (6)	28 (7)
	5.00	12500	12062	13 (9)	42 (3)
8-MBAA	0.15	370	361	4 (6)	28 (4)
	0.44	1111	1032	7 (10)	41 (15)
	1.33	3333	3451	6 (8)	50 (5)
	4.00	10000	9626	8 (6)	71 (7)
7,12-DMBAA	0.41	1027	1169	1 (2)	35 (2)
	1.23	3083	3080	11 (4)	40 (2)
	3.70	9250	9665	15 (9)	42 (6)
	11.10	27750	31204	39 (20)	70 (14)

[a] Percentage of embryos exhibiting one or more malformations.
[b] Microinjections were not performed for this treatment.
Standard deviation of each data point is presented in parentheses.
BAA = benz[a]anthracene; 4-MBAA = 4-methylbenz[a]anthracene; 8-MBAA = 8-methylbenz[a]anthracene; 7,12-DMBAA = 7,12-dimethylbenz[a]anthracene; N/A = not applicable.

Table 7.2: *Calculated lethal doses (nanograms per gram of egg) of BAA, 4-MBAA, 8-MBAA, and 7,12-DMBAA in zebrafish embryos until 15 dpf.*

Chemical	LD_{20}	LD_{50}
BAA	4715 (2792–8184)	26129 (19142–46451)
4-MBAA	3102 (1479–4898)	25776 (12823–58210)[a]
8-MBAA	1142 (485–2259)	4979 (3221–8649)
7,12-DMBAA	2326 (998–6839)	20402 (10375–41783)

[a]Value extrapolated by GraphPad Prism 9 software due to mortality not reaching 50%
The 95% confidence intervals are given in parentheses.
BAA = benz[a]anthracene; 4-MBAA = 4-methylbenz[a]anthracene; 8-MBAA = 8-methylbenz[a]anthracene; 7,12-DMBAA = 7,12-dimethylbenz[a]anthracene; dpf = days postfertilization; LD_{20}/LD_{50} = 20% and 50% lethal doses.

Table 7.3: *Relative potencies of BAA, 4-MBAA, 8-MBAA, and 7,12-DMBAA in vivo, in vitro, and in the literature to BAA.*

Chemical	*In vivo* ReP to BAA	*In vitro* ReP to BAA	EROD ReP to BAA
BAA	1.0	1.0	1.0[a]
4-MBAA	1.0	5.1	N/A
8-MBAA	5.6	18.4	8.0[a]
7,12-DMBAA	1.4	0.6	2.0[a]

[a] *Relative potencies were calculated from data presented by Barron et al. (2004).*
All relative potencies were calculated based on nanomolar concentrations (nanomoles per gram of egg or nanomolar). Relative potencies to 2,3,7,8-tetrachlorodibenzo-p-dioxin are provided in Supporting Information, Table VI.2.
BAA = benz[a]anthracene; 4-MBAA = 4-methylbenz[a]anthracene; 8-MBAA = 8-methylbenz[a]anthracene; 7,12-DMBAA = 7,12-dimethylbenz[a]anthracene; ReP = relative potency; EROD = ethoxyresorufin-o-deethylase; N/A = not available.

Table 7.4: *Calculated effect concentrations (nanomolar) for activation of the zebrafish aryl hydrocarbon receptor 2 by BAA, 4-MBAA, 8-MBAA, and 7,12-DMBAA in vitro.*

Chemical	$EC_{threshold}$	EC_{20}	EC_{50}	EC_{80}
BAA	0.1	0.3 (0.1–0.8)	2.2 (0.4–11.5)	17.7 (1.1–299.2)
4-MBAA	0.1	0.3 (0.2–0.4)	0.8 (0.6–1.2)	2.8 (1.5–5.5)
8-MBAA	0.1	0.1 (0.0–0.1)	0.2 (0.0–1.0)	0.85 (0.1–13.1)
7,12-DMBAA	0.3	1.5 (1.1–2.2)	6.22 (4.0–9.7)	25.5 (11.6–56.1)

The 95% confidence intervals are given in parentheses.
BAA = benz[a]anthracene; 4-MBAA = 4-methylbenz[a]anthracene; 8-MBAA = 8-methylbenz[a]anthracene; 7,12-DMBAA = 7,12-dimethylbenz[a]anthracene;
ECthreshold = effect concentration threshold; ECx = x% effect concentration.

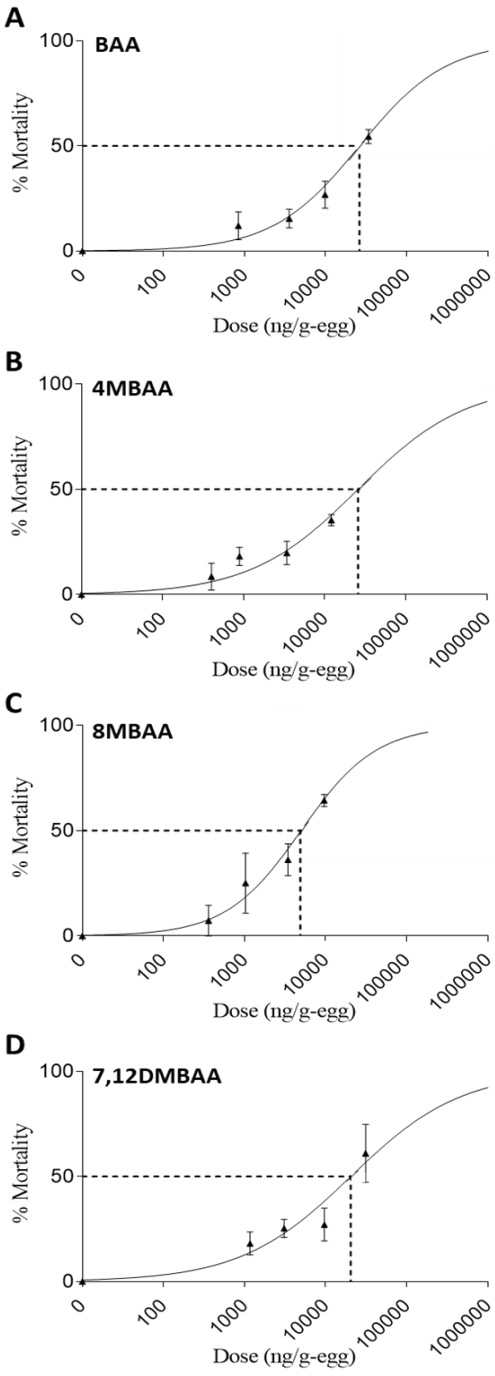

Figure 7.1: *Dose–response curves of early–life stage mortality observed in zebrafish embryos prior to 15 days postfertilization following exposure to BAA (A), 4-MBAA (B), 8-MBAA (C), or 7,12-DMBAA (D).*

Percent mortality was normalized to zero in the control. All mortality values are shown in Table 7.1. Data are presented as mean ± standard error based on three replicate studies, each with n = 24 embryos per dose of chemical. Median lethal doses are indicated as dotted lines. BAA = benz[a]anthracene; 4-MBAA = 4-methylbenz[a]anthracene; 8-MBAA = 8-methylbenz[a]anthracene; 7,12-DMBAA = 7,12-dimethylbenz[a]anthracene.

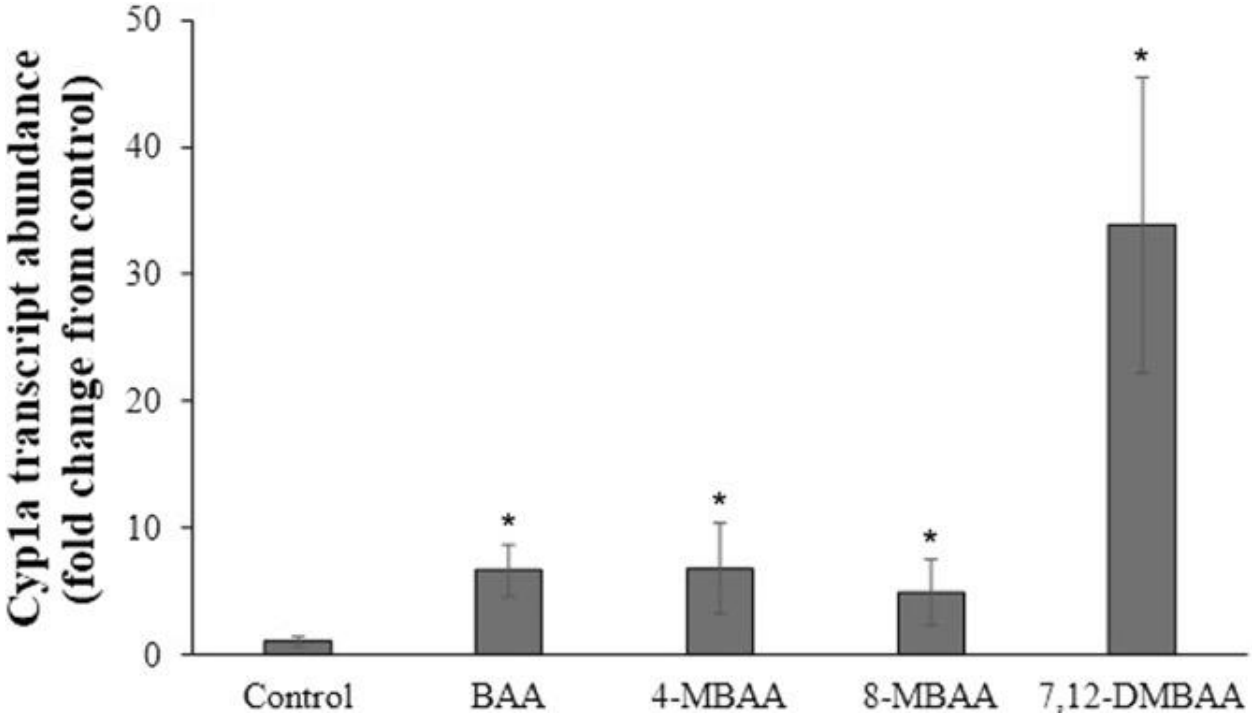

Figure 7.2: *Effect of exposure to the median lethal dose (LD_{50}) of BAA, 4-MBAA, 8-MBAA, or 7,12-DMBAA on the messenger RNA abundance of cyp1a in zebrafish embryos collected at 120 h postfertilization.*

Data are expressed as the mean ± standard deviation relative to the process control. Statistical significance ($p < 0.05$, one-way analysis of variance with Dunnett's post hoc test) is indicated by an asterisk. The LD50 values are given in Table 7.2. cyp1a = cytochrome P450 1a; BAA = benz[a]anthracene; 4-MBAA = 4-methylbenz[a]anthracene; 8-MBAA = 8-methylbenz[a]anthracene; 7,12-DMBAA = 7,12-dimethylbenz[a]anthracene.

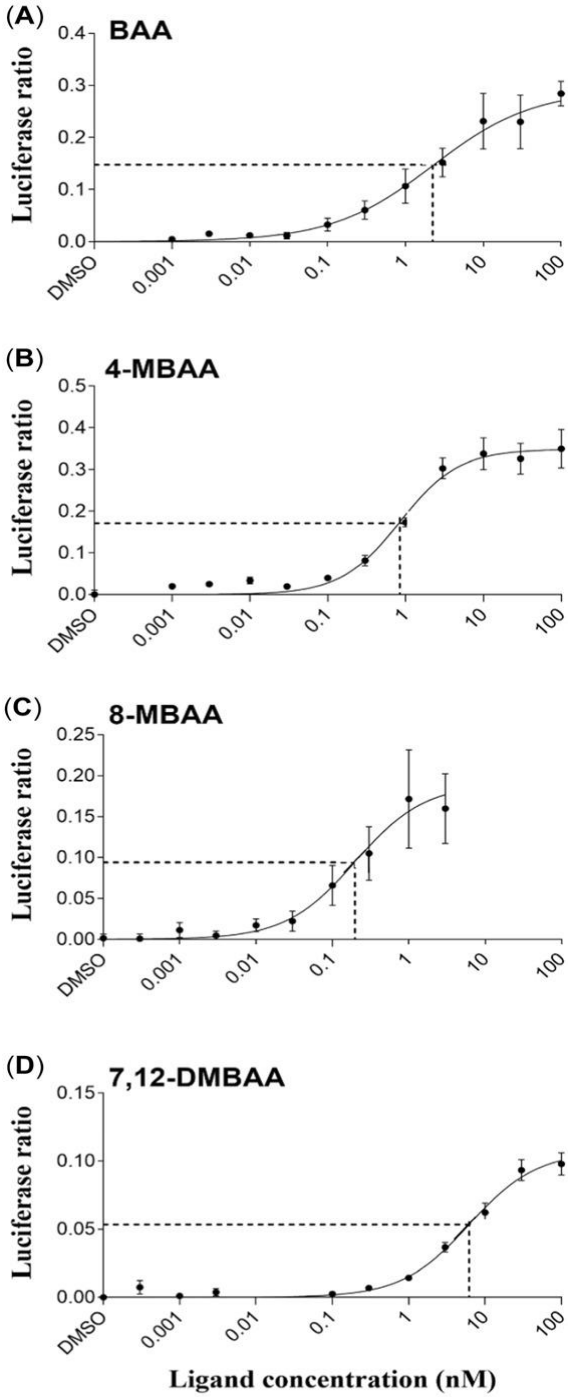

Figure 7.3: *Dose–response curves of COS-7 cells transfected with aryl hydrocarbon receptor 2 of zebrafish following exposure to BAA (A), 4-MBAA (B), 8-MBAA (C), or 7,12-DMBAA (D). Data are presented as mean ± standard error based on three independent assays, each performed with four replicates per concentration of chemical. Median effect concentrations are represented as dotted lines. BAA = benz[a]anthracene; 4-MBAA = 4-methylbenz[a]anthracene; 8-MBAA = 8-methylbenz[a]anthracene; 7,12-DMBAA = 7,12-dimethylbenz[a]anthracene; DMSO = dimethylsulfoxide.*

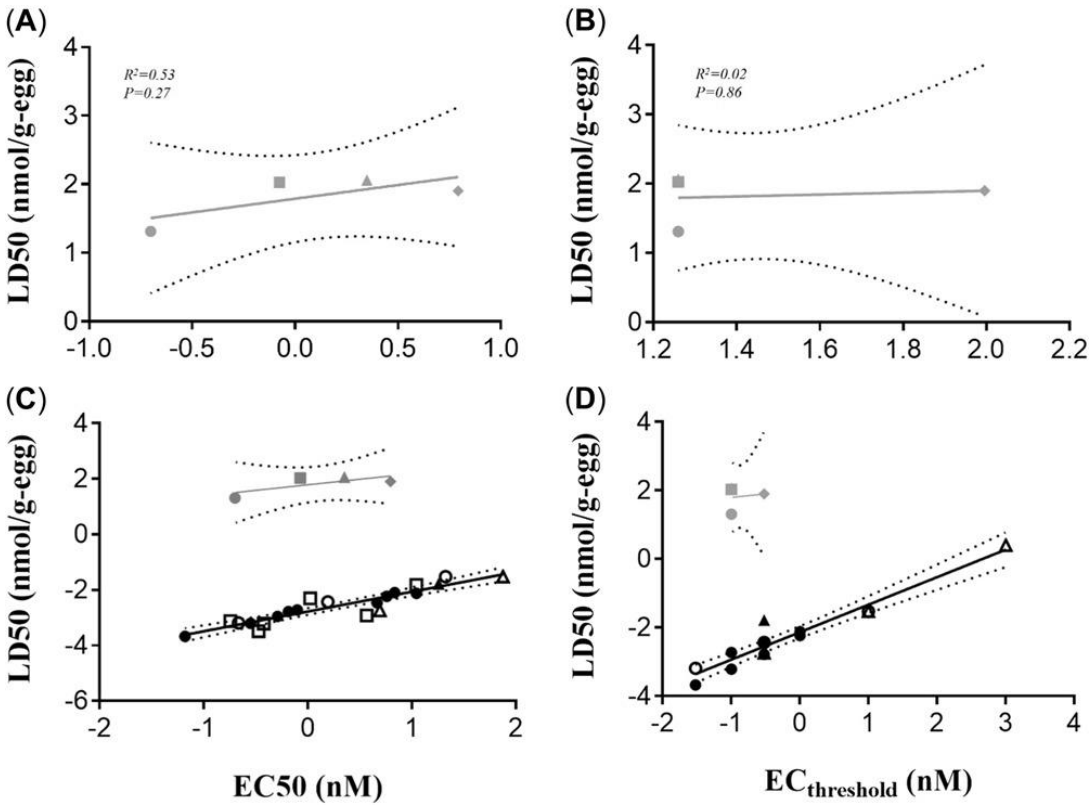

Figure 7.4: *Linear regressions for early–life stage mortality (median lethal dose) against sensitivity to activation (EC50 [A] or ECthreshold [B]) of the zebrafish aryl hydrocarbon receptor 2 by BAA (triangle), 4-MBAA (square), 8-MBAA (circle) and 7,12-DMBAA (diamond).*
All values are shown elsewhere (Table 7.2 and Table 7.4). Best-fit line and 95% confidence interval are shown. Linear regressions for polycyclic aromatic hydrocarbons (gray) alongside corresponding linear regressions for dioxin-like compounds (black [Doering et al., 2018, 2020]) for comparison (C, D). Equations for the lines are $y = 0.4003x + 1.788$ *(A) and* $y = 0.1371x + 1.627$ *(B).* LD_{50} *= median lethal dose;* EC_{50} *= median effect concentration;* $EC_{threshold}$ *= effect concentration threshold.*

7.6 References

(1) Latimer, J.; Zheng, J. The sources, transport, and fate of PAHs in the marine environment. In *Pahs: An ecotoxicological perspective*, 2003.

(2) Alegbeleye, O. O.; Opeolu, B. O.; Jackson, V. A. Polycyclic aromatic hydrocarbons: A critical review of environmental occurrence and bioremediation. *Environ Manage* **2017**, *60* (4), 758-783. DOI: 10.1007/s00267-017-0896-2

(3) Shen, H.; Huang, Y.; Wang, R.; Zhu, D.; Li, W.; Shen, G.; Wang, B.; Zhang, Y.; Chen, Y.; Lu, Y.; Chen, H.; Li, T.; Sun, K.; Li, B.; Liu, W.; Liu, J.; Tao, S. Global atmospheric emissions of polycyclic aromatic hydrocarbons from 1960 to 2008 and future predictions. *Environ Sci Technol* **2013**, *47* (12), 6415-6424. DOI: 10.1021/es400857z

(4) Wallace, S. J.; de Solla, S. R.; Head, J. A.; Hodson, P. V.; Parrott, J. L.; Thomas, P. J.; Berthiaume, A.; Langlois, V. S. Polycyclic aromatic compounds (PACs) in the canadian environment: Exposure and effects on wildlife. *Environ Pollut* **2020**, *265* (Pt B), 114863. DOI: 10.1016/j.envpol.2020.114863

(5) Barron, M. G.; Carls, M. G.; Heintz, R.; Rice, S. D. Evaluation of fish early life-stage toxicity models of chronic embryonic exposures to complex polycyclic aromatic hydrocarbon mixtures. *Toxicol Sci* **2004**, *78* (1), 60-67. DOI: 10.1093/toxsci/kfh051

(6) Vignet, C.; Devier, M. H.; Le Menach, K.; Lyphout, L.; Potier, J.; Cachot, J.; Budzinski, H.; Begout, M. L.; Cousin, X. Long-term disruption of growth, reproduction, and behavior after embryonic exposure of zebrafish to PAH-spiked sediment. *Environ Sci Pollut Res Int* **2014**, *21* (24), 13877-13887. DOI: 10.1007/s11356-014-2585-5

(7) Brinkmann, M.; Koglin, S.; Eisner, B.; Wiseman, S.; Hecker, M.; Eichbaum, K.; Thalmann, B.; Buchinger, S.; Reifferscheid, G.; Hollert, H. Characterisation of transcriptional responses to dioxins and dioxin-like contaminants in roach (Rutilus rutilus) using whole transcriptome analysis. *Sci Total Environ* **2016**, *541*, 412-423. DOI: 10.1016/j.scitotenv.2015.09.087

(8) Doering, J. A.; Tang, S.; Peng, H.; Eisner, B. K.; Sun, J.; Giesy, J. P.; Wiseman, S.; Hecker, M. High conservation in transcriptomic and proteomic response of white sturgeon to equipotent concentrations of 2,3,7,8-TCDD, PCB 77, and benzo[*a*]pyrene. *Environ Sci Technol* **2016**, *50* (9), 4826-4835. DOI: 10.1021/acs.est.6b00490

(9) Clark, B. W.; Matson, C. W.; Jung, D.; Di Giulio, R. T. AHR2 mediates cardiac teratogenesis of polycyclic aromatic hydrocarbons and PCB-126 in Atlantic killifish (Fundulus heteroclitus). *Aquat Toxicol* **2010**, *99* (2), 232-240. DOI: 10.1016/j.aquatox.2010.05.004

(10) Elonen, G. E.; Spehar, R. L.; Holcombe, G. W.; Johnson, R. D.; Fernandez, J. D.; Erickson, R. J.; Tietge, J. E.; Cook, P. M. Comparative toxicity of 2,3,7,8-tetrachlorodibenzo-p-dioxin to seven freshwater fish species during early life-stage development. *Environmental Toxicology and Chemistry* **1998**, *17* (3), 472-483. DOI: 10.1002/etc.5620170319

(11) Van Tiem, L. A.; Di Giulio, R. T. AHR2 knockdown prevents PAH-mediated cardiac toxicity and XRE- and ARE-associated gene induction in zebrafish (Danio rerio). *Toxicol Appl Pharmacol* **2011**, *254* (3), 280-287. DOI: 10.1016/j.taap.2011.05.002

(12) Geier, M. C.; Chlebowski, A. C.; Truong, L.; Massey Simonich, S. L.; Anderson, K. A.; Tanguay, R. L. Comparative developmental toxicity of a comprehensive suite of polycyclic aromatic hydrocarbons. *Arch Toxicol* **2018**, *92* (2), 571-586. DOI: 10.1007/s00204-017-2068-9

(13) Incardona, J. P. Molecular mechanisms of crude oil developmental toxicity in fish. *Arch Environ Contam Toxicol* **2017**, *73* (1), 19-32. DOI: 10.1007/s00244-017-0381-1
(14) Agency for toxic substances and disease registry. ATSDR's substance priority list. 2017.
(15) Achten, C.; Hofmann, T. Native polycyclic aromatic hydrocarbons (PAH) in coals - a hardly recognized source of environmental contamination. *Sci Total Environ* **2009**, *407* (8), 2461-2473. DOI: 10.1016/j.scitotenv.2008.12.008
(16) Hodson, P. V. The toxicity to fish embryos of PAH in crude and refined oils. *Arch Environ Contam Toxicol* **2017**, *73* (1), 12-18. DOI: 10.1007/s00244-016-0357-6
(17) Headley, J.; Akre, C.; Conly, F. M.; Peru, K. M.; Dickson, L. Preliminary characterization and source assessment of PAHs in tributary sediments of the Athabasca River, Canada. *Environmental Forensics* **2001**, *2* (4), 335-345. DOI: 10.1006/enfo.2001.0064
(18) Kurek, J.; Kirk, J. L.; Muir, D. C.; Wang, X.; Evans, M. S.; Smol, J. P. Legacy of a half century of Athabasca Oil Sands development recorded by lake ecosystems. *Proc Natl Acad Sci U S A* **2013**, *110* (5), 1761-1766. DOI: 10.1073/pnas.1217675110
(19) Laender, F. D.; Hammer, J.; Hendriks, A. J.; Soetaert, K.; Janssen, C. Combining monitoring data and modeling identifies PAHs as emerging contaminants in the Arctic. *Environmental science & technology* **2011**, *45* (20), 9024-9029.
(20) Lin, H.; Morandi, G. D.; Brown, R. S.; Snieckus, V.; Rantanen, T.; Jorgensen, K. B.; Hodson, P. V. Quantitative structure-activity relationships for chronic toxicity of alkyl-chrysenes and alkyl-benz[*a*]anthracenes to Japanese medaka embryos (Oryzias latipes). *Aquat Toxicol* **2015**, *159*, 109-118. DOI: 10.1016/j.aquatox.2014.11.027
(21) Fang, J.; Dong, S.; Boogaard, P. J.; Rietjens, I. M.; Kamelia, L. Developmental toxicity testing of unsubstituted and methylated 4-and 5-ring polycyclic aromatic hydrocarbons using the zebrafish embryotoxicity test. *Toxicology In Vitro* **2022**, *80*, 105312.
(22) Barron, M. G.; Heintz, R.; Rice, S. D. Relative potency of PAHs and heterocycles as aryl hydrocarbon receptor agonists in fish. In *Marine Environmental Research*, 2004, 2004; 2004; Vol. 58, pp 95-100. DOI: 10.1016/j.marenvres.2004.03.001.
(23) Lille-Langoy, R.; Jorgensen, K. B.; Goksoyr, A.; Pampanin, D. M.; Sydnes, M. O.; Karlsen, O. A. Substituted two- to five-ring polycyclic aromatic compounds are potent agonists of Atlantic cod (Gadus morhua) aryl hydrocarbon receptors ahr1a and ahr2a. *Environ Sci Technol* **2021**, *55* (22), 15123-15135. DOI: 10.1021/acs.est.1c02946
(24) Evans, M.; Davies, M.; Janzen, K.; Muir, D.; Hazewinkel, R.; Kirk, J.; de Boer, D. PAH distributions in sediments in the oil sands monitoring area and western Lake Athabasca: Concentration, composition and diagnostic ratios. *Environmental Pollution* **2016**, *213*, 671-687.
(25) Doering, J. A.; Farmahin, R.; Wiseman, S.; Beitel, S. C.; Kennedy, S. W.; Giesy, J. P.; Hecker, M. Differences in activation of aryl hydrocarbon receptors of white sturgeon relative to lake sturgeon are predicted by identities of key amino acids in the ligand binding domain. *Environmental Science & Technology* **2015**, *49* (7), 4681-4689.
(26) Farmahin, R.; Manning, G. E.; Crump, D.; Wu, D.; Mundy, L. J.; Jones, S. P.; Hahn, M. E.; Karchner, S. I.; Giesy, J. P.; Bursian, S. J.; Zwiernik, M. J.; Fredricks, T. B.; Kennedy, S. W. Amino acid sequence of the ligand-binding domain of the aryl hydrocarbon receptor 1 predicts sensitivity of wild birds to effects of dioxin-like compounds. *Toxicol Sci* **2013**, *131* (1), 139-152. DOI: 10.1093/toxsci/kfs259
(27) Idowu, I.; Francisco, O.; Thomas, P. J.; Johnson, W.; Marvin, C.; Stetefeld, J.; Tomy, G. T. Validation of a simultaneous method for determining polycyclic aromatic compounds and

alkylated isomers in biota. *Rapid Commun Mass Spectrom* **2018**, *32* (3), 277-287. DOI: 10.1002/rcm.8035
(28) Seiler, T. B.; Best, N.; Fernqvist, M. M.; Hercht, H.; Smith, K. E.; Braunbeck, T.; Mayer, P.; Hollert, H. PAH toxicity at aqueous solubility in the fish embryo test with danio rerio using passive dosing. *Chemosphere* **2014**, *112*, 77-84. DOI: 10.1016/j.chemosphere.2014.02.064
(29) Lane, T.; Green, D.; Bluhm, K.; Raes, K.; Janz, D. M.; Liber, K.; Hecker, M. In ovo exposure of fathead minnow (Pimephales promelas) to selenomethionine via maternal transfer and embryo microinjection: A comparative study. *Aquat Toxicol* **2019**, *216*, 105299. DOI: 10.1016/j.aquatox.2019.105299
(30) Organisation for Economic Cooperation and Development. *Test no. 210: Fish, early-life stage toxicity test*; 2013. DOI: https://doi.org/10.1787/9789264203785-en.
(31) Pfaffl, M. W. A new mathematical model for relative quantification in real-time RT–PCR. *Nucleic acids research* **2001**, *29* (9), e45-e45.
(32) Fujita, K. K.; Xia, Z.; Tomy, G.; Montina, T.; Wiseman, S. ^{1}H NMR based metabolomic profiling of early life stage zebrafish (Danio rerio) exposed to a water-soluble fraction of weathered sediment-bound diluted bitumen. *Aquat Toxicol* **2021**, *232*, 105766. DOI: 10.1016/j.aquatox.2021.105766
(33) Van Essen, D.; Devoy, C.; Miller, J.; Jones, P. D.; Wiseman, S. Effects of the brominated flame retardant, TBCO, on development of zebrafish (Danio rerio) embryos. *Chemosphere* **2021**, *266*, 129195.
(34) Farmahin, R.; Wu, D.; Crump, D.; Herve, J. C.; Jones, S. P.; Hahn, M. E.; Karchner, S. I.; Giesy, J. P.; Bursian, S. J.; Zwiernik, M. J.; Kennedy, S. W. Sequence and in vitro function of chicken, ring-necked pheasant, and Japanese quail AHR1 predict in vivo sensitivity to dioxins. *Environ Sci Technol* **2012**, *46* (5), 2967-2975. DOI: 10.1021/es2043992
(35) Doering, J. A.; Farmahin, R.; Wiseman, S.; Kennedy, S. W.; Giesy, J. P.; Hecker, M. Functionality of aryl hydrocarbon receptors (AHR1 and AHR2) of White Sturgeon (Acipenser Transmontanus) and implications for the risk assessment of dioxin-like compounds. *Environ Sci Technol* **2014**, *48* (14), 8219-8226. DOI: 10.1021/es502054h
(36) Tanguay, R. L.; Abnet, C. C.; Heideman, W.; Peterson, R. E. Cloning and characterization of the zebrafish (Danio rerio) aryl hydrocarbon receptor. *Biochim Biophys Acta* **1999**, *1444* (1), 35-48. DOI: 10.1016/s0167-4781(98)00252-8
(37) Han, D.; Nagy, S. R.; Denison, M. S. Comparison of recombinant cell bioassays for the detection of ah receptor agonists. *Biofactors* **2004**, *20* (1), 11-22. DOI: 10.1002/biof.5520200102
(38) Rushing, S. R.; Denison, M. S. The silencing mediator of retinoic acid and thyroid hormone receptors can interact with the aryl hydrocarbon (AH) receptor but fails to repress ah receptor-dependent gene expression. *Arch Biochem Biophys* **2002**, *403* (2), 189-201. DOI: 10.1016/s0003-9861(02)00233-3
(39) Xia, Z.; Idowu, I.; Kerr, E.; Klaassen, N.; Assi, H.; Bray, H.; Marvin, C.; Thomas, P. J.; Stetefeld, J.; Tomy, G. T. New approaches to reduce sample processing times for the determination of polycyclic aromatic compounds in environmental samples. *Chemosphere* **2021**, *274*, 129738. DOI: 10.1016/j.chemosphere.2021.129738
(40) Magnusson, B.; Örnemark, U. *The fitness for purpose of analytical methods – a laboratory guide to method validation and related topics (2nd ed)*; 2014.
(41) Doering, J. A.; Giesy, J. P.; Wiseman, S.; Hecker, M. Predicting the sensitivity of fishes to dioxin-like compounds: Possible role of the aryl hydrocarbon receptor (AHR) ligand

binding domain. *Environmental Science and Pollution Research* **2013**, *20*, 1219-1224.
(42) King-Heiden, T. C.; Mehta, V.; Xiong, K. M.; Lanham, K. A.; Antkiewicz, D. S.; Ganser, A.; Heideman, W.; Peterson, R. E. Reproductive and developmental toxicity of dioxin in fish. *Mol Cell Endocrinol* **2012**, *354* (1-2), 121-138. DOI: 10.1016/j.mce.2011.09.027
(43) Tillitt, D. E.; Buckler, J. A.; Nicks, D. K.; Candrl, J. S.; Claunch, R. A.; Gale, R. W.; Puglis, H. J.; Little, E. E.; Linbo, T. L.; Baker, M. Sensitivity of Lake Sturgeon (Acipenser fulvescens) early life stages to 2,3,7,8-tetrachlorodibenzo-p-dioxin and 3,3',4,4',5-pentachlorobiphenyl. *Environ Toxicol Chem* **2017**, *36* (4), 988-998. DOI: 10.1002/etc.3614
(44) Hahn, M. E. Aryl hydrocarbon receptors: Diversity and evolution. *Chem Biol Interact* **2002**, *141* (1-2), 131-160. DOI: 10.1016/s0009-2797(02)00070-4
(45) Carlson, E. A.; Li, Y.; Zelikoff, J. T. Exposure of Japanese medaka (Oryzias latipes) to Benzo[*A*]Pyrene Suppresses Immune Function And Host Resistance Against Bacterial challenge. *Aquat Toxicol* **2002**, *56* (4), 289-301. DOI: 10.1016/s0166-445x(01)00223-5
(46) Fang, X.; Corrales, J.; Thornton, C.; Clerk, T.; Scheffler, B. E.; Willett, K. L. Transcriptomic changes in zebrafish embryos and larvae following benzo[*a*]pyrene exposure. *Toxicol Sci* **2015**, *146* (2), 395-411. DOI: 10.1093/toxsci/kfv105
(47) Head, J. A.; Jeffery, R. W.; Farmahin, R.; Kennedy, S. W. Potency of polycyclic aromatic hydrocarbons (PAHs) for induction of ethoxyresorufin-o-deethylase (EROD) activity in hepatocyte cultures from chicken, pekin duck, and greater scaup. *Environ Sci Technol* **2015**, *49* (6), 3787-3794. DOI: 10.1021/acs.est.5b00125
(48) Kuhnert, A.; Vogs, C.; Seiwert, B.; Aulhorn, S.; Altenburger, R.; Hollert, H.; Kuster, E.; Busch, W. Biotransformation in the zebrafish embryo -temporal gene transcription changes of cytochrome P450 enzymes and internal exposure dynamics of the AHR binding xenobiotic benz[*a*]anthracene. *Environ Pollut* **2017**, *230*, 1-11. DOI: 10.1016/j.envpol.2017.04.083
(49) Le Bihanic, F.; Sommard, V.; Perrine de, L.; Pichon, A.; Grasset, J.; Berrada, S.; Budzinski, H.; Cousin, X.; Morin, B.; Cachot, J. Environmental concentrations of benz[*a*]anthracene induce developmental defects and DNA damage and impair photomotor response in Japanese medaka larvae. *Ecotoxicol Environ Saf* **2015**, *113*, 321-328. DOI: 10.1016/j.ecoenv.2014.12.011
(50) Franci, C. D.; Aleksieva, A.; Boulanger, E.; Brandenburg, J.; Johnston, T.; Malinova, A.; Head, J. A. Potency of polycyclic aromatic hydrocarbons in chicken and Japanese quail embryos. *Environ Toxicol Chem* **2018**, *37* (6), 1556-1564. DOI: 10.1002/etc.4099
(51) Buckler, J.; Candrl, J. S.; McKee, M. J.; Papoulias, D. M.; Tillitt, D. E.; Galat, D. L. Sensitivity of shovelnose sturgeon (Scaphirhynchus platorynchus) and pallid sturgeon (S. Albus) early life stages to 3,3', 4,4',5-pentachlorobiphenyl and 2, 3, 7, 8-tetrachlorodibenzo-p-dioxin exposure. *Environmental Toxicology and Chemistry* **2015**, *34* (6), 1417-1424.
(52) Doering, J. A.; Wiseman, S.; Giesy, J. P.; Hecker, M. A cross-species quantitative adverse outcome pathway for activation of the aryl hydrocarbon receptor leading to early life stage mortality in birds and fishes. *Environ Sci Technol* **2018**, *52* (13), 7524-7533. DOI: 10.1021/acs.est.8b01438
(53) Karchner, S. I.; Franks, D. G.; Kennedy, S. W.; Hahn, M. E. *The molecular basis for differential dioxin sensitivity in birds: Role of the aryl hydrocarbon receptor*; 2006.
(54) Wirgin, I.; Roy, N. K.; Loftus, M.; Chambers, R. C.; Franks, D. G.; Hahn, M. E. Mechanistic basis of resistance to PCBs in Atlantic tomcod from the Hudson River. *Science* **2011**, *331* (6022), 1322-1325.
(55) Doering, J. A.; Dubiel, J.; Wiseman, S. Predicting early life stage mortality in birds and fishes

from exposure to low-potency agonists of the aryl hydrocarbon receptor: A cross-species quantitative adverse outcome pathway approach. *Environ Toxicol Chem* **2020**, *39* (10), 2055-2064. DOI: 10.1002/etc.4816

(56) Zhang, R.; Wang, X.; Zhang, X.; Song, C.; Letcher, R. J.; Liu, C. Polychlorinated diphenylsulfides activate aryl hydrocarbon receptor 2 in zebrafish embryos: Potential mechanism of developmental toxicity. *Environ Sci Technol* **2018**, *52* (7), 4402-4412. DOI: 10.1021/acs.est.8b00366

(57) Frakes, R. A.; Zeeman, C. Q.; Mower, B. Bioaccumulation of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) by fish downstream of pulp and paper mills in maine. *Ecotoxicol Environ Saf* **1993**, *25* (2), 244-252. DOI: 10.1006/eesa.1993.1023

(58) Hahn, M. E.; Stegeman, J. J. Regulation of cytochrome P4501A1 in teleosts: Sustained induction of CYP1A1 mRNA, protein, and catalytic activity by 2,3,7,8-tetrachlorodibenzofuran in the marine fish stenotomus chrysops. *Toxicol Appl Pharmacol* **1994**, *127* (2), 187-198. DOI: 10.1006/taap.1994.1153

(59) Whyte, J. J.; Jung, R. E.; Schmitt, C. J.; Tillitt, D. E. Ethoxyresorufin-o-deethylase (EROD) activity in fish as a biomarker of chemical exposure. *Crit Rev Toxicol* **2000**, *30* (4), 347-570. DOI: 10.1080/10408440091159239

(60) Budzinski, H.; Mazéas, O.; Tronczynski, J.; Désaunay, Y.; Bocquené, G.; Claireaux, G. Link between exposure of fish (Solea solea) to PAHs and metabolites: Application to the "Erika" oil spill. *Aquatic Living Resources* **2004**, *17* (3), 329-334. DOI: 10.1051/alr:2004040

(61) Beyer, J.; Jonsson, G.; Porte, C.; Krahn, M. M.; Ariese, F. Analytical methods for determining metabolites of polycyclic aromatic hydrocarbon (PAH) pollutants in fish bile: A review. *Environ Toxicol Pharmacol* **2010**, *30* (3), 224-244. DOI: 10.1016/j.etap.2010.08.004

(62) Loerracher, A. K.; Braunbeck, T. Cytochrome P450-dependent biotransformation capacities in embryonic, juvenile and adult stages of zebrafish (Danio rerio)-a state-of-the-art review. *Arch Toxicol* **2021**, *95* (7), 2299-2334. DOI: 10.1007/s00204-021-03071-7

(63) Otte, J. C.; Schultz, B.; Fruth, D.; Fabian, E.; van Ravenzwaay, B.; Hidding, B.; Salinas, E. R. Intrinsic xenobiotic metabolizing enzyme activities in early life stages of zebrafish (Danio rerio). *Toxicol Sci* **2017**, *159* (1), 86-93. DOI: 10.1093/toxsci/kfx116

(64) Farmahin, R.; Crump, D.; O'Brien, J. M.; Jones, S. P.; Kennedy, S. W. Time-dependent transcriptomic and biochemical responses of 6-formylindolo [3,2-b] carbazole (FICZ) and 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) are explained by AHR activation time. *Biochemical Pharmacology* **2016**, *115*, 134-143.

(65) Farmahin, R.; Crump, D.; Kennedy, S. W. Sensitivity of avian species to the aryl hydrocarbon receptor ligand 6-formylindolo [3, 2-b] carbazole (ficz). *Chemico-Biological Interactions* **2014**, *221*, 61-69.

(66) Barron, M. G.; Holder, E. Are exposure and ecological risks of PAHs underestimated at petroleum contaminated sites? *Human and Ecological Risk Assessment: An International Journal* **2003**, *9* (6), 1533-1545. DOI: 10.1080/10807030390251029

Chapter 8: Conclusion and Future Directions

8.1 Conclusion

My research aimed to develop methods to extract, separate, identify, and quantify complex mixtures of PACs, especially HPAHs, and their numerous isomers in different environmental matrices. For the last two decades, PACs have seen renewed interest by the global scientific community as important environmental contaminants. Among all PACs, the 16 US EPA priority PAHs still receive most of the focus. Limitations for other sub-classes of PACs, especially HPAHs, includes the lack of authentic analytical standards and the challenge in separation and identification of co-eluting compounds. However, there is continuous exposure to PACs for biological systems, both wildlife and humans, because of urbanization/population expansion and an associated global reliance on fossil fuels. The toxicity of some sub-classes of PACs are not well-studied, especially APAHs and HPAHs.

To my knowledge, there is a lack of a unified analytical method for the entire PACs family, either to quantify them by analytical instrumentation or fully validated extraction methods. This is due in part to the chemical complexity of PACs and to the myriad theoretically possible structural isomers. My research was able to address some of the knowledge gaps acknowledged here and within the scientific community on both exhaustive extraction and accurate quantification of PACs.

My research on identification of HPAHs from AOSR samples by GC-HRMS was able to illustrate the importance of studying this novel class of contaminants and unearth the environmental occurrence of many new HPAHs. To further address the paucity of unified

analytical methods for PACs, I fully validated two analytical methods of extraction and clean-up for PACs following Eurachem guidelines in an ISO 17025 accredited laboratory. The first method aimed to extract PACs from solid samples, which involved a one-step extraction ASE method and dSPE clean-up for biota samples. The second method focused on extracting PACs from semi-liquid samples, especially avian eggs. Both validated methods improve on extraction efficiencies, streamline the extraction process and reduce the volume of solvents, which makes the sample processing more environmentally friendly and also cost-effective.

In addition, I have also studied different approaches for quantifying PACs, which offers some suggestion on how to best quantify PACs with good data quality objectives. With my unified quantification method, the discrepancies of interlaboratory studies can be mitigated.

The analytical methodologies that I developed also contribute to the toxicology study. For toxicity properties of PACs, I have collaborated with coworkers to assess both *in vivo* and *in vitro* toxicity of APAHs and determine the relationship between alkylation and health effect. The toxicity of PACs is affected by the degree of alkylation and position of alkylation.

Although my research was able to address some of the issues in the field namely issues related to a lack of fully validated analytical methods and a uniform quantification method for PACs, one of the major drawbacks that still exists is the paucity of authentic analytical standards, especially for HPAHs. This has limited the identification of more HPAHs and determination of isomer-specific concentrations of HPAHs when detected.

While I have detected HPAHs from the Canadian environment, the resolution of current state-of-art analytical instrumentation, *e.g.*, GC × GC-HRToF-MS, is still challenged to separate

structural isomers of HPAHs. Some structural isomers, such as Cl-Phe and Cl-Ant, coelute and cannot be resolved even with the superior separation abilities of GC × GC.

One of the main strengths of my research is that it offers validated and unified extraction and quantification methods of many commercially available PACs. The validated extraction method improves on the extraction efficiencies and makes extraction "greener" by using significantly smaller solvent volumes.

Finally, the impact of my research is that it has caused a paradigm shift in what PAC-like compounds are being monitored in the Canadian environment. Increasingly, our collaborators at the Environment and Climate Change Canada are reporting on the compounds I detected which ultimately will help regulators and policy-makers in Canada decide on the hazards these compounds may pose to the environment.

8.2 Future Directions

Collaboration with synthetic researchers is essential for making more analytical standards, which will play an important role in isomer-specific studies and toxicological risk assessments. Having a unified list of PACs, such as 16 US EPA PAHs, will contribute significantly to related research.

Standardized approach for quantification of PACs is urgently needed, especially for APAHs, HPAHs, and heterocyclic compounds. The lack of a standardized methods for quantification has limited the use of published data, such as geographical and temporal comparison of PAC concentration in certain matrix.

The toxicological data and risk assessment for novel PACs also needs to be studied. Besides conventional *in vivo* and *in vitro* methods, reliable predictive non-animal toxicity testing model, *i.e.*, *in silico* methods, are needed. *In silico* methods may help the screening of toxic compounds and the development of toxicity assessments on complex mixtures.

Current research has not emphasized of the importance of 2D chromatography. The separation power of 2D chromatography can play a more central role in quantitative assessment, especially in congener-specific determination of PACs. Advancement of analytical instrumentation is crucial for future PAC analyses. There are some PAC sub-classes, such as heterocyclic PACs (S-, N-, and O-based PAHs), halogenated APAHs and halogenated heterocyclic PACs that are very much understudied. Naturally, there are likely many more sub-classes of PACs that are present in the environment. Therefore, advanced analytical instrumentation with better resolving power will be tremendous for not only PAC research but other fields in environmental research.

The development of new standard reference materials (SRMs) is also needed for PAC analyses. There is a limited number of PAC compounds identified in commercially available SRMs. In addition, SRMs, as positive controls, are especially important for interlaboratory studies and assessing data quality.

Overall, a systematic approach of analysing and investigating PACs should be established for various aspects of PACs study, including occurrence (environmental compartment, geography), source, distribution (mobility), persistence, fate and toxicity.[1]

8.3 References

(1) Marvin, C. H.; Tomy, G. T.; Thomas, P. J.; Holloway, A. C.; Sandau, C. D.; Idowu, I.; Xia, Z. Considerations for prioritization of polycyclic aromatic compounds as environmental contaminants. *Environ Sci Technol* **2020**, *54* (23), 14787-14789. DOI: 10.1021/acs.est.0c04892

Appendix I. Additional Metrics from Chapter 2: Methodology

Table I.1: *Nomenclature, EI fragmentation patterns, measured experimental mass of the HPAH standards determined using GC-MS/MS and GC-HRTOF-MS.*

Compound	Formula	CAS number[a]	GC-MS/MS			GC-HRTOF-MS		
			$M^{+\bullet}$[b]	Quantifying transition	Qualifying transition	$M^{+\bullet}$[b]	Confirmer	Fragmentation pattern
1-Br-Nap	$C_{10}H_7Br$	90-11-9	205.9	205.9 → 127.1	205.9 → 77.0	205.9684	127.0518	$[M–Br]^+$
2-Br-Nap	$C_{10}H_7Br$	580-13-2	205.9	205.9 → 127.1	205.9 → 77.0	205.9756	127.0603	$[M–Br]^+$
9-Cl-Fle	$C_{13}H_9Cl$	6630-65-5	199.9	165.0 → 165.0[c]	199.9 → 165.0	200.0386	165.0697	$[M–Cl]^+$
1,4-Br_2-Nap	$C_{10}H_6Br_2$	83-53-4	286.1	286.1 → 126.0	286.1 → 204.9	285.8853	126.0642	$[M–2Br]^{+\bullet}$
2,7-Br_2-Nap	$C_{10}H_6Br_2$	58556-75-5	286.1	286.1 → 126.0	286.1 → 204.9	285.8821	126.0438	$[M–2Br]^{+\bullet}$
2-Cl-Fle	$C_{13}H_9Cl$	2523-44-6	199.9	165.0 → 165.0[c]	199.9 → 165.0	200.0382	165.0697	$[M–Cl]^+$
5-Br-Ace	$C_{12}H_9Br$	2051-98-1	234.1	234.1 → 153.1	234.1 → 234.1[c]	231.9883	153.0696	$[M–Br]^+$
9-Br-Fle	$C_{13}H_9Br$	1940-57-4	246.1	165.0 → 165.0[c]	246.1 → 165.0	243.9880	165.0697	$[M–Br]^+$
2-Br-Fle	$C_{13}H_9Br$	1133-80-8	246.1	165.0 → 165.0[c]	246.1 → 165.0	243.9882	165.0702	$[M–Br]^+$
9-Cl-Phe	$C_{14}H_9Cl$	947-72-8	212.0	212.0 → 176.0	212.0 → 151.0	212.0392	176.0623	$[M–HCl]^{+\bullet}$
1-Cl-Ant	$C_{14}H_9Cl$	4985-70-0	212.0	212.0 → 176.0	212.0 → 151.0	212.0393	176.0624	$[M–HCl]^{+\bullet}$
2-Cl-Ant	$C_{14}H_9Cl$	17135-78-3	212.0	212.0 → 176.0	212.0 → 151.0	212.0393	176.0624	$[M–HCl]^{+\bullet}$
9-Cl-Ant	$C_{14}H_9Cl$	716-53-0	212.0	212.0 → 176.0	212.0 → 151.0	212.0390	176.0622	$[M–HCl]^{+\bullet}$
2,7-Cl_2-Fle	$C_{13}H_8Cl_2$	7012-16-0	234.0	234.0 → 199.1	234.0 → 234.0[c]	234.0000	199.0310	$[M–Cl]^+$
1,2-Br_2-Any	$C_{12}H_6Br_2$	13019-33-5	310.1	310.1 → 149.9	310.1 → 228.9	309.8811	150.0464	$[M–2Br]^{+\bullet}$
3-Br-Phe	$C_{14}H_9Br$	715-50-4	256.0	256.0 → 177.1	256.0 → 151.1	255.9891	176.0625	$[M–HBr]^{+\bullet}$
9-Br-Phe	$C_{14}H_9Br$	573-17-1	256.0	256.0 → 177.1	256.0 → 151.1	255.9889	176.0625	$[M–HBr]^{+\bullet}$
2-Br-Phe	$C_{14}H_9Br$	62162-97-4	256.0	256.0 → 177.1	256.0 → 151.1	255.9889	176.0625	$[M–HBr]^{+\bullet}$

1-Br-Ant	$C_{14}H_9Br$	7397-92-4	256.0	256.0 → 177.1	256.0 → 151.1	255.9893	176.0626	$[M–HBr]^{+\bullet}$
2-Br-Ant	$C_{14}H_9Br$	7321-27-9	256.0	256.0 → 177.1	256.0 → 151.1	255.9892	176.0624	$[M–HBr]^{+\bullet}$
9-Br-Ant	$C_{14}H_9Br$	1564-64-3	256.0	256.0 → 177.1	256.0 → 151.1	255.9889	176.0622	$[M–HBr]^{+\bullet}$
1,4-Cl_2-Ant	$C_{14}H_8Cl_2$	66259-12-9	246.0	246.0 → 176.1	246.0 → 211.2	246.0004	176.0623	$[M–2Cl]^{+\bullet}$
3,9-Cl_2-Phe	$C_{14}H_8Cl_2$	7473-66-7	246.0	246.0 → 176.1	246.0 → 211.2	245.9997	176.0618	$[M–2Cl]^{+\bullet}$
1,5-Cl_2-Ant	$C_{14}H_8Cl_2$	6406-96-8	246.0	246.0 → 176.1	246.0 → 211.2	246.0005	176.0623	$[M–2Cl]^{+\bullet}$
9,10-Cl_2-Ant	$C_{14}H_8Cl_2$	605-48-1	246.0	246.0 → 176.1	246.0 → 211.2	245.9996	176.0619	$[M–2Cl]^{+\bullet}$
1,9-Cl_2-Phe	$C_{14}H_8Cl_2$	1006693-48-6	246.0	246.0 → 176.1	246.0 → 211.2	245.9997	176.0618	$[M–2Cl]^{+\bullet}$
9,10-Cl_2-Phe	$C_{14}H_8Cl_2$	17219-94-2	246.0	246.0 → 176.1	246.0 → 211.2	245.9999	176.0618	$[M–2Cl]^{+\bullet}$
5,6-Br_2-Ace	$C_{12}H_8Br_2$	19190-91-1	312.2	312.1 → 152.1	312.1 → 233.1	311.8974	152.0621	$[M–2Br]^{+\bullet}$
2,7-Br_2-Fle	$C_{13}H_8Br_2$	16433-88-8	324.1	324.1 → 243.0	324.1 → 163.9	323.8981	242.9810	$[M–Br]^{+}$
3-Cl-Flu	$C_{16}H_9Cl$	25911-51-7	236.0	236.0 → 201.1	236.0 → 236.0[c]	236.0387	200.0619	$[M–HCl]^{+\bullet}$
8-Cl-Flu	$C_{16}H_9Cl$	145730-31-0	236.0	236.0 → 201.1	236.0 → 236.0[c]	236.0385	200.0618	$[M–HCl]^{+\bullet}$
1-Cl-Pyr	$C_{16}H_9Cl$	34244-14-9	236.0	236.0 → 201.1	236.0 → 236.0[c]	236.0388	200.0618	$[M–HCl]^{+\bullet}$
3,9,10-Cl_3-Phe	$C_{14}H_7Cl_3$	800409-57-8	282.0	282.0 → 282.0[c]	282.0 → 246.7	279.9604	210.0230	$[M–2Cl]^{+\bullet}$
3-Br-Flu	$C_{16}H_9Br$	13438-50-1	279.9	279.9 → 200.1	279.9 → 279.9[c]	279.9883	200.0621	$[M–HBr]^{+\bullet}$
1,8-Br_2-Ant	$C_{14}H_8Br_2$	131276-24-9	336.1	336.1 → 176.0	336.1 → 254.9	335.8981	176.0625	$[M–2Br]^{+\bullet}$
9,10-Br_2-Ant	$C_{14}H_8Br_2$	523-27-3	336.1	336.1 → 176.0	336.1 → 254.9	335.8980	176.0624	$[M–2Br]^{+\bullet}$
1,3-Cl_2-Flu	$C_{16}H_8Cl_2$	82520-76-1	269.9	269.9 → 269.9[c]	269.9 → 235.0	269.9999	200.0620	$[M–2Cl]^{+\bullet}$
2,7-Br_2-Phe	$C_{14}H_8Br_2$	62325-30-8	336.1	336.1 → 176.0	336.1 → 254.9	335.8973	176.0622	$[M–2Br]^{+\bullet}$
4-Br-Pyr	$C_{16}H_9Br$	1732-26-9	279.9	279.9 → 200.1	279.9 → 279.9[c]	279.9886	200.0623	$[M–HBr]^{+\bullet}$
9,10-Br_2-Phe	$C_{14}H_8Br_2$	15810-15-8	336.1	336.1 → 176.0	336.1 → 254.9	335.8984	176.0622	$[M–2Br]^{+\bullet}$
1-Br-Pyr	$C_{16}H_9Br$	1714-29-0	279.9	279.9 → 200.1	279.9 → 279.9[c]	279.9892	200.0627	$[M–HBr]^{+\bullet}$
3,8-Cl_2-Flu	$C_{16}H_8Cl_2$	25911-52-8	270.0	269.9 → 269.9[c]	269.9 → 235.0	269.9999	200.0622	$[M–2Cl]^{+\bullet}$
3,6,9-Cl_3-Phe	$C_{14}H_7Cl_3$	1173180-74-9	282.0	282.0 → 282.0[c]	282.0 → 246.7	279.9606	210.0229	$[M–2Cl]^{+\bullet}$

3,4-Cl_2-Flu	$C_{16}H_8Cl_2$	108079-33-0	270.0	269.9 → 269.9[c]	269.9 → 235.0	269.9994	200.0617	$[M–2Cl]^{+\bullet}$
9-Br-1,5-Cl_2-Ant	$C_{14}H_7BrCl_2$	201406-34-0	326.0	326.0 → 209.9	326.0 → 244.8	326.9094	210.0237	$[M–BrCl]^{+\bullet}$
7-Cl-BaA	$C_{18}H_{11}Cl$	20268-52-4	262.0	262.0 → 226.1	262.0 → 262.0[c]	262.0549	226.0781	$[M–HCl]^{+\bullet}$
6-Cl-Chr	$C_{18}H_{11}Cl$	95791-46-1	262.0	262.0 → 226.1	262.0 → 262.0[c]	262.0542	226.0775	$[M–HCl]^{+\bullet}$
1,5,9,10-Cl_4-Ant	$C_{14}H_6Cl_4$	82843-47-8	316.0	316.0 → 316.0[c]	316.0 → 244.0	315.9201	243.9851	$[M–2H2Cl]^{+\bullet}$
Cl_3-Pyr	$C_{16}H_7Br_3$	n/a	304.0	303.8 → 234.2	303.8 → 268.8	303.9609	234.0223	$[M–2Cl]^{+\bullet}$
1,8-Br_2-Pyr	$C_{16}H_8Br_2$	38303-35-4	360.0	360.0 → 200.1	360.0 → 280.8	359.8976	200.0624	$[M–2Br]^{+\bullet}$
1,6-Br_2-Pyr	$C_{16}H_8Br_2$	27973-29-1	360.0	360.0 → 200.1	360.0 → 280.8	359.8979	200.0624	$[M–2Br]^{+\bullet}$
7-Br-BaA	$C_{18}H_{11}Br$	32795-84-9	305.9	305.9 → 226.1	305.9 → 305.9	306.0048	226.0779	$[M–HBr]^{+\bullet}$
4-Br-BaA	$C_{18}H_{11}Br$	61921-39-9	305.9	305.9 → 226.1	305.9 → 305.9	306.0049	226.0783	$[M–HBr]^{+\bullet}$
6,12-Cl_2-Chr	$C_{18}H_{10}Cl_2$	144757-71-1	296.0	296.0 → 226.2	296.0 → 260.1	296.0151	226.0776	$[M–2Cl]^{+\bullet}$
7,12-Cl_2-BaA	$C_{18}H_{10}Cl_2$	63021-10-3	296.0	296.0 → 226.2	296.0 → 260.1	296.0164	226.0782	$[M–2Cl]^{+\bullet}$
Cl_4-Pyr	$C_{16}H_8Br_4$	n/a	340.0	340.0 → 340.0[c]	340.0 → 267.8	339.9187	267.9838	$[M–2H2Cl]^{+\bullet}$
Cl_4-Flu	$C_{16}H_8Br_4$	n/a	340.0	340.0 → 340.0[c]	340.0 → 267.8	339.9185	267.9840	$[M–2H2Cl]^{+\bullet}$
6-Cl-BaP	$C_{20}H_{11}Cl$	21248-01-1	285.9	285.9 → 285.9[c]	285.9 → 249.8	286.0544	250.0776	$[M–2Cl]^{+\bullet}$
7,11-Br_2-BaA	$C_{18}H_{10}Br_2$	1006693-50-0	385.8	385.8 → 226.0	385.8 → 385.8[c]	385.9136	226.0780	$[M–2Br]^{+\bullet}$
2,3,9,10-Br_4-Ant	$C_{14}H_6Cl_4$	82843-47-8	494.1	494.1 → 494.1[c]	173.9 → 173.9[c]	493.7140	174.0459	$[M–4Br]^{+\bullet}$
7,12-Br_2-BaA	$C_{18}H_{10}Br_2$	152678-24-5	385.8	385.8 → 226.0	385.8 → 385.8[c]	385.9135	226.0778	$[M–2Br]^{+\bullet}$
Cl-Per	$C_{20}H_{11}Cl$	n/a	285.9	285.9 → 285.9[c]	285.9 → 249.8	286.0543	250.0775	$[M–2Cl]^{+\bullet}$
4,7-Br_2-BaA	$C_{18}H_{10}Br_2$	94210-35-2	385.8	385.8 → 226.0	385.8 → 385.8[c]	385.9159	226.0780	$[M–2Br]^{+\bullet}$
5,7-Br_2-BaA	$C_{18}H_{10}Br_2$	1006693-52-2	385.8	385.8 → 226.0	385.8 → 385.8[c]	385.9139	226.0781	$[M–2Br]^{+\bullet}$
6-Br-BaP	$C_{20}H_{11}Br$	21248-00-0	332.1	331.9 → 331.9[c]	331.9 → 251.2	332.0029	250.0784	$[M–HBr]^{+\bullet}$

[a] *CAS number= Chemical Abstracts Service registry number*

[b] *$M^{+\bullet}$= m/z of molecular ion*

[c] *pseudo-transition*

Appendix II. Additional Metrics from Chapter 3: Identification of Halogenated Polycyclic Aromatic Hydrocarbons in Biological Samples from Alberta Oil-Sands Region

Table II.1: *List of Chemicals.*

IUPAC Name	Acronym	CAS number[a]	Source	Address
1-chloropyrene	1-Cl-Pyr	34244-14-9	Cambridge Isotope	Tewksbury, MA, USA
7-bromobenz[*a*]anthracene	7-Br-BaA	32795-84-9	Cambridge Isotope	Tewksbury, MA, USA
7-chlorobenz[*a*]anthracene	7-Cl-BaA	20268-52-4	Cambridge Isotope	Tewksbury, MA, USA
9-chloroanthracene	9-Cl-Ant	716-53-0	Cambridge Isotope	Tewksbury, MA, USA
9-chlorophenanthrene	9-Cl-Phe	947-72-8	Cambridge Isotope	Tewksbury, MA, USA
7,12-dichlorobenz[*a*]anthracene	7,12-Cl_2-BaA	63021-10-3	Cambridge Isotope	Tewksbury, MA, USA
1-bromopyrene	1-Br-Pyr	1714-29-0	Sigma Aldrich	St Louis, MO, USA
1-chloroanthracene	1-Cl-Ant	4985-70-0	Sigma Aldrich	St Louis, MO, USA
1,5-dichloroanthracene	1,5-Cl_2-Ant	6406-96-8	Sigma Aldrich	St Louis, MO, USA
2-bromofluorene	2-Br-Fle	1133-80-8	Sigma Aldrich	St Louis, MO, USA
2,7-dibromofluorene	2,7-Br_2-Fle	16433-88-8	Sigma Aldrich	St Louis, MO, USA
3-bromophenanthrene	3-Br-Phe	715-50-4	Sigma Aldrich	St Louis, MO, USA
5-bromoacenaphthene	5-Br-Ana	2051-98-1	Sigma Aldrich	St Louis, MO, USA
9-bromophenanthrene	9-Br-Phe	573-17-1	Sigma Aldrich	St Louis, MO, USA
9,10-dibromoanthracene	9,10-Br_2-Ant	523-27-3	Sigma Aldrich	St Louis, MO, USA
9,10-dibromophenanthrene	9,10-Br_2-Phe	15810-15-8	Sigma Aldrich	St Louis, MO, USA
d_8-naphthalene		1146-65-2	Cambridge Isotope	Tewksbury, MA, USA
d_8-acenaphthylene		93951-97-4	Cambridge Isotope	Tewksbury, MA, USA
d_{10}-acenaphthene		15067-26-2	Cambridge Isotope	Tewksbury, MA, USA
d_{10}-fluorene		81103-79-9	Cambridge Isotope	Tewksbury, MA, USA
d_{10}-phenanthrene		1517-22-2	Cambridge Isotope	Tewksbury, MA, USA
d_{10}-pyrene		1718-52-1	Cambridge Isotope	Tewksbury, MA, USA
d_{12}-benz(*a*)anthracene		1718-53-2	Cambridge Isotope	Tewksbury, MA, USA
d_{12}-chrysene		1719-03-5	Cambridge Isotope	Tewksbury, MA, USA
d_{12}-benzo(*b*)fluoranthene		93951-98-5	Cambridge Isotope	Tewksbury, MA, USA

d_{12}-benzo(*k*)fluoranthene	93952-01-3	Cambridge Isotope	Tewksbury, MA, USA
d_{12}-benzo(*a*)pyrene	63466-71-7	Cambridge Isotope	Tewksbury, MA, USA
d_{12}-indeno(1,2,3-*c,d*) pyrene	203578-33-0	Cambridge Isotope	Tewksbury, MA, USA
d_{14}-dibenz(*a,h*)anthracene	13250-98-1	Cambridge Isotope	Tewksbury, MA, USA
d_{12}-benzo(*g,h,i*)perylene	93951-66-7	Cambridge Isotope	Tewksbury, MA, USA
Naphthalene	91-20-3	Cambridge Isotope	Tewksbury, MA, USA
Acenaphthylene	208-96-8	Cambridge Isotope	Tewksbury, MA, USA
Acenaphthene	83-32-9	Cambridge Isotope	Tewksbury, MA, USA
Fluorene	86-73-7	Cambridge Isotope	Tewksbury, MA, USA
Phenanthrene	85-01-8	Cambridge Isotope	Tewksbury, MA, USA
Pyrene	129-00-0	Cambridge Isotope	Tewksbury, MA, USA
Benz(*a*)anthracene	56-55-3	Cambridge Isotope	Tewksbury, MA, USA
Chrysene	218-01-9	Cambridge Isotope	Tewksbury, MA, USA
Benzo(*b*)fluoranthene	205-99-2	Cambridge Isotope	Tewksbury, MA, USA
Benzo(*k*)fluoranthene	207-08-9	Cambridge Isotope	Tewksbury, MA, USA
Benzo(*a*)pyrene	50-32-8	Cambridge Isotope	Tewksbury, MA, USA
Indeno(1,2,3-*c,d*) pyrene	193-39-5	Cambridge Isotope	Tewksbury, MA, USA
Dibenz(*a,h*)anthracene	53-70-3	Cambridge Isotope	Tewksbury, MA, USA
Benzo(*g,h,i*)perylene	191-24-2	Cambridge Isotope	Tewksbury, MA, USA

[a] *CAS number = Chemical Abstracts Service registry number*

Table II.2: *Nomenclature, EI fragmentation patterns, mean measured experimental mass and mean mass accuracy of the HPAC standards determined using GC-HRTOF-MS.*

Compound	r_t (min)[a]	Fragmentation pattern	DBE[b]	Theoretical mass (amu)[c]	Mean measured mass ± SD (amu)[d]	Mean mass accuracy ± SD (ppm)
5-Br-Ana	12.21	$M^{+\bullet}$	8	231.9882	231.9880±0.0001	-0.8046±0.3193
$C_{12}H_9Br$		$[M–Br]^+$		**153.0699**	153.0698±0.0001	-0.6141±0.2697
2-Br-Fle	13.49	$M^{+\bullet}$	9	243.9882	243.9879±0.0001	-1.3935±0.5249
$C_{13}H_9Br$		$[M–Br]^+$		**165.0699**	165.0698±0.0001	-0.6179±0.1931
9-Cl-Phe	14.88	$M^{+\bullet}$	10	212.0387	212.0386±0.0001	-0.6052±0.4402
$C_{14}H_9Cl$		$[M–HBr]^{+\bullet}$		176.0621	176.0620±0.0001	-0.1515±0.5212
1-Cl-Ant	15.04	$M^{+\bullet}$	10	**212.0387**	212.0386±0.0001	-0.5502±0.4075
$C_{14}H_9Cl$		$[M–HCl]^{+\bullet}$		176.0621	176.0620±0.0001	-0.1515±0.4827
9-Cl-Ant	15.20	$M^{+\bullet}$	10	**212.0387**	212.0386±0.0001	-0.7310±0.4380
$C_{14}H_9Cl$		$[M–HCl]^{+\bullet}$		176.0621	176.0620±0.0001	-0.3029±0.2757
3-Br-Phe	16.63	$M^{+\bullet}$	10	255.9882	255.9880±0.0001	-0.7617±0.5278
$C_{14}H_9Br$		$[M–HBr]^{+\bullet}$		**176.0621**	176.0620±0.0001	-0.0568±0.3921
9-Br-Phe	16.90	$M^{+\bullet}$	10	255.9882	255.9880±0.0001	-0.7292±0.4217
$C_{14}H_9Br$		$[M–HBr]^{+\bullet}$		**176.0621**	176.0620±0.0001	-0.3029±0.1958
1,5-Cl_2-Ant	18.68	$M^{+\bullet}$	10	**245.9998**	245.9995±0.0001	-0.9350±0.3736
$C_{14}H_8Cl_2$		$[M–2Cl]^{+\bullet}$		176.0621	176.0619±0.0001	-0.8520±0.2927
2,7-Br_2-Fle	19.76	$M^{+\bullet}$	9	321.8987	323.8962±0.0002	-1.7289±0.5996
$C_{13}H_8Br_2$		$[M–Br-HBr]^+$		**163.0542**	163.0542±0.0001	0.1838±0.2877
1-Cl-Pyr	21.63	$M^{+\bullet}$	12	**236.0387**	236.0386±0.0001	-0.6849±0.5021
$C_{16}H_9Cl$		$[M–Cl]^+$		201.0699	201.0698±0.0001	-1.8319±0.2287
9,10-Br_2-Ant	23.59	$M^{+\bullet}$	10	335.8967	335.8964±0.0001	-1.1015±0.2909
$C_{14}H_8Br_2$		$[M–2Br]^{+\bullet}$		**176.0621**	176.0621±0.0001	0.2130±0.2389
9,10-Br_2-Phe	23.86	$M^{+\bullet}$	10	335.8967	335.8964±0.0001	-1.1015±0.2909
$C_{14}H_8Br_2$		$[M–2Br]^{+\bullet}$		**176.0621**	176.0621±0.0001	0.2130±0.2389
1-Br-Pyr	23.87	$M^{+\bullet}$	12	279.9882	279.9880±0.0002	1.0715±0.7805
$C_{16}H_9Br$		$[M–Br]^+$		**201.0699**	201.0696±0.0001	-1.2433±0.4813

7-Cl-BaA	26.99	$M^{+\bullet}$	13	**262.0544**	262.0541±0.0002	-0.9387±0.4739
$C_{18}H_{11}Cl$		$[M–HCl]^{+\bullet}$		226.0777	226.0778±0.0001	0.4512±0.1910
7-Br-BaA	28.60	$M^{+\bullet}$	13	306.0039	306.0034±0.0001	-1.3970±0.4726
$C_{18}H_{11}Br$		$[M–HBr]^{+\bullet}$		**226.0777**	226.0776±0.0002	-0.5861±0.7284
7,12-Cl_2-BaA	29.53	$M^{+\bullet}$	13	**296.0154**	296.0150±0.0006	-1.5033±1.9878
$C_{18}H_{10}Cl_2$		$[M–2Cl]^{+\bullet}$		226.0777	226.0777±0.0002	-0.0111±0.4327

[a] *r_t = retention times (min)*
[b] *DBE=double bond equivalence*
[c] *m/z value in bold signifies the base peak in the EI full scan mass spectra*
[d] *mean and standard deviation (SD) is of 5 replicate injections of the standard.*

Appendix III. Additional Metrics from Chapter 4: New Approaches to Reduce Sample Processing Times for the Determination of Polycyclic Aromatic Compounds in Environmental Samples

***Table III.1**: List of Chemicals.*

IUPAC Name	Acronym	CAS number[a]	Source	Address
1,2-dibromoacenaphthylene	1,2-Br_2-Any	13019-33-5	Sigma Aldrich	St Louis, MO, USA
1,4-dichloroanthracene	1,4-Cl_2-Ant	66259-12-9	Sigma Aldrich	St Louis, MO, USA
1,5,9,10-tetrachloroanthracene	1,5,9,10-Cl_4-Ant	82843-47-8	Sigma Aldrich	St Louis, MO, USA
1,5-dichloroanthracene	1,5-Cl_2-Ant	6406-96-8	Sigma Aldrich	St Louis, MO, USA
1,6-dibromopyrene	1,6-Br_2-Pyr	27973-29-1	Matrix Scientific	Columbia, SC, USA
1,8-dibromoanthracene	1,8-Br_2-Ant	131276-24-9	Tokyo Chemical Industries	Tokyo, Japan
1-bromopyrene	1-Br-Pyr	1714-29-0	Sigma Aldrich	St Louis, MO, USA
1-chloroanthracene	1-Cl-Ant	4985-70-0	Sigma Aldrich	St Louis, MO, USA
1-chloropyrene	1-Cl-Pyr	34244-14-9	Cambridge Isotope	Tewksbury, MA, USA
2,3,9,10-tetrabromoanthracene	2,3,9,10-Br_4-Ant	82843-47-8	Sigma Aldrich	St Louis, MO, USA
2,7-dibromofluorene	2,7-Br_2-Fle	16433-88-8	Sigma Aldrich	St Louis, MO, USA
2,7-dibromophenanthrene	2,7-Br_2-Phe	62325-30-8	Tokyo Chemical Industries	Tokyo, Japan
2,7-dichlorofluorene	2.7-Cl_2-Fle	7012-16-0	Toronto Research Chemicals	Toronto, ON, Canada
2-bromophenanthrene	2-Br-Ant	7321-27-9	Tokyo Chemical Industries	Tokyo, Japan
2-bromofluorene	2-Br-Fle	1133-80-8	Sigma Aldrich	St Louis, MO, USA
2-chlorofluorene	2-Cl-Fle	2523-44-6	Matrix Scientific	Columbia, SC, USA
3-bromofluoranthene	3-Br-Flu	13438-50-1	Tokyo Chemical Industries	Tokyo, Japan
3-bromophenanthrene	3-Br-Phe	715-50-4	Sigma Aldrich	St Louis, MO, USA
4-bromobenz[*a*]anthracene	4-Br-BaA	61921-39-9	Tokyo Chemical Industries	Tokyo, Japan
4-bromopyrene	4-Br-Pyr	1732-26-9	Tokyo Chemical Industries	Tokyo, Japan
5,6-dibromo-1,2-dihydroacenaphthylene	5,6-Br_2-Ana	19190-91-1	Matrix Scientific	Columbia, SC, USA
5-bromoacenaphthene	5-Br-Ana	2051-98-1	Sigma Aldrich	St Louis, MO, USA
7,12-dichlorobenz[*a*]anthracene	7,12-Cl_2-BaA	63021-10-3	Cambridge Isotope	Tewksbury, MA, USA
7-chlorobenz[*a*]anthracene	7-Cl-BaA	20268-52-4	Cambridge Isotope	Tewksbury, MA, USA
9,10-dibromophenanthrene	9,10-Br_2-Phe	15810-15-8	Sigma Aldrich	St Louis, MO, USA
9-bromo-1,5-dichloroanthracene	9-Br-1,5-Cl_2-Ant	201406-34-0	Sigma Aldrich	St Louis, MO, USA
9-bromophenanthrene	9-Br-Phe	573-17-1	Sigma Aldrich	St Louis, MO, USA
9-Chloroanthracene	9-Cl-Ant	716-53-0	Cambridge Isotope	Tewksbury, MA, USA

9-Chlorophenanthrene	9-Cl-Phe	947-72-8	Cambridge Isotope	Tewksbury, MA, USA
d_8-naphthalene		1146-65-2	Cambridge Isotope	Tewksbury, MA, USA
d_8-acenaphthylene		93951-97-4	Cambridge Isotope	Tewksbury, MA, USA
d_{10}-acenaphthene		15067-26-2	Cambridge Isotope	Tewksbury, MA, USA
d_{10}-fluorene		81103-79-9	Cambridge Isotope	Tewksbury, MA, USA
d_{10}-phenanthrene		1517-22-2	Cambridge Isotope	Tewksbury, MA, USA
d_{10}-fluoranthene		206-44-0	Cambridge Isotope	Tewksbury, MA, USA
d_{10}-pyrene		1718-52-1	Cambridge Isotope	Tewksbury, MA, USA
d_{12}-benz(*a*)anthracene		1718-53-2	Cambridge Isotope	Tewksbury, MA, USA
d_{12}-chrysene		1719-03-5	Cambridge Isotope	Tewksbury, MA, USA
d_{12}-benzo(*b*)fluoranthene		93951-98-5	Cambridge Isotope	Tewksbury, MA, USA
d_{12}-benzo(*k*)fluoranthene		93952-01-3	Cambridge Isotope	Tewksbury, MA, USA
d_{12}-benzo(*a*)pyrene		63466-71-7	Cambridge Isotope	Tewksbury, MA, USA
d_{12}-indeno(1,2,3-*c,d*) pyrene		203578-33-0	Cambridge Isotope	Tewksbury, MA, USA
d_{14}-dibenz(*a,h*)anthracene		13250-98-1	Cambridge Isotope	Tewksbury, MA, USA
d_{12}-benzo(*g,h,i*)perylene		93951-66-7	Cambridge Isotope	Tewksbury, MA, USA
Naphthalene		91-20-3	Accustandard Inc.	New Haven, CT, USA
Acenaphthylene		208-96-8	Accustandard Inc.	New Haven, CT, USA
Acenaphthene		83-32-9	Accustandard Inc.	New Haven, CT, USA
Fluorene		86-73-7	Accustandard Inc.	New Haven, CT, USA
Phenanthrene		85-01-8	Accustandard Inc.	New Haven, CT, USA
Anthracene		120-12-7	Accustandard Inc.	New Haven, CT, USA
Fluoranthene		206-44-0	Accustandard Inc.	New Haven, CT, USA
Pyrene		129-00-0	Accustandard Inc.	New Haven, CT, USA
Benz(*a*)anthracene		56-55-3	Accustandard Inc.	New Haven, CT, USA
Chrysene		218-01-9	Accustandard Inc.	New Haven, CT, USA
Benzo(*b*)fluoranthene		205-99-2	Accustandard Inc.	New Haven, CT, USA
Benzo(*k*)fluoranthene		207-08-9	Accustandard Inc.	New Haven, CT, USA
Benzo(*a*)pyrene		50-32-8	Accustandard Inc.	New Haven, CT, USA
Indeno(1,2,3-*c,d*) pyrene		193-39-5	Accustandard Inc.	New Haven, CT, USA
Dibenz(*a,h*)anthracene		53-70-3	Accustandard Inc.	New Haven, CT, USA

Benzo(*g,h,i*)perylene	191-24-2	Accustandard Inc.	New Haven, CT, USA
d_{10}-anthracene	120-12-7	Accustandard Inc.	New Haven, CT, USA
1,2,5,6-tetramethylnaphthalene	2131-43-3	Chiron AS	Trondheim, Norway
1,2,6,9-tetramethylphenanthrene	204256-39-3	Chiron AS	Trondheim, Norway
1,2,6-trimethylphenanthrene	30436-55-6	Chiron AS	Trondheim, Norway
1,3,6-trimethylchrysene	1586755-28-3	Chiron AS	Trondheim, Norway
1,3-dimethylphenanthrene	16664-45-2	Chiron AS	Trondheim, Norway
1,4,6,7-tetramethylnaphthalene	13764-18-6	Chiron AS	Trondheim, Norway
1,6-dimethylnaphthalene	575-43-9	Chiron AS	Trondheim, Norway
1,7-dimethylfluorene	442-66-0	Chiron AS	Trondheim, Norway
1,7-dimethylphenanthrene	483-87-4	Chiron AS	Trondheim, Norway
1,8-dimethylphenanthrene	7372-87-4	Chiron AS	Trondheim, Norway
1-methylfluorene	1730-37-6	Chiron AS	Trondheim, Norway
1-methylnaphthalene	90-12-0	AccuStandard Inc.	New Haven, CT, USA
1-methylphenanthrene	832-69-9	AccuStandard Inc.	New Haven, CT, USA
1-methylpyrene	2381-21-7	Chiron AS	Trondheim, Norway
2,3,5-trimethylnaphthalene	2245-38-7	Chiron AS	Trondheim, Norway
2,6-dimethylnaphthalene	581-42-0	AccuStandard Inc.	New Haven, CT, USA
2-methylchrysene	3351-32-4	Chiron AS	Trondheim, Norway
2-methylnaphthalene	91-57-6	AccuStandard Inc.	New Haven, CT, USA
2-methylphenanthrene	2531-84-2	AccuStandard Inc.	New Haven, CT, USA
3,6-mimethylphenanthrene	1576-67-6	Chiron AS	Trondheim, Norway
3-methylphenanthrene	832-71-3	Chiron AS	Trondheim, Norway
4,5-dimethylpyrene	15679-25-1	Chiron AS	Trondheim, Norway
4-methylpyrene	3353-12-06	Chiron AS	Trondheim, Norway
7-methylbenzo[*a*]pyrene	63041-77-0	Chiron AS	Trondheim, Norway
9-methylphenanthrene	883-20-5	Chiron AS	Trondheim, Norway
1-methyl-7-isopropylphenanthrene (Retene)	483-65-8	Chiron AS	Trondheim, Norway

[a] *CAS number = Chemical Abstracts Service registry number*

Table III.2: *Mass spectrometric parameters for HPAH analysis: MS/MS ion transitions and collision energy (CE).*

Acronym	Quantification transition	Confirmation transition	Confirmation transition 2	CE (eV)
1,2-Br_2-Any	310.0→149.9	310.0→228.9		25
1,4-Cl_2-Ant	246.0→176.1	246.0→211.2	246.0→246.0	25
1,5,9,10-Cl_4-Ant	316.0→316.0	316.0→244.0	316.0→280.9	25
1,5-Cl_2-Ant	246.0→175.9	246.0→210.0	246.0→246.0	25
1,6-Br_2-Pyr	360.1→200.0	360.1→279.0		25
1,8-Br_2-Ant	335.9→176.0	335.9→256.9		25
1-Br-Pyr	279.9→201.0	279.9→279.9		25
1-Cl-Ant	212.0→176.0	212.0→151.0	212.0→212.0	25
1-Cl-Pyr	236.0→201.1	236.0→236.0	236.0→200.1	15
2,3,9,10-Br_4-Ant	494.1→494.1	173.9→173.9		15
2,7-Br_2-Fle	324.0→243.0	324.0→163.9	324.0→324.0	15
2,7-Br_2-Phe	335.9→176.0	335.9→256.9		25
2.7-Cl_2-Fle	234.0→199.1	234.0→234.0		15
2-Br-Ant	256.0→177.1	256.0→151.1	256.0→256.0	15
2-Br-Fle	165.0→165.0	165.0→164.0	243.9→165.0	5
2-Cl-Fle	164.9→164.9	199.9→165.0		5
3-Br-Flu	279.9→200.1	279.9→279.9		25
3-Br-Phe	256.0→177.1	256.0→151.1	256.0→256.0	15
4-Br-BaA	308.0→225.9	308.0→308.0		25
4-Br-Pyr	279.9→200.1	279.9→279.9		25
5,6-Br_2-Ana	312.1→152.1	312.1→233.1	312.1→312.1	15
5-Br-Ana	234.1→153.1	234.1→234.1	153.1→153.1	25
7,12-Cl_2-BaA	296.0→226.2	296.0→260.1	296.0→296.0	25
7-Cl-BaA	261.9→226.1	262.0→262.0		25

9,10-Br_2-Phe	335.9→176.0	335.9→256.9		25
9-Br-1,5-Cl_2-Ant	326.0→209.9	326.0→244.8	326.0→326.0	15
9-Br-Phe	256.0→177.1	256.0→151.1	256.0→256.0	15
9-Cl-Ant	212.0→176.0	212.0→151.0	212.0→212.0	25
9-Cl-Phe	212.0→176.0	212.0→151.0	212.0→212.0	25

Appendix IV. Additional Metrics from Chapter 5: Microbead Beating Extraction of Avian Eggs for Polycyclic Aromatic Compounds

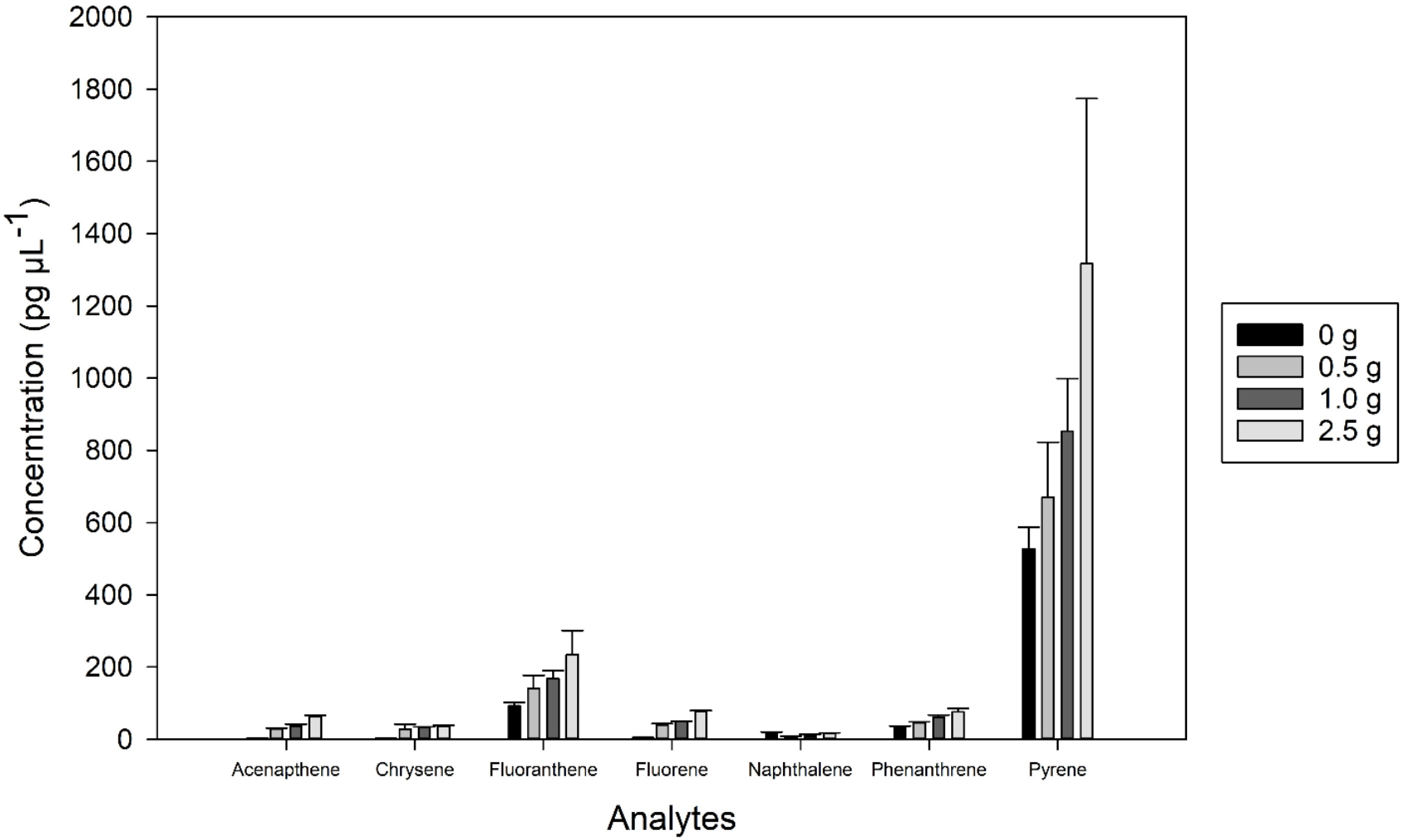

Figure IV.1: *Mean concentrations ± standard deviation (pg μL^{-1}, n = 3) of selected PAHs in method blanks using different weights of microbeads in our high-density tubes.*

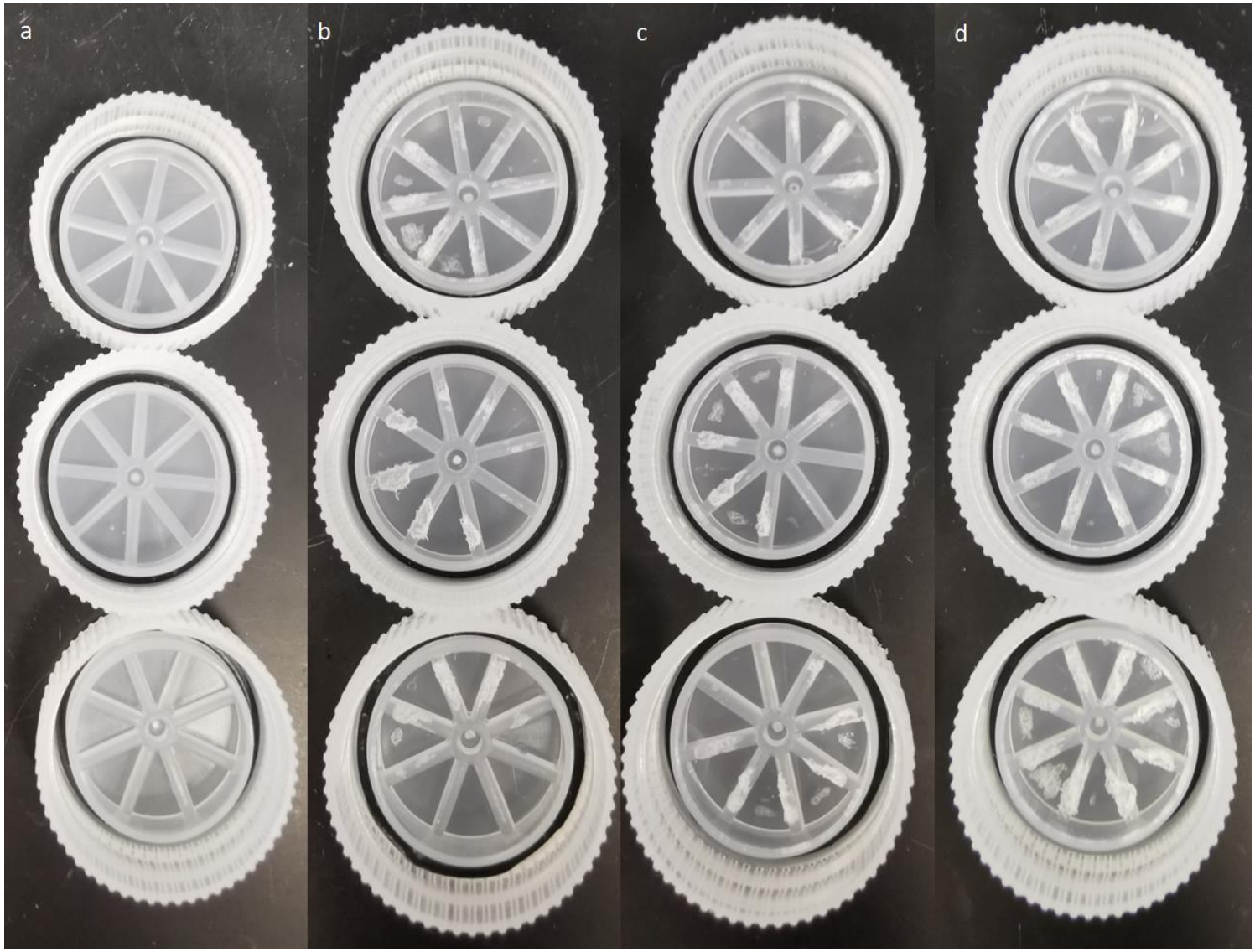

Figure IV.2: *The effect of microbead weight (a: 0g, b: 0.5g, c: 1.0g and d: 2.5g) on the integrity of lids. Note the white color on some of the lids indicating the indentation caused by impact of microbeads on the lids.*

Appendix V. Additional Metrics from Chapter 6: Comparison of Different Approaches to Quantify Substituted Polycyclic Aromatic Compounds

Table V.1: *List of individual substituted PACs.*

No.	Individual Substituted Alkylated PACs
1	1-methylnaphthalene
2	2-methylnaphthalene
3	2,6-dimethylnaphthalene
4	2,3,5-trimethylnaphthalene
5	1,4,6,7-tetramethylnaphthalene
6	1-methylfluorene
7	1,7-dimethylfluorene
8	5-methylchrysene
9	1,3,6-trimethylchrysene
10	1-methylpyrene
11	4-methylpyrene
12	4,5-dimethylpyrene
13	1-methylphenanthrene
14	2-methylphenanthrene
15	3-methylphenanthrene
16	9-methylphenanthrene
17	1,3-dimethylphenanthrene
18	2,6-dimethylphenanthrene
19	1,7-dimethylphenanthrene
20	1,8-dimethylphenanthrene
21	3,6-dimethylphenanthrene
22	1,2,6-trimethylphenanthrene
23	1,2,6,9-tetramethylphenanthrene
24	7-methylbenzo(*a*)pyrene
25	dibenzothiophene
26	4-methyldibenzothiophene
27	2,8-dimethyldibenzothiophene
28	2,4,7-trimethyldibenzothiophene

Table V.2: *Substituted PACs used as external standards.*

Compound Cluster	Quantifying Compound
C_1-Naphthalenes	1-methylnaphthalene/2-methylnaphthalene
C_2-Naphthalenes	2,6-dimethylnaphthalene
C_3-Naphthalenes	2,3,5-trimethylnaphthalene
C_4-Naphthalenes	1,4,6,7-tetramethylnaphthalene
C_1-Fluorenes	1-methylfluorene
C_2;C_3-Fluorenes	1,7-dimethylfluorene
C_1-Chrysene/C_2-Benzanthracenes/Chrysenes/Triphenylenes	5-methylchrysene
C_3-;C_4-Benzanthracenes/Chrysenes/Triphenylenes	1,3,6-trimethylchrysene
C_1-Pyrenes/Fluoranthenes	4-methylpyrene
C_2-;C_3-;C_4-Pyrenes/Fluoranthenes	4,5-dimethylpyrene
C_1-Phenanthrenes/Anthracenes	3-methylphenanthrene
C_2-Phenanthrenes/Anthracenes	1,3-dimethylphenanthrene
C_3-Phenanthrene/Anthracenes	1,2,6-trimethylphenanthrene
C_4-Phenanthrenes/Anthracenes	1,2,6,9-tetramethylphenanthrene
C_1-;C_2-Benzo(*a*)pyrenes	7-methylbenzo(*a*)pyrene
C_1-Dibenzothiophenes	4-methyl dibenzothiophene
C_2-Dibenzothiophenes	2,8-dimethyldibenzothiophene
C_3-;C_4-Dibenzothiophenes	2,4,7-trimethyldibenzothiophene

Table V.3: *Average relative response factor (ARRF) for 16 USEPA PAHs in our standards that was used to quantify the 3 SRMs.*

Analytes	**SRM 1944[1]**	**SRM 1597a[2]**	**SRM 2779[3]**
Acenaphthene	1.17	1.18	1.02
Acenaphthylene	1.05	1.50	1.02
Anthracene	1.62	2.11	1.47
Benz(*a*)anthracene	1.50	1.35	1.42
Benzo(*a*)pyrene	1.57	1.63	1.69
Benzo(*b*)fluoranthene	1.54	1.76	1.62
Benzo(*g,h,i*)perylene	1.50	1.48	1.50
Benzo(*k*)fluoranthene	1.53	1.81	1.28
Chrysene	1.52	1.47	1.17
Dibenz(*a,h*)anthracene	1.21	1.22	1.17
Fluoranthene	1.68	1.51	1.47
Fluorene	0.50	0.78	0.51
Indeno(1,2,3-*c,d*)pyrene	1.28	1.21	1.24
Naphthalene	0.42	0.31	0.36
Phenanthrene	1.75	2.12	1.65
Pyrene	1.97	1.80	1.70

[1] These are the ARRFs from our standard that was used to quantify SRM 1944.
[2] These are the ARRFs from our standard that was used to quantify SRM 1597a.
[3] These are the ARRFs from our standard that was used to quantify SRM 2779.

Table V.4: *Closest eluting mass labeled PAHs for each group of substituted PACs.*

Substituted PAH cluster	Deuterated PAH
C_1-Naphthalene	d_{10}-Naphthalene
C_2-Naphthalene	d_{10}-Acenapthylene
C_3-Naphthalene	d_{10}-Phenanthrene
C_4-Naphthalene	d_{10}-Phenanthrene
C_1-Phenanthrene	d_{10}-Phenanthrene
C_2-Phenanthrene	d_{10}-Fluoranthene
C_3-Phenanthrene	d_{10}-Pyrene
C_4-Phenanthrene	d_{10}-Pyrene
C_1-Dibenzothiophenes	d_{10}-Phenanthrene
C_2-Dibenzothiophenes	d_{10}-Fluoranthene
C_3-Dibenzothiophenes	d_{10}-Pyrene
C_4-Dibenzothiophenes	d_{10}-Pyrene
C_1-Fluoranthenes/Pyrenes	d_{10}-Pyrene
C_2-Fluoranthenes/Pyrenes	d_{12}-Benz(*a*)anthracene
C_3-Fluoranthenes/Pyrenes	d_{12}-Benz(*a*)anthracene
C_4-Fluoranthenes/Pyrenes	d_{12}-Benz(*a*)anthracene
C_1-Benzanthracenes/Chrysenes/Triphenylenes	d_{12}-Chrysene
C_2-Benzanthracenes/Chrysenes/Triphenylenes	d_{12}-Chrysene
C_3-Benzanthracenes/Chrysenes/Triphenylenes	d_{10}-Benzo(*a*)pyrene
C_4-Benzanthracenes/Chrysenes/Triphenylenes	d_{10}-Benzo(*a*)pyrene
C_1-Fluorenes	d_{10}-Fluorene
C_2-Fluorenes	d_{10}-Phenanthrene
C_3-Fluorenes	d_{10}-Phenanthrene
C_1-Benzo(*a*)pyrene	d_{12}-Benzo(*a*)pyrene
C_2-Benzo(*a*)pyrene	d_{12}-Benzo(*a*)pyrene

Table V.5: *Average relative response factor (ARRF) for substituted PACs in our standards that was used to quantify the 3 SRMs.*

Analytes	Average relative response factor (Method 2&3)			Average relative response factor (Method 4)		
	SRM 1944[1]	**SRM 1597a[2]**	**SRM 2779[3]**	**SRM 1944[1]**	**SRM 1597a[2]**	**SRM 2779[3]**
2-methylnaphthalene	1.43	1.21	1.37	0.58	0.46	0.75
1-methylnaphthalene	1.31	1.10	1.20	0.53	0.42	0.66
3-methylphenanthrene	0.97	1.04	1.11	0.65	0.52	0.65
2-methylphenanthrene	0.87	1.10	1.12	0.65	0.55	0.66
9- & 4-methylphenanthrene	1.09	1.07	1.14	0.72	0.54	0.67
1-methylphenanthrene	1.03	1.07	1.10	0.68	0.53	0.65
2,6-dimethylphenanthrene	0.91	0.84	-	0.60	0.41	-
1,7-dimethylphenanthrene	0.49	0.57	0.59	0.33	0.28	0.34
1,8-dimethylphenanthrene	0.47	0.49	-	0.31	0.24	-
4-methylpyrene	1.54	1.77	1.90	0.34	0.43	0.58
1-methylpyrene	1.42	1.69	1.82	0.32	0.41	0.56
2,6-dimethylnaphthalene	-	0.84	-	-	0.32	-
Dibenzothiophene	-	1.02	1.16	-	0.25	0.35
4-methyldibenzothiophene	-	2.48	-	-	0.60	-
Benzo[*b*]naphtho[1,2-*d*]thiophene	-	1.02	-	-	0.29	-
Benzo[*b*]naphtho[2,3-*d*]thiophene	-	1.16	-	-	0.33	-
C_2-Naphthalenes (2,6-dimethylnaphthalene)	-	-	0.91	-	-	0.50
C_3-Naphthalenes (2,3,5-trimethylnaphthalene)	-	-	0.82	-	-	0.44
C_4-Naphthalenes (1,2,5,6-tetramethylnaphtalene)	-	-	0.79	-	-	0.43
C_1-Phenanthrenes/Anthracenes (3-methylphenanthrene)	-	-	1.11	-	-	0.65
C_2-Phenanthrenes/Anthracenes (1,3-dimethylphenanthrene)	-	-	0.76	-	-	0.45

C_3-Phenanthrenes/Anthracenes (1,2,6-trimethylphenanthrene)	-	-	1.23	-	-	0.71
C_4-Phenanthrenes/Anthracenes (1,2,6,9-tetramethylphenanthrene)	-	-	0.53	-	-	0.31
C_1-Benzanthracenes/Chrysenes/Triphenylenes (6-methylchrysene)	-	-	0.04	-	-	0.01
C_2-Benzanthracenes/Chrysenes/Triphenylenes (6-methylchrysene)	-	-	0.12	-	-	0.12
C_3-Benzanthracenes/Chrysenes/Triphenylenes (1,3,6-trimethylchrysene)	-	-	0.19	-	-	0.04
C_4-Benzanthracenes/Chrysenes/Triphenylenes (1,3,6-trimethylchrysene)	-	-	0.19	-	-	0.04
C_1-Fluorenes (1-methylfluorene)	-	-	1.82	-	-	0.53
C_2-Fluorenes (1,7-dimethylfluorene)	-	-	1.36	-	-	0.42
C_3-Fluorenes (1.7-dimethylfluorene)	-	-	1.36	-	-	0.42
C_1-Dibenzothiophenes (4-methyldibenzothiophene)	-	-	2.73	-	-	0.84
C_2-Dibenzothiophenes (2,8-dimethyldibenzothiophene)	-	-	0.76	-	-	0.22
C_3-Dibenzothiophenes (2,4,7-trimethyldibenzothiophene)	-	-	1.43	-	-	0.44
C_4-Dibenzothiophenes (2,4,7-trimethyldibenzothiophene)	-	-	1.43	-	-	0.44
C_1-Fluoranthenes/Pyrenes (4-methylpyrene)	-	-	1.90	-	-	0.58
C_2-Fluoranthenes/Pyrenes (4,5-dimethylpyrene)	-	-	1.23	-	-	0.38
C_3-Fluoranthenes/Pyrenes (4,5-dimethylpyrene)	-	-	1.23	-	-	0.38

C_4-Fluoranthenes/Pyrenes (4,5-dimethylpyrene)	-	-	1.23	-	-	0.38

[1] *These are the ARRFs from our standard that was used to quantify SRM 1944.*
[2] *These are the ARRFs from our standard that was used to quantify SRM 1597a.*
[3] *These are the ARRFs from our standard that was used to quantify SRM 2779.*

Table V.6: *Certified and measured concentrations (mean ± standard deviation, mg/kg) of 16 US-EPA PAHs in 3 standard reference materials determined using the isotope dilution approach to quantitation and GC/MS/MS using multiple reaction monitoring for detection.*

Analytes	Certified/ Reference Concentrations (mg/kg)			Measured Concentrations (mg/kg)		
	SRM 1944	SRM 1597a	SRM 2779	SRM 1944[1] (%) [c]	SRM 1597a[2] (%) [c]	SRM 2779[3] (%) [c]
Acenaphthene	0.39 ± 0.03 [b]	7.63 ± 0.26 [a]	n/a	0.31 ± 0.03 (-19.4%)	7.76 ± 0.39 (1.6%)	22.33 ± 0.79 (n/a)
Acenaphthylene	n/a	263.00 ± 7.00 [a]	8.09 ± 0.10 [b]	0.86 ± 0.05 (n/a)	221.27 ± 15.78 (-15.9%)	12.39 ± 0.59 (53.2%)
Anthracene	1.13 ± 0.07 [b]	107.00 ± 3.00 [a]	3.42 ± 0.59 [a]	1.28 ± 0.05 (13.5%)	102.03 ± 5.83 (-4.6%)	4.75 ± 0.10 (39.0%)
Benz(*a*)anthracene	4.72 ± 0.11 [a]	98.10 ± 2.30 [a]	7.03 ± 0.85 [a]	3.68 ± 0.18 (-22.1%)	78.27 ± 6.45 (-20.2%)	7.15 ± 0.83 (1.7%)
Benzo(*a*)pyrene	4.30 ± 0.13 [a]	93.50 ± 1.40 [a]	1.36 ± 0.35 [b]	3.45 ± 0.23 (-19.9%)	69.98 ± 2.65 (-25.2%)	1.26 ± 0.20 (-7.6%)
Benzo(*b*)fluoranthene	3.87 ± 0.42 [a]	66.10 ± 4.40 [a]	5.62 ± 0.34 [a]	3.40 ± 0.32 (-12.1%)	52.64 ± 3.80 (-20.4%)	4.97 ± 0.72 (-11.6%)
Benzo(*g,h,i*)perylene	2.84 ± 0.10 [a]	50.5 ± 0.60 [a]	2.11 ± 0.26 [a]	2.59 ± 0.15 (-8.8%)	41.71 ± 1.85 (-17.4%)	2.07 ± 0.29 (-1.8%)
Benzo(*k*)fluoranthene	2.30 ± 0.20 [a]	41.20 ± 0.40 [a]	0.66 ± 0.28 [b]	2.34 ± 0.16 (1.6%)	38.09 ± 3.76 (-7.6%)	0.61 ± 0.09 (-7.2%)
Chrysene	4.87 ± 0.10 [a]	66.20 ± 5.30 [a]	47.40 ± 1.70 [a]	5.22 ± 0.33 (7.4%)	70.64 ± 3.72 (6.7%)	53.69 ± 2.73 (13.3%)
Dibenz(*a,h*)anthracene	0.424 ± 0.069 [a]	6.93 ± 0.40 [a]	0.574 ± 0.091 [a]	0.79 ± 0.04 (85.9%)	8.13 ± 0.62 (17.3%)	1.77 ± 0.11 (208%)
Fluoranthene	8.92 ± 0.32 [a]	327.00 ± 7.00 [a]	4.36 ± 0.40 [a]	7.99 ± 0.30 (-10.4%)	273.83 ± 18.74 (-16.3%)	3.45 ± 0.39 (-20.8%)
Fluorene	0.48 ± 0.04 [b]	145.00 ± 4.00 [a]	145.00 ± 43 [b]	0.39 ± 0.02 (-17.9%)	118.58 ± 7.66 (-18.2%)	144.48 ± 1.30 (0.4%)
Indeno(1,2,3-*c,d*)pyrene	2.78 ± 0.10 [a]	55.50 ± 0.80 [a]	0.48 ± 0.14 [b]	2.62 ± 0.12 (-5.8%)	41.09 ± 3.08 (-26.0%)	0.26 ± 0.08 (-45.7%)

Naphthalene	1.28 ± 0.04 [b]	1030.00 ± 100 [a]	855.00 ± 46.00 [a]	1.71 ± 0.05 (33.8%)	908.16 ± 114.73 (-11.8%)	879.21 ± 23.21 (2.8%)
Phenanthrene	5.27 ± 0.22 [a]	454.00 ± 7.00 [a]	258.00 ± 27.00 [a]	5.92 ± 0.23 (12.3%)	396.86 ± 25.01 (-12.6%)	273.75 ± 11.36 (6.1%)
Pyrene	9.70 ± 0.42 [a]	240.00 ± 7.00 [a]	14.81 ± 0.39 [a]	8.19 ± 0.36 (-15.6%)	204.63 ± 16.16 (-14.7%)	10.13 ± 1.04 (-31.6%)

[1] *Seven replicate measurements*
[2] *Three replicate measurements*
[3] *Three replicate measurements*
[a] *Certified Mass Fractions*
[b] *Reference Mass Fraction*
[c] *Percentage difference*

Table V.7: *Certified and measured concentrations (mean ± standard deviation, mg/kg) of APAHs in 3 standard reference materials determined using the external calibration approach to (**Method 1**) quantitation and GC/MS/MS using multiple reaction monitoring for detection.*

Analytes	Certified/Reference Concentrations (mg/kg)			Measured Concentrations (mg/kg)		
	SRM 1944	SRM 1597a	SRM 2779	SRM 1944[1] (%) [c]	SRM 1597a[2] (%) [c]	SRM 2779[3] (%) [c]
2-methylnaphthalene	0.74 ± 0.06 [b]	95.00 ± 2.90 [b]	1630.00 ± 50.00 [a]	0.88 ± 0.07 (18.7%)	128.36 ± 7.80 (35.1%)	1740.39 ± 139.07 (6.8%)
1-methylnaphthalene	0.47 ± 0.02 [b]	43.90 ± 1.80 [b]	1140.00 ± 20.00 [a]	0.46 ± 0.02 (-3.0%)	42.77 ± 3.18 (-2.6%)	1211.39 ± 93.23 (6.3%)
3-methylphenanthrene	2.10 ± 0.10 [b]	15.80 ± 0.80 [a]	206.00 ± 32.00 [a]	2.23 ± 0.16 (6.2%)	15.96 ± 0.91 (3.6%)	209.30 ± 15.65 (1.6%)
2-methylphenanthrene	1.90 ± 0.06 [b]	19.10 ± 1.10 [a]	230.00 ± 14.00 [a]	1.88 ± 0.11 (-1.2%)	20.07 ± 1.35 (5.08%)	241.80 ± 20.09 (5.1%)
9- & 4-methylphenanthrene	1.60 ± 0.20 [b]	5.31 ± 0.50 [a]	232.00 ± 19.00 [a]	1.51 ± 0.10 (-5.9%)	10.61 ± 0.33 (67.1%)	269.86 ± 23.69 (16.3%)
1-methylphenanthrene	1.70 ± 0.10 [b]	9.23 ± 0.22 [a]	169.00 ± 10.00 [a]	1.39 ± 0.10 (-18.2%)	9.05 ± 1.96 (-2.0%)	191.30 ± 16.17 (13.2%)
2,6-dimethylphenanthrene	0.79 ± 0.02 [b]	1.06 ± 0.24 [b]	n/a	0.49 ± 0.02 (-37.8%)	1.20 ± 0.07 (13.6%)	n/a n/a
1,7-dimethylphenanthrene	0.62 ± 0.02 [b]	1.43 ± 0.10 [b]	110.00 ± 12.00 [b]	0.56 ± 0.04 (-10.1%)	1.23 ± 0.07 (-13.8%)	101.88 ± 13.22 (-7.4%)
1,8-dimethylphenanthrene	0.24 ± 0.01 [b]	0.26 ± 0.05 [b]	n/a	0.25 ± 0.02 (5.0%)	0.37 ± 0.03 (43.9%)	n/a n/a
4-methylpyrene	1.44 ± 0.03 [b]	5.13 ± 0.36 [b]	21.60 ± 1.50 [b]	1.20 ± 0.06 (-16.5%)	3.93 ± 0.25 (-23.5%)	20.26 ± 2.87 (-6.2%)
1-methylpyrene	1.29 ± 0.03 [b]	4.60 ± 1.10 [b]	12.10 ± 1.80 [b]	0.93 ± 0.05 (-28.1%)	4.12 ± 0.29 (-10.4%)	15.34 ± 1.11 (26.8%)
2,6-dimethylnaphthalene	n/a	5.75 ± 0.63 [b]	n/a	n/a n/a	9.16 ± 0.72 (59.3%)	n/a n/a
Dibenzothiophene	n/a	17.70 ± 0.40 [b]	51.8 ± 2.10 [a]	n/a n/a	31.30 ± 2.13 (76.8%)	51.28 ± 4.85 (-1.0%)
4-methyldibenzothiohene	n/a	1.37 ± 0.08 [b]	n/a	n/a n/a	1.38 ± 0.07 (1.0%)	n/a n/a

Benzo[*b*]naphtho[2,3-*d*]thiophene	n/a	3.68 ± 0.59 [b]	n/a	n/a n/a	3.21 ± 0.27 (-12.7%)	n/a n/a
Benzo[*b*]naphtho[1,2-*d*]thiophene	n/a	2.41 ± 0.21[b]	n/a	n/a n/a	2.03 ± 0.19 (-15.9%)	n/a n/a
C_2-Naphthalenes	n/a	n/a	2170.00 ± 360.00 [b]	n/a n/a	n/a n/a	3989.28 ± 308.97 (83.8%)
C_3-Naphthalenes	n/a	n/a	1380.00 ± 270.00 [b]	n/a n/a	n/a n/a	3323.01 ± 321.27 (140.8%)
C_4-Naphthalenes	n/a	n/a	700.00 ± 130.00 [b]	n/a n/a	n/a n/a	695.45 ± 43.34 (-0.6%)
C_1-Phenanthrenes/Anthracenes	n/a	n/a	670.00 ± 90.00 [b]	n/a n/a	n/a n/a	931.21 ± 76.04 (39.0%)
C_2-Phenanthrenes/Anthracenes	n/a	n/a	630.00 ± 60.00 [b]	n/a n/a	n/a n/a	1003.40 ± 130.28 (59.3%)
C_3-Phenanthrenes/Anthracenes	n/a	n/a	400.00 ± 50.00 [b]	n/a n/a	n/a n/a	657.22 ± 68.43 (64.3%)
C_4-Phenanthrenes/Anthracenes	n/a	n/a	200.00 ± 30.00 [b]	n/a n/a	n/a n/a	134.48 ± 20.25 (-32.8%)
C_1-Benzanthracenes/Chrysenes/Triphenylenes	n/a	n/a	110.00 ± 7.00 [b]	n/a n/a	n/a n/a	306.65 ± 14.44 (178.8%)
C_2-Benzanthracenes/Chrysenes/Triphenylenes	n/a	n/a	130.00 ± 18.00 [b]	n/a n/a	n/a n/a	467.50 ± 64.20 (259.6%)
C_3-Benzanthracenes/Chrysenes/Triphenylenes	n/a	n/a	93.00 ± 12.00 [b]	n/a n/a	n/a n/a	107.49 ± 4.49 (15.6%)
C_4-Benzanthracenes/Chrysenes/Triphenylenes	n/a	n/a	71.00 ± 16.00 [b]	n/a n/a	n/a n/a	19.55 ± 4.11 (-78.5%)
C_1-Fluorenes	n/a	n/a	300.00 ± 60.00 [b]	n/a n/a	n/a n/a	474.49 ± 60.23 (58.2%)
C_2-Fluorenes	n/a	n/a	380.00 ± 30.00 [b]	n/a n/a	n/a n/a	584.91 ± 58.54 (53.9%)
C_3-Fluorenes	n/a	n/a	270.00 ± 40.00 [b]	n/a n/a	n/a n/a	133.48 ± 13.16 (-50.6%)
C_1-Dibenzothiophenes	n/a	n/a	130.00 ± 20.00 [b]	n/a n/a	n/a n/a	208.75 ± 22.70 (60.6%)
C_2-Dibenzothiophenes	n/a	n/a	160.00 ± 20.00 [b]	n/a n/a	n/a n/a	390.01 ± 46.73 (143.8%)

C_3-Dibenzothiophenes	n/a	n/a	110.00 ± 10.00 [b]	n/a n/a	n/a n/a	262.14 ± 32.80 (138.3%)
C_4-Dibenzothiophenes	n/a	n/a	56.00 ± 10.00 [b]	n/a n/a	n/a n/a	59.73 ± 8.26 (6.7%)
C_1-Fluoranthenes/Pyrenes	n/a	n/a	67.00 ± 7.00 [b]	n/a n/a	n/a n/a	149.48 ± 9.43 (123.1%)
C_2-Fluoranthenes/Pyrenes	n/a	n/a	130.00 ± 10.00 [b]	n/a n/a	n/a n/a	177.06 ± 20.32 (36.2%)
C_3-Fluoranthenes/Pyrenes	n/a	n/a	120.00 ± 20.00 [b]	n/a n/a	n/a n/a	39.85 ± 3.79 (-66.8%)
C_4-Fluoranthenes/Pyrenes	n/a	n/a	87.00 ± 21.00 [b]	n/a n/a	n/a n/a	35.97 ± 5.66 (-58.7%)

[1] *Seven replicate measurements*
[2] *Three replicate measurements*
[3] *Three replicate measurements*
[a] *Certified Mass Fractions*
[b] *Reference Mass Fraction*
[c] *Percentage difference*

Table V.8: *Certified and measured concentrations (mean ± standard deviation, mg/kg) of APAHs in 3 standard reference materials determined using the external calibration approach (**Method 2**) to quantitation and GC/MS/MS using multiple reaction monitoring for detection.*

Analytes	Certified/Reference Concentrations (mg/kg)			Measured Concentrations (mg/kg)		
	SRM 1944	SRM 1597a	SRM 2779	SRM 1944[1] (%) [c]	SRM 1597a[2] (%) [c]	SRM 2779[3] (%) [c]
2-methylnaphthalene	0.74 ± 0.06 [b]	95.00 ± 2.90 [b]	1630.00 ± 50.00 [a]	0.85 ± 0.06 (15.2%)	132.17 ± 8.05 (39.1%)	1736.96 ± 139.03 (6.6%)
1-methylnaphthalene	0.47 ± 0.02 [b]	43.90 ± 1.80 [b]	1140.00 ± 20.00 [a]	0.45 ± 0.02 (-3.8%)	46.06 ± 3.31 (1.5%)	1232.23 ± 94.98 (8.1%)
3-methylphenanthrene	2.10 ± 0.10 [b]	15.80 ± 0.80 [a]	206.00 ± 32.00 [a]	2.34 ± 0.16 (11.4%)	19.20 ± 0.96 (9.9%)	223.05 ± 16.70 (8.3%)
2-methylphenanthrene	1.90 ± 0.06 [b]	19.10 ± 1.10 [a]	230.00 ± 14.00 [a]	2.01 ± 0.12 (5.6%)	23.54± 1.43 (11.8%)	255.70 ± 21.27 (11.2%)
9- & 4-methylphenanthrene	1.60 ± 0.20 [b]	5.31 ± 0.50 [a]	232.00 ± 19.00 [a]	1.61 ± 0.11 (0.6%)	13.11 ± 0.36 (82.7%)	284.10 ± 24.98 (22.5%)
1-methylphenanthrene	1.70 ± 0.10 [b]	9.23 ± 0.22 [a]	169.00 ± 10.00 [a]	1.48 ± 0.11 (-12.7%)	10.91 ± 0.47 (7.3%)	203.05 ± 17.19 (20.2%)
2,6-dimethylphenanthrene	0.79 ± 0.02 [b]	1.06 ± 0.24 [b]	n/a	0.55 ± 0.02 (-29.8%)	1.30 ± 0.06 (-1.6%)	n/a n/a
1,7-dimethylphenanthrene	0.62 ± 0.02 [b]	1.43 ± 0.10 [b]	110.00 ± 12.00 [b]	0.60 ± 0.04 (-2.6%)	1.59 ± 0.07 (-14.0%)	107.34 ± 13.95 (-2.4%)
1,8-dimethylphenanthrene	0.24 ± 0.01 [b]	0.26 ± 0.05 [b]	n/a	0.28 ± 0.02 (15.7%)	0.47 ± 0.03 (29.8%)	n/a n/a
4-methylpyrene	1.44 ± 0.03 [b]	5.13 ± 0.36 [b]	21.60 ± 1.50 [b]	1.31 ± 0.07 (-9.2%)	4.23 ± 0.27 (-17.5%)	20.70 ± 2.99 (-4.2%)
1-methylpyrene	1.29 ± 0.03 [b]	4.60 ± 1.10 [b]	12.10 ± 1.80 [b]	1.01 ± 0.05 (-21.6%)	4.46 ± 0.31 (-3.0%)	15.54 ± 1.12 (28.4%)
2,6-dimethylnaphthalene	n/a	5.75 ± 0.63 [b]	n/a	n/a n/a	10.93 ± 0.72 (59.3%)	n/a n/a
Dibenzothiophene	n/a	17.70 ± 0.40 [b]	51.8 ± 2.10 [a]	n/a n/a	33.26 ± 2.26 (87.9%)	51.30 ± 4.85 (-1.0%)
4-methyldibenzothiohene	n/a	1.37 ± 0.08 [b]	n/a	n/a n/a	1.50 ± 0.08 (9.4%)	n/a n/a

Benzo[*b*]naphtho[2,3-*d*]thiophene	n/a	3.68 ± 0.59 [b]	n/a	n/a n/a	3.91 ± 0.28 (-7.0%)	n/a n/a
Benzo[*b*]naphtho[1,2-*d*]thiophene	n/a	2.41 ± 0.21[b]	n/a	n/a n/a	2.15 ± 0.20 (-12.4%)	n/a n/a
C_2-Naphthalenes	n/a	n/a	2170.00 ± 360.00 [b]	n/a n/a	n/a n/a	4211.75± 326.79 (94.1%)
C_3-Naphthalenes	n/a	n/a	1380.00 ± 270.00 [b]	n/a n/a	n/a n/a	2867.55 ± 277.60 (107.8%)
C_4-Naphthalenes	n/a	n/a	700.00 ± 130.00 [b]	n/a n/a	n/a n/a	584.82 ± 36.84 (-16.5%)
C_1-Phenanthrenes/Anthracenes	n/a	n/a	670.00 ± 90.00 [b]	n/a n/a	n/a n/a	1009.21 ± 82.60 (50.7%)
C_2-Phenanthrenes/Anthracenes	n/a	n/a	630.00 ± 60.00 [b]	n/a n/a	n/a n/a	1050.54 ± 137.08 (66.8%)
C_3-Phenanthrenes/Anthracenes	n/a	n/a	400.00 ± 50.00 [b]	n/a n/a	n/a n/a	568.72 ± 58.46 (42.2%)
C_4-Phenanthrenes/Anthracenes	n/a	n/a	200.00 ± 30.00 [b]	n/a n/a	n/a n/a	115.66 ± 17.81 (-42.2%)
C_1-Benzanthracenes/Chrysenes/Triphenylenes	n/a	n/a	110.00 ± 7.00 [b]	n/a n/a	n/a n/a	381.52 ± 18.04 (246.8%)
C_2-Benzanthracenes/Chrysenes/Triphenylenes	n/a	n/a	130.00 ± 18.00 [b]	n/a n/a	n/a n/a	422.0 ± 57.03 (224.6%)
C_3-Benzanthracenes/Chrysenes/Triphenylenes	n/a	n/a	93.00 ± 12.00 [b]	n/a n/a	n/a n/a	89.35 ± 3.30 (-3.9%)
C_4-Benzanthracenes/Chrysenes/Triphenylenes	n/a	n/a	71.00 ± 16.00 [b]	n/a n/a	n/a n/a	18.19 ± 4.40 (-80.0%)
C_1-Fluorenes	n/a	n/a	300.00 ± 60.00 [b]	n/a n/a	n/a n/a	464.07 ± 59.21 (54.7%)
C_2-Fluorenes	n/a	n/a	380.00 ± 30.00 [b]	n/a n/a	n/a n/a	578.61 ± 58.19 (52.3%)
C_3-Fluorenes	n/a	n/a	270.00 ± 40.00 [b]	n/a n/a	n/a n/a	131.97 ± 13.44 (-51.1%)
C_1-Dibenzothiophenes	n/a	n/a	130.00 ± 20.00 [b]	n/a n/a	n/a n/a	209.28 ± 22.88 (61.0%)
C_2-Dibenzothiophenes	n/a	n/a	160.00 ± 20.00 [b]	n/a n/a	n/a n/a	371.86 ± 44.72 (132.4%)

C_3-Dibenzothiophenes	n/a	n/a	110.00 ± 10.00 [b]	n/a n/a	n/a n/a	275.81 ± 34.67 (150.7%)
C_4-Dibenzothiophenes	n/a	n/a	56.00 ± 10.00 [b]	n/a n/a	n/a n/a	62.45 ± 8.79 (11.5%)
C_1-Fluoranthenes/Pyrenes	n/a	n/a	67.00 ± 7.00 [b]	n/a n/a	n/a n/a	151.78 ± 9.26 (126.5%)
C_2-Fluoranthenes/Pyrenes	n/a	n/a	130.00 ± 10.00 [b]	n/a n/a	n/a n/a	174.62 ± 19.77 (34.3%)
C_3-Fluoranthenes/Pyrenes	n/a	n/a	120.00 ± 20.00 [b]	n/a n/a	n/a n/a	38.83 ± 4.28 (-67.6%)
C_4-Fluoranthenes/Pyrenes	n/a	n/a	87.00 ± 21.00 [b]	n/a n/a	n/a n/a	34.84 ± 4.66 (-60.0%)

[1]*Seven replicate measurements*
[2]*Three replicate measurements*
[3]*Three replicate measurements*
[a]*Certified Mass Fractions*
[b]*Reference Mass Fraction*
[c]*Percentage difference*

Table V.9: *Certified and measured concentrations (mean ± standard deviation, mg/kg) of APAHs in 3 standard reference materials determined using the external calibration approach (**Method 3**) to quantitation and GC/MS/MS using multiple reaction monitoring for detection.*

Analytes	Certified/Reference Concentrations (mg/kg)			Measured Concentrations (mg/kg)		
	SRM 1944	SRM 1597a	SRM 2779	SRM 1944[1] (%) [c]	SRM 1597a[2] (%) [c]	SRM 2779[3] (%) [c]
2-methylnaphthalene	0.74 ± 0.06 [b]	95.00 ± 2.90 [b]	1630.00 ± 50.00 [a]	0.43 ± 0.02 (-41.8%)	84.88 ± 3.29 (-10.7%)	1331.62 ± 47.90 (-18.3%)
1-methylnaphthalene	0.47 ± 0.02 [b]	43.90 ± 1.80 [b]	1140.00 ± 20.00 [a]	0.25 ± 0.02 (-46.6%)	41.88 ± 2.40 (-4.6%)	944.83 ± 31.28 (-17.1%)
3-methylphenanthrene	2.10 ± 0.10 [b]	15.80 ± 0.80 [a]	206.00 ± 32.00 [a]	1.88 ± 0.12 (-10.5%)	15.45 ± 0.83 (0.3%)	185.97 ± 7.28 (-9.7%)
2-methylphenanthrene	1.90 ± 0.06 [b]	19.10 ± 1.10 [a]	230.00 ± 14.00 [a]	1.60 ± 0.10 (-15.7%)	19.52 ± 1.33 (2.19%)	213.12 ± 9.72 (-7.3%)
9- & 4-methylphenanthrene	1.60 ± 0.20 [b]	5.31 ± 0.50 [a]	232.00 ± 19.00 [a]	1.29 ± 0.08 (-19.1%)	10.59 ± 0.36 (66.8%)	236.82 ± 13.11 (2.1%)
1-methylphenanthrene	1.70 ± 0.10 [b]	9.23 ± 0.22 [a]	169.00 ± 10.00 [a]	1.19 ± 0.08 (-30.1%)	9.04 ± 0.46 (-2.0%)	169.26 ± 8.56 (0.2%)
2,6-dimethylphenanthrene	0.79 ± 0.02 [b]	1.06 ± 0.24 [b]	n/a	0.58 ± 0.03 (-26.3%)	1.06 ± 0.06 (-0.4%)	n/a n/a
1,7-dimethylphenanthrene	0.62 ± 0.02 [b]	1.43 ± 0.10 [b]	110.00 ± 12.00 [b]	0.63 ± 0.5 (2.3%)	1.25 ± 0.08 (-12.6%)	92.59 ± 5.41 (-15.8%)
1,8-dimethylphenanthrene	0.24 ± 0.01 [b]	0.26 ± 0.05 [b]	n/a	0.29 ± 0.03 (21.7%)	0.32 ± 0.03 (23.3%)	n/a n/a
4-methylpyrene	1.44 ± 0.03 [b]	5.13 ± 0.36 [b]	21.60 ± 1.50 [b]	1.29 ± 0.05 (-10.6%)	4.38 ± 0.23 (-14.7%)	19.79 ± 1.97 (-8.4%)
1-methylpyrene	1.29 ± 0.03 [b]	4.60 ± 1.10 [b]	12.10 ± 1.80 [b]	1.00 ± 0.06 (-22.8%)	4.61 ± 0.23 (0.3%)	12.13 ± 1.52 (0.2%)
2,6-dimethylnaphthalene	n/a	5.75 ± 0.63 [b]	n/a	n/a n/a	8.59 ± 0.51 (49.4%)	n/a n/a
Dibenzothiophene	n/a	17.70 ± 0.40 [b]	51.8 ± 2.10 [a]	n/a n/a	30.40 ± 1.61 (71.8%)	42.27 ± 2.95 (-18.4%)
4-methyldibenzothiohene	n/a	1.37 ± 0.08 [b]	n/a	n/a n/a	1.37 ± 0.05 (0.0%)	n/a n/a

Benzo[*b*]naphtho[2,3-*d*]thiophene	n/a	3.68 ± 0.59 [b]	n/a	n/a n/a	3.53 ± 0.16 (-4.0%)	n/a n/a
Benzo[*b*]naphtho[1,2-*d*]thiophene	n/a	2.41 ± 0.21[b]	n/a	n/a n/a	2.18 ± 0.11 (-9.7%)	n/a n/a
C_2-Naphthalenes	n/a	n/a	2170.00 ± 360.00 [b]	n/a n/a	n/a n/a	3229.00 ± 108.37 (48.8%)
C_3-Naphthalenes	n/a	n/a	1380.00 ± 270.00 [b]	n/a n/a	n/a n/a	2453.85 ± 83.99 (77.8%)
C_4-Naphthalenes	n/a	n/a	700.00 ± 130.00 [b]	n/a n/a	n/a n/a	487.67 ± 15.34 (-30.3%)
C_1-Phenanthrenes/Anthracenes	n/a	n/a	670.00 ± 90.00 [b]	n/a n/a	n/a n/a	841.54 ± 39.30 (25.6%)
C_2-Phenanthrenes/Anthracenes	n/a	n/a	630.00 ± 60.00 [b]	n/a n/a	n/a n/a	905.92 ± 46.07 (43.8%)
C_3-Phenanthrenes/Anthracenes	n/a	n/a	400.00 ± 50.00 [b]	n/a n/a	n/a n/a	512.34 ± 22.46 (28.1%)
C_4-Phenanthrenes/Anthracenes	n/a	n/a	200.00 ± 30.00 [b]	n/a n/a	n/a n/a	102.04 ± 13.25 (49.0%)
C_1-Benzanthracenes/Chrysenes/Triphenylenes	n/a	n/a	110.00 ± 7.00 [b]	n/a n/a	n/a n/a	276.33 ± 34.04 (151.2%)
C_2-Benzanthracenes/Chrysenes/Triphenylenes	n/a	n/a	130.00 ± 18.00 [b]	n/a n/a	n/a n/a	303.5 ± 31.19 (133.5%)
C_3-Benzanthracenes/Chrysenes/Triphenylenes	n/a	n/a	93.00 ± 12.00 [b]	n/a n/a	n/a n/a	91.35 ± 7.45 (-1.8%)
C_4-Benzanthracenes/Chrysenes/Triphenylenes	n/a	n/a	71.00 ± 16.00 [b]	n/a n/a	n/a n/a	15.51 ± 0.85 (-82.3%)
C_1-Fluorenes	n/a	n/a	300.00 ± 60.00 [b]	n/a n/a	n/a n/a	393.21 ± 27.33 (31.1%)
C_2-Fluorenes	n/a	n/a	380.00 ± 30.00 [b]	n/a n/a	n/a n/a	474.96 ± 35.76 (25.0%)
C_3-Fluorenes	n/a	n/a	270.00 ± 40.00 [b]	n/a n/a	n/a n/a	109.84 ± 7.89 (-59.3%)
C_1-Dibenzothiophenes	n/a	n/a	130.00 ± 20.00 [b]	n/a n/a	n/a n/a	171.75 ± 13.10 (32.1%)
C_2-Dibenzothiophenes	n/a	n/a	160.00 ± 20.00 [b]	n/a n/a	n/a n/a	334.12 ± 31.15 (108.8%)

C_3-Dibenzothiophenes	n/a	n/a	110.00 ± 10.00 [b]	n/a n/a	n/a n/a	254.75 ± 22.04 (131.6%)
C_4-Dibenzothiophenes	n/a	n/a	56.00 ± 10.00 [b]	n/a n/a	n/a n/a	61.79 ± 7.71 (10.34%)
C_1-Fluoranthenes/Pyrenes	n/a	n/a	67.00 ± 7.00 [b]	n/a n/a	n/a n/a	127.65 ± 21.49 (90.52%)
C_2-Fluoranthenes/Pyrenes	n/a	n/a	130.00 ± 10.00 [b]	n/a n/a	n/a n/a	172.98 ± 23.82 (33.06%)
C_3-Fluoranthenes/Pyrenes	n/a	n/a	120.00 ± 20.00 [b]	n/a n/a	n/a n/a	41.36 ± 4.87 (65.74%)
C_4-Fluoranthenes/Pyrenes	n/a	n/a	87.00 ± 21.00 [b]	n/a n/a	n/a n/a	42.27 ± 2.95 (18.41%)

[1]*Seven replicate measurements*
[2]*Three replicate measurements*
[3]*Three replicate measurements*
[a]*Certified Mass Fractions*
[b]*Reference Mass Fraction*
[c]*Percentage difference*

Table V.10: *Certified and measured concentrations (mean ± standard deviation, mg/kg) of APAHs in 3 standard reference materials determined using the average relative response factor (ARRF) approach to quantitation (**Method 4**) and GC/MS/MS using multiple reaction monitoring for detection.*

Analytes	Certified/ Reference Concentrations (mg/kg)			Measured Concentrations (mg/kg)		
	SRM 1944	SRM 1597a	SRM 2779	SRM 1944[1] (%) [c]	SRM 1597a[2] (%) [c]	SRM 2779[3] (%) [c]
2-methylnaphthalene	0.74 ± 0.06 [b]	95.00 ± 2.90 [b]	1630.00 ± 50.00 [a]	0.64 ± 0.09 (-14.1%)	92.51 ± 8.75 (-2.6%)	1224.56 ± 119.19 (-24.9%)
1-methylnaphthalene	0.47 ± 0.02 [b]	43.90 ± 1.80 [b]	1140.00 ± 20.00 [a]	0.37 ± 0.05 (-22.1%)	45.59 ± 4.99 (3.9%)	864.30 ± 86.15 (-24.2%)
3-methylphenanthrene	2.10 ± 0.10 [b]	15.80 ± 0.80 [a]	206.00 ± 32.00 [a]	1.62 ± 0.23 (-22.9%)	11.84 ± 1.35 (-23.1%)	169.15 ± 23.72 (-17.9%)
2-methylphenanthrene	1.90 ± 0.06 [b]	19.10 ± 1.10 [a]	230.00 ± 14.00 [a]	1.40 ± 0.14 (-26.4%)	14.97 ± 1.85 (-21.6%)	194.01 ± 26.08 (-15.6%)
9- & 4-methylphenanthrene	1.60 ± 0.20 [b]	5.31 ± 0.50 [a]	232.00 ± 19.00 [a]	1.15 ± 0.16 (-28.0%)	8.02 ± 0.71 (26.3%)	215.41 ± 29.26 (-7.2%)
1-methylphenanthrene	1.70 ± 0.10 [b]	9.23 ± 0.22 [a]	169.00 ± 10.00 [a]	0.99 ± 0.14 (-41.7%)	6.94 ± 0.72 (-24.8%)	153.79 ± 20.01 (-9.0%)
2,6-dimethylphenanthrene	0.79 ± 0.02 [b]	1.06 ± 0.24 [b]	n/a	0.48 ± 0.08 (-39.1%)	0.87 ± 0.11 (-18.2%)	n/a n/a
1,7-dimethylphenanthrene	0.62 ± 0.02 [b]	1.43 ± 0.10 [b]	110.00 ± 12.00 [b]	0.56 ± 0.14 (-9.1%)	1.01 ± 0.12 (-29.5%)	84.20 ± 10.90 (-23.5%)
1,8-dimethylphenanthrene	0.24 ± 0.01 [b]	0.26 ± 0.05 [b]	n/a	0.25 ± 0.07 (3.9%)	0.31 ± 0.03 (18.5%)	n/a n/a
4-methylpyrene	1.44 ± 0.03 [b]	5.13 ± 0.36 [b]	21.60 ± 1.50 [b]	0.90 ± 0.21 (-37.6%)	2.91± 0.23 (-43.2%)	5.76 ± 0.41 (-73.3%)
1-methylpyrene	1.29 ± 0.03 [b]	4.60 ± 1.10 [b]	12.10 ± 1.80 [b]	0.76 ± 0.15 (-41.2%)	3.08 ± 0.21 (-33.1%)	3.52 ± 0.28 (-70.9%)
2,6-dimethylnaphthalene	n/a	5.75 ± 0.63 [b]	n/a	n/a n/a	9.68 ± 1.11 (68.3%)	n/a n/a
Dibenzothiophene	n/a	17.70 ± 0.40 [b]	51.8 ± 2.10 [a]	n/a n/a	20.25 ± 1.01 (14.4%)	37.54 ± 4.00 (-27.5%)
4-methyldibenzothiohene	n/a	1.37 ± 0.08 [b]	n/a	n/a n/a	0.90 ± 0.06 (-34.2%)	n/a n/a

Benzo[*b*]naphtho[2,3-*d*]thiophene	n/a	2.41 ± 0.21 [b]	n/a	n/a n/a	1.48 ± 0.22 (-38.5%)	n/a n/a
Benzo[*b*]naphtho[1,2-*d*]thiophene	n/a	3.68 ± 0.59 [b]	n/a	n/a n/a	2.40 ± 0.34 (-34.7%)	n/a n/a
C_2-Naphthalenes	n/a	n/a	2170.00 ± 360.00 [b]	n/a n/a	n/a n/a	2938.32 ± 295.16 (35.4%)
C_3-Naphthalenes	n/a	n/a	1380.00 ± 270.00 [b]	n/a n/a	n/a n/a	2274.85 ± 211.01 (64.8%)
C_4-Naphthalenes	n/a	n/a	700.00 ± 130.00 [b]	n/a n/a	n/a n/a	452.69 ± 42.21 (-35.3%)
C_1-Phenanthrenes/Anthracenes	n/a	n/a	670.00 ± 90.00 [b]	n/a n/a	n/a n/a	764.36 ± 102.23 (14.1%)
C_2-Phenanthrenes/Anthracenes	n/a	n/a	630.00 ± 60.00 [b]	n/a n/a	n/a n/a	822.96 ± 108.34 (30.6%)
C_3-Phenanthrenes/Anthracenes	n/a	n/a	400.00 ± 50.00 [b]	n/a n/a	n/a n/a	473.62 ± 62.62 (18.4%)
C_4-Phenanthrenes/Anthracenes	n/a	n/a	200.00 ± 30.00 [b]	n/a n/a	n/a n/a	103.03 ± 12.01 (-48.5%)
C_1-Benzanthracenes/Chrysenes/Triphenylenes	n/a	n/a	110.00 ± 7.00 [b]	n/a n/a	n/a n/a	316.28 ± 53.84 (187.53%)
C_2-Benzanthracenes/Chrysenes/Triphenylenes	n/a	n/a	130.00 ± 18.00 [b]	n/a n/a	n/a n/a	353.66 ± 39.97 (172.0 %)
C_3-Benzanthracenes/Chrysenes/Triphenylenes	n/a	n/a	93.00 ± 12.00 [b]	n/a n/a	n/a n/a	108.71 ± `0.37 (16.9 %)
C_4-Benzanthracenes/Chrysenes/Triphenylenes	n/a	n/a	71.00 ± 16.00 [b]	n/a n/a	n/a n/a	71.01 ± 4.02 (22.0 %)
C_1-Fluorenes	n/a	n/a	300.00 ± 60.00 [b]	n/a n/a	n/a n/a	170.91 ± 20.25 (-43.0%)
C_2-Fluorenes	n/a	n/a	380.00 ± 30.00 [b]	n/a n/a	n/a n/a	194.99 ± 23.05 (-48.7%)
C_3-Fluorenes	n/a	n/a	270.00 ± 40.00 [b]	n/a n/a	n/a n/a	44.92 ± 6.21 (-83.4%)
C_1-Dibenzothiophenes	n/a	n/a	130.00 ± 20.00 [b]	n/a n/a	n/a n/a	161.39 ± 23.82 (24.1%)
C_2-Dibenzothiophenes	n/a	n/a	160.00 ± 20.00 [b]	n/a n/a	n/a n/a	295.12 ± 29.90 (84.5%)

C_3-Dibenzothiophenes	n/a	n/a	110.00 ± 10.00 [b]	n/a n/a	n/a n/a	210.07 ± 25.00 (91.0%)
C_4-Dibenzothiophenes	n/a	n/a	56.00 ± 10.00 [b]	n/a n/a	n/a n/a	50.78 ± 8.48 (-9.3%)
C_1-Fluoranthenes/Pyrenes	n/a	n/a	67.00 ± 7.00 [b]	n/a n/a	n/a n/a	38.85 ± 5.09 (-42.0%)
C_2-Fluoranthenes/Pyrenes	n/a	n/a	130.00 ± 10.00 [b]	n/a n/a	n/a n/a	50.60 ± 6.30 (-61.1%)
C_3-Fluoranthenes/Pyrenes	n/a	n/a	120.00 ± 20.00 [b]	n/a n/a	n/a n/a	12.65 ± 1.40 (-89.5%)
C_4-Fluoranthenes/Pyrenes	n/a	n/a	87.00 ± 21.00 [b]	n/a n/a	n/a n/a	10.87 ± 2.59 (-87.5%)

[1]*Seven replicate measurements*
[2]*Three replicate measurements*
[3]*Three replicate measurements*
[a]*Certified Mass Fractions*
[b]*Reference Mass Fraction*
[c]*Percentage difference*

Table V.11: *Certified and measured concentrations (mean ± standard deviation, mg/kg) of APAHs in 3 standard reference materials determined using the relative response factor (RRF) approach to quantitation (**Method 5**) and GC/MS/MS using multiple reaction monitoring for detection.*

Analytes	**Certified/Reference Concentrations (mg/kg)**			**Measured Concentrations (mg/kg)**		
	SRM 1944	**SRM 1597a**	**SRM 2779**	**SRM 1944[1] (%) [c]**	**SRM 1597a[2] (%) [c]**	**SRM 2779[3] (%) [c]**
2-methylnaphthalene	0.74 ± 0.06 [b]	95.00 ± 2.90 [b]	1630.00 ± 50.00 [a]	1.99 ± 0.32 (168.6%)	120.17 ± 11.36 (26.5%)	2574.34 ± 250.56 (57.9%)
1-methylnaphthalene	0.47 ± 0.02 [b]	43.90 ± 1.80 [b]	1140.00 ± 20.00 [a]	1.04 ± 0.17 (122.3%)	54.01 ± 5.91 (23.0%)	1597.16 ± 159.21 (40.1%)
3-methylphenanthrene	2.10 ± 0.10 [b]	15.80 ± 0.80 [a]	206.00 ± 32.00 [a]	1.24 ± 0.16 (-41.1%)	4.61 ± 0.42 (-75.8%)	66.84 ± 9.38 (-67.6%)
2-methylphenanthrene	1.90 ± 0.06 [b]	19.10 ± 1.10 [a]	230.00 ± 14.00 [a]	1.09 ± 0.15 (-42.4%)	4.99 ± 0.62 (-73.9%)	77.16 ± 10.37 (-66.5%)
9- & 4-methylphenanthrene	1.60 ± 0.20 [b]	5.31 ± 0.50 [a]	232.00 ± 19.00 [a]	0.90 ± 0.12 (-43.6%)	3.22 ± 0.23 (-58.8%)	87.39 ± 11.87 (-62.3%)
1-methylphenanthrene	1.70 ± 0.10 [b]	9.23 ± 0.22 [a]	169.00 ± 10.00 [a]	0.85 ± 0.11 (-49.8%)	2.25 ± 0.23 (-75.7%)	60.58 ± 7.88 (-64.2%)
2,6-dimethylphenanthrene	0.79 ± 0.02 [b]	1.06 ± 0.24 [b]	n/a	0.25 ± 0.03 (-67.9%)	0.22 ± 0.03 (-79.4%)	n/a n/a
1,7-dimethylphenanthrene	0.62 ± 0.02 [b]	1.43 ± 0.10 [b]	110.00 ± 12.00 [b]	0.22 ± 0.03 (-63.8%)	0.17 ± 0.02 (-87.9%)	17.56 ± 2.27 (-84.0%)
1,8-dimethylphenanthrene	0.24 ± 0.01 [b]	0.26 ± 0.05 [b]	n/a	0.09 ± 0.02 (-62.6%)	0.045 ± 0.004 (-82.6%)	n/a n/a
4-methylpyrene	1.44 ± 0.03 [b]	5.13 ± 0.36 [b]	21.60 ± 1.50 [b]	0.41 ± 0.04 (-71.7%)	0.74 ± 0.06 (-85.6%)	1.42 ± 0.10 (-93.4%)
1-methylpyrene	1.29 ± 0.03 [b]	4.60 ± 1.10 [b]	12.10 ± 1.80 [b]	0.31 ± 0.03 (-75.9%)	0.75 ± 0.05 (-83.8%)	0.83 ± 0.07 (-93.1%)
2,6-dimethylnaphthalene	n/a	5.75 ± 0.63 [b]	n/a	n/a n/a	8.79 ± 1.00 (52.3%)	n/a n/a
Dibenzothiophene	n/a	17.70 ± 0.40 [b]	51.80 ± 1.20 [a]	n/a n/a	3.06 ± 0.15 (-82.7%)	13.81 ± 2.20 (-73.3%)
4-methyldibenzothiohene	n/a	1.37 ± 0.08 [b]	n/a	n/a n/a	0.33 ± 0.02 (-75.8%)	n/a n/a

Benzo[*b*]naphtho[2,3-*d*]thiophene	n/a	2.41 ± 0.21 [b]	n/a	n/a n/a	0.26 ± 0.04 (-89.4%)	n/a n/a
Benzo[*b*]naphtho[1,2-*d*]thiophene	n/a	3.68 ± 0.59 [b]	n/a	n/a n/a	0.47 ± 0.07 (-87.2%)	n/a n/a
C_2-Naphthalenes	n/a	n/a	2170.00 ± 360.00 [b]	n/a n/a	n/a n/a	4127.49 ± 414.61 (90.2%)
C_3-Naphthalenes	n/a	n/a	1380.00 ± 270.00 [b]	n/a n/a	n/a n/a	2816.76 ± 261.28 (104.1%)
C_4-Naphthalenes	n/a	n/a	700.00 ± 130.00 [b]	n/a n/a	n/a n/a	544.97 ± 50.81 (-22.1%)
C_1-Phenanthrenes/Anthracenes	n/a	n/a	670.00 ± 90.00 [b]	n/a n/a	n/a n/a	302.05 ± 40.40 (-54.9%)
C_2-Phenanthrenes/Anthracenes	n/a	n/a	630.00 ± 60.00 [b]	n/a n/a	n/a n/a	222.25 ± 29.30 (-64.7%)
C_3-Phenanthrenes/Anthracenes	n/a	n/a	400.00 ± 50.00 [b]	n/a n/a	n/a n/a	204.02 ± 26.98 (-49.0%)
C_4-Phenanthrenes/Anthracenes	n/a	n/a	200.00 ± 30.00 [b]	n/a n/a	n/a n/a	17.66 ± 2.25 (-91.2%)
C_1-Benzanthracenes/Chrysenes/Triphenylenes	n/a	n/a	110.00 ± 7.00 [b]	n/a n/a	n/a n/a	2.27 ± 0.39 (-97.9%)
C_2-Benzanthracenes/Chrysenes/Triphenylenes	n/a	n/a	130.00 ± 18.00 [b]	n/a n/a	n/a n/a	6.97 ± 1.11 (-94.6%)
C_3-Benzanthracenes/Chrysenes/Triphenylenes	n/a	n/a	93.00 ± 12.00 [b]	n/a n/a	n/a n/a	3.71 ± 0.35 (-96.0%)
C_4-Benzanthracenes/Chrysenes/Triphenylenes	n/a	n/a	71.00 ± 16.00 [b]	n/a n/a	n/a n/a	0.51 ± 0.10 (-99.4%)
C_1-Fluorenes	n/a	n/a	300.00 ± 60.00 [b]	n/a n/a	n/a n/a	50.47 ± 5.98 (-83.2%)
C_2-Fluorenes	n/a	n/a	380.00 ± 30.00 [b]	n/a n/a	n/a n/a	45.53 ± 5.38 (-88.0%)
C_3-Fluorenes	n/a	n/a	270.00 ± 40.00 [b]	n/a n/a	n/a n/a	10.49 ± 1.45 (-96.1%)
C_1-Dibenzothiophenes	n/a	n/a	130.00 ± 20.00 [b]	n/a n/a	n/a n/a	148.31 ± 21.89 (14.1%)
C_2-Dibenzothiophenes	n/a	n/a	160.00 ± 20.00 [b]	n/a n/a	n/a n/a	71.09 ± 7.20 (-55.6%)

C_3-Dibenzothiophenes	n/a	n/a	110.00 ± 10.00 [b]	n/a n/a	n/a n/a	101.64 ± 12.10 (-7.6%)
C_4-Dibenzothiophenes	n/a	n/a	56.00 ± 10.00 [b]	n/a n/a	n/a n/a	24.57 ± 4.10 (-56.1%)
C_1-Fluoranthenes/Pyrenes	n/a	n/a	67.00 ± 7.00 [b]	n/a n/a	n/a n/a	9.67 ± 1.10 (-85.6%)
C_2-Fluoranthenes/Pyrenes	n/a	n/a	130.00 ± 10.00 [b]	n/a n/a	n/a n/a	8.05 ± 1.00 (-93.8%)
C_3-Fluoranthenes/Pyrenes	n/a	n/a	120.00 ± 20.00 [b]	n/a n/a	n/a n/a	2.01 ± 0.22 (-98.3%)
C_4-Fluoranthenes/Pyrenes	n/a	n/a	87.00 ± 21.00 [b]	n/a n/a	n/a n/a	1.64 ± 0.41 (-98.1%)

[1]*Seven replicate measurements*
[2]*Three replicate measurements*
[3]*Three replicate measurements*
[a]*Certified Mass Fractions*
[b]*Reference Mass Fraction*
[c]*Percentage difference*

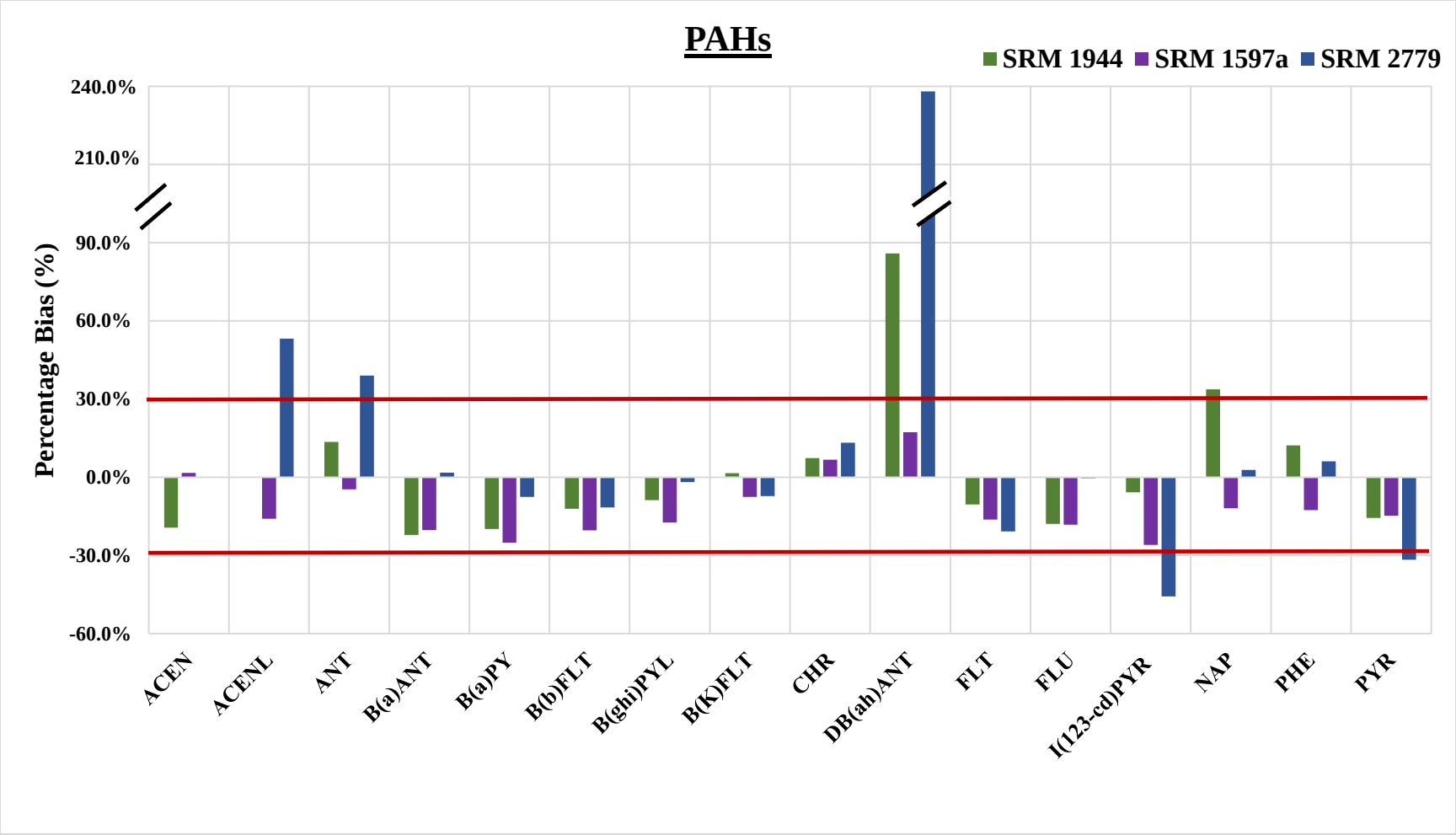

Figure V.1: *Percentage bias for 16 USEPA PAHs in extract of all 3 SRMs with the acceptable limits.*

Appendix VI. Additional Metrics from Chapter 7: Alkylation of Benz[a]anthracene Affects Toxicity to Early‐Life Stage Zebrafish and *In Vitro* Aryl Hydrocarbon Receptor 2 Transactivation in a Position‐Dependent Manner

Table VI.1: *Method performance characteristics of the six target analytes extracted using the sample workflow described in Section 7.3.2. and analysis using GC/MS/MS.*

Chemical	Recovery (%)	Precision (% RSD[c])	LOD[a] (pg µL^{-1})	LOQ[b] (pg µL^{-1})	LOD[a] (ng/g-egg)	LOQ[b] (ng/g-egg)
4-MBAA	94.2	6.1	11.5	38.2	57.5	191.0
7,12-DMBAA	89.0	7.4	13.2	44.0	66.0	220.0
8-MBAA	100.0	7.5	15.1	50.2	75.5	251.0
BAA	113.5	3.4	7.7	25.7	38.5	128.5

[a] *limit of detection*
[b] *limit of quantification*
[c] *relative standard deviation*

Table VI.2: *Relative potencies of tested chemicals in vivo, in vitro, and in the literature to TCDD.*

All RePs were calculated based on nanomolar concentrations (nmol/g-egg or nM).

Chemical	*in vivo* ReP to TCDD	*in vitro* ReP to TCDD	EROD ReP to TCDD
TCDD	1.0[a]	1.0[b]	1.0[c]
BAA	0.00007[a]	1.0[b]	0.0002[c]
4-MBAA	0.00008[a]	5.1[b]	N/A[d]
8-MBAA	0.0004[a]	18.4[b]	0.0016[c]
7,12-DMBAA	0.0001[a]	0.6[b]	0.0004[c]

[a] *RePs calculated based on the LD50 for early life-stage toxicity of TCDD in zebrafish from Elonen et al. (1998).*
[b] *RePs calculated based on the EC50 for activation of zebrafish AhR2 by TCDD in the in vitro AhR transactivation assay from Zhang et al. (2018).*
[c] *RePs previously published by Barron et al. (2004).*
[d] *Data not available (N/A).*

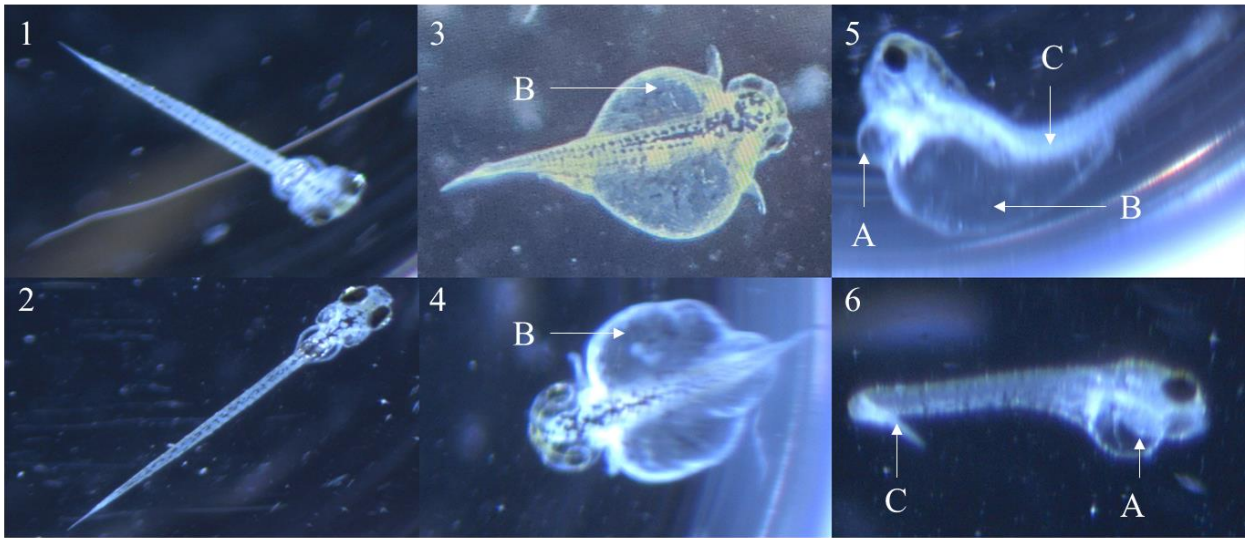

Figure VI.1: *Representative images of control larvae (1–2) and larvae exposed to BAA, 4-MBAA, 8-MBAA, or 7,12-DMBAA (3–6). Larvae exposed to PAHs exhibited malformations such as pericardial edema (A), yolk sac edema (B), and spinal curvatures (C) at an elevated rate.*