ABSTRACT

Development and Application of High-Throughput Sample Preparation Methods for Solid Matrices

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This research presents the development and application of high-throughput sample preparation methods for the analysis of organic and inorganic contaminants from solid samples. The approach presented evaluates conventional methods to identify potential areas of improvement. In this sense, conventional methods serve as a framework for the development of high-throughput sample preparation methods. In general, improvements include expansion of target analyte list thereby increase the environmental applicability, reduction of sample preparation steps, and as a result, reduction of sample preparation time.

The analytical bottleneck is often associated with sample preparation, especially in the analysis of organic contaminants from environmental samples. Many environmental analytical chemistry methods can be broken down into one or more sample preparation steps followed by one or more chemical analysis steps. Improvement of historical methods has focused on the development of advance instrumentation (i.e. focusing on the chemical analysis). However, recent efforts have focused on the overall

reduction of time and/or steps associated with sample preparation. For example, post-extraction cleanup adsorbents can be incorporated into the pressurized liquid extraction step to perform a selective pressurized liquid extraction (SPLE). SPLE methods significantly reduced sample preparation time, solvent requirements, and waste production.

Specific examples presented in this dissertation include: 1) the development and application of SPLE methods for the analysis of organic contaminants from sediments and biological tissues; 2) the development and application of a simplified acid digestion method for the analysis of mercury and selenium in rare samples of Pacific walrus (*Odobenus rosmarus divergens*) muscle (*L. dorsi*). These examples illustrate the approach for the development of high-throughput sample preparation methods that have successfully combined techniques into a single method, and/or eliminated post-extraction cleanup steps. The availability of these methods increases laboratory's capacity and preparedness to analyze rapidly large volumes of samples. These methods could find use in routine analysis and monitoring studies of environmental samples, food and pharmaceutical industries, agriculture, toxicology studies, and forensic sciences among others. Lastly, this dissertation discusses a few opportunities that lay ahead for improvement and development in sample preparation.

Development and Application of High-Throughput Sample Preparation Methods for Solid Matrices

by

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A Dissertation

Approved by The Institute of Ecological, Earth, and Environmental Sciences

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Submitted to the Graduate Faculty of
Baylor University in Partial Fulfillment of the
Requirements for the Degree
of

Doctor of Philosophy

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ACKNOWLEDGMENTS

The author would like to express her sincere thanks to her advisor, Dr. Usenko, for his insightful and thoughtful guidance. Special thanks to the Doctoral Committee members; Dr. Bruce, Dr. Chambliss, Dr. Sheesley, and Dr. Trumble for their support and relevant suggestions for the development of this dissertation. The author would like to thank her coauthors, Dr. Bigorgne, Dr. Brooks, Dr. Dehn, Dr. Matson, E. Oziolor, Dr. Robinson, Dr. Subedi, Dr. Trumble, and Dr. Williams for their collaboration and significant contributions to this research work. The author is thankful for the support of The Institute of Ecological, Earth, and Environmental Sciences and the Department of Environmental Science at Baylor University. The author would like to state her deepest gratitude to past and present lab coworkers, Dr. Subedi, Dr. Robinson, and A. Clark for the challenging research discussions. Also, thanks to family and friends for their love, support, and trust.

ABBREVIATIONS

ASE	Accelerated Solvent Extraction
BC	Black Carbon
CCV	Calibration Curve Verification
CRM	Certified Reference Material
CVAFS	Cold Vapor Atomic Fluorescence Spectrophotometer
dry wt	Dry Weight
DCM	Dichloromethane
dl-PCB	Dioxin-like Polychlorinated Biphenyls
ECNI/MS	Electron Capture Negative Ionization Mass Spectrometry
EPA (USEPA)	Environmental Protection Agency
GB	Gangs Bayou
GC/MS	Gas Chromatography Mass Spectrometry
GC-EI/MS	Gas Chromatography Electron Ionization Mass Spectrometry
GPC-UV	Gel Permeation Chromatography-Ultraviolet
HpCDD/F	Heptachlorinated Dibenzo-*p*-dioxins/furans
HRGC	High Resolution Gas Chromatography
HRMS	High Resolution Mass Spectrometry
HSC	Houston Ship Channel
HX	n-Hexanes
HxCDD/F	Hexachlorinated Dibenzo-*p*-dioxins/furans

ICP-MS	Inductively Coupled Plasma Mass Spectrometry
MDL	Method Detection Limit
MS/MSD	Matrix Spike (Matrix Spike Duplicate)
ND	Non-Detect (Concentration below MDL)
OC/TOC	Organic Carbon (Total Organic Carbon)
OCDD/F	Octachlorinated Dibenzo-*p*-dioxins/furans
PAH	Polycyclic Aromatic Hydrocarbons
PB	Patrick Bayou
PC	Principal Component
PCB	Polychlorinated Biphenyl
PCDD/F	Polychlorinated Dibenzo-*p*-dioxins/furans
PeCDD/F	Pentachlorinated Dibenzo-*p*-dioxins/furans
PLE	Pressurized Liquid Extraction
RPD	Relative Percent Difference
SD	Standard Deviation
SIM	Selective Ion Monitoring
SIR	Selective Ion Recording
SJRWP	San Jacinto River Waste Pits
SPLE	Selective Pressurized Liquid Extraction
SRM	Standard Reference Material
TCDD/F	Tetrachlorinated Dibenzo-*p*-dioxins/furans
TCEQ	Texas Commission on Environmental Quality
THg	Total Mercury

TOC	Total Organic Carbon
TOL	Toluene
VB	Vince Bayou
wet wt	Wet Weight
WHO	World Health Organization

CHAPTER ONE

Introduction

Improvement of historical methods has focused on the development of advance instrumentation (i.e. focusing on the chemical analysis) while maintaining conventional sample preparation protocols. Sample preparation is typically one of the most time-consuming and labor-intensive steps of conventional analytical techniques. Overall, chemical analysis can benefit from improved sample preparation methods that reduce time, contamination, analyte loss, and variability of results. Many environmental analytical chemistry methods can be broken down into one or more sample preparation steps followed by one or more chemical analysis steps. Typical sample preparation methods consist of sample homogenization, extraction, cleanup, and concentration. Currently, there are several major techniques for extraction of organic and inorganic contaminants from solid samples; each with its own advantages and disadvantages, e.g. Soxhlet extraction, acid digestion, microwave extraction, ultrasonic extraction, pressurized liquid extraction (PLE), and supercritical fluid extraction (Richter et al. 1996, Bjorklund et al. 2000, Camel 2001, Sporring et al. 2005, Schantz 2006). The purpose of the extraction is to dissolve the analytes of interest from the matrix and into a solvent that will be adequate for the intended analytical technique.

Over the last 30 years, initial improvements in sample preparation for analysis of organic contaminants focused on the automation of the extraction step. Most improvements originate from Soxhlet extraction, which has been the traditional extraction method for over a hundred years and many laboratories still use it for routine

analysis (Jensen and Andraos 2007). New techniques have built upon Soxhlet to shorten extraction time, reduce solvents, and automate the process (Luque de Castro and García-Ayuso 1998). For example, automated Soxhlet extraction, a technique commercially introduced in the 1980s, reduced extraction time and solvent by placing the sample in contact with the boiling solvent followed by a rinsing step. In other efforts to reduce extraction time, the Soxhlet apparatus has been combined with ultrasound or microwave extraction (Luque de Castro and Priego-Capote 2010).

Further improvements to extraction technology resulted in extraction techniques that operate at high temperature and pressure, such as supercritical fluid extraction, pressurized microwave assisted extraction, and pressurized liquid extraction (PLE). Some advantages of performing the extraction at higher temperatures are: enhanced mass transfer, decrease solvent viscosity and surface tension, increased analyte solubility and diffusion rates (e.g. solubility of anthracene increases 13-fold as temperature increases from 50 to 150 °C), and weaker solute-matrix interactions (Richter et al. 1996). In order to perform extractions at temperatures above the extraction solvent's boiling point, while maintaining it in a liquid state, these new techniques operate at high pressures (i.e. 1500 – 2000 psi for PLE) (Richter et al. 1996). Thermo Scientific™ Dionex™ commercially introduced PLE, also known as pressurized fluid extraction and accelerated solvent extraction, in 1996. The main advantages of PLE over Soxhlet are a significant reduction in extraction time (from several hours to a few minutes), automation, lower solvent volume, reduction in personnel's exposure to chemicals, and a more exhaustive extraction (Schantz 2006). This advantages are also important during method development and optimization of the extraction parameters (e.g. temperature, extraction

time, number of extraction cycles, and solvents) for a new matrix and/or analytes of interest (Richter et al. 1996). In the past 5 to 10 years, several environmental chemical laboratories have focused not only on the optimization of the extraction (i.e. PLE) but also on the reduction or elimination of post-extraction cleanup steps. Typical post-extraction cleanup techniques used in the analysis of organic contaminants from environmental samples include silica and florisil column chromatography, size exclusion chromatography (i.e. gel permeation chromatography (GPC)), acid – base partitioning, and oxidation/reduction (EPA 1996c). In their review of PLE, Bjorklund et al. identified post-extraction cleanup as one of the disadvantages of the method (Bjorklund et al. 2000). Post-extraction cleanup steps lengthen the amount of time required for sample preparation, increase waste (i.e. organic solvents), personnel exposure to solvents, opportunities for analyte loss or contamination, instrumental training, and laboratory space requirements. Several post-extraction cleanup steps may be necessary to remove potential interferences found in extracts of environmental samples. Without adequate sample cleanup, analytical methods may require additional instrument maintenance and downtime.

Several conventional extraction methods, such as those reported by the Environmental Protection Agency (EPA), can serve as a framework (Figure 1.1) for the development and optimization of high-throughput sample preparation techniques (Usenko et al. 2013). These conventional post-extraction cleanup techniques provide important information regarding potential bulk matrix interferences, specific interferences, and molecular interferences that may affect quantitation of the target analytes. For example, GPC or florisil packed columns are typically used to remove lipid

interferences present in the extracts. Sulfur can be extracted from sediments, soils, and sludge and is typically removed using activated copper, silver impregnated silica column, or GPC. Polychlorinated biphenyls (PCBs) are potential molecular interferences of polychlorinated dibenzo-*p*-dioxins and polychlorinated dibenzofurans (PCDD/Fs) and are typically fractionated, post-extraction, using an activated carbon column. High-throughput methods must address the removal of these and other potential interferences while eliminating/reducing the number of steps required during sample preparation.

In recent years, high-throughput sample preparation methods have incorporated typical post-extraction cleanup adsorbents within the extraction step. These new methods take advantage of the configuration of PLE systems by placing the adsorbents into the extraction cell beneath the sample homogenate. The result is an automated selective pressurized liquid extraction (SPLE) of target analytes without extraction of potential interferences (Figure 1.2). SPLE methods have focus on reducing or eliminating time-consuming post-extraction cleanup steps. Several adsorbents (e.g. acidic and neutral silica, florisil, acidic and basic alumina) have been successfully incorporated into SPLE for the analysis of diverse analytes (e.g. PCDD/Fs, PCBs, pesticides, pharmaceutical, personal care products) from various environmental matrices (e.g. fish, sediments, blubber, whale earwax) (Murphy et al. 1996, Björklund et al. 2001, Gomez-Ariza et al. 2002, Subedi et al. 2011, Trumble et al. 2012, Robinson et al. 2013, Oziolor et al. 2014). The intrinsic advantages of incorporating extraction and cleanup include: the elimination/reduction of additional cleanup instrumentation and labware, laboratory space requirements, hood space requirements, personnel training, exposure to chemicals, and opportunities for sample contamination and analyte loss (Usenko et al. 2013).

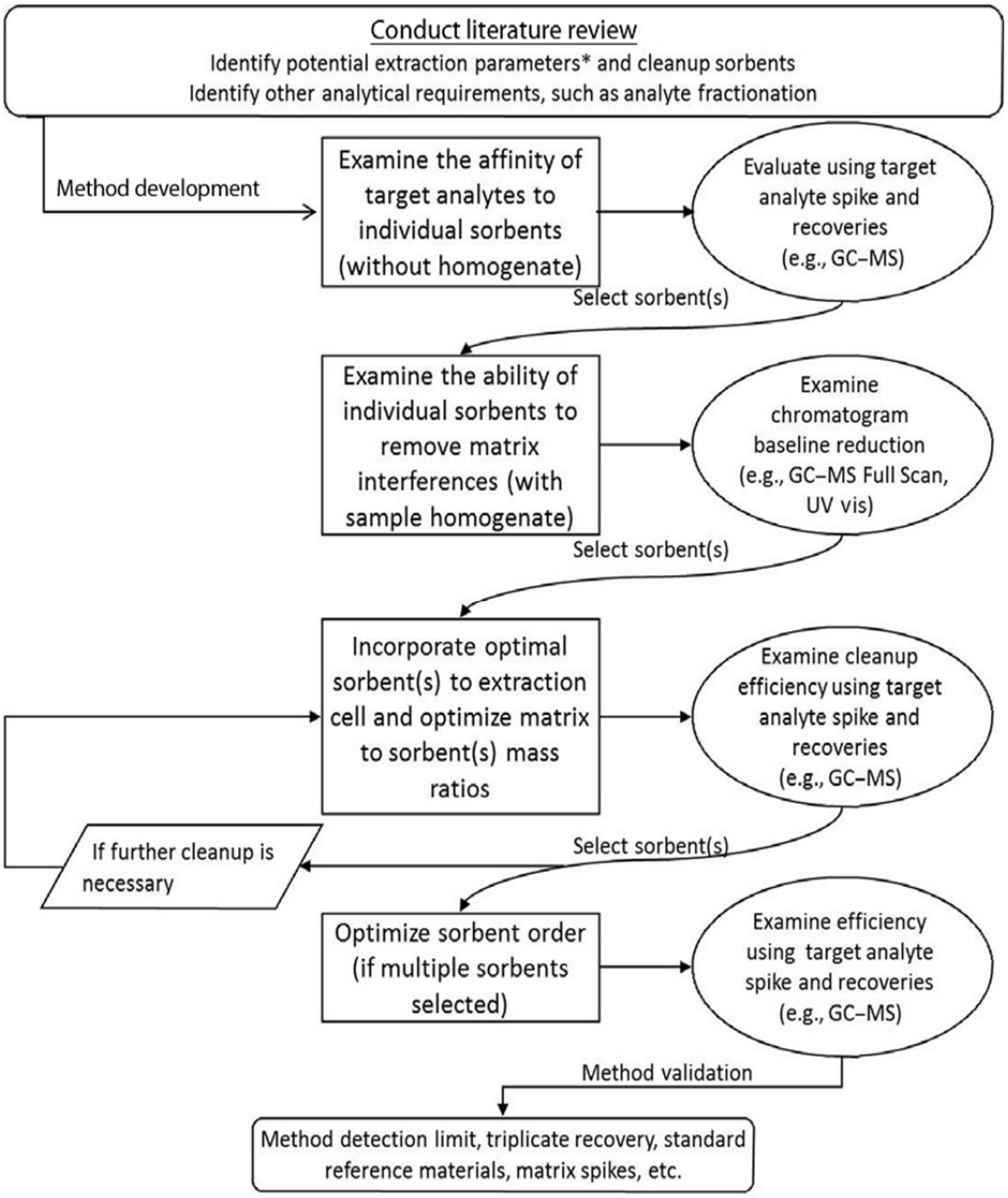

Figure 1.1. A proposed flowchart for SPLE method development and validation.
*Extraction parameters such as temperature, pressure, number and duration of cycles, and solvent(s) may need to be optimized for certain analytes and/or matrices.
(Usenko et al. 2013)

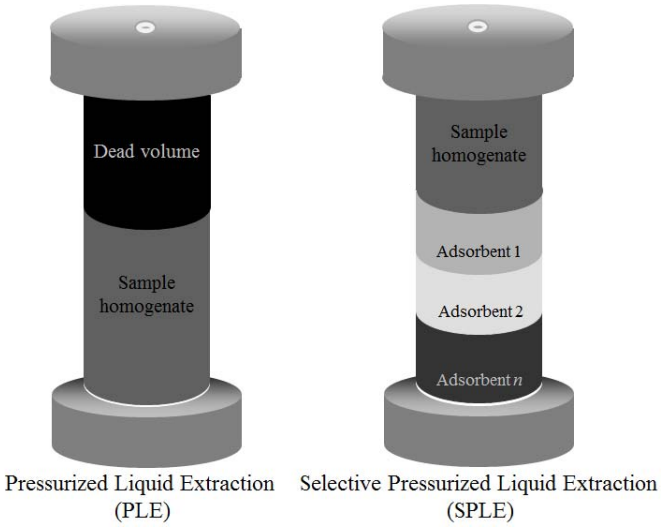

Figure 1.2. Pressurized liquid extraction (PLE) and Selective PLE (SPLE).

Sample preparation and analysis throughput can also be optimized by combining two or more conventional sample preparation techniques, used for multiple classes of contaminants, into a single sample preparation method. For instance, conventional analysis of PCDD/Fs and PCBs follows two separate methods EPA 1613 for PCDD/Fs and EPA 1668B for PCBs (EPA 1994a, EPA 2008). The general steps of each method are sample homogenization, extraction (typically Soxhlet or PLE), a series of cleanup steps (chromatographic columns and GPC), and analysis. These conventional methods can served as a framework to develop a single sample preparation method that allows for the extraction of an expanded target analyte list from a single sample (Subedi et al. 2011, Aguilar et al. 2014, Subedi et al. 2014). Using these methods as a starting point, specific adsorbents (e.g. Carbopak^{TM}) can be incorporated into the extraction cell for the fractionation of select classes of target analytes (e.g. PCDD/Fs from dl-PCBs) thus allowing their analysis while eliminating potential molecular interferences (Haglund et al. 2007, Subedi and Usenko 2012, Subedi et al. 2014). Because PCDD/Fs can be formed as

byproducts during PCBs manufacturing, are structurally similar, and have similar physicochemical properties it would be advisable to monitor all classes of compounds when analyzing environmental samples. Although commercial production and distribution of PCBs in the United States is heavily restricted, there are still legacy sources, such as transformers, containing PCBs. Current sources of PCDD/Fs and PCBs include incineration, re-volatilization from contaminated areas, and clandestine disposal of contaminated wastes.

Due to their hydrophobic and lipophilic characteristics, in the aquatic environment PCDD/Fs and PCBs will preferentially accumulate in sediments and adipose tissues of aquatic organisms. Sediment cores had been used to look at historical deposition of contaminant concentrations (Usenko et al. 2007). Contaminants found in sediments are typically bioavailable to benthic organisms through pore water and direct ingestion (e.g. filter feeders). Once contaminants have accumulated in benthic organisms, they have the potential to bioaccumulate throughout the food web into higher trophic levels. In addition, benthic organisms can affect the distribution and availability of contaminants in sediments through resuspension, mixing, and burrowing. Historic and current uses/emissions and the ability of these chemicals to partition to sediments have resulted in contamination of many aquatic ecosystems, including several Superfund sites. Identification, assessment, and monitoring of these contaminated sites are necessary to develop and evaluate the success of remediation efforts and understand the contaminants' transport in these systems. However, there are several analytical challenges associated with the detailed characterization and monitoring of contaminated sites. Mainly, the ability to obtain timely results to support the decision-making process as well as the cost

associated with the routine monitoring. SPLE methods that reduce sample preparation time and increase the number of target analytes extracted from a single sample would benefit routine analysis and monitoring studies of organic contaminants.

Analysis of inorganic contaminants, such as mercury and metals, in solid matrices typically consists of digestion, centrifugation or filtration, to remove non-digested material, dilution, and analysis (EPA 1996a, EPA 2002). Concentrated and dilute acids and mixtures of acids are used for the digestion of the solid matrix and will depend on the type of matrix (e.g. biological tissue, sediment, sludge, soil) and the target analyte (e.g. mercury, metals). Different instrumentation is available to perform the digestion from heating blocks and acid digestion vessels to automated microwave closed digestion apparatus. Typically, two separate methods are employed for the digestion of mercury and metals from biological tissues: EPA 1631 for mercury and EPA 3050B for metals (EPA 1996a, EPA 2002). Analogous to the extraction of organic contaminants the similitudes in the methods can be used to develop a single sample preparation method with an expanded target analyte list. In this particular case, the first step in sample preparation consists on the acid digestion of the sample in order to bring into solution the analytes of interest (mercury or metals). From that point forward, aliquots of the digestate can be separated for further treatment (i.e. oxidation of mercury species to Hg(II)) and dilution prior to analysis by the corresponding methods. Similar to the analysis of organic contaminants, a few PLE methods have been used for the analysis of organometallic and metals from oils. However; additional instrumentation and method development is necessary for their application in trace analysis (Alonso-Rodríguez et al. 2006, Moreda-Piñeiro et al. 2006, Mato-Fernández et al. 2007, Moreda-Piñeiro et al. 2007a, Moreda-

Piñeiro et al. 2007b, Carballo-Paradelo et al. 2012). The development of a single digestion method for mercury and metals from biological tissues would result in a high-throughput method by eliminating a second sample preparation technique. Utilizing a single method would also reduce the volume of acid needed and opportunities for sample loss and contamination. The availability of a single digestion method is also advantageous when the amount of sample is limited, such as when analyzing marine mammal tissue.

Sample availability is a limiting factor when conducting monitoring studies of contaminants in rare samples such as walrus' tissues. For example, it may be desirable to monitor mercury along with other metals and essentials elements (e.g. selenium); particularly when performing toxicological assessments (AMAP 2011). Mercury is a ubiquitous global pollutant with annual atmospheric emissions of approximately 5000 – 6000 tons per year from both natural and anthropogenic sources (AMAP 2011, Driscoll et al. 2013). In the atmosphere, mercury reaches higher latitudes and colder regions, such as the Arctic, via long-range atmospheric transport. In the aquatic environment, sulfate-reducing bacteria methylates mercury to methylmercury, which is bioavailable to aquatic organisms and accumulates through the food web (Morel et al. 1998, AMAP 2011). Bioaccumulation of mercury and other metals in Arctic organisms, such as marine mammals, is of particular importance due to their known role as sentinel species and their use in the sustenance of Native human populations (FWS 2007, Welfinger-Smith et al. 2011, Trumble et al. 2013). However, assessing and monitoring the presence of mercury and metals in tissues of marine mammals has several analytical challenges due to the limited access to the samples. The development of sample preparation methods that

allow the identification of several analytes from a single sample would help to overcome some of these analytical challenges.

The objectives of this dissertation are 1) to provide a framework for the development of high-throughput sample preparation methods for solid matrices; 2) to develop high-throughput sample preparation methods for the analysis organic and inorganic contaminants in solid matrices; and 3) to apply these methods to the analysis of environmental samples. The following pages present examples of the application of this framework for the development and application of high-throughput sample preparation methods and their applications to three different monitoring studies. Chapter Two presents the development and application of a SPLE method for the simultaneous automated extraction of three classes of environmental contaminants from sediment samples (i.e. PCDD/Fs and PCBs). Chapter Three presents an example of the application of SPLE methods for extraction and analysis of PCDD/Fs and PCBs from biological and sediment samples collected at two Superfund sites in Houston, TX. Chapter Four updates the concentrations of mercury and selenium in skeletal muscle of Pacific walrus (*Odobenus rosmarus divergens*) and presents a simplified digestion method for the extraction of mercury, selenium, cadmium, and arsenic from these samples. To conclude, Chapter Five outlines the current challenges and future trends on the development and applications of high-throughput extraction methods of environmental contaminants.

CHAPTER TWO

Development and Application of a Novel Method for High-throughput Determination of PCDD/Fs and PCBs in Sediments[1]

This chapter published as: Aguilar, L.; Williams, E. S.; Brooks, B. W.; Usenko, S. Development and Application of a Novel Method for High-throughput Determination of PCDD/Fs and PCBs in Sediments. *Env Toxicol Chem* **2014**.

Abstract

A selective pressurized liquid extraction technique was developed for the simultaneous extraction of polychlorinated dibenzo-*p*-dioxins/dibenzofurans (PCDD/Fs) and dioxin-like polychlorinated biphenyls (dl-PCBs) from contaminated sediments. The final method incorporated cleanup adsorbents (Florisil, alumina, and silica) into the extraction cell in a 1:1 ratio of matrix to individual adsorbent (w/w). Sulfur, a common interference found in sediments, was successfully removed by placing activated copper in the extraction bottle prior to extraction. No additional post extraction cleanup was required and sample throughput was reduced to 2.5 h per sample. Target analytes were quantified using high-resolution gas chromatography/electron-capture negative ionization mass spectrometry and verified by high-resolution gas chromatography/high-resolution mass spectrometry. Though mean analyte recoveries (n=3) of PCDD/Fs and dl-PCBs were 84±5.8% and 70±8.4%, respectively, mean surrogate recoveries for all PCDD/Fs using this novel method were greatly improved compared with US Environmental

[1]Reprinted with permission from [Aguilar, L.; Williams, E. S.; Brooks, B. W.; Usenko, S. Development and Application of a Novel Method for High-throughput Determination of PCDD/Fs and PCBs in Sediments. *Env. Toxicol. Chem*. **2014**. Copyright © 2014 SETAC.

Protection Agency (USEPA) method 1613 (~25–155%) and USEPA method 8290a (40–135%). After development, the method was used to examine surficial sediment samples from the San Jacinto River waste pits, a Superfund site in Houston, Texas, USA. In all samples, PCDD/Fs and dl-PCBs were detected, and the contaminant concentrations ranged over five orders of magnitude.

Introduction

Polychlorinated dibenzo-*p*-dioxins/dibenzofurans (PCDD/Fs) and dioxin-like polychlorinated biphenyls (dl-PCBs) are structurally analogous chemicals. They are not easily degraded and have low water solubility, low vapor pressure, and high octanol/water partitioning coefficients. Because of these characteristics, they are considered toxic, persistent in the environment, and bioaccumulative [1]. Polychlorinated dibenzo-*p*-dioxins/dibenzofurans are unintentionally formed *de novo* or from chlorinated precursors, such as chlorobenzene and PCBs, during combustion processes (natural and anthropogenic), manufacturing of chemicals, and pulp and paper bleaching operations, among other sources [2]. In contrast, PCBs were intentionally produced starting in 1929 for commercial uses such as coolants, flame retardants, plasticizers, adhesives, and transfer agents in carbonless copy paper, among other applications (manufacturing has been banned in the United States since 1979) [1,3]. There are 75 PCDD, 135 PCDF and 209 PCB congeners, and their toxicity profiles to aquatic life and human health vary greatly. Of particular toxicological interest are seven PCDD and ten PCDF congeners with chlorine substitution in positions 2, 3, 7, and 8, and 12 PCB congeners with *ortho* and *meta* chlorine substitution [1,4].

The traditional methodology for analysis of PCDD/Fs and PCBs involves extraction (Soxhlet or pressurized liquid extraction [PLE]) followed by several column chromatography and gel permeation chromatography (GPC) cleanup steps [5–8].

Although, postextraction cleanup is time-consuming—thus resulting in high costs associated with labor, solvents, and time to project completion—it is necessary to reduce interferences. Removing interferences results in improved detection and quantitation of analytes while reducing instrument maintenance and downtime [9]. In recent years, there has been a shift toward developing methods that remove interferences without increasing sample preparation time. For example, postextraction cleanup may be successfully reduced or eliminated by incorporating cleanup adsorbents within the extraction cell [10–17]. Different terms are used to describe methods to simultaneously perform extraction and cleanup, such as selective pressurized liquid extraction (SPLE), enhanced pressurized liquid extraction, and in-cell cleanup; we will use the term SPLE in the present study.

Selective pressurized liquid extraction methods reduce sample preparation time and therefore are more efficient than postextraction cleanup methods for analysis of a large number of samples, implementation monitoring programs, and time-sensitive projects. The SPLE method described in the present study was developed and used for the analysis of sediment samples from the San Jacinto River Waste Pits (SJRWP) Superfund site, a 20-acre impoundment located in Houston, Texas, USA [18]. The SJRWP were built in the mid-1960s to dispose of the pulp and paper waste that resulted from chlorine paper bleaching operations. The chlorinated waste contained PCDD/Fs that were formed as byproducts during paper bleaching. Dredging to the San Jacinto River in the 1970s and 1980s caused partial subsidence of the pits, causing contamination of the

local environment [18]. Previous studies identified PCDD/Fs and PCBs in biota, sediments, and water of the Houston Ship Channel and SJRWP [19–25]. In fact, the SJRWP have been identified as the primary source of PCDD/Fs in the region, but these previous studies included only two sediment samples taken at one location within the SJRWP [19,20]. The SJRWP were placed on the US Environmental Protection Agency (USEPA) National Priority List of Superfund sites in 2008, and the remedial investigation and feasibility study began in 2010 [18].

The objectives of the present study were to develop a high-throughput extraction method for the characterization of three contaminant classes (PCDD/Fs and dl-PCBs) and to incorporate postextraction cleanup steps into the extraction step to develop a SPLE method. The SPLE method consists of an automated, single-step, selective extraction and cleanup of PCDD/Fs and dl-PCBs from sediments. This SPLE method was used for the analysis of 15 sediment samples collected at the SJRWP Superfund site, prior to remediation efforts that started in February 2011 [26].

Materials and Methods

Chemicals

Chemicals were purchased from commercial vendors at reagent grade or better and stored in accordance with the manufacturer's recommendations. Basic alumina, silver nitrate (10% weight on silica gel), Florisil1, and copper powder were purchased from Sigma-Aldrich; silica gel, sodium sulfate, acetone, toluene, dichloromethane, and n-hexanes were purchased from BDH Chemicals. Standard reference material 1944 (SRM 1944) New York/New Jersey Waterway Sediment was purchased from the National

Institute of Standards and Technology. Native and isotopically labeled PCDD/Fs and dl-PCBs analytes, surrogates, and internal standard were purchased from AccuStandard. Isotopically labeled surrogates of all PCDD/F congeners were used as their quantitation standards. Isotopically labeled surrogates of PCB-77, 81, 126, and 169 were used as PCB quantitation standards, and the internal standard was [$^{13}C_{12}$]PCB-189. The target analytes selected are those included in the 2005 World Health Organization (WHO) reevaluation of human and mammalian toxic equivalent factors of dioxins and dioxin-like compounds [4].

Sediment Samples Collection and Characterization

Surficial sediment samples (~100 g wet wt, top 5 cm) were collected in triplicate in August 2010, prior to remediation, from the SJRWP Superfund site at 15 different locations. Sampling was conducted following standard USEPA and Texas Commission on Environmental Quality protocols. All sampling and laboratory equipment was cleaned prior to use. Laboratory equipment was washed, rinsed with deionized water, baked at 350 °C for 12 h, and solvent-rinsed (acetone and n-hexanes) prior to use. In general, sediment that was in direct contact with sampling equipment was not transferred to collection vessels. The sampling equipment was cleaned, rinsed with deionized water, and rinsed with ambient water between samples to remove bulk material and avoid cross-contamination. Surface sediment samples were collected with a stainless steel Ponar sampler, homogenized, and stored in pre-cleaned amber glass jars at 4 °C until analysis. Prior to analysis, the samples were stirred with a stainless steel spatula to ensure homogenization and then divided into aliquots for sediment characterization (total organic carbon [TOC], black carbon, and moisture content) and chemical analysis.

Additional surficial sediment samples were collected offsite, approximately 1 km downstream, and used for method development, spike and recovery analysis, and calculation of method detection limits (MDLs). These samples exhibit physical characteristics similar to those of the sediment at the SJRWP but had concentrations of PCDD/Fs and dl-PCBS that were undetectable or below the MDL, with the exception of octachlorodibenzo-*p*-dioxin (OCDD).

Moisture content, TOC, and black carbon were determined for all sediment samples using traditional methods [27,28]. Briefly, moisture content percentage was determined by drying an aliquot of sediment sample (~5 g in triplicate) at 110 °C until constant weight was achieved. The dry weight percentage was calculated as the ratio of dry wt to wet wt, multiplied by 100. Sediment aliquots were dried to constant weight at 60 °C and use for TOC and black carbon analysis. The dried sediment was ground with mortar and pestle to a free-flowing powder. Aliquots of dried-ground sediment were used for TOC and black carbon determination (~50 mg and 5 mg, respectively). For TOC analysis, the dried-ground sediment was placed in Ag capsules and treated with HCl (1N) until all inorganic carbon was removed. The acid was allowed to evaporate, and once the sediment was free of acid it was re-wrapped in Sn capsules. For black carbon analysis, the dried-ground sediment was placed in Ag capsules in a muffle furnace at 200 8C for 18 h. After cooling down, the samples were treated with HCl (1N) to remove inorganic carbon. The HCl was added in 25-µL increments, until all inorganic carbon was removed, and allowed to evaporate before the samples were wrapped in Sn capsules. Black carbon and TOC and analyses were performed using a Flash EA 1112 Series (ThermoQuest).

Selective Pressurized Liquid Extraction

The finalized method for the SPLE of PCDD/Fs and dl-PCBs from sediment samples was conducted as follows. Sodium sulfate was pre-cleaned by baking at 500 °C for 12 h and allowed to cool before use. Silica, alumina, and Florisil were pre-cleaned with toluene in two static cycles for 5 min at 100 °C and 1500 psi (50% rinse volume) using the accelerated solvent extractor (ASE 350; Thermo Scientific). Sediment samples (~10 g wet wt) were homogenized to a fine powder with the pre-cleaned sodium sulfate using mortar and pestle. The homogenized sediment samples were placed on top of pre-cleaned silica, alumina, and Florisil (1:1 sample-to-individual adsorbents ratio, w/w) in a 100-mL extraction cell (Figure 2.1). Sample extraction parameters were similar to those used for adsorbent pre-cleaning with the exception of rinse volume, which was increased to 75%. Samples were spiked in the extraction cell with isotopically labeled surrogate standards to correct for analyte loss during sample preparation. Samples spiked with surrogates were allowed to come to equilibrium for approximately 60 min before extraction. The extraction of target analytes and removal of potential interferences was performed in a single automated step. Sulfur was removed from the sediment extracts using copper powder (~3 g in 5mL toluene), which was previously activated with 20% (v/v) nitric acid, and subsequently rinsed with deionized water, acetone, and n-hexanes. The activated copper was placed in the extraction bottle with ~3mL of toluene prior to extraction, without further increment to sample preparation or extraction time. The sulfur was allowed to bind to the activated copper for 30 min as extracts were introduced into the extraction bottle and allowed to cool. Sediment extracts were decanted and concentrated under a gentle stream of nitrogen to approximately 0.2 mL using a

TurboVap II (Caliper Life Sciences) and then transferred to a 2-mL amber vial with a 500-µL glass insert and spiked with isotopically labeled internal standard before chemical analysis.

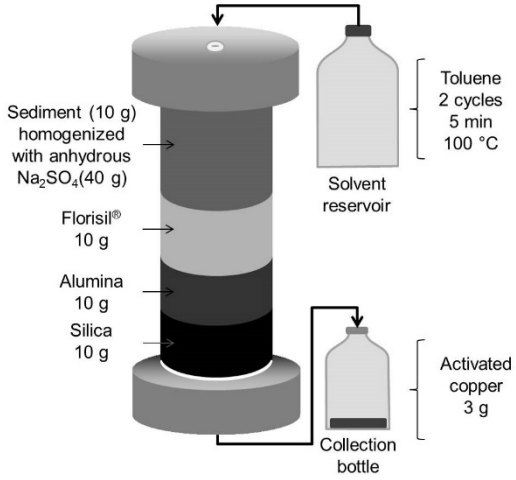

Figure 2.1. Accelerated solvent extraction cell with cleanup adsorbents for finalized selective pressurized liquid extraction of polychlorinated dibenzo-*p*-dioxins/dibenzofurans and dioxin-like polychlorinated biphenyls from sediments.

Selective Pressurized Liquid Extraction Optimization

The SPLE technique was optimized for extraction solvents and adsorbents type and mass ratio. Silica, alumina, and Florisil were selected as cleanup adsorbents and copper as sulfur retainer based on cleanup steps from USEPA method 1613 [6]. Silica, alumina, and Florisil were placed in the extraction cell, whereas activated copper was placed inside the extraction bottle at different sample to individual adsorbent ratios (1:1, 1:0.5, 1:0.2, and 1:0.25 w/w). Gel permeation chromatography–ultraviolet detection (GPC-UV) and full-scan gas chromatography–electron impact ionization/mass spectrometry (GC-EI/MS) were used to verify the efficiency of adsorbents to retain interferences from a 10-g aliquot of homogenized sediment; both methods have been described previously [13,29]. In addition to copper, silver nitrate-impregnated silica was

also evaluated as an adsorbent to remove sulfur. The efficiency of extraction using different extraction solvents (dichloromethane:n-hexanes [1:1]; toluene) was also evaluated. Analyte recoveries were verified by electron-capture negative chemical ionization/mass spectrometry (ECNI/MS) as describe below.

Chemical Analysis

The instrumental parameters and settings used for the quantitation of PCDD/Fs and dl-PCBs have been previously described [13]. Briefly, helium (99.999%) was used as carrier gas, and chromatographic separation of target analytes was achieved using a capillary DB-Dioxin column (60 m, 0.25 mm, 0.25 mm; Agilent Technologies). Target analytes were quantified using high-resolution gas chromatography (HRGC)–ECNI/MS in SIM (Agilent 7890 GC coupled to Agilent 5975 MSD). We quantified 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) and verified all other target analytes using HRGC–highresolution mass spectrometry (HRMS) in SIR mode (Agilent 6890N GC coupled to Fisons Vg ProSpec) [13]. Calibration curve verification (mid-point of calibration curve) standards were run every third sample to verify instrument performance. When calibration curve verification recoveries were not within 25%, instrument maintenance was performed. Method and laboratory blanks were also run with each batch to check for contamination and carry-over. Target analytes were identified based on their retention times and ratios of qualitative to quantitative ion responses.

Recovery Analysis and Method Detection Limits

Spike and recovery experiments were performed as part of method development using surficial sediment samples collected approximately 1 km downstream from the

SJRWP. The homogenized sediment samples were spiked with target analytes (calibration curve verification level) and allowed to equilibrate for approximately 1 h in the extraction cell. Isotopically labeled surrogate standards and internal standard were spiked into the extracts before chemical analysis. The entire analytical method was evaluated through triplicate spike and recovery experiments, as described above, and analysis of SRM 1944 (n=3). Samples of SRM 1944 were spiked with isotopically labeled surrogate standards and allowed to equilibrate for approximately 1 h before extraction. Isotopically labeled internal standard was spiked into the SRM 1944 extracts before chemical analysis.

Method detection limits were statistically determined using seven replicates of sediment samples (~10 g) spiked with target analytes (second-lowest calibration point) prior to extraction and allowed to equilibrate for 1 h. Method detection limit samples were extracted using SPLE and analyzed as previously described. The MDLs were calculated using the one-sided Student's *t*-statistic (99% confidence, six degrees of freedom, $t_{0.99=}3.143$) multiplied by the standard deviation of the quantified concentrations. The MDLs represent the lowest, non-zero, analyte concentration that can be reported (99% confidence) for a defined matrix [30].

Environmental Samples: SJRWP Characterization and Spatial Distribution of Contaminants

The newly developed method was used for the analysis of PCDD/Fs and dl-PCBs in surficial sediments from the SJRWP Superfund site. Calibration curve verifications and laboratory reagent blanks were included in the sample batch after every third sample to monitor instrument performance. Adsorbent and method blanks were used to

determine background concentrations of PCDD/Fs and dl-PCBs and were deducted from sample concentrations. Matrix spiked and matrix spiked duplicates were also analyzed and consisted of sediment samples spiked, prior to extraction, with target analytes (calibration curve verification level).

Principal component analysis was used to evaluate differences in concentration profiles among sampling locations within the SJRWP. Principal component analysis was generated using the PRINCOMP procedure of SAS® software, Version 9.2. Principal component analysis has the advantage of reducing the dimensionality of the data and projecting it into aggregated components that can explain the majority of the variability present in the dataset. Individual analyte concentrations were zero normalized (mean=0; standard deviation=1) to minimize statistical bias caused by the large range of concentrations found at the site.

Results and Discussions

Selective Pressurized Liquid Extraction Optimization

Extraction solvent optimization. Dichloromethane:*n*-hexanes (1:1 v/v) and toluene were evaluated as extraction solvents using the sample extraction parameters described above in Selective Pressurized Liquid Extraction Optimization. The efficiency of each solvent to extract PCDD/Fs and dl-PCBs was determined through analyte spike and recovery experiments (n=1). Recoveries of PCDD/Fs and dl-PCBs for each solvent, prior to and after adsorbent optimization, are presented in Table 2.1. Extractions performed prior to adsorbent optimization using dichloromethane:*n*-hexanes (1:1) yielded <60% recoveries for most target analytes, whereas extraction with the planar solvent

toluene, using the same parameters, resulted in >90% recovery of PCDD/Fs and >60% recovery of dl-PCBs. Toluene was further evaluated using two static cycles (5 min), and recoveries of dl-PCBs increased to >70%. Toluene was used for adsorbent optimization, and the finalized method, including all adsorbents, was evaluated in triplicate. The finalized SPLE method, which consisted of two cycles of toluene at 100 °C and included all adsorbents in the extraction cell, yielded PCDD/Fs and dl-PCBs analyte recoveries of 84±5.8% and 70±8.4%, respectively (Table 2.1).

Table 2.1. Pressurized liquid extraction solvent optimization

	Extraction conditions			% Recovery ± SD	
n	Solvent	Cycles	Temperature (°C)	PCDD/Fs	dl-PCBs
1	DCM:HX (1:1)	1 (5 min)	100	55 ± 17	39 ± 16
1	TOL	1 (5 min)	100	95 ± 8.1	66 ± 13
1	TOL	2 (5 min)	100	96 ± 7.3	75 ± 8.4
3	TOL	2 (5 min)	100	84 ± 5.8	70 ± 8.4

SD=Standard deviation; DCM=dichloromethane; HX=*n*-hexanes; TOL=toluene; PCDD/Fs=polychlorinated dibenzo-*p*-dioxins/dibenzofurans; dl-PCBs=dioxin-like polychlorinated biphenyls.

Removal of bulk interferences. High-throughput extraction techniques allow for the removal of additional postextraction cleanup steps while maintaining extract cleanliness. Silica, alumina, and Florisil are used in USEPA method 1613 and have been successfully incorporated into the extraction cell to retain potential interferences and yield clean extracts [6,13,14,31,32]. The incorporation of silica and alumina into the extraction cell (1:1 sample to individual adsorbent ratio, w/w) resulted in1 the removal or reduction some bulk interferences and low m/z fragments (Figure 2.2 and 2.3). Florisil

(1:1 sample to individual adsorbent ratio, w/w) removed or reduced the remaining bulk background interferences as corroborated by full scan GC/MS (Figure 2.3).

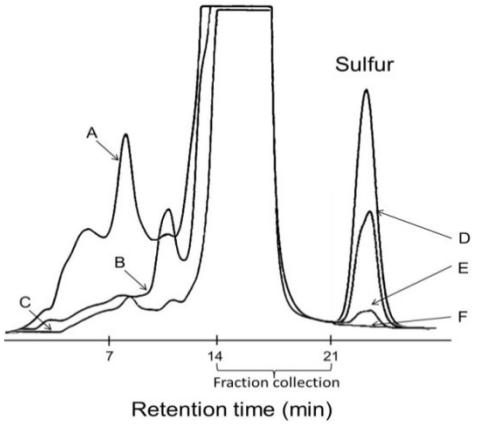

Figure 2.2. Gel permeation chromatography–ultraviolet detection GPC-UV chromatograms of selective pressurized liquid extraction, A=sediment (10 g) – sodium sulfate homogenate; B=sediment homogenate, silica, and alumina (1:1:1); C=sediment homogenate, silica, alumina, and Florisil (1:1:1:1); D=removal of sulfur with $AgNO_3$-silica (1:0.5) inside the extraction cell; E=removal of sulfur using $AgNO_3$-silica (1:1) inside the extraction cell; F=removal of sulfur using activated copper (1:0.1) inside the extraction bottle.

Packed columns of silver nitrate–impregnated silica and acid activated copper are used to remove sulfur from sediment samples [8,33–37]. To remove postextraction cleanup steps, the addition of silver nitrate into the extraction cell was evaluated. Silver nitrate was placed inside the extraction cell prior to extraction. The efficiency of sulfur removal by silver nitrate was verified by GPC-UV in which sulfur eluted from the column at approximately 22 min (Figure 2.2). Using silver nitrate at sample-to-adsorbent ratios of 1:0.5 and 1:1 w/w removed approximately 50% and 90% of the sulfur present in the sample, respectively (Figure 2.2D and 2.2E). A higher sample-to-silver nitrate ratio was not evaluated because of limited space inside the extraction cell. Activated copper was also evaluated for sulfur removal using GPC-UV. For logistics activated copper

powder was placed into the extraction bottle at a sample to copper ratio of 1:0.1 w/w. The addition of copper to the extraction bottle resulted in the successful removal of sulfur present in the extracts (Figure 2.2F). Thus, activated copper placed in the extraction bottle was used to successfully remove sulfur interferences from the extracts without adding appreciable time to the sample preparation step.

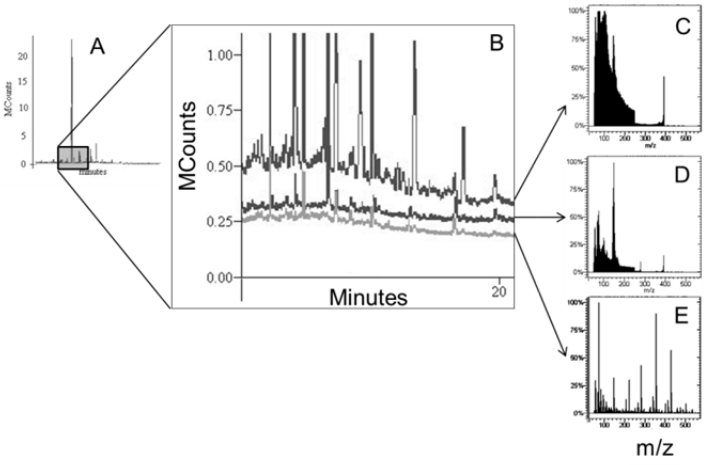

Figure 2.3. Electron ionization full-scan chromatograms and background spectra. Acquisition range 50-500 m/z. (A) Full scan chromatograms, (B) Detail of full-scan chromatograms' baseline, (C) sediment homogenate (10 g), (D) sediment homogenate, silica, alumina (1:1:1), and (E) sediment homogenate, silica, alumina, and Florisil (1:1:1:1)

Recovery Analysis and Method Detection Limits

Target analyte recoveries of the analytical method were determined by triplicate spike and recovery experiments of approximately 10-g sediment samples. The mean triplicate recoveries (n=3) of PCDD/Fs and dl-PCBs were 84±5.8% and 70±8.4%, respectively. The finalized method was also evaluated in triplicate using SRM 1944. Measured concentrations were compared with reference and certified values for PCDD/Fs and dl-PCBs, respectively. The PCDD/Fs and PCBs were extracted from 5.0 g and 0.5 g of SRM 1944 (n=3), respectively, and spiked with isotopically labeled

surrogate standards. Seven dl-PCBs were detected in SRM 1944 (PCB-77, 105, 114, 118, 156, 157, and 167) in concentrations ranging from 1.42 µg/kg to 62.9 µg/kg dry wt. Standard reference material 1944 provides certified values for PCB-105, 118, and 156. Analytical results using the SPLE method were within the expanded uncertainty of certified values, with the exception of PCB-156 (SPLE concentration 10.2±1.2 µg/kg; certified value 6.52±0.66 µg/kg). The SRM 1944 reference values were provided for all PCDD/Fs and ranged from 0.019 µg/kg to 5.80 µg/kg dry wt. Measured concentrations of PCDD/Fs ranged from 0.022 µg/kg to 4.58 µg/kg dry wt. The SRM 1944 2,3,7,8-TCDD concentration using SPLE was 0.209±0.039 µg/kg dry weight, compared with reference value of 0.133±0.009 µg/kg dry weight. Differences between reference and measured concentrations may be the result of differences in extraction and cleanup methods employed (i.e., Sohxlet or PLE and postextraction cleanup vs SPLE). As a result of interferences present in the SRM 1944, 1,2,3,7,8-pentachlorodibenzo-*p*-dioxin (PeCDD) and 1,2,3,7,8,9-hexachlorodibenzo-*p*-dioxin (HxCDD) were not detected. Statistically derived MDL values for PCDDs, PCDFs, and dl-PCBs were 21.9±23.7 pg/g, 12.5±3.49 pg/g, and 32.2±33.2 pg/g dry wt, respectively. The MDL for 2,3,7,8-TCDD was 22.67 pg/g dry wt as determined using HRGC-HRMS.

Environmental Samples: SJRWP characterization

Contaminant concentrations in the SJRWP. The method was used for the assessment of concentrations of PCDD/Fs and dl-PCBs in surficial sediments collected at the SJRWP Superfund site. Quality control and assurance protocols were followed and included laboratory reagent blanks and calibration curve verifications. Matrix spiked and

matrix spiked duplicates were analyzed to determine the precision of the method in surficial sediment samples. The mean (n=2) surrogate relative percent difference in matrix spiked and matrix spiked duplicate fortified sediment samples was 11.2±11.8%. The mean (n=2) surrogate relative percent difference of PCDDs, PCDFs, and dl-PCBs in matrix spiked and matrix spiked duplicates were 17.2±19.0%, 8.00±4.74%, and 8.69±3.85%, respectively. Mean (n=15) surrogate recovery of all surrogates measured in surficial sediment samples was 72.4±15.9% (PCDDs 74.3±6.0%; PCDFs 66.9±16.4%; dl-PCBs 82.8±23.4%).

Polychlorinated dibenzo-*p*-dioxins/dibenzofurans and dl-PCBs were detected in all surficial sediments samples from the SJRWP, and their concentrations ranged over five orders of magnitude (Figure 2.4). In general, dl-PCBs concentrations were greater than those of PCDDs and PCDF (Figure 2.5). In all 15 sediment samples, 2,3,7,8-TCDD, 2,3,7,8-tetrachlorodibenzofuran (TCDF), OCDD were detected. In six samples or fewer, PCB-81, PCB-169, 2,3,4,6,7,8-hexachlorodibenzofuran (HxCDF), and the penta, hexa, and hepta chlorinated PCDDs were detected. The highest concentrations of all congeners (except OCDD) were detected in the northern edge of the SJRWP in S09, S10, and S11. The lowest concentrations were found in S14, located in the southern edge of the SJRWP (Table 2). The contaminant profile of surficial sediments of the SJRWP was dominated by the presence of PCB-118 (contributing approximately 40% to total contaminant concentrations) followed by PCB-105 and 2,3,7,8-TCDF (approximately 15% and 11% mean contribution, respectively). However, S14 exhibit a different contaminant profile, with OCDD contributing approximately 60% to total concentrations. In addition, the highest concentration of OCDD (1190 pg/g dry wt) was found in S14. Principal

component analysis was used to evaluate the variance in the contaminant profile in sediment at each sampling site. The first two principal components PC1and PC2 explain 81% of the variance in the data and confirm that the concentration profile at S14 differs from the rest of the SJRWP samples (Figure 2.6). Dry weight concentrations of PCB-118 ranged from 290 pg/g to 220 000 pg/g with mean 34 000±66 000 pg/g and a median concentration of 5600 pg/g. Mean PCB-118 concentrations at each sampling site were approximately 12 times higher than those of 2,3,7,8-TCDD. Concentrations of 2,3,7,8-TCDD in the SJRWP ranged from 4.0 pg/g dry weight to 15 000 pg/g dry weight, with mean 2800±5200 pg/g dry weight and a median value of 530 pg/g dry weight (Table 2.2).

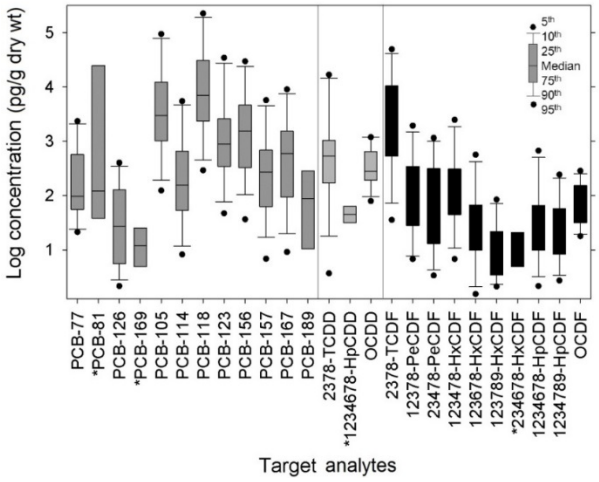

Figure 2.4. Concentrations of various congeners in sediment from the San Jacinto River Waste Pits Superfund site, Texas, USA, prior to remedial action. Asterisk indicates detection frequency <40%.

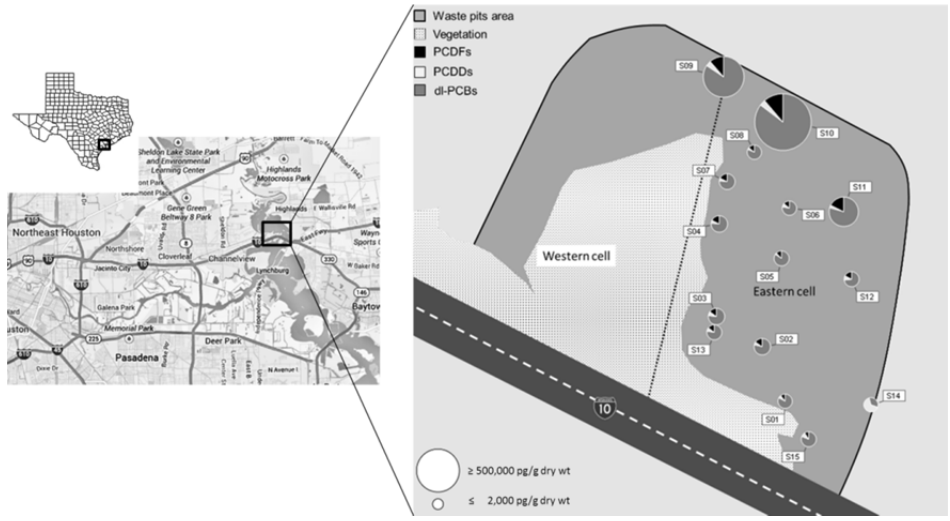

Figure 2.5. Sampling site locations and contaminant concentrations at the San Jacinto River Waste Pits Superfund site, Texas, USA, prior to remedial action. Dash line represents approximate division between Western and Eastern cells.4

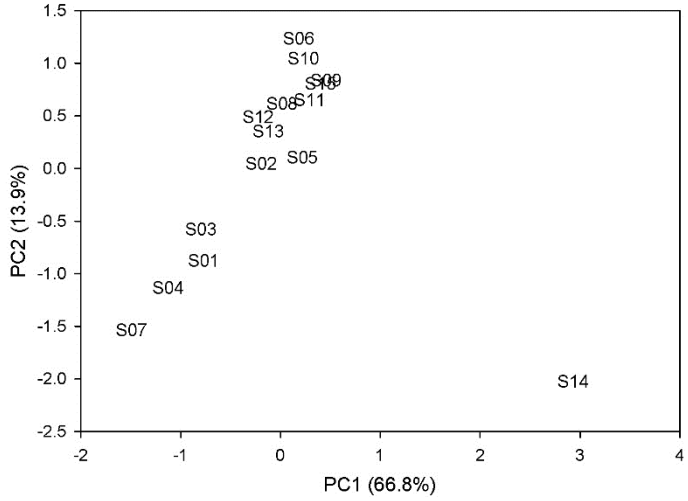

Figure 2.6. Principal component (PC) 1 and 2 for sediment concentrations from the San Jacinto River Waste Pits Superfund site, Texas, USA. S=sample site.

Table 2.2. Contaminant concentrations, prior to remediation, of surficial sediments from the San Jacinto River Waste Pits Superfund site, TX, USA

	Detection frequency	*pg g^{-1} dw*					
		Min. conc.[a]	*Max. conc.*	*Mean ± SD*			*Median*
PCBs							
PCB-77	14/15	21.3	2,350	387	±	698	86.8
PCB-81	3/15	38	24,900	1,670	±	6,430	120
PCB-105	15/15	124	94,300	14,100	±	27,600	2,300
PCB-114	15/15	8.2	5,480	805	±	1,610	125
PCB-118	15/15	292	225,000	34,400	±	66,300	5,650
PCB-123	15/15	47.2	34,600	5,180	±	10,100	882
PCB-126	14/15	2.16	403	68.4	±	115	20.5
PCB-156	15/15	36.6	29,700	5,050	±	8,590	1,120
PCB-157	15/15	6.87	5,710	920	±	1,640	178
PCB-167	15/15	9.16	9,030	1,440	±	2,590	258
PCB-169	4/15	2.02	28.4	4.31	±	9.15	17.1
PCB-189	15/15	0.92	317	97.6	±	120	24.7
PCDFs							
2,3,7,8-TCDF	15/15	35.7	49,500	8,700	±	15,100	1,970
1,2,3,7,8-PeCDF	14/15	6.79	1,940	303	±	557	57.0
2,3,4,7,8-PeCDF	14/15	3.39	1,150	186	±	342	31.8
1,2,3,4,7,8-HxCDF	14/15	6.79	2,480	392	±	699	125
1,2,3,6,7,8-HxCDF	14/15	1.54	565	90.2	±	159	29.8
1,2,3,7,8,9-HxCDF	12/15	2.12	84.7	13.8	±	23.7	3.98
2,3,4,6,7,8-HxCDF	4/15	3.88	22.8	3.51	±	7.09	13.0
1,2,3,4,6,7,8-HpCDF	14/15	2.16	673	102	±	189	33.3
1,2,3,4,7,8,9-HpCDF	12/15	2.73	243	38.9	±	69.5	13.1
OCDF	11/15	17.9	286	63.2	±	79.1	32.0
PCDDs							
2,3,7,8-TCDD	15/15	3.71	16,800	2,740	±	5,210	534
1,2,3,7,8-PeCDD	0/15	-	-	-	±	-	-
1,2,3,4,7,8-HxCDD	0/15	-	-	-	±	-	-
1,2,3,6,7,8-HxCDD	0/15	-	-	-	±	-	-
1,2,3,7,8,9-HxCDD	2/15	48.3	362	27.3	±	93.3	205
1,2,3,4,6,7,8-HpCDD	6/15	29.5	72.5	19.0	±	26.0	44.9
OCDD	15/15	79.3	1,190	447	±	358	279

[a] Excluding non-detects

TOC and black carbon normalization. Black carbon and TOC were determined for all surficial sediment samples (Supplemental Data, Table S2). Mean black carbon-to-TOC ratio was 9.8±1.2% for all sampling sites except S09, S10, and S11 (mean 4.1±0.88%). The TOC fractions at S09, S10, and S11 were higher than the rest of the samples, thus resulting in lower black carbon-to-TOC ratios. In addition, the highest contaminant loads corresponded to these samples. The evidence suggests that the high concentrations of contaminants found at these sampling sites are associated with the organic fraction of the sediment. It is also worth mentioning that these sampling sites are located on the northern edge of the SJRWP area (S09, S10, S11; Figure 2.6), suggesting that mixing and re-suspension of sediment, characteristic of tidally influenced sites, might have extended the contaminated area beyond the original perimeter of the SJRWP. The surficial sediment samples were collected from the eastern cell area of the SJRWP; however, the western cell of the SJRWP, an area covered by vegetation, was not sampled. Regression analysis between TOC and total contaminant concentration shows a linear relationship (r2¼0.89, p<0.001) between the 2 variables when all sites are included in the analysis (Supplemental Data, Figure S1A). However, when statistical outliers (S09, S10, and S11) are removed from the analysis, there is no linear relationship between the variables (r^2=0.127, p=0.256). Similar results are found for regression analysis between black carbon and total contaminant concentrations (Supplemental Data, Figure S1B). These results are consistent with previous studies of PCDD/Fs in similar locations in the Houston Ship Channel [19,22]. It is likely that the high contaminant concentrations may be the result of mixing and resuspension of contaminated sediments with high organic

matter content and not the result of contaminant partitioning into organic or black carbon fractions of the sediment.

Conclusions

The incorporation of adsorbents into the extraction cell resulted in an automated single-step extraction and cleanup method for the analysis of PCDD/Fs and dl-PCBs in surficial sediments. Mean surrogate recoveries of PCDD/Fs and dl-PCBs. were improved using SPLE (845.8% and 708.4%, respectively) from those reported on USEPA method 1613 (PCDD/Fs, 2–155%) and USEPA method 8290a (PCBs, 40–135%). To our knowledge, this is the first single-step automated extraction and cleanup method of PCDD/Fs and dl-PCBs from sediments. The incorporation of activated copper into the extraction bottle resulted in the removal of sulfur interferences from the extracts, thus eliminating the need for additional postextraction cleanup. The resulting method was successfully applied for the analysis of contaminated surficial sediments from the SJRWP. Polychlorinated dibenzo-p-dioxins/dibenzofurans and dl-PCBs were detected in all samples; and 2,3,7,8-TCDD and 2,3,7,8-TCDF were detected in all sediment samples as expected because of the use of the SJRWP to dispose of waste from pulp bleaching operations. Contaminant concentrations ranged over 5 orders of magnitude with dl-PCBs dominating the contaminant profile at the site. There was no relationship between total contaminant concentrations and TOC or black carbon when highly contaminated outliers were excluded from the analysis. However, this may be the result of the particular characteristics of the site, such as tidal influence, continuous dredging activities, sediment resuspension and mixing. The presence of higher concentrations of contaminants in the

periphery of the site, near the main channel of the San Jacinto River, could suggests the need to study adjacent habitats beyond the original perimeter of contamination

Acknowledgements

The authors are thankful for the financial support by the Texas Commission on Environmental Quality and Texas Environmental Health Institute (Project Ref. 32370108). We thank to B. Subedi and J. Berninger for assistance with collecting sediment samples from the San Jacinto River Waste Pits Superfund site in Houston, Texas, USA. The authors are grateful to A. Ramirez at the Baylor Mass Spectrometry Center and S. Dworkin at Baylor University for providing access to VG Prospec and Flash EA 1112 Series, respectively. The authors also thank R. Sheesley for her support with black carbon analysis.

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Supporting Information

For: Development and Application of a Novel Method for High-throughput Determination of PCDD/Fs and PCBs in Sediments [1]

L. Aguilar, E. S. Williams, B. W. Brooks, S. Usenko

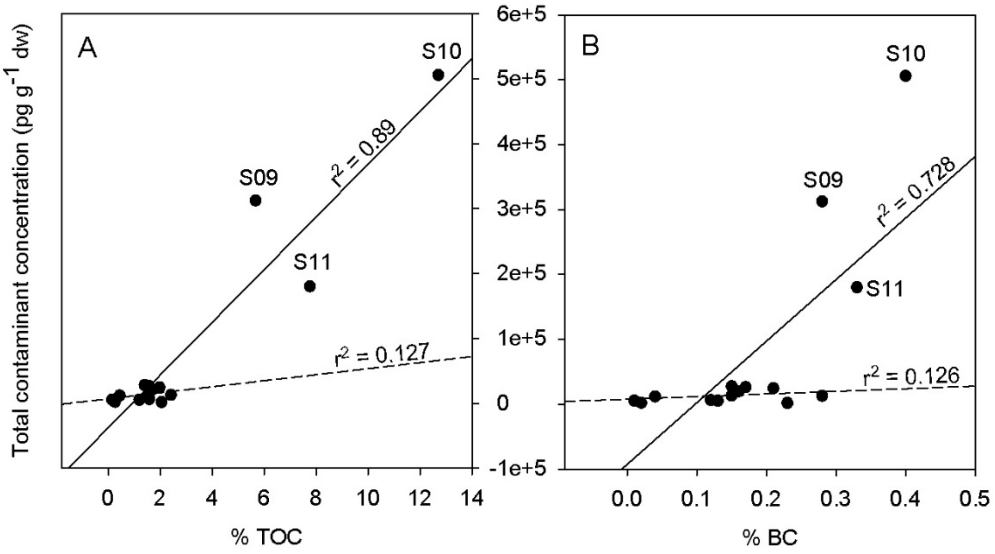

Figure S2.1. Linear regression analysis between total contaminant concentrations and (A) %TOC and (B) %BC in surficial sediment samples of the San Jacinto River Waste Pits Superfund site, TX, USA. The solid regression line includes all samples and the dash line excludes outliers S09, S10 and S11. TOC = Total organic carbon; BC = Black carbon.

[1]Reprinted with permission from [Aguilar, L.; Williams, E. S.; Brooks, B. W.; Usenko, S. Development and Application of a Novel Method for High-throughput Determination of PCDD/Fs and PCBs in Sediments. *Env. Toxicol. Chem*. **2014**. Copyright © 2014 SETAC.

Table S2.1. Sediment characteristics by sampling site from the San Jacinto River Waste Pits Superfund site, TX, USA, prior to remedial action.

Sample ID	Moisture %	TOC %	BC %	BC/TOC %
S01	20	0.26	0.02	9.0
S02	51	1.40	0.15	11.0
S03	57	1.58	0.17	11.0
S04	44	1.97	0.21	11.0
S05	23	0.44	0.04	8.0
S06	38	1.19	0.13	11.0
S07	37	1.64	0.16	10.0
S08	18	0.15	0.01	9.0
S09	34	5.67	0.28	5.0
S10	51	12.7	0.40	3.0
S11	48	7.76	0.33	4.0
S12	63	2.41	0.28	11.0
S13	57	1.53	0.15	9.0
S14	46	2.05	0.23	11.0
S15	56	1.58	0.12	8.0

TOC = total organic carbon; BC = black carbon

CHAPTER THREE

Applications of SPLE Methods

Excerpts from this chapter published as:
Subedi, B.; Aguilar, L.; Williams, E. S.; Brooks, B. W.; Usenko, S. Selective Pressurized Liquid Extraction Technique Capable of Analyzing Dioxins, Furans, and PCBs in Clams and Crab Tissue. *B Environ Contam Tox* **2013**. *92* (4), 460-465[1]
and
Oziolor, E. M.; Bigorgne, E.; Aguilar, L.; Usenko, S.; Matson, C. W. Evolved Resistance to PCB- and PAH-induced Cardiac Teratogenesis and Reduced CYP1A Activity in Gulf Killifish (*Fundulus grandis*) Populations from the Houston Ship Channel, Texas. *Aquat Toxicol* **2014**. *150*, 210-219[2]

In Subedi et al. 2014 *Selective Pressurized Liquid Extraction Technique Capable of Analyzing Dioxins, Furans, and PCBs in Clams and Crab Tissue*; the author contributed to sample preparation, discussion of results, and manuscript preparation.

In Oziolor et al. 2014 *Evolved Resistance to PCB- and PAH-induced Cardiac Teratogenesis and Reduced CYP1A Activity in Gulf Killifish (Fundulus grandis) Populations from the Houston Ship Channel, Texas*; the author contributed to sample preparation and analysis, discussion and interpretation of results, and manuscript preparation.

[1]Reprinted with permission from [Subedi, B..; Aguilar, L. Williams, E. S.; Brooks, B. W.; Usenko, S. Selective Pressurized Liquid Extraction Technique Capable of Analyzing Dioxins, Furans, and PCBs in Clams and Crab Tissue. *B Environ Contam Tox* **2013**. 92 (4), 460-465]

[2]Reprinted with permission from [Oziolor, E. M.; Bigorgne, E.; Aguilar, L.; Usenko, S.; Matson, C. W. Evolved Resistance to PCB- and PAH-induced Cardiac Teratogenesis and Reduced CYP1A Activity in Gulf Killifish (*Fundulus grandis*) Populations from the Houston Ship Channel, Texas. *Aquat Toxicol* **2014**. 150, 210-219]

Introduction

Selective pressurized liquid extraction of contaminants from solid matrices

Historical methods of extraction of organic contaminants used in routine analysis include automated and conventional Soxhlet extraction and pressurized liquid extraction (PLE) (EPA 1994b, EPA 1996b, EPA 2007a). Traditionally, these methods required extensive post-extraction cleanup prior to analysis resulting in lenghty sample preparation. However, over the past 5 to 10 years several research groups combined PLE and typical post-extraction cleanup adsorbents into a single automated step. These new methods result in faster, cleaner, high-througput sample preparation, reduced time and solvents required, exposure to chemicals, and overall analysis cost. Their development is usually based on conventional methods and incorporate typical post-extraction adsorbents into the extraction cell. The adsorbents retain potential matrix interferences while allowing the extraction of target anlytes for a selective PLE (SPLE). In addition, certain adsorbents can be incorporated into the extraction cell to perform typical post-extraction fractionation steps (Haglund et al. 2007, Subedi and Usenko 2012, Subedi et al. 2014). For example, Subedi et al. followed the recomendations from EPA method 1613 (EPA 1994a) to incorporate the necessary sorbents required for the SPLE and fractionation of PCDD/Fs and dl-PCBs from clams and crabs (Subedi et al. 2014).

> Using EPA method 1613(EPA 1994a) as a guide, silica gel, florisil, carbopack/celite, and alumina, were identified as the necessary cleanup adsorbents. Silica gel and alumina have been used to retain lipids, while florisil has been shown to remove large biomolecules (Subedi and Usenko 2012). Carbopack, dispersed in celite, is typically used to fractionate planar compounds (i.e. PCDD/Fs) from the non-planar compounds (i.e. PCBs). Typically, PCDD/Fs are eluted from the carbopack using a planar solvent such as toluene (EPA 1994a). Matrix interferences were retained

> by the adsorbents, while target analytes were allowed to pass through the adsorbent layers and collected in a collection bottle. This high-throughput analytical method incorporates a PLE technique which combines the required cleanup techniques into a single automated step. (Subedi et al. 2014)

The automation and simplification of the sample preparation protocol reduces sample handling and opportunities for sample loss and contamination. This results in narrow ranges of overall method recoveries. For example, surrogate recoveries of PCDD/Fs reported by method EPA 1613 are <25 to >155% while recoveries using SPLE from sediments are 84 ± 5.8% (Aguilar et al. 2014) and from fish composites 85 ± 3.0% (Subedi and Usenko 2012). Similarly, Subedi et al. reported mean recoveries of PCDDs in clams and crabs were 94 ± 2.3 % and 94 ± 3.0 %; and PCDFs were 84 ± 1.6 % and 92 ± 2.8 %, respectively using SPLE with in-cell fractionation (Subedi et al. 2014).

Application of SPLE methods to environmental samples

In addition, the high-throughput characteristic of SPLE methods increase laboratory preparedness and capacity. SPLE methods are useful during projects that require analysis of multiple samples with rapid turnaround times. For example, Subedi and Usenko (Subedi and Usenko 2012) and Aguilar et al. (Oziolor et al. 2014) developed SPLE methods for the automated simultaneous extraction of PCDD/Fs and dl-PCBs from fish tissues and surficial sediments, respectively. This two recently developed SPLE methods (Subedi and Usenko 2012, Oziolor et al. 2014) were used as part of an ecotoxicology study, to extract PCDD/Fs and dl-PCBs from whole body Gulf killifish (*Fundulus grandis*) and surficial sediments from two Superfund sites and a reference site in the Houston Ship Channel (HSC) in Houston, TX (Oziolor et al. 2014).

The HSC is a heavily polluted estuarine zone, which contains about 40% of the nation's oil refineries (Howell et al. 2011). Increased loads of anthropogenic contaminants have been found throughout the HSC in sediment, water and tissue samples (Suarez et al. 2006, Lakshmanan et al. 2010). Several locations along the HSC are on the National Priorities List, including Patrick Bayou (EPA ID: TX0000605329) and Vince Bayou (Part of US Oil Recovery; EPA ID: TXN000607093) (Fig. 3.1). As part of the Superfund program, these locations were selected for possible remedial actions because of their high levels of contamination with pesticides, PCBs, PCDD/Fs and PAHs. Previous reports have documented levels of total PCDD/Fs reaching 1 mg/kg, while PCBs and PAHs averaged 10 mg/kg and 50 mg/kg, respectively, in sediments along the HSC (Anchor QEA, 2010). PCDD/Fs vary spatially, and have been reported at concentrations of 360–690 ng/g organic carbon (OC) and 0.69–5.3 ng/g lipid in fish (Howell et al. 2011). While PCDD/Fs are observed to decrease toward the mouth of the channel, PCB concentrations tend to increase, reaching levels between 14 and 19 μg/g OC and 0.42 and 31 μg/g lipid in fish (Howell et al. 2011). (Oziolor et al. 2014)

PCBs have also been found in high concentrations (~37 pg/g) in channel catfish (Ictalurus punctatus) tissues collected from the San Jacinto River Waste Pits Superfund site in the HSC (Subedi and Usenko 2012). These classes of contaminants are persistent, bioaccumulative and toxic, and in addition have been present in the HSC for over 50 years, resulting in chronic exposure of resident aquatic species (Yeager et al. 2007). The HSC is also contaminated with high levels of PAHs, as a result of point and nonpoint source pollution, including both petrogenic and pyrogenic sources (Howell et al. 2011). Gangs Bayou (GB) was selected as a reference because it is relatively isolated from the HSC, but is still within Galveston Bay.(Oziolor et al. 2014)

Materials and Methods

All chemicals were purchased from commercial vendors at reagent grade or higher and stored in accordance with the manufacturer's recommendations. The target analyte list included seven dioxins, ten furans, and twelve dioxin-like PCBs. Isotopically labeled versions of PCDD/Fs and DL-PCBs congeners were used as surrogate standards. $^{13}C_{12}$-PCB189 was used as internal standard for PCDD/Fs and DL-PCBs. PCDD/Fs and DL-PCBs standards were purchased from Wellington Laboratories (Guelph, ON, Canada). Basic alumina, Celite®, Carbopak™, Florisil®, and copper powder were purchased from Sigma–Aldrich;

silica gel, sodium sulfate, toluene (TOL), dichloromethane (DCM), and n-hexanes (HX) were purchased from BDH Chemicals (West Chester, PA, USA). Target analytes were extracted from fish and sediments using an accelerated solvent extractor (ASE; ASE 350 Dionex–Thermo Fisher Scientific, Sunnyvale, CA, USA). PCDD/Fs and DL-PCBs were extracted from fish using a previously described selective pressurized liquid extraction (SPLE) method (Subedi and Usenko 2012). Briefly, an aliquot of 1 g was taken from whole-fish homogenates and further homogenized with anhydrous sodium sulfate to remove moisture, until the homogenate had the consistency of a free flowing powder. The homogenates were placed on top of pre-cleaned adsorbents layered in a 100 mL ASE cell. DL-PCBs were extracted using DCM:HX and PCDD/Fs were subsequently extracted using TOL. PCDD/Fs and DL-PCBs were extracted from sediment using a previously developed SPLE method (Oziolor et al. 2014). Briefly, an aliquot of 1 g of sediment was homogenized with anhydrous sodium sulfate, to remove moisture and placed on top of pre-cleaned adsorbents layered in a 100 mL ASE cell. PCDD/Fs and DL-PCBs were extracted using TOL. Extraction conditions were 100 °C, 1500 psi, 5 min static time, and 75% flush volume. The instrumental parameters and settings used for the quantitation of PCDD/Fs and DL-PCBs have been previously described (Subedi and Usenko 2012). Target analytes were separated and quantified using high-resolution gas chromatography coupled with electron capture negative ionization mass spectrometry using selective ion monitoring (HRGC–ECNI/MS). The GC system used was an Agilent 7890A coupled with 5975C MSD (Santa Clara, CA, USA). Chromatographic separation was performed using a capillary DB-Dioxin (60 m × 0.25 mm × 0.15 μm) column (J & W Scientific, USA). (Oziolor et al. 2014)

Quality assurance and control protocols were followed and have been previously described (Subedi and Usenko 2012). Briefly, calibration curve verification standards (CCV, mid-point of calibration curve) were analyzed after every third sample to monitor instrument performance. Matrix spiked samples (MS, MSD), laboratory blanks, and reagent blanks were also analyzed. Target analytes were identified based on their retention time and quantitative to qualitative ions ratios (±20%). Due to the reduced sensitivity of HRGC-ECNI/MS, 2,3,7,8-TCDD and OCDD were excluded from the target analyte list. Percent moisture was determined for sediment samples and percent lipid was determined for fish samples following standard protocols (Lauenstein and Cantillo 1998). In summary, percent moisture content was determined by drying an aliquot of sediment to constant weight at 110 °C. Percent lipid was determined gravimetrically by extracting

an aliquot of homogenized fish with DCM at 100 °C. The extracts were concentrated to 5 mL, and an aliquot of 3 mL was weighed on a dried, tared beaker. (Oziolor et al. 2014)

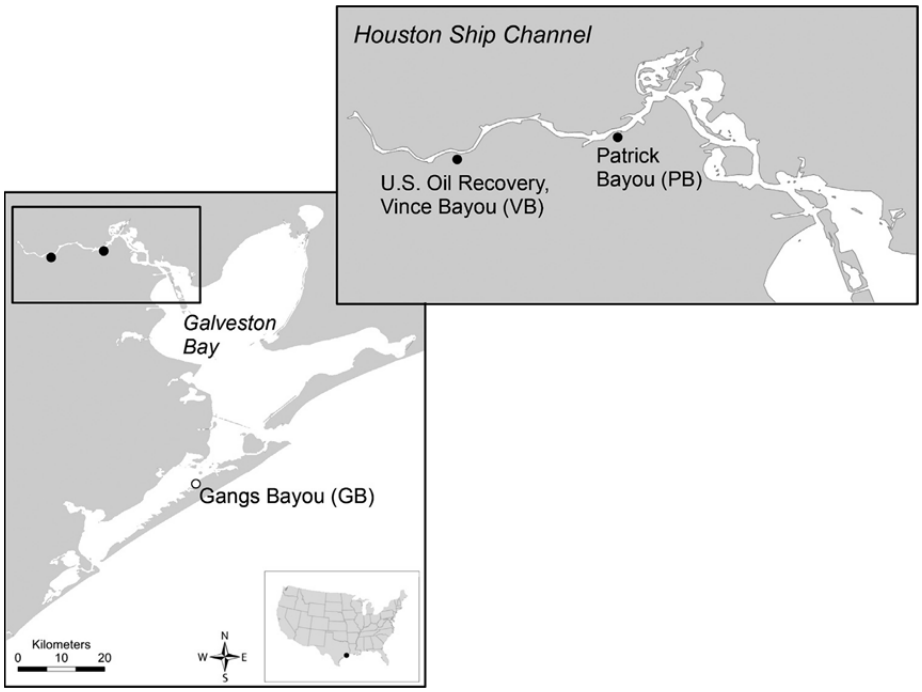

Figure 3.1. Map of the Galveston Bay with sampling locations from the Houston Ship Channel (PB and VB) and the reference location on Galveston island (GB). (Oziolor et al. 2014)

Results

Fish collected from all three sampling locations had detectable levels of the contaminants analyzed (Fig. 3.2A). GB fish contained lower total PCBs (*t*-test: GB vs. PB, $p < 0.0001$; GB vs. VB, $p = 0.03$; PB vs. VB, $p < 0.0001$) and comparable PCDD/F concentrations (*t*-test: GB vs. PB, $p = 0.56$; GB vs. VB, $p = 0.03$; PB vs. VB, $p = 0.053$) to the contaminated sites (Table S3.1). Sediment concentrations showed lower levels of both PCBs and PCDD/Fs in GB, with the highest contamination observed at PB (Fig. 3.2B, Table S3.2). Male fish were also observed to contain higher loads of contaminants than females, adding to the variability of sample concentrations. (Oziolor et al. 2014)

Sediment and fish concentrations showed agreement between the sites with the highest PCBs concentrations found at PB followed by VB. PCDD/Fs concentrations were

slightly higher in sediments of the Superfund sites, PB and VB, than the reference site GB. PCBs concentrations were higher than PCDD/Fs concentrations in fish samples from all locations and sediments of PB and VB (Fig. 3.2).

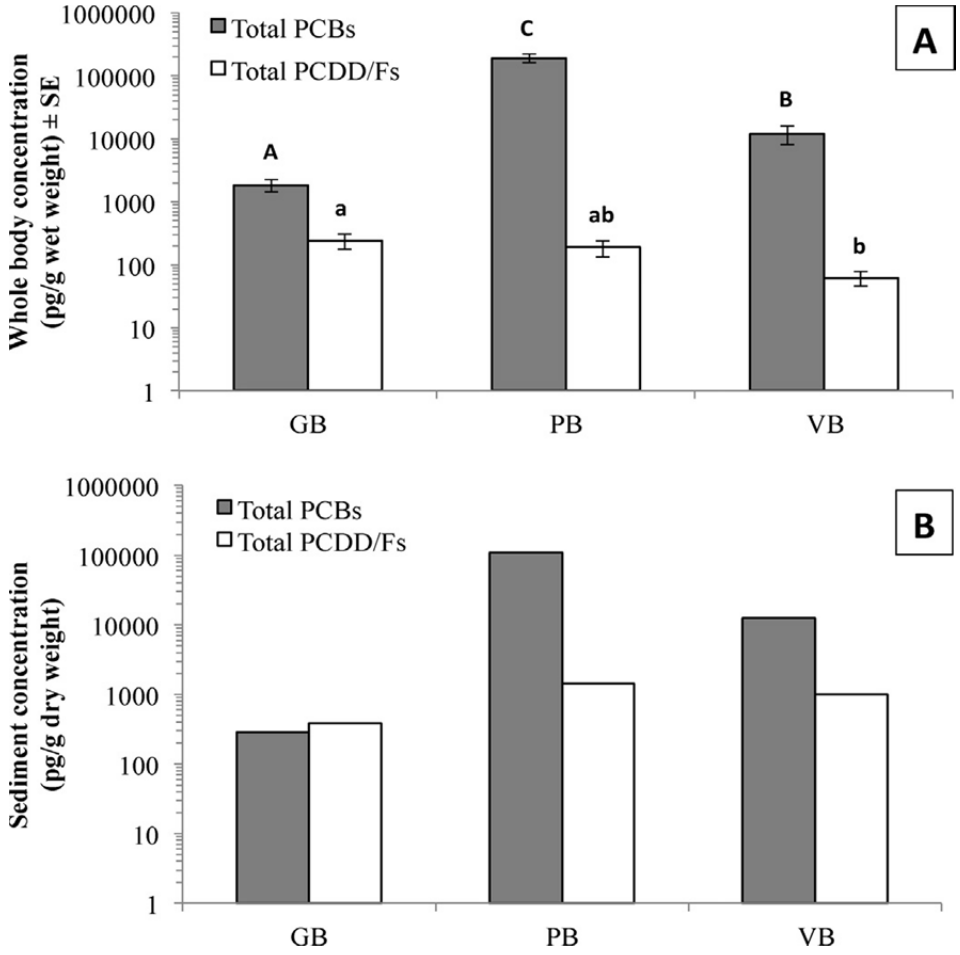

Figure 3.2. PCB and PCDD/F concentrations in fish and sediment. Part A: Wet weight concentrations for total PCBs and PCDD/Fs in fish homogenates from sampled sites. Letters denote statistically different means. Total PCB concentrations differed subtantially between locations (*t*-test: GB vs. PB, $p < 0.0001$; GB vs. VB, $p = 0.03$; PB vs.VB, $p < 0.0001$), while total PCDD/F concentrations were of comparable magnitude (*t*-test: GB vs. PB, $p = 0.56$; GB vs. VB, $p = 0.03$; PB vs. VB, $p = 0.053$). Part B: Dry weight sediment concentrations for total PCBs and PCDD/Fs collected at sampling sites. GB shows lower concentrations of both classes of compounds than contaminated sites. (Oziolor et al. 2014)

Conclusions

Even though the purpose of the study was to investigate the effects of chronic exposure of PCBs and PAHs in the genetic variability of *F. grandis*, PCDD/Fs were also reported due to their historic presence in the Houston Ship Channel. The availability of an automated single step extraction method for all three classes of contaminants was also a determining factor in the decision to include PCDD/Fs in the final target analyte list.

> Sediment concentrations at PB and VB were consistent with the high contamination reported by the TCEQ and previous literature (Howell et al., 2011; Lakshmanan et al., 2010; Subedi and Usenko,2012). Concentrations of PCDD/Fs at GB were surprising and may have been caused by a more recent contamination of the site, since we found no previous reports of environmental contamination in that area. Represented concentrations were derived from one sampling period, which may not be extensive enough to describe the profile of the population and location. While we may have sampled at a hot spot of contamination, the rest of the bayou may be less polluted. (Oziolor et al. 2014)

The presence of these contaminants in sediments of all sites reveals a concentration baseline of PCDD/Fs and PCBs exposure for all *F. grandis* populations. Further monitoring of the sediments at reference site, GB, is necessary to gain information of the extent of contamination of the area. Screening several points within GB would provide a clearer picture of the distribution of contaminants and exposure of *F. grandis* populations.

Supporting Information

For: Evolved Resistance to PCB- and PAH-induced Cardiac Teratogenesis and Reduced CYP1A Activity in Gulf Killifish (*Fundulus grandis*) Populations from the Houston Ship Channel, Texas.[3]

E. M. Oziolor, E. Bigorgne, L. Aguilar, S. Usenko, C. W. Matson

Table S3.1. Whole body wet weight toxin concentrations in fish collected from three sampling locations.

			Mean concentrations in fish pg/g wet wt			Standard Error		
		Analytes	GB	PB	VB	GB	PB	VB
Polychlorinated	Biphenyls	PCB-81 (TA)	ND	310	23	ND	130	9.3
		PCB-77 (TA)	ND	4,100	39	ND	1,700	16
		PCB-123 (TA)	76	6,400	850	31	2,600	350
		PCB-118 (TA)	560	37,000	5,300	230	15,000	2,200
		PCB-114 (TA)	28	1,500	620	11	600	250
		PCB-105 (TA)	260	21,000	1,600	100	8,500	660
		PCB-126 (TA)	9.0	110	25	3.6	47	10
		PCB-167 (TA)	25	680	330	10	280	140
		PCB-156 (TA)	66	2,300	1,500	27	950	620
		PCB-157 (TA)	17	390	180	7.0	160	72
		PCB-169 (TA)	6.5	18	5.7	2.6	7.4	2.3
		PCB-189 (TA)	8.0	63	50	3.2	26	20
	Dibenzofurans	2378-TCDF (TA)	62	37	38	25	15	15
		12378-PeCDF (TA)	7.4	8.5	ND	3.0	3.5	ND
		23478-PeCDF (TA)	8.4	13	ND	3.4	5.2	ND
		123478-HxCDF (TA)	7.4	7.9	ND	3.0	3.2	ND
		123678-HxCDF (TA)	7.4	7.9	ND	3.0	3.2	ND
		123789-HxCDF (TA)	7.4	9.0	ND	3.0	3.7	ND
		234678-HxCDF (TA)	8.4	10	ND	3.4	3.9	ND
		1234678-HpCDF (TA)	8.4	8.7	2.9	3.4	3.6	1.2
		1234789-HpCDF (TA)	7.4	11	ND	3.0	4.3	ND
		OCDF (TA)	22	ND	ND	9.0	ND	ND
	Dibenzo-p-dioxins	12378-PeCDD (TA)	ND	ND	ND	ND	ND	ND
		123478-HxCDD (TA)	9.5	12	ND	3.9	4.8	ND
		123678-HxCDD (TA)	20	20	ND	8.2	8.3	ND
		123789-HxCDD (TA)	26	23	ND	11	9.4	ND
		1234678-HpCDD (TA)	ND	ND	ND	ND	ND	ND

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Table S3.2. Dry weight normalized concentrations for toxins in sediment collected from three sampling locations. ND: Concentrations below method detection limits.

			Concentration pg/g dw		
			GB	PB	VB
Polychlorinated	Biphenyls	PCB-81 (TA)	ND	ND	ND
		PCB-77 (TA)	ND	3,300	260
		PCB-123 (TA)	ND	8,800	1,190
		PCB-118 (TA)	140	56,000	5,500
		PCB-114 (TA)	ND	1,200	90
		PCB-105 (TA)	ND	28,000	1,900
		PCB-126 (TA)	33	360	58
		PCB-167 (TA)	18	2,300	480
		PCB-156 (TA)	37	1,500	2,600
		PCB-157 (TA)	26	1,500	260
		PCB-169 (TA)	15	10	ND
		PCB-189 (TA)	18	250	140
	Dibenzofurans	2378-TCDF (TA)	69	130	140
		12378-PeCDF (TA)	15	10	24
		23478-PeCDF (TA)	11	ND	16
		123478-HxCDF (TA)	ND	18	47
		123678-HxCDF (TA)	ND	23	ND
		123789-HxCDF (TA)	ND	ND	ND
		234678-HxCDF (TA)	ND	ND	ND
		1234678-HpCDF (TA)	29	87	55
		1234789-HpCDF (TA)	ND	23	13
		OCDF (TA)	120	1,000	230
	Dibenzo-p-dioxins	12378-PeCDD (TA)	ND	ND	ND
		123478-HxCDD (TA)	ND	ND	ND
		123678-HxCDD (TA)	ND	ND	34
		123789-HxCDD (TA)	ND	ND	ND
		1234678-HpCDD (TA)	130	100	430

CHAPTER FOUR

Development and Application of a High-Throughput Digestion Method of Mercury and Selenium in Pacific Walrus (*Odobenus rosmarus divergens*) Muscle Tissue

Aguilar, L.; Trumble, S. J.; Dehn, L-A.; Usenko, S. (In preparation for *Mar Pollut Bull*)

Abstract

This study presents the development and application of a high-throughput digestion method for the analysis of Mercury (Hg) and Selenium (Se) from a single sample of skeletal muscle of Pacific walrus (*Odobenus rosmarus divergens*). Hg is a global pollutant capable of undergoing long-range transport and accumulation in Arctic ecosystems. Se is an essential element and has a relatively high binding affinity for Hg. Improvement of conventional EPA methods presented here focused on sample preparation (i.e. tissue digestion) and utilized traditional instrumentation. This method reduces sample preparation and requirements by ~50% when compared to performing two separate digestions following conventional methods. The suitability of the method was evaluated by triplicate spike and recovery experiments and analysis of standard reference material (SRM 1946). The method was applied to the analysis of Hg and Se concentrations from 28 Pacific walrus from Savoonga and Gambell, Alaska, collected during the 2009 and 2010 subsistence hunts. Hg and Se were quantified in all samples with concentrations ranging from 9 to 32 and 900 to 7,300 μg/kg, respectively. This study contributes to fill in the vast gap in information of Hg and Se concentrations in tissues of Pacific walrus. Se:Hg molar ratios in skeletal muscle of Pacific walrus are reported for the first time with an average molar ratio of 85 to 880.

Introduction

There are two conventional methods for the digestion and analysis of Mercury (Hg; EPA method 1631) and Selenium (Se; EPA method 3050B) in biological tissues (EPA 1996a, EPA 2002). However, when analyzing rare samples, such as tissues from Arctic marine mammals, it is not possible to perform two separate analysis due to the limited sample availability. In these cases, conventional methods can be used as a framework for the development of new sample preparation methods that expand the target analyte list to include all analytes of interest. In this particular case, EPA methods 1631 and 3050B can be used as a template for the development of a single digestion method for Hg and Se from biological tissues.

Hg is a naturally occurring element, found in ores mainly bound to sulfur as cinnabar and as an impurity in coal and metals. It is released into the environment from anthropogenic (e.g. fossil fuel combustion, mining, industrial processes) as well as natural (e.g. re-emission from oceans, geogenic, biomass burning, and weathering) sources (Bigham et al. 2006, Pirrone et al. 2010). Primary anthropogenic sources account for approximately 20-30% of the estimated annual global atmospheric emissions, (Pirrone et al. 2010, AMAP 2011, Driscoll et al. 2013) with two thirds being emitted from fossil fuel combustion (Pacyna et al. 2006). Estimating global Hg concentration trends is a very complex and colossal task. Through the years, Hg concentrations in sediment cores, peat deposits, and air records have been used to estimate global trends (AMAP 2011). Analysis of Hg in lake sediment cores from the United States (Minnesota and Wisconsin) showed that atmospheric Hg inputs have increase >3 times since pre-industrial times, with an average annual increase of ~2% (Swain et al. 1992). Similarly,

researchers have attempted to reconstruct worldwide trends from direct atmospheric measurements. Slemr et al., found an increase of 1.2-1.5% per year in atmospheric Hg concentration from 1977-1990 (Slemr and Langer 1992, Slemr et al. 2011). From 1990 – 1994 Hg concentrations dropped 5% per year and remained steady from 1996 – 2001 (Slemr et al. 1995, Slemr et al. 2011). Subsequent estimates have shown decline or no discernable trend in background concentrations at high and mid-latitudes (1996-2009; (Ebinghaus et al. 2011); 2000-2009; (Berg et al. 2013, Cole et al. 2013)). These observations are in contrast with estimates of increasing global anthropogenic emissions; highlighting the need for further monitoring of shifts in known regional sources (i.e. North America and Asia) (Sunderland et al. 2008, Pacyna et al. 2010, Pirrone et al. 2010). Gaseous elemental mercury (Hg^0) has an atmospheric residence time of approximately one year, which allows for its long-range atmospheric transport to remote regions such as the Arctic (Driscoll et al. 2013). In the atmosphere, Hg^0 is oxidized to ionic Hg (Hg(II)), in the presence of sunlight and halogens, and removed by dry and wet deposition (Lindberg et al. 2002). In the aquatic environment, Hg is found mainly as Hg(II) and methyl mercury (CH_3Hg). Hg(II) has a high affinity for sulfides and under anoxic conditions sulfate-reducing bacteria methylate Hg(II) present in water and sediments to CH_3Hg (Morel et al. 1998). CH_3Hg is bioavailable to most aquatic organisms and known to bioaccumulate and biomagnify along marine food webs (Atwell et al. 1998, Dehn et al. 2006b, Seixas et al. 2014a). CH_3Hg is a neurotoxin, and can cross the blood-brain, placenta, and intestinal tract barriers becoming a systemic contaminant in the body. Long-lived marine mammals, such as walrus (*Odobenus rosmarus*), accumulate Hg throughout their entire life (Born et al. 1981, Taylor et al. 1989,

Warburton and Seagars 1993, Wagemann and Stewart 1994, Outridge et al. 2002, FWS 2007). Approximately, 90% of the Hg that accumulates in muscle tissue of marine mammals, such as walrus, is in the form of CH_3Hg (Wagemann et al. 1998, Woshner et al. 2001, Dehn et al. 2006b).

Selenium (Se) is an essential trace element in vertebrates, including marine mammals and humans, and is a component of 25 proteins (selenoproteins) associated with thyroid hormone function and antioxidant activities in humans (Taylor et al. 2009). Se deficiency can cause cardiac and skeletal muscle disorders in humans and cattle (Rederstorff et al. 2006); while high concentrations of Se cause developmental effects in birds and fish (Hamilton 2004). The main pathway of Se intake in the marine environment is through the food web (Hamilton 2004) where Se bioaccumulates at low trophic levels (Kehrig et al. 2013, Seixas et al. 2014b).

In recent studies, it was elucidated that Hg acts as an inhibitor of selenoproteins (Raymond and Ralston 2009, Ralston and Raymond 2010, Ralston and Raymond 2014). Ionic Hg has a high binding affinity for Se. As a result, Hg binds to selenide, thus limiting the availability of Se for selenoprotein synthesis. Se:Hg molar ratios close to, or lower than, 1:1 are an indicator of compromise selenoprotein synthesis. Therefore, concentrations of Se in surplus of Hg (molar ratios > 1:1) are necessary to maintain protein homeostasis in the organism. Koeman (1973, 1975), observed a positive correlation, and a 1:1 molar ratio, between Hg and Se concentrations in liver, kidney, and brain tissues of Arctic marine mammals (Koeman et al. 1973, Koeman et al. 1975). Given its role in Hg toxicity it is advisable to report Se concentrations, as well as Se:Hg molar ratios, when assessing Hg concentrations.

Temporal and spatial information on Hg and Se concentrations in Arctic marine mammals is scarce. There is a particularly large gap in reported concentrations of these elements in tissues of Pacific walrus (*Odobenus rosmarus divergens*) of Alaska, USA (Born et al. 1981, Taylor et al. 1989, Warburton and Seagars 1993, Ponce 1997, Dehn et al. 2006b, Welfinger-Smith et al. 2011). The majority of the studies that report concentrations of Hg and Se analyzed liver and kidney tissues; with only two studies presenting concentration of Hg and one of Se in muscle tissue (Born et al. 1981, Welfinger-Smith et al. 2011). Marine mammals, such as walrus, are a significant part of the subsistence harvest of Native Alaskan (FWS 2007, Welfinger-Smith et al. 2011). Therefore, it is important to monitor contaminant concentrations in the tissues of these traditional food sources.

The objectives of this study are: 1) to develop a method for the digestion of total mercury (THg) and Se from skeletal muscle (*L. dorsi*) of Pacific walrus; 2) to apply this method for the analysis of skeletal muscle of Pacific walrus; and 3) to provide Se:Hg ratios for muscle tissue of Pacific walrus.

Materials and Methods

Chemicals

Chemicals and standards were purchased from commercial vendors at reagent grade and stored in accordance with manufacturer's recommendations. Concentrated nitric acid, hydrochloric acid, and stannous chloride, were purchased from BDH (Radnor, PA, USA); potassium bromide and hydroxylamine hydrochloride were purchased from EMD Millipore (Billerica, MA, USA). Standard Reference Material® 1946 (SRM 1946)

Lake Superior fish tissue was purchased from the National Institute of Standards and Technology (Gaithersburg, MD, USA). THg, Se, and Rh standards were purchase from Perkin-Elmer (Waltham, MA, USA).

Sampling

Pacific walrus' skeletal muscle (*L. dorsi*) was collected during the spring 2009 (n=21) and 2010 (n=7) subsistence hunts in Savoonga and Gambell, Alaska (Figure 4.1). Muscle samples were collected and placed in 2 mL cryovials and stored on dry ice until shipped to Baylor University. Samples were stored at -80 °C until analysis.

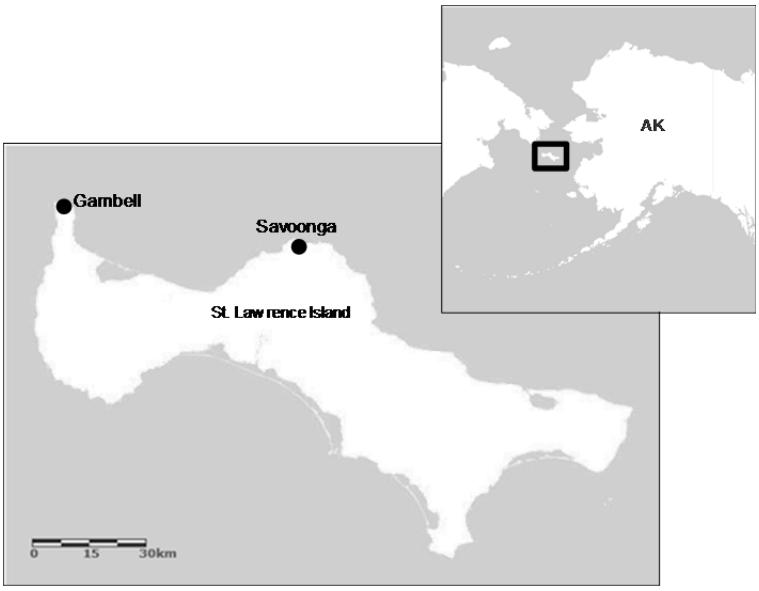

Figure 4.1. Locations where subsistence-harvested Pacific walrus were collected.

Total Mercury (THg) and Se Analysis

Due to the rarity of the samples and ultra-trace concentrations levels there was a need for a high-throughput digestion method capable of extracting Hg and Se from a single sample. EPA methods 1631 (Hg) and 3050B (metals including Se) were used as a starting point for the development of a single digestion method for Pacific walrus muscle

tissue (EPA 1996a, EPA 2002). The final method was conducted as follows: an aliquot of 0.5 g of walrus' skeletal muscle tissue was digested at ~70 °C in 5 mL concentrated nitric acid (70% w/w) for three hours under reflux. After digestion, the solubilized samples were allowed to cool down and an aliquot of 2 mL was reserved for elemental analysis. The remaining 3 mL were diluted to 40 mL with BrCl (0.02 N) and allowed to cool. An aliquot of 4 mL was further diluted to 40 mL with nanopure water (18.2 MΩ). The diluted digestate was analyzed for Hg using a Tekran 2600 cold vapor atomic fluorescence (CVAFS) mercury analyzer (Tekran, Knoxville, TN) with dual stage gold preconcentration. The aliquot reserved for elemental analysis was diluted to 75 mL with nanopure water. The nitric acid concentration of the diluted sample was 2% w/w. Se was analyzed by inductively coupled plasma mass spectrometry (ICP-MS) using the ELAN 9000 ICP-MS (Perkin-Elmer, Waltham, MA), with ^{103}Rh as internal standard. Instrumental parameters and monitoring ions are presented in Table S4.1.

Recovery Analysis, Method Detection Limits, and Quality Assurance and Control

Quality control protocols recommended by EPA method 1631 were followed to demonstrate accuracy and precision of the method, monitor matrix interferences, and confirm adequate instrument performance. The suitability of the digestion method to extract THg and Se was evaluated by triplicate spike and recovery experiments (using store bought beef as a surrogate matrix) and analysis in triplicate of a standard reference material (SRM 1946). Briefly, an aliquot of 0.5 g was spiked with 4 ng of mercury and 2.5 µg of Se and digested as previously described. SRM 1946 was also digested following the method described above and THg and Se concentrations were compared to certified and reference concentrations, respectively, reported in the certificate of analysis

and the variation reported as relative percent difference (RPD). Method detection limits (MDL) were statistically derived by measuring seven replicates of beef muscle tissue spiked with 0.4 ng of mercury and 250 ng of each Se (EPA 1986). MDL represent the lowest concentration that can be reported as non-zero with 99% confidence (EPA 1986). Mean triplicate recoveries (± SD), SRM 1946 concentrations and RPD, and MDL values are presented below. Duplicate samples, laboratory reagents blanks, method blanks, ongoing precision and recovery samples, and calibration check verification (CCV; 4th point of a 7-points calibration curve) standards were analyzed every 10 samples for Hg and every five samples for Se analysis.

Statistical Analysis

After log transforming data to ensure normality, linear regression analysis was employed to evaluate trends between concentrations of Hg and Se in muscle of Pacific walrus. Pearson's product moment correlation was used to evaluate the relationship between concentrations of Hg and Se for all samples, by year, and by location. Differences in wet weight concentrations of Hg and Se by sampling year or location were examined using student *t*-tests ($\alpha = 0.05$).

Results and Discussion

Conventional methods were used as a framework to develop a new method for the simultaneous digestion of THg and Se from skeletal muscle of Pacific walrus. The new method combined the two conventional methods used for THg and Se analysis (EPA 1631 and EPA 3050B) and thus reduced sample preparation time by ~50% (Figure 4.2). The new method allows the analysis of THg and Se from a single sample aliquot. The

suitability of the digestion method for both THg and Se analysis in biological tissue was evaluated by triplicate spike and recovery experiments of a surrogate matrix (beef muscle) and SRM 1946. Triplicate spike and recovery results showed good reproducibility of the method for THg (101% ± 4.5; standard deviation) and Se (85% ± 7.7; standard deviation) (Table 4.1). Measured concentrations of THg and Se in SRM 1946 were in agreement with certified/reference concentrations and showed good reproducibility (Table 4.1). This method was applied to the analysis of THg and Se in skeletal muscle tissues of 28 Pacific walruses. Age metadata was only available for 15 samples (10 samples harvested in 2009 and 5 samples in 2010) with ages ranging from 8 – 34 years. Mean age was 15.3 years and median age was 12 years.). Gender metadata was only available for 13 samples: 11 females (n = 6, 2009; n = 5, 2010) and two males (2010). Method specific MDL values were statistically calculated to determine the lowest non-zero concentration for THg and Se that can be reported with 95% confidence (Table 4.1). MDLs for THg and Se were 0.98 and 220 µg/kg wet wt, respectively.

Table 4.1 Triplicate recoveries, method detection limits, and standard reference material analysis using the modified mercury and selenium digestion method.

Analytes	**Percent recovery ± SD (n=3)**	**MDL µg/kg wet wt (n=7)**	**SRM 1946 RPD (n=3)**	**Certified/reference concentration mg/kg wet wt[a]**	**Mean measured concentration mg/kg wet wt[d] (n=3)**
Total Hg	101 ± 4.5	0.98	2	0.433 ± 0.009[b]	0.444 ± 0.022
Se	85 ± 7.7	220	26	0.491 ± 0.043[c]	0.621 ± 0.066

SD: Standard deviation; MDL: Method detection limits; RPD: Relative percent difference; [a]Unweighted mean ± expanded uncertainty about the mean (95% confidence); [b]Certified value; [c]Reference value; [d]Mean concentration ± standard deviation

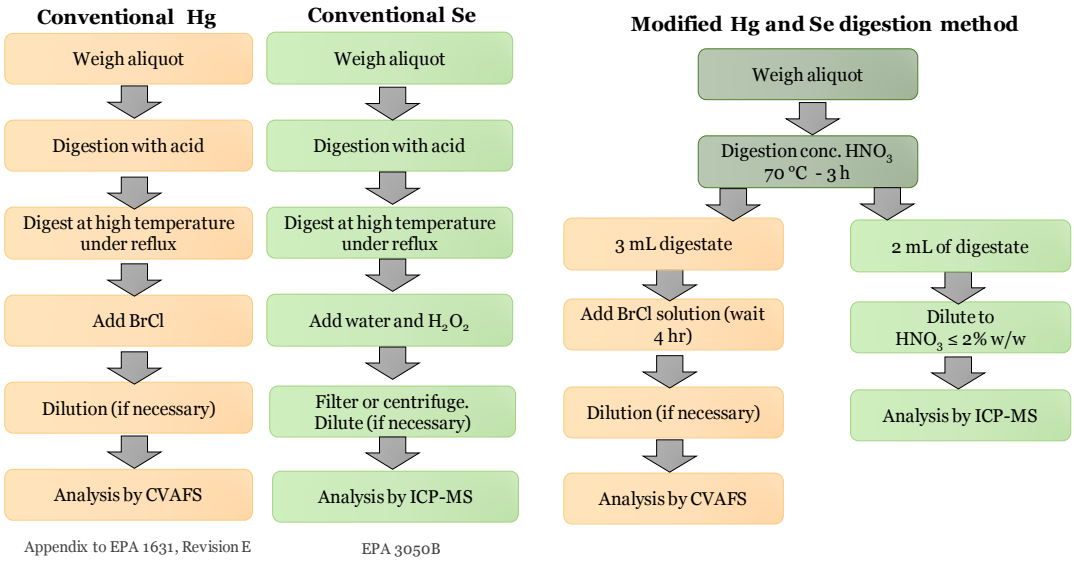

Figure 4.2 Conventional and modified digestion methods of biological tissues for mercury and selenium analysis.

Concentrations of THg and elements in Pacific walrus muscle

THg and Se were quantified, at above MDL levels, in all skeletal muscle samples (n = 28) of Pacific walrus (Table 4.2). No trend or correlation was observed between THg or Se concentrations and age; *t*-tests (df = 26, α = 0.05) showed no significant difference between concentrations of THg or Se and sampling location or year. Wet weight concentrations of THg and Se ranged from 9 – 32 and 900 – 7,300 µg/kg, respectively. Mean concentrations of THg in muscle of Pacific walruses (Table 4.2) from 2009 and 2010 (n = 28, [THg] = 0.02 ± 0.01 mg/kg wet weight (wet wt)) are comparable to those from 1971 (n = 6, [THg] = 0.02 ± 0.01 mg/kg wet wt) and 2005 – 2009 (n = 10, [THg] = 0.004 ± 0.007 mg/kg wet wt) (Born et al. 1981, Ponce 1997, Welfinger-Smith et al. 2011). To the best of our knowledge, this report presents the highest sample size for analysis of THg concentrations in muscle of Pacific walrus. In addition, this is the second report of Se concentrations in muscle of Pacific walrus. Mean Se concentrations from

2009 – 2010, this study, were similar (n = 28, [Se] = 3.3 ± 1.5 mg/kg wet wt) to those from 2005-2009 (n = 26, [Se] = 2.7 ± 1.9 mg/kg wet wt) (Welfinger-Smith et al. 2011).

Table 4.2. Mean concentrations (±SD) of THg and Se in muscle of Pacific and Atlantic walrus

Element	Subspecies	Year	mg kg^{-1} wet wt		n	Source
THg	Pacific walrus	1971	0.02	(±0.01)	6	(Born et al. 1981)
	Pacific walrus	2003-2009	0.004	(±0.007)	10	(Welfinger-Smith et al. 2011)
	Pacific walrus	2009-2010	0.02	(±0.01)	28	This study
	Atlantic walrus	1975-1977	0.08	(±0.05)	58	(Born et al. 1981)
	Atlantic walrus	1982-1988	0.11	(±0.13)	113	(Wagemann and Stewart 1994)
	Atlantic walrus	1988-1990	0.11	(±0.02)	3	(Atwell et al. 1998)
	Atlantic walrus	1992	0.11	(±0.14)	90-113	(Muir et al. 1999)
Se	Pacific walrus	2003-2009	2.7	(±1.90)	26	(Welfinger-Smith et al. 2011)
	Pacific walrus	2009-2010	3.26	(±1.50)	28	This study
	Atlantic walrus	1982-1988	3.33	(±1.52)	113	(Wagemann and Stewart 1994)
Cd	Pacific walrus	2003-2009	0.08	(±0.09)	5	(Welfinger-Smith et al. 2011)
	Pacific walrus	2009-2010	0.26	--	1	This study*
	Atlantic walrus	1982-1988	0.14	(±0.13)	114	(Wagemann and Stewart 1994)

*Above MDL concentrations were measured in 1 out of 28 samples analyzed.

The limited amount of temporal data of THg and Se concentrations in muscle of Pacific walrus contributes to the difficulty in assessing temporal trends. In Greenland, Riget et al. observed that overall mean Hg concentrations have remained constant from 1977 to 2003 in tissues of Atlantic walruses (Riget et al. 2007, Rigét et al. 2011). However, there is spatial variation in temporal trends of Hg concentrations in other marine mammals, such as ringed seals, with some areas showing increasing trends, while others decrease or remain constant (Braune et al. 2005, Rigét et al. 2011). Similarly, analysis of THg in beluga whale's teeth showed an increasing trend from pre-industrial, 1450-1650, to modern, 1993, concentrations with higher concentrations observed in

western areas (Braune et al. 2005). McHuron et al. observed geographical differences in THg and Se concentrations in hair and blood of harbor seals located 200 km apart (McHuron et al. 2014). Possible causes of spatial differences in concentrations may include changes in diet, feeding areas, regional contamination, river outflows, and permafrost melting among others.

THg and Se concentrations were used to calculate Se:Hg molar ratios in muscle of Pacific walrus. In all samples, Se concentrations were an at least three orders of magnitude greater than THg concentrations. Previous studies have reported a correlation between THg and Se concentrations in tissues of marine mammals (Koeman et al. 1975). Pearson's correlation coefficient and linear regression analysis were used to evaluate correlations and trends between concentrations of THg and Se in muscle (Figure 4.3). THg and Se concentrations had a significant positive correlation ($r = 0.47$, $p = 0.012$, $n = 28$) for all samples. Mean molar ratios of Se:Hg were 428 ± 166 and ranged from 85 to 880. Walrus are benthic feeders, with clams constituting most of their diet, although in rare occasions walruses may prey on seals (Dehn et al. 2006a). Se:Hg molar ratios of mollusks from Greenland were 150 ± 50; n = 45 (Dietz et al. 2000). Kehrig et al., reported differences in THg concentrations among three species of mollusks from a tropical marine environment (Kehrig et al. 2006). Median concentrations of THg and Se in mollusks of Alaska (2001-2012) ranged from 0.009-0.035 and 0.06-1.6 mg/kg wet wt, respectively (Health 2012). Given that, Hg and Se uptake is primarily through the diet, concentrations in mollusks will influence THg and Se concentrations in Pacific walrus tissues.

Conclusions

Conventional methods for digestion of Hg and metals from biological tissues served as a framework for the development of a single digestion method of Hg and Se from Pacific walrus' skeletal muscle. This new method significantly reduced sample preparation time and sample aliquot requirements. Given that this method was based on conventional methods for digestion of biological tissues, there is opportunity for its application to other biological matrices. In addition, there is opportunity to expand the number of analytes that can be analyzed from each sample aliquot. The aliquot reserved for Se analysis, could be further treated to ensure the complete digestion of other metals (e.g. H_2O_2 treatment). Ongoing and future monitoring studies of Hg and metals will benefit from the availability of this method and its future improvements. The main advantages of lower sample preparation costs and sample requirements translate in lower costs associated with analysis.

Results from the application of this method contributed to the available dataset on Hg and Se concentrations in marine mammals. In particular, these results will contribute to the current state of Hg and Se concentrations in Pacific walrus. In order to construct robust temporal trends it is necessary to continue monitoring these and other metal concentrations in marine mammals. Furthermore, continuous monitoring studies would provide information regarding the effects of regional and global environmental changes in contaminant concentrations of Arctic marine mammals.

Acknowledgements

The authors are thankful to the C. Gus Glasscock, Jr. fund for excellence in environmental sciences for the financial support. The authors are grateful to the Mass Spectrometry Center at Baylor University for providing access to the Elan 9000 ICP-MS.

Supporting Information

Table S4.1. ICP-MS instrument parameters and monitoring ions

ICP-MS parameters	
ICP RF power	1300
Nebulizer gas flow	0.91
Lens voltage	8.25
Scan mode	Peak Hopping
Dwell time	50 ms
Number of replicates	3
Total integration time	3000 ms
Element	*Analytical mass*
Se	81.917

CHAPTER FIVE

Conclusions

Sample preparation is one of the main bottlenecks in many analytical techniques. Historical methods for organic (EPA 1613 and 1668B) and inorganic (EPA 1631 and 3050B) contaminants, are time consuming, labor intensive, and/or require large volumes of solvents or acids. However, historical methods served as a framework for the development of the high-throughput sample preparation methods that reduced sample preparation time, volume of solvents, number of steps, exposure to hazardous chemicals, opportunities for sample contamination, and analyte loss. The examples presented in this dissertation are an outline for future development of high-throughput sample preparation methods for organic and inorganic contaminants. In summary, conventional methods and advances in instrumentation are integrated to developed high-throughput sample preparation methods.

Over the past 20 years, there have been significant improvements to extraction methods, particularly for organic contaminants. The first generation of pressurized liquid extraction (PLE) methods focused on the extraction of target analytes from the sample homogenate. The second generation of PLE methods, namely selective PLE (SPLE) focused on the simultaneous extraction of target analytes and retainment of potential interferences. This was achieved through the incorporation of extraction adsorbents into the extraction cell. The use of automated PLE systems has allowed the manipulation of extraction parameters; resulting in methods tailored to specific matrices and analytes.

Conventional methods for the analysis of organic contaminants, such as EPA method 1631 and 1668B, require extensive post-extraction cleanup to remove potential analytical interferences. These conventional methods were used as a framework to develop the methods presented in Chapters Two and Three. These methods utilized PLE instrumentation by incorporating typical post-extraction cleanup adsorbents into the extraction step. The resulting SPLE methods reduced overall analysis time by ~90% when compared to Soxhlet extraction (18-24 h) with post-extraction cleanup (2-4 h) and multiple concentration steps (2-4 h). Overall, the SPLE methods reduced the number of sample preparation steps, increased the number of target analytes, and provided a platform for future SPLE methods for organic contaminants in sediment and biological tissues. Sediment samples in particular presented a few analytical challenges. First, sediment is a complex matrix that requires the elimination of a wide range of interferences. The removal of these potential interferences from the extract was accomplished through the incorporation of multiple adsorbents (i.e. silica, alumina, and florisil) into the extraction cell. Second, elemental sulfur, also a potential interference, must be removed prior to analysis. This step was also successfully incorporated through the addition of activated copper powder into the collection bottle of the PLE system. Third, conventional methods typically fractionate PCDD/Fs from dl-PCBs to eliminate the possibility of molecular interferences. A couple of methods have attempted the use of in-cell fractionation; Chuang et al., reported lower than 40% recoveries for PCDD/Fs while Do et al., reported only recoveries of PCDD/Fs using a modified PLE extraction cell (Chuang et al. 2009, Do et al. 2013). Further development is required to achieve complete fractionation of PCDD/Fs and dl-PCBs extracted from sediment samples while

maintaining quantifiable recoveries for all analytes. Although, the SPLE method presented in Chapter Two does not incorporate a fractionation step, PCDD/Fs and dl-PCBs are simultaneously extracted from a single sample. This and other SPLE methods can be used as stepping-stones to further improve automated extraction and fractionation of analytes from sediment samples (Subedi and Usenko 2012, Aguilar et al. 2014, Subedi et al. 2014).

Due to their high-throughput capability, these methods are suitable for their application in long-term monitoring studies, or in projects that require analysis of a large number of samples. For example, SPLE methods can be used to identify contaminant sources, to monitor the extent of contamination, or to assess the success of remediation efforts (e.g. Superfund sites). Besides the rapid turn-around time, high-throughput methods increase the laboratory preparedness to respond rapidly to unexpected projects, such as during environmental emergencies, which increase workload and expedite results. Overall, high-throughput sample preparation methods provide decision makers with timely and reliable results in a cost-effective manner. Examples of the advantages of applications of SPLE methods for analysis of organic contaminants in sediment samples were presented in Chapters Two and Three. The SPLE method allowed the extraction of PCDD/Fs and dl-PCBs from contaminated sediments in three Superfund sites located in the Houston Ship Channel (HSC) in Houston, TX. When analyzing sediment samples it is typical to obtain sample composites of the area of interest and report average contaminant concentrations. This approach is necessary when using traditional sample preparation methods as the analysis of a large number of samples is often logistically not feasible. However, when using SPLE methods the analysis (including all quality control protocols)

of a large number of sediment samples (n=15) can be completed in a few days (4-5 days). For example, the detailed analysis of the SJRWP showed that the distribution of the contaminant concentrations in sediment was not homogeneous. The highest concentrations were observed in 3 out of 15 samples and corresponded to the northern perimeter of the SJRWP. This observation would have not been possible by analyzing sample composites. Moreover, composite samples do not provide evidence of the extent of the spatial contamination.

The availability of high-throughput sample preparation methods, like SPLE, promotes collaboration among different research groups. Laboratories are better equipped to participate in collaboration studies, such as toxicology studies, without hindering their main research activities. SPLE methods for sediment and biological tissue were applied for the analysis of PCDD/Fs and dl-PCBs in three sediment and 18 fish collected at two Superfund sites in Houston, TX. Results from the chemical analysis of these samples were employed to assess the effects of these contaminants in *F. grandis*, and has been published elsewhere (Oziolor et al. 2014). PCBs had been previously detected in sediment samples from these two Superfund sites (Patrick Bayou (PB), Vince Bayou (VB)), and the Superfund site SJRWP (Chapter Two). PB is located approximately 9 km away from the SJRWP (Chapter Two), while VB is approximately 10 km away from PB (Figure 5.1). Comparison of total contaminant concentrations among Superfund sites show that PB had the highest concentrations followed by SJRWP and Vince Bayou. At each site, total dl-PCB concentrations (Σdl-PCB) constituted over 80% of the total contaminant load with Σdl-PCB being responsible for 99% of the contaminant concentrations at PB (Figure 5.2). Between 60 and 85% of Σdl-PCB concentrations was

attributed to pentachlorinated PCBs, particularly PCB-118 (40-50% of total dl-PCB concentration at each site). PCDD/Fs and dl-PCBs were also detected in sediment from a reference site (Gangs Bayou (GB)) in Galveston, TX. Contaminant concentrations at this location were at low pg/g dry wt levels (Figure 5.2).

Figure 5.1 Location of Superfund sites San Jacinto River Waste Pits (SJRWP), Patrick Bayou (PB), and Vince Bayou (VB) in the Houston Ship Channel, Houston, TX.

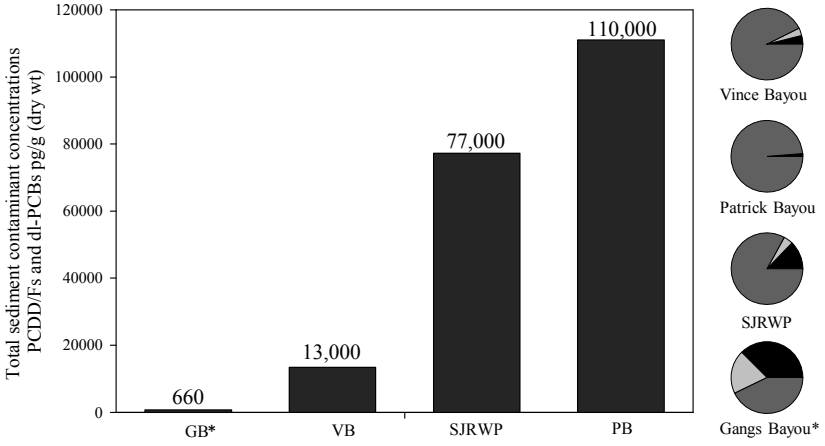

Figure 5.2 Total contaminant concentrations at three Superfund sites and reference site in Houston, TX. Gangs Bayou (GB), Vince Bayou (VB), San Jacinto River Waste Pits (SJRWP), and Patrick Bayou (PB). Pie charts show contribution of PCDD (light gray), PCDF (black), and dl-PCB (dark gray) concentrations to total contaminant concentrations at each location. SJRWP shows average concentrations of n=15 samples. *Reference site in Galveston, TX.

Conventional methods for the analysis of inorganic contaminants, such as EPA method 1613 and 3050B, require extensive handling of samples and acids, thus increasing personnel exposure to hazardous chemicals and opportunities for sample contamination and analyte loss. These conventional methods were used as a framework to develop the method presented in Chapter Four. These methods were combined to include mercury and selenium in a single digestion method. The resulting digestion method reduced overall analysis time by ~50% when compared to employing separate digestions for mercury (~12 h) and metals (~4 h). Overall, the combined digestion method reduced the number of sample preparation steps, increased the number of target analytes, and provided a platform for future high-throughput methods for inorganic contaminants in biological tissues. This high-throughput digestion method was specifically design for its application to the analysis of mercury and selenium in skeletal muscle (*L. dorsi*) of Pacific walrus (*Odobenus rosmarus divergens*). This method allowed the analysis of mercury and selenium from a single sample aliquot of Pacific walrus muscle. The main challenge was utilizing a single digestion method that was compatible for two separate analytical techniques (i.e. Cold Vapor Atomic Fluorescence Spectrometry and Inductively Coupled Plasma Mass Spectrometry). The conventional digestion methods were compared to identify a divergent point from which two aliquots of the digestate could be reserved for each individual analysis. The initial digestion of the tissue was performed with concentrated nitric. After separating the digestates aliquots for each analysis, mercury digestates were treated with BrCl to oxidize all mercury species. This method has opportunity for further incorporation of additional target analytes (other metals) and application to other biological tissues (e.g. blubber, liver). Subsequent

treatment(s) (e.g. hydrogen peroxide, other acids) to the aliquot reserved for metal analysis could improve recoveries of additional metal species present in the sample. This method also presents a particular example of the benefits of expanding the number of target analytes than can be extracted from a single sample. In general, tissues from marine mammals are rare samples. Although, there is global interest in monitoring a wide range of organic and inorganic pollutants in the Arctic, analysts must choose a few analysis that can be achieve with the limited samples available. Monitoring studies benefit greatly from methods that allow the identification of several target analytes from a single sample aliquot. Further improvement of this method to include additional target analytes will be beneficial to future monitoring studies of mercury and metals in Arctic marine organisms. The results presented in Chapter Four are an initial response to the hiatus in mercury monitoring studies in Pacific walrus in Alaska. These results are a starting point to fill in the gap in spatial and temporal information of mercury and selenium concentrations in walrus populations in Alaska. Future studies would provide a larger sample size to assess correlations between mercury/selenium and age or gender of P. walruses and to assess spatial and temporal changes in contaminant concentrations.

There are several areas of opportunity for further improvement of sample preparation techniques. One important point to keep in mind is that the instrumentation utilized for SPLE methods was not initially design or intended for the simultaneous extraction-cleanup step. Therefore, there are opportunities for the improvement and development of SPLE instrumentation for easier optimization of extraction/cleanup parameters. One of the most commonly employed PLE devices is commercially available under the trade name Accelerated Solvent Extractor (ASE, Thermo Fisher). Programing

settings in the ASE 350 control the extraction temperature, number and time of static cycles, flush volume, purge time, and solvent or mixture of solvents (up to three) to be used. However, sequential extractions, such as when performing fractionation of analytes, require two separate extractions; meaning that the extraction oven has to cool down and start over. This heat-cool process would add significant time to the SPLE method when incorporating several fractionation steps. Improvements to the instrumentation's heating and solvent delivery controls would result in streamlined fractionation methods.

Although, in current SPLE methods the main function of the adsorbents incorporated into the extraction cell is the retention of potential interferences, there is opportunity to explore other applications. For instance, adsorbents could be used in a similar way to a chromatographic column to allow for the sequential fractionation of analytes. For example, PCDD/Fs have been successfully fractionated from dl-PCBs when incorporating activated carbon (i.e. Carbopak^{TM}) into the extraction cell in an analogous way to post-extraction column fractionation (EPA 1994a, Haglund et al. 2007, Subedi and Usenko 2012, Subedi et al. 2014) (Subedi et al. 2014). There is an opportunity for the development of new adsorbents custom-made to retain specific chemicals followed by their subsequent "elution" with the appropriate solvent. This could be applied to characterize the sample along with the extraction of analytes of interest. For example, lipids retained in a sorbent during extraction of target analytes could be subsequently extracted and even fractionated from a single sample. Thus, lipid content and determination of fatty acids could be obtained from the same sample aliquot as contaminant concentrations. This would increase the opportunities for collaborative research, as one laboratory's interferences may be another laboratory's target analytes.

Fractionation could be expanded to include organic and inorganic species (such as heavy metals) from a single sample, increasing dramatically the information obtained from a single sample aliquot. Different solvents could be used for the sequential extraction of organic and inorganic target analytes from a single matrix. This would be a paradigm shift from traditional methods for analysis of organic and inorganic contaminants that are frequently present in environmental samples. Method development for simultaneous SPLE extraction of organic and inorganic analytes can use previous PLE and SPLE methods for organic analytes and the few available methods for organometallic species as a framework (Alonso-Rodríguez et al. 2006, Moreda-Piñeiro et al. 2006, Mato-Fernández et al. 2007, Moreda-Piñeiro et al. 2007a, Moreda-Piñeiro et al. 2007b, Carballo-Paradelo et al. 2012). PLE and SPLE of heavy metals from solid matrices is a relatively new area with many opportunities for further development of instrumentation and methods.

In summary, the methods reported in this dissertation can serve as basis for further development and improvement of sample preparation techniques. New SPLE methods should strive for the complete removal of interferences without post-extraction cleanup. There are many opportunities for future development of analytical methods for environmental samples and more will appear as advancements in technology and adsorbents become available. Moreover, it can be expected that other areas of research, e.g. food, pharmaceutical, forensic sciences, adopt these high-throughput methods in their routine analysis.

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