Defining Biota-Sediment Accumulation Factors for the San Jacinto River Waste Pits, Texas
Final Report

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Submitted by

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Table of Contents

List of Figures 4

List of Tables 6

1.0 Introduction 8

1.1 San Jacinto River Waste Pits Site History Summary 8

1.2 Dioxins, Furans, and PCBs 9

2.0 Sampling Methods and Results 10

2.1 Sediment 10

2.2 Clams, Crab, and Fish 12

3.0 Analysis and Results 16

3.1 Sediment 16

3.1.1 Physical Characterization 16

3.1.2 Analysis of Dioxins, Furans, and PCBs in Sediment 17

3.2 Clams, Crab, and Fish 29

3.2.1 Lipid Content 29

3.2.2 Analysis of Dioxins, Furans, and PCBs in Tissue 29

3.2.3 Comparison to EPA Method 1316B 32

3.2.4 Isotopic Composition of C and N 33

3.3 Data Summary for Sediment, Clams, Crab, and Fish 33

3.3.1 Summary Description of Data for Dioxins, Furans, and PCBs 33

3.3.2 Quality Assurance Summary 49

4.0 Food Web 50

4.1 Trophic Analysis 50

5.0 Bioaccumulation of Dioxins, Furans, and PCBs in the SJRWP 55

5.1 Gobas/Arnot Model of Bioaccumulation 57

5.2 Calculation of Site-Specific Biota-Sediment Accumulation Factors in the SJRWP 66

6.0 QSAR Modeling of BSAF Values 74

7.0 Conclusions 80

8.0 References 87

List of Figures

Figure 1. Locations of sediment sampling at the San Jacinto River Waste Pits, Texas in August 2010..... 12

Figure 2. Locations and methods employed for fish and shellfish sampling at the San Jacinto River Waste Pits, Texas, in August 2010. 14

Figure 3. Accelerated solvent extraction cell with cleanup sorbents for enhanced pressurized liquid extraction. 20

Figure 4. GPC-UV chromatograms of ePLE. (A) Sediment (~10 g) – sodium sulfate homogenate, (B) sediment homogenate, silica, and alumina (1:1:1), and (C) sediment homogenate, silica, alumina, and Florisil® (1:1:1:1) 21

Figure 5. Electron Impact Ionization-full scan chromatograms and background spectra. Acquisition range 50-500 m/z. (A) Sediment homogenate (10g), (B) Sediment homogenate, silica, alumina (1:1:1), and (C) sediment homogenate, silica, alumina, and Florisil® (1:1:1:1).. 22

Figure 6. GPC-UV chromatograms of sulfur removal from sediment (~10 g). (A) GPC calibration mix standard showing sulfur peak at 22 minutes, (B) Removal of sulfur with $AgNO_3$ (10 g) inside an extraction cell, (C) removal of sulfur using $AgNO_3$ (5 g) inside an extraction cell, (D) removal of sulfur using activated copper (1 g) inside an extraction bottle. 23

Figure 7. Surficial sediment concentrations (pg/g dw) of 2,3,7,8-TCDD, 2,3,7,8-TCDF, and ΣPCBs collected at the San Jacinto River Waste Pits, Texas, in August 2010. 39

Figure 8. Relationship between trophic positions of the Mad Island March Preserve, Texas, based on stable isotope analysis (A) and stomach content analysis (B) (Akin and Winemiller 2006), and trophic positions of the San Jacinto River Waste Pits, Texas, based on stable isotope analysis..... 53

Figure 9. Fishers in the San Jacinto River Waste Pits during site visit by TCEQ, TDSHS and Baylor personnel in the fall of 2009. Black drum, red drum, mullet, sheepshead and hard clams were observed in possession of fishers. 55

Figure 10. Quantitative structure activity relationship model of biota-sediment accumulation factors for PCBs in black drum at the San Jacinto River Waste Pits Site. 78

Figure 11. Quantitative structure activity relationship model of biota-sediment accumulation factors for PCBs in clams at the San Jacinto River Waste Pits Site. 79

Figure 12. Quantitative structure activity relationship model of biota-sediment accumulation factors for PCBs in blue crab at the San Jacinto River Waste Pits Site. 80

List of Tables

Table 1. Organisms collected, identified and enumerated during sampling at the San Jacinto River Waste Pits, Texas in August 2010. 15

Table 2. Target analyte list for the present study. 18

Table 3. Target analytes, monitored ions, triplicate recoveries, and statistically-derived method detection limits. 27

Table 4. Detection frequency for dioxins, furans, and PCBs in sediment, clams, crab, and fish 33

Table 5. Summary statistics for dioxins, furans, and PCBs measured in sediment (dry weight normalized) 35

Table 6. Summary statistics for dioxins, furans, and PCBs measured in sediment (wet weight normalized) 36

Table 7. Summary statistics for dioxins, furans, and PCBs measured in sediment (TOC normalized) 37

Table 8. Summary statistics for dioxins, furans, and PCBs measured in clams (wet weight normalized) 41

Table 9. Summary statistics for dioxins, furans, and PCBs measured in clams (lipid weight normalized) 42

Table 10. Summary statistics for dioxins, furans, and PCBs measured in crab (wet weight normalized) 44

Table 11. Summary statistics for dioxins, furans, and PCBs measured in crab (lipid weight normalized) 45

Table 12. Summary statistics for dioxins, furans, and PCBs measured in fish (wet weight normalized) 47

Table 13. Summary statistics for dioxins, furans, and PCBs measured in fish (lipid weight normalized) 48

Table 14. Percent relative standard deviation of surrogates measured in matrix spikes and matrix spike duplicate samples for sediment, clams, crab, and fish. 49

Table 15. Percent surrogate recoveries for sediment, clams, crab, and fish. 50

Table 16. Taxa for which stable isotope analysis was performed for trophic position after sampling of the San Jacinto River Waste Pits, Texas in August 2010. 51

Table 17. A comparison of modeled and observed concentrations of polychlorinated dioxins, furans, and biphenyls near the San Jacinto River Waste Pits 62

Table 18. Site-specific preliminary biota sediment accumulation factors for dioxins, furans, and PCBs at the San Jacinto River Waste Pits 68

Table 19. A comparison of biota sediment accumulation factors for the San Jacinto River Waste Pits and the US Army Corps of Engineers database..... 71

Table 20. Trophic level-normalized biota sediment accumulation factor values for the San Jacinto River Waste Pits 72

1.0 Introduction

1.1 San Jacinto River Waste Pits Site History Summary

The San Jacinto River Waste Pits (SJRWP) are located on the San Jacinto River between Channelview and Highlands, Texas. The waste pits were utilized in the 1960's-70's to dispose of pulp and paper waste. Previous studies have identified polychlorodibenzo-p-dioxins (dioxins), polychlorodibenzofurans (furans), and dioxin-like polychlorinated biphenyls (PCBs) in the sediment and aquatic organisms of the Houston Ship Channel (HSC). From the late 1960s to the early 1980s, process sludge from paper manufacturing was deposited on a site adjacent to the southern San Jacinto River. Over time, dredging in the area promoted subsidence of the site, which in turn caused contamination and spreading of dioxins and furans into the surrounding aqueous environment. Fishing advisories were issued in 1990 for much of the HSC and upper Galveston Bay estuary to cover concerns regarding consumption of finfish, speckled trout, catfish, and crab and the associated exposures to dioxins, furans, and PCBs (http://gbic.tamug.edu/ss/faq.html). The SJRWP was placed on the US EPA National Priority List of Superfund sites in 2008. The remedial investigation/feasibility study began in 2010.

The Texas Environmental Health Institute (TEHI), a collaborative effort between Texas Department of State Health Services (TDSHS) and Texas Commission on Environmental Quality (TCEQ), supported the present research project after Baylor's Center for Reservoir and Aquatic Systems Research (CRASR) responded to a request for proposals (RFP). The RFP offered support for research efforts to understand bioaccumulation of dioxins, furans, and PCBs at the SJRWP. Thus, the current study explored bioaccumulation through several research objectives, based on the scope of the RFP and available funding. Thus, the present study was not designed to perform or support robust health or ecological risk assessments, support remedial

decision making, or extend spatially beyond the immediate area of the SJRWP site. The specific research objectives of the current study included: 1) measurement of concentrations of dioxins, furans, and PCBs in fish, invertebrates, and sediment samples at the SJRWP using a novel extraction methodology, 2) estimation of site-specific biota sediment accumulation factors (BSAF) values for targeted invertebrates and fish, 3), delineation of trophic position of sampled food web members at the SJRWP site using stable isotope approaches, 4) modeling of bioaccumulation of dioxins, furans, and PCBs using the Gobas/Arnot framework (which underpins many regulatory bioaccumulation tools, including TrophicTrace) that was informed by isotopic delineation of trophic position, 5) explore use of quantitative structure activity relationship (QSAR) models for BSAF values, and 6) establishment of a combined approach to determine site-specific BSAFs for other contaminated sites.

1.2 Dioxins, Furans, and PCBs

Dioxins, furans, and dioxin-like PCBs enter into the environment as byproducts of municipal and industrial waste incinerators (Lustenhouwer, Olie et al. 1980), manufacturing of chlorophenols (Rappe, Andersson et al. 1987), and pulp and paper mill effluents (Clement, Suter et al. 1989). Once in the environment, these chlorinated compounds are considered persistent, bioaccumulative, and toxic (USEPA 1994; Wan et al. 2005; Van den Berg et al. 2006; Garabrant et al. 2009). Dioxins, furans, and PCBs have been measured in sediments (Yeager, Santschi et al. 2007), fish (Wan, Jones et al. 2010), birds (Braune and Simon 2003), human serum, adipose tissue (Patterson, Todd et al. 1994) and other environmental matrices using a wide variety of analytical methodologies, such as EPA 1613, EPA 1668, EPA 8290A, and European Standard Method EN1948-1/2/3 (USEPA 1994; USEPA 2003; USEPA 2007). The routine analysis of

dioxins, furans, and PCBs in complex matrices such as sediment and clams typically require the removal of more polar and nonpolar interferences. Often these analytical methods utilize gel permeation chromatography (GPC) or column chromatography techniques such as silica gel cleanup. Analytical methods that utilize multiple individual cleanup techniques are time-, cost-, space-, and labor-intensive, and they increase the potential for loss of target analytes and surrogates during sample preparation (Subedi and Usenko 2012).

2.0 Sampling Methods and Results

The SJRWP site originally included three pits: A, B, and C. Pit A became covered in vegetation, and the berms surrounding pits B and C eventually subsided allowing open access to the San Jacinto River. The entire submerged area in the San Jacinto River is about 100m x 200m (~21,000m^2). This area is an estuarine tidal zone, which makes the environment highly dynamic, with fluctuations in water levels and salinity that vary both daily and seasonally. Additionally, the site is part of a highly industrialized zone of the river. The complexity of the site makes it difficult to predict what fish and shellfish species will be present within the site on any given sampling occasion. To address this challenge, we performed baseline biological surveys on several occasions during the project period to identify organisms and associated prey. We initially developed a targeted list of specific edible fish and shellfish that were anticipated to be present at the SJRWP site for field collection and laboratory analysis.

2.1 Sediment

A stratified random sampling approach was employed to collect sediment samples within the pits and the adjacent river channel. This area was partitioned to 10m^2 blocks, which were

randomly selected for sampling. Sediment sampling was conducted by petite ponar, which collected sediment from the upper 4 to 10 cm (TCEQ 2007, 2008). At each sampling location triplicate petite ponar samples were collected and composited (**Figure 1**). In addition, global positioning system (GPS) coordinates of each sampling location were recorded using a GPS Pathfinder® Pro XR receiver that provided sub-meter accuracy and real-time differential correction in the field. Data was stored on a Trimble® GeoXT handheld unit.

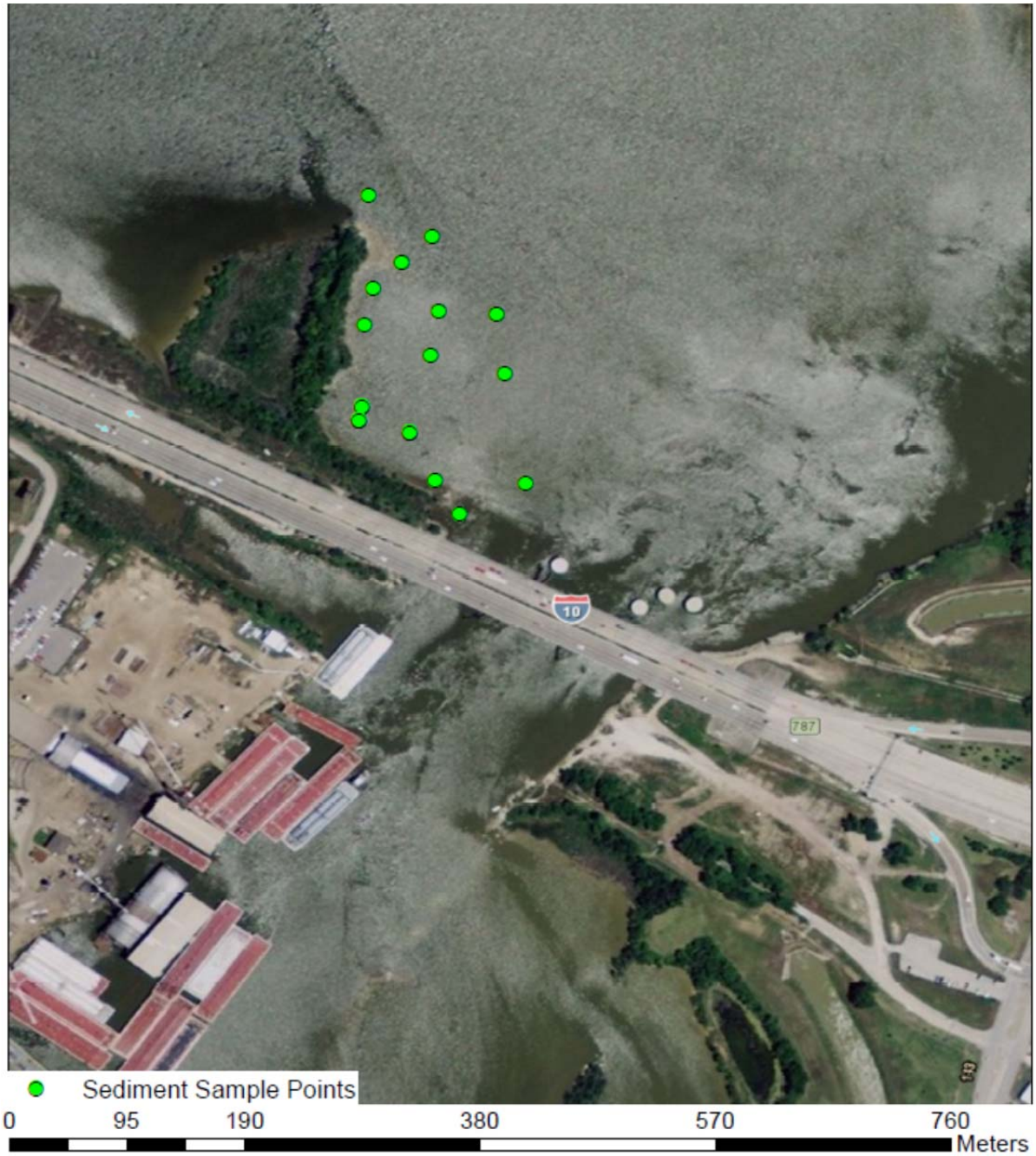

Figure 1. Locations of sediment sampling at the San Jacinto River Waste Pits, Texas in August 2010.

2.2 Clams, Crab, and Fish

A number of different collection methods were employed to collect the diverse biota present at the site. These included gill nets, baited traps, cast nets, seines, and hook and line

(**Figure 2**). The length and weight of each organism collected was recorded along with species, collection method, GPS coordinates, and time. To be considered for collection an individual organism must meet a minimum size requirement for adult fish as designated by the TCEQ Surface Water Quality Monitoring Procedures Manual (TCEQ 2007, 2008). An effort was thus made to ensure that all individuals of a single species were of similar size (within 15 to 20% of total length); however, for some species this was not possible. More information on sample selection and compositing is provided below. All fish collection activities conducted during the present study complied with a Baylor University animal care and use protocol and requirements of our scientific collection permit from Texas Parks and Wildlife Department.

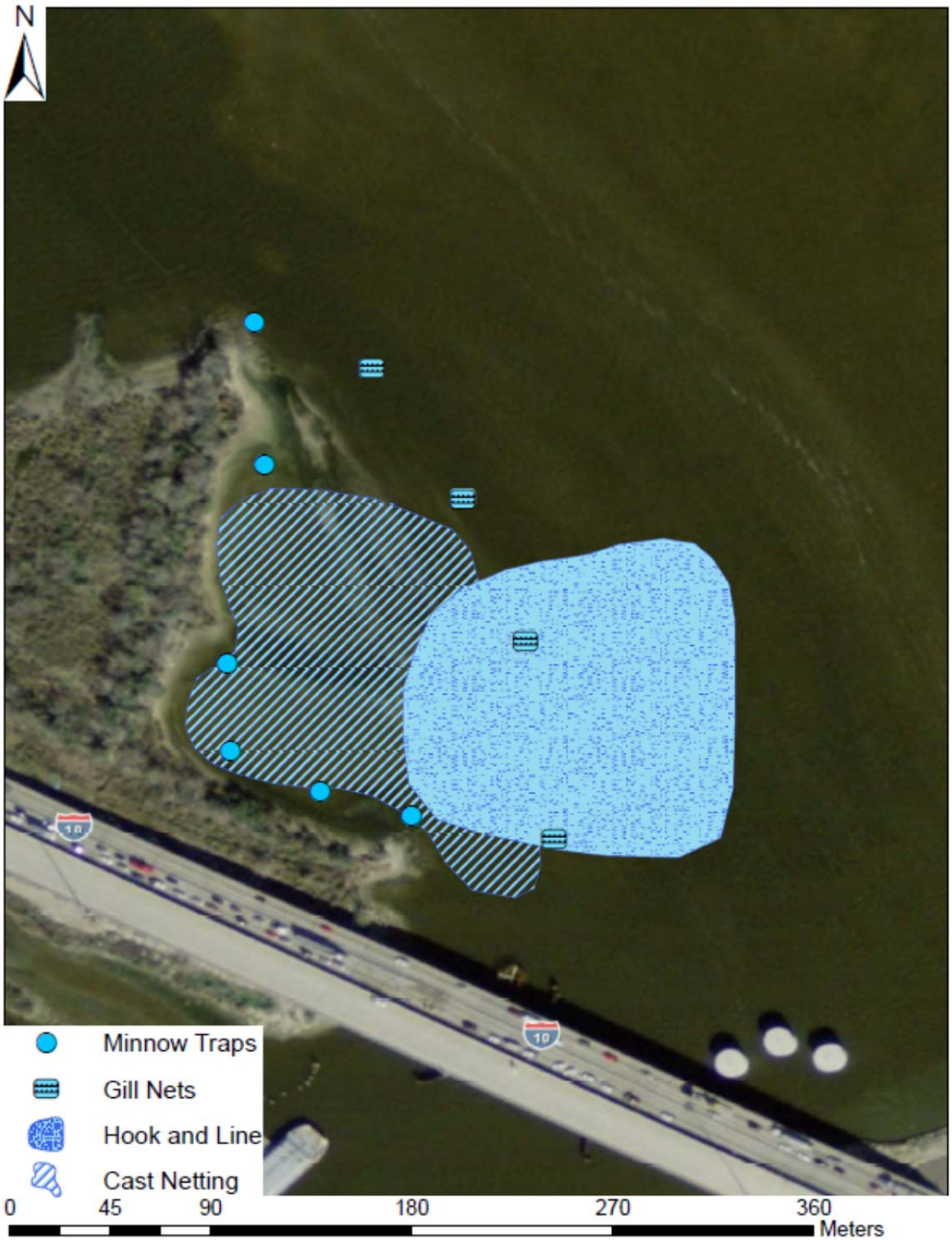

Figure 2. Locations and methods employed for fish and shellfish sampling at the San Jacinto River Waste Pits, Texas, in August 2010.

Unfortunately, the scope of the study and analytical costs associated with the determination of dioxins, furans, and PCBs in tissue and sediment precluded examination of numerous edible species or broader spatial sampling approaches typically employed for BSAF calculations. Thus, given the scope of the study, we examined collection events and then selected organisms and sediments collected from an August 2010 sampling event, in consultation with TEHI project managers, to examine site-specific BSAFs for three edible species (clams, blue crab, and black drum) residing at the SJRWP site. Additional prey species were collected at lower trophic levels to support isotopic discrimination of food web structure and trophic transfer modeling. Results from biological sampling during August 2010 are provided in **Table 1**.

Table 1. Organisms collected, identified and enumerated during sampling at the San Jacinto River Waste Pits, Texas in August 2010.

Group	Scientific Name	Common Name	Total Number
Crustacean	*Callinectes sapidus*	Blue crab	37
Crustacean	*Palaemonetes pugio*	Grass shrimp	6
Crustacean	*Pinead sp.*	Pinead shrimp	23
Mollusk	*Mercenaria spp*	Clam	26
Fish	*Arius felis*	Hardhead catfish	2
Fish	*Bagre marinus*	Gafftopsail catfish	8
Fish	*Brevoortia patronus*	Menhaden	110
Fish	*Caranax sp.*	Jack	1
Fish	*Cyprinodon variegatus*	Sheepshead minnow	2
Fish	*Dorsoma cepedianum*	Gizzard shad	5
Fish	*Elops saurus*	Ladyfish	28
Fish	*Fundulus grandis*	Gulf killifish	132
Fish	*Fundulus majalis*	Longnose kilifish	5
Fish	*Lagodon rhomboides*	Pin fish	6
Fish	*Menidia beryllina*	Inland silverside	121
Fish	*Mugil cephalus*	Striped mullet	248
Fish	*Paralichthys albigutta*	Gulf flounder	2
Fish	*Poecilia latipinna*	Molly	5
Fish	*Pogonias cromis*	Black drum	31
Fish	*Sciaenops ocellatus*	Red drum	20

All samples were frozen immediately after collection and processing, and then were transported from the field to Baylor University, where they were stored at -20°C with backup power supplies prior to analysis. A chain of custody form was used to track each sample from the field to the laboratory.

3.0 Analysis and Results

3.1 Sediment

3.1.1 *Physical Characterization*

Aliquots of each sediment sample were used to measure moisture content, total carbon (TC), black carbon (BC), and total organic carbon (TOC). Moisture content, TOC, BC, and TC were determined for all sediment samples using traditional methods (Gustafsson and Gschwend 1997; Gustafsson, Haghseta et al. 1997). Percent moisture content was determined by drying an aliquot of sediment sample (~5 g in triplicate) at 110 °C until constant weight. Percent moisture content was calculated as the ratio of the difference in weight of wet sample to dry sample by the weight of dry sample times 100.

Aliquots of sediment samples were dried to constant weight at 60 °C and were ground with mortar and pestle to a free-flowing powder. Aliquots of dried-ground sediment were used for TC, TOC, and BC determination (~50, ~50, and ~5 mg, respectively). TC, TOC, and BC analysis was performed using a Flash EA 1112 Series (ThermoQuest, Waltham, MA). Prior to analysis, aliquots used for TC analysis were wrapped in Sn foil. Aliquots of dried-ground sediment designated for TOC analysis were placed in Ag capsules and treated with HCl (1N) to remove inorganic carbon. Once the sediment was free of acid, it was wrapped in Sn capsules. Aliquots designated for BC analysis were placed in Ag capsules in a muffle furnace at 200 °C for

18 hours. After cooling down, the samples were treated with HCl (1N) to remove inorganic carbon. Again, when the sediment samples were acid free they were wrapped in Sn capsules.

A summary of the sediment samples collected and their physical characteristics is provided in Appendix A.

3.1.2 *Analysis of Dioxins, Furans, and PCBs in Sediment*

3.1.2.1 Chemicals and materials

Chemicals were purchased from commercial vendors at reagent grade or better and stored in accordance with manufacturer's recommendations. Basic alumina, silver nitrate (10% weight on silica gel), celite, Carbopak™, Florisil®, and copper powder were purchased from Sigma-Aldrich (St Louis, MO); silica gel, sodium sulfate, toluene (TOL), dichloromethane (DCM), and n-hexanes (HEX) were purchased from BDH Chemicals (West Chester, PA). Standard Reference Material 1944 New York/New Jersey Waterway Sediment (SRM 1944) was purchased from the National Institute of Standards and Technology (Gaithersburg, MD). Native and isotopically labeled dioxin, furan, and PCB analytes, surrogates, and internal standard were purchased from AccuStandard (New Haven, CT). Isotopically labeled versions of dioxin and furan congeners were used as their respective surrogate standards, $^{13}C_{12}$ PCB-77, 81, 126, and 169 were used as PCBs surrogate standards, and $^{13}C_{12}$ PCB-189 was used as an internal standard.

The target analytes are presented in **Table 2** and included all compounds on the 2005 World Health Organization (WHO) reevaluation of human and mammalian toxic equivalency factors (TEF) of dioxins and dioxin-like compounds (Van den Berg, Birnbaum et al. 2006). The 2005 WHO lists includes 7 dioxins, 10 furans, and 12 laterally-substituted PCBs congeners.

Table 2. Target analyte list for the present study.

Target Analytes	CAS #	TEF*
Dioxins		
2378-TCDD Tetrachlorodibenzo-p-dioxin	1746-01-6	1
12378-PeCDD Pentachlorodibenzo-p-dioxin	40321-76-4	1
123678-HxCDD Hexachlorodibenzo-p-dioxin	39227-28-6	0.1
123478-HxCDD Hexachlorodibenzo-p-dioxin	57653-85-7	0.1
123789-HxCDD Hexachlorodibenzo-p-dioxin	19408-74-3	0.1
1234678-HpCDD Heptachlorodibenzo-p-dioxin	35822-46-9	0.01
12346789-OCDD Octachlorodibenzo-p-dioxin	3268-87-9	0.0003
Furans		
2378-TCDF Tetrachlorodibenzofuran	51207-31-9	0.1
12378-PeCDF Pentachlorodibenzofuran	57117-41-6	0.03
23478-PeCDF Pentachlorodibenzofuran	57117-31-4	0.03
123678-HxCDF Hexachlorodibenzofuran	70648-26-9	0.1
123789-HxCDF Hexachlorodibenzofuran	57117-44-9	0.1
123478-HxCDF Hexachlorodibenzofuran	72918-21-9	0.1
234678-HxCDF Hexachlorodibenzofuran	60851-34-5	0.1
1234678-HpCDF Hexachlorodibenzofuran	67562-39-4	0.01
1234789-HpCDF Heptachlorodibenzofuran	55673-89-7	0.01
12346789-OCDF Octachlorodibenzofuran	39001-02-0	0.0003
PCBs		
33'44'-TeCB Tetrachlorobiphenyl (PCB 77)	32598-13-3	0.0001
344'5-TCB Tetrachlorobiphenyl (PCB 81)	70362-50-4	0.0003
233'44'-PeCB Pentachlorobiphenyl (PCB 105)	32598-14-4	0.00003
2344'5-PeCB Pentachlorobiphenyl (PCB 114)	74472-37-0	0.00003
23'44'5-PeCB Pentachlorobiphenyl (PCB 118)	31508-00-6	0.00003
2'344'5-PeCB Pentachlorobiphenyl (PCB 123)	65510-44-3	0.00003
33'44'5-PeCB Pentachlorobiphenyl (PCB 126)	57465-28-8	0.1
233'44'5-HxCB Hexachlorobiphenyl (PCB 156)	38380-08-4	0.00003
233'44'5'-HxCB Hexachlorobiphenyl (PCB 157)	69782-90-7	0.00003
23'44'55'-HxCB Hexachlorobiphenyl (PCB 167)	52663-72-6	0.00003
33'44'55'-HxCB Hexachlorobiphenyl (PCB 169)	32774-16-6	0.03
233'44'55'-HpCB Heptachlorobiphenyl (PCB 189)	39635-31-9	0.00003

*Toxic Equivalency Factor (TEF) World Health Organization, 2005

3.1.2.2 Enhanced pressurized liquid extraction

A method for the enhanced pressurized liquid extraction (ePLE) of dioxins, furans, and PCBs from sediment samples was developed as part of the research aims of this project (Subedi and Usenko 2012). Sodium sulfate was pre-cleaned by baking at 500 °C for 12 h and allowed to cool prior to use. Silica, alumina, and Florisil® were pre-cleaned with TOL under the following accelerated solvent extractor (ASE) conditions: Two 5-min static cycles at 100 °C and 1,500 psi with a 50% rinse volume. Sediment samples (~10 g ww) were homogenized with the pre-cleaned sodium sulfate using mortar and pestle until dry. The homogenized sediment samples were placed on top of pre-cleaned silica, alumina, and Florisil® (1:1 sample to sorbent ratio) in a 100 ml ASE cell (**Figure 3**). Samples were spiked in the ASE 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 ~20 min prior to extraction. The extraction of target analytes and removal of interferences was performed in a single automated step using an ASE-350 (Dionex – Thermo Fisher Scientific, Sunnyvale, CA). Sulfur was removed from the sediment extracts using activated copper powder (~3 g in 5 mL TOL), which was previously activated with 20% (v/v) nitric acid, and subsequently rinsed with deionized water, acetone, and HEX. Within the ASE collection bottle, sample extract was allowed to interact with the copper powder for 30 min after extraction to remove sulfur. PLE conditions were the similar to those used for sorbent pre-cleaning with the exception of rinse volume that was increased to 75%. The ASE extracts were concentrated under a gentle stream of nitrogen to ~0.2 mL using a Turbo Vap II (Caliper Life Sciences, Hopkinton, MA) then transferred to a 2-mL amber vial with a 500-µL glass insert and spiked with isotopically-labeled internal standard prior to chemical analysis.

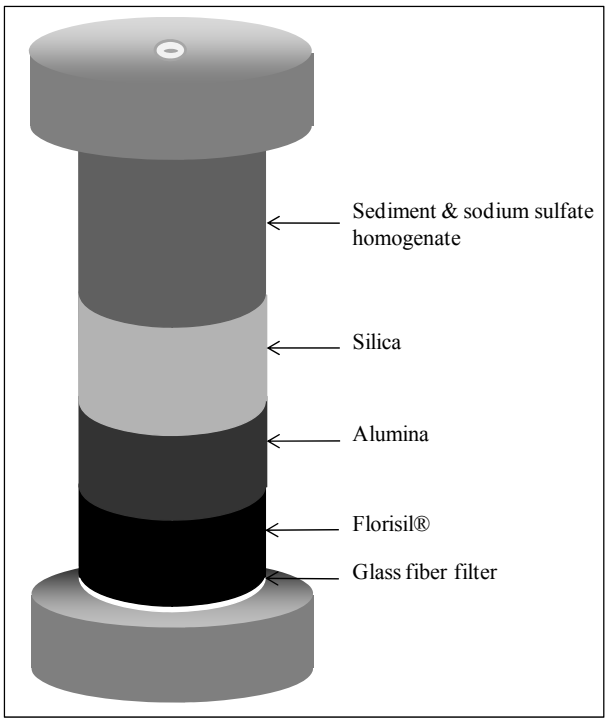

Figure 3. Accelerated solvent extraction cell with cleanup sorbents for enhanced pressurized liquid extraction.

3.1.2.3 Removal of bulk interferences

Silica, alumina, and Florisil® were added to the extraction cell in order to perform the extraction and cleanup in a single automated step. Aliquots of sediment (~10 g) homogenized with sodium sulfate were placed on top of pre-cleaned sorbents (1:1) and extracted under the conditions previously described. Extract cleanliness was evaluated with GPC-UV technique and full scan gas chromatography/mass spectrometry (GC-MS) in electron impact ionization (EI) mode. Silica, alumina, and Florisil® significantly retained extractable potential interferences (**Figure 4**). The ePLE method eliminated the need for further extract cleanup. Full scan GC-MS chromatograms and spectra were used to evaluate the ePLE efficiency at removing bulk interferences. Silica and alumina (1:1) removed some compounds with low mass-to-charge ratios (i.e., low m/z) while the combination of silica, alumina, and Florisil® (1:1:1) efficiently removed

most of the bulk background interferences (**Figure 5**). Efficient cleanup of extracts is important to avoid potential interferences and to reduce instrument maintenance.

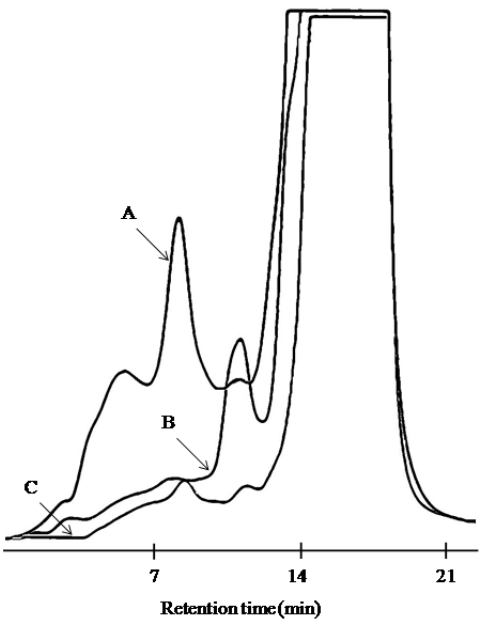

Figure 4. GPC-UV chromatograms of ePLE. (A) Sediment (~10 g) – sodium sulfate homogenate, (B) sediment homogenate, silica, and alumina (1:1:1), and (C) sediment homogenate, silica, alumina, and Florisil® (1:1:1:1)

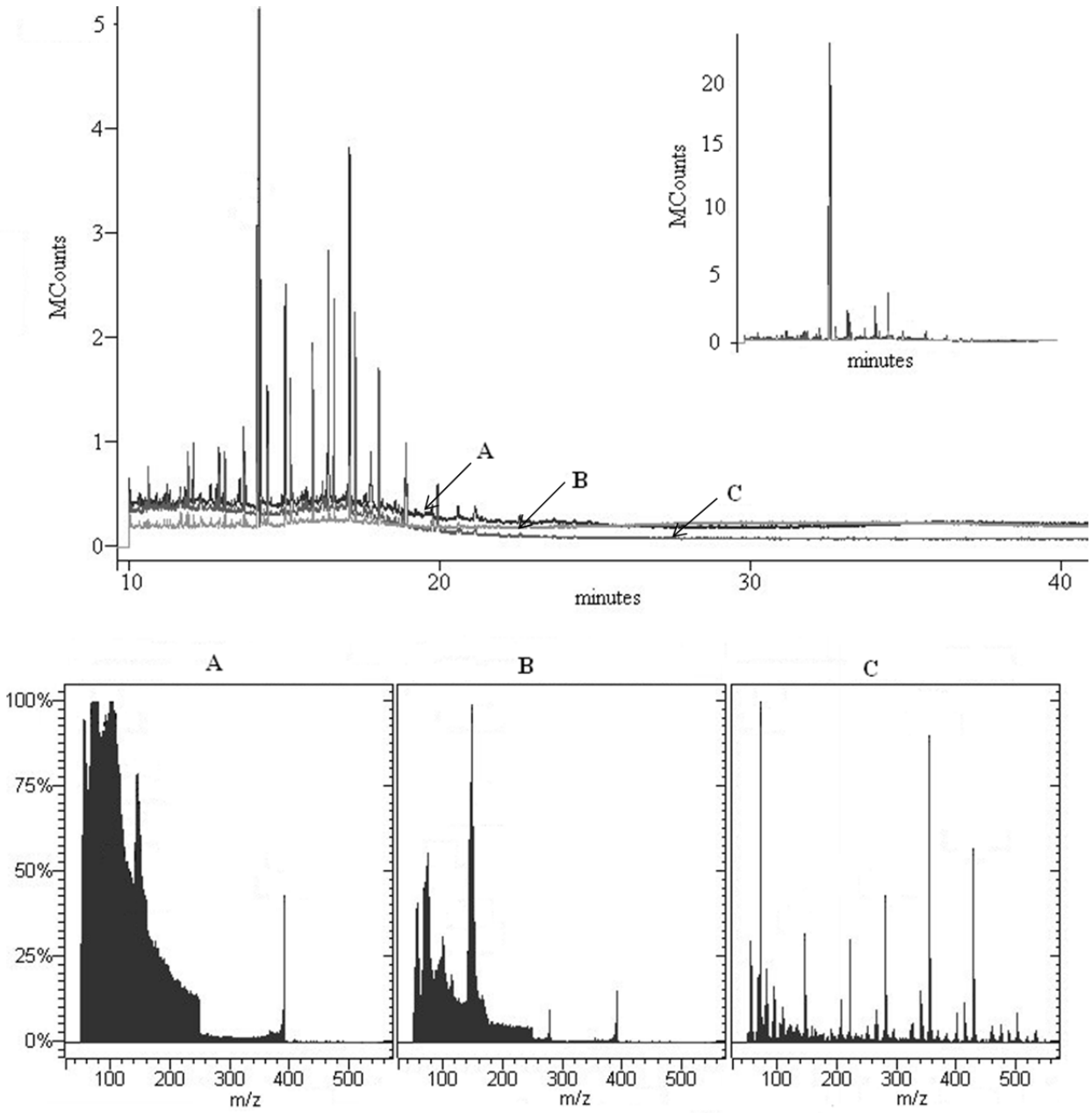

Figure 5. Electron Impact Ionization-full scan chromatograms and background spectra. Acquisition range 50-500 m/z. (A) Sediment homogenate (10g), (B) Sediment homogenate, silica, alumina (1:1:1), and (C) sediment homogenate, silica, alumina, and Florisil® (1:1:1:1)

3.1.2.4 Removal of sulfur interferences optimization

The efficiency of silver nitrate impregnated silica and acid-activated copper powder to remove elemental sulfur from sediment samples was verified by GPC. Silver nitrate (5 and 10g) was placed inside the extraction cell while activated copper powder (~1 g) was placed inside the extraction bottle (**Figure 6**). Elemental sulfur elutes from the GPC column at ~22 min (**Figure 6**A). Silver nitrate failed to retain the sulfur extracted from the sample (**Figure 6**B and C) while activated copper powder successfully removed the sulfur present in the extract (**Figure 6**D). The amount of copper powder required for the sediment samples was determined by GPC-UV. Activated copper was more effective at removing sulfur interferences than silver nitrate when integrated into the ePLE technique (**Figure 6**). In addition, copper is more affordable than silver nitrate impregnated silica.

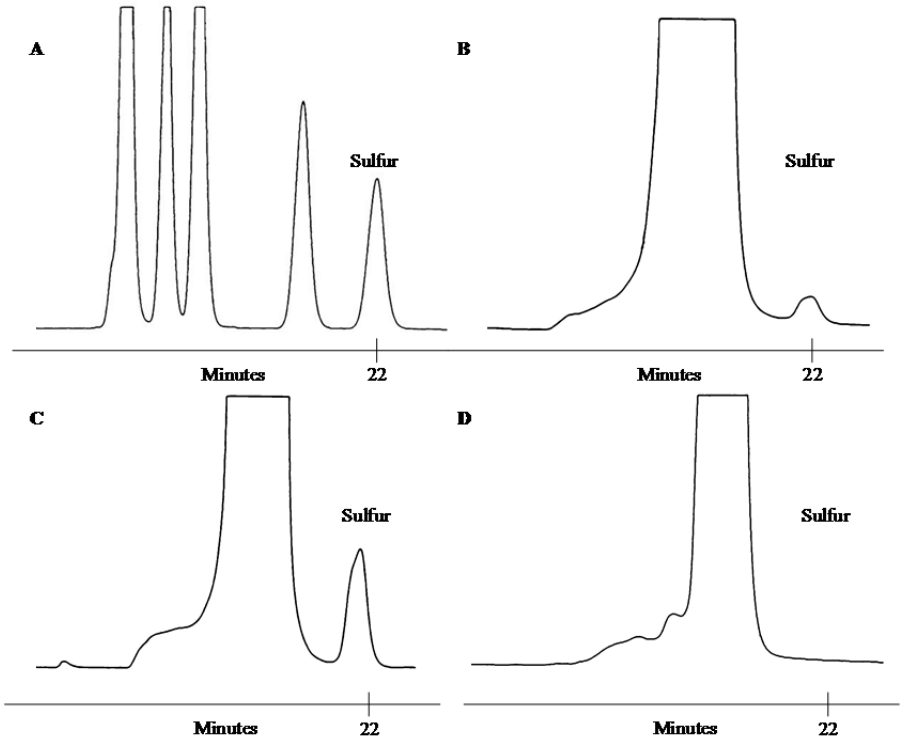

Figure 6. GPC-UV chromatograms of sulfur removal from sediment (~10 g). (A) GPC calibration mix standard showing sulfur peak at 22 minutes, (B) Removal of sulfur with $AgNO_3$ (10 g) inside an extraction cell, (C) removal of sulfur using $AgNO_3$ (5 g) inside an extraction cell, (D) removal of sulfur using activated copper (1 g) inside an extraction bottle.

3.1.2.5 Separation of dioxins and furans from PCBs in sediment

Carbopak™/celite is used to separate dioxins and furans, from PCBs by passing the concentrated extract through a column and eluting with DCM:HEX (1:1 v/v) followed by TOL. Subedi and Usenko successfully separated dioxins and furans from PCBs in fish tissue samples by incorporating carbopak™/celite into the extraction cell (Subedi and Usenko 2012). First, PCBs were extracted with one cycle DCM:HEX (1:1) while dioxins and furans were preferentially retained by the carbopak™. Subsequently, dioxins and furans were extracted with one cycle TOL extraction (Subedi and Usenko 2012). This technique was applied to sediment samples to separate dioxins and furans from PCBs. The efficiency of the separation was evaluated through analyte recovery experiments. Sediment (10 g) was homogenized with sodium sulfate, placed on top of the pre-cleaned sorbents, and fortified with target analytes. Carbopak™/celite (1:0.05:0.5) was placed inside the extraction cell with silica, alumina, and Florisil® (1:1:1:1) and pre-cleaned as previously described. The sample was extracted with one cycle DCM:HEX (1:1) followed by a one cycle TOL extraction. The DCM:HEX and TOL extracts were collected and analyzed separately. Recoveries for dioxins, furans, and PCBs from the DCM:HEX (1:1) extraction were 59 ± 17.7% and 47 ± 3.3%, respectively. The TOL extraction yielded average dioxins, furans, and PCBs recoveries of 25 ± 4.2% and 31 ± 4.5%, respectively. Unlike fish samples dioxins and furans in sediment were not efficiently separated from PCBs using the technique described by Subedi and Usenko. Similarly, Chuang et al. were not able to quantitatively recover dioxins and furans from sediment and soil extracted with DCM (120 °C) followed by TOL (125 °C) when including carbopak™/celite within the extraction cell (Chuang, Van Emon et al. 2009).

3.1.2.6 Contaminant analysis in sediment

The analytical instrumentation used for the analysis of dioxins, furans, and PCBs in solid matrices has been previously described (Subedi and Usenko 2012). Briefly, analyses were performed using high resolution gas chromatography coupled with electron capture negative ionization mass spectrometry (HRGC–ECNI/MS). An Agilent 7890A (Agilent Technologies Inc., Santa Clara, CA, USA) GC system coupled with 5975C inert XL EI/CI MSD triple axis detector and 7683B series injector was used. One microliter of the sample extract was injected in pulsed splitless injection mode at 250 °C and 60 psi. Injection pulse pressure was maintained for 0.74 min and followed by 40 mL min^{-1} purge flow to split vent at 0.75 min. Initial flow rate of helium carrier gas (99.999%) was 2.88 mL min^{-1}. Methane (99.999%) was used as a chemical ionization buffer gas. Chromatographic separation was performed using a capillary DB-Dioxin (60 m × 0.25 mm × 0.15 μm) column (J & W Scientific, USA). The GC temperature program comprised of initial temperature of 140 °C, 10 °C min^{-1} temperature ramp to 240 °C, hold for 10 min, and the final ramp at 5 °C min^{-1} to 260 °C. The thermal auxiliary transfer line and source temperatures were 250 °C while the quadrupole temperature was 200 °C.

Target analyte concentrations were verified using HRGC–high resolution mass spectrometry (HRMS) using an Agilent 6890N GC system coupled with VG Prospec (Fisons Instrument, Manchester, UK) and 7683 series injector and autosampler. One microliter sample was injected in splitless injection mode at 260 °C and 60 psi. Injection was purged for 2 min at 40 mL min^{-1}. The constant flow rate of helium carrier gas (99.999%) was maintained throughout the run with an initial flow rate of 1.5 mL min^{-1}. Chromatographic separation was performed using a capillary DB-Dioxin column as described above. The GC temperature program comprised of initial temperature of 140 °C, 10 °C min^{-1} temperature ramp to 220 °C, hold for 10

min, and the final ramp at 5 °C min^{-1} to 265 °C. The final temperature was held for 34 min with a total run time of 61 min. The transfer line and source temperatures were 250 °C. Qualitative and quantification ions were monitored at electron energy of 35 eV and a mass resolution of 10,000. The trap and emission currents were 650 μA and 1.57 mA, respectively.

Isotope dilution mass spectrometry was utilized for the quantification of target analytes. Two chlorine isotope ions were monitored for qualitative and quantification purpose through ECNI (**Table 3**) as well as HRMS with selective ion monitoring (SIM) mode for each analyte, surrogate, and internal standard. Due to reduced sensitivity through ECNI technique, 2,3,7,8-TCDD and $^{13}C_{12}$-2,3,7,8-TCDD were quantified using HRMS.

Table 3. Target analytes, monitored ions, triplicate recoveries, and statistically-derived method detection limits.

Analytes	Monitored ions (m/z) HRGC–ECNI/MS	Triplicate Recoveries ± RSD			MDL ($pg\ g^{-1}$)
		Clam	Crab	Fish	
Dioxins					
2378-TCDD[a]	321.9860, 319.6730			84 ± 11	28
12378-PeCDD	355.9, 357.9	83 ± 2.3	92 ± 2.9	86 ± 4.4	4.84
123478-HxCDD	389.8, 391.8	83 ± 5.2	94 ± 4.0	87 ± 1.5	5.11
123678-HxCDD	389.8, 391.8	84 ± 2.1	95 ± 4.3	84 ± 2.1	5.17
123789-HxCDD	389.8, 391.8	90 ± 2.8	88 ± 2.1	89 ± 5.8	5.15
1234678-HpCDD	423.8, 425.7	111 ± 4.0	101 ± 1.0	88 ± 2.2	9.66
OCDD	459.7, 457.7	111 ± 2.5	91 ± 3.8	100 ± 7.4	43.9
Furans					
2378-TCDF	305.9, 303.9	73 ± 2.7	86 ± 5.0	76 ± 2.9	9.45
12378-PeCDF	339.9, 341.9	83 ± 1.9	91 ± 1.8	87 ± 1.7	2.53
23478-PeCDF	339.9, 341.9	69 ± 1.9	88 ± 2.6	72 ± 1.4	2.83
123478-HxCDF	373.8, 375.8	84 ± 1.6	92 ± 1.8	80 ± 3.6	2.74
123678-HxCDF	373.8, 375.8	82 ± 1.5	90 ± 2.0	80 ± 2.8	2.74
123789-HxCDF	373.8, 375.8	80 ± 0.9	91 ± 3.8	79 ± 2.3	3.38
234678-HxCDF	373.8, 375.8	92 ± 1.2	97 ± 4.5	89 ± 3.4	3.63
1234678-HpCDF	407.8, 409.8	89 ± 1.1	93 ± 2.7	86 ± 2.3	1.96
1234789-HpCDF	407.8, 409.8	92 ± 1.8	95 ± 1.9	91 ± 0.4	2.17
OCDF	443.8, 441.7	93 ± 1.2	95 ± 1.5	93 ± 3.4	4.13
PCBs					
PCB 77	289.9, 291.9	84 ± 2.0	66 ± 3.8	91 ± 2.0	3.29
PCB 81	289.9, 291.9	82 ± 4.3	65 ± 4.7	90 ± 2.3	3.2
PCB 105	325.9, 327.9	100 ± 5.4	75 ± 11	103 ± 2.8	N/A
PCB 114	325.9, 327.9	93 ± 4.0	74 ± 4.0	93 ± 3.0	3.61
PCB 118	325.9, 327.9	91 ± 3.4	64 ± 7.7	93 ± 2.8	N/A
PCB 123	325.9, 327.9	88 ± 3.1	74 ± 13	94 ± 4.7	N/A
PCB 126	325.9, 327.9	91 ± 1.5	75 ± 6.8	91 ± 3.3	3.39
PCB 156	359.8, 361.8	94 ± 1.2	75 ± 5.1	95 ± 1.6	N/A
PCB 157	359.8, 361.8	96 ± 1.8	81 ± 2.6	96 ± 1.5	10.1
PCB 167	359.8, 361.8	94 ± 1.4	84 ± 2.9	91 ± 3.3	8.28
PCB 169	359.8, 361.8	94 ± 1.4	80 ± 2.3	90 ± 0.8	2.5
PCB 189	393.8, 395.8	97 ± 0.4	85 ± 1.1	91 ± 1.0	2.62

[a]Samples analyzed through HRGC–HRMS
N/A refers non-applicable explaining the background concentrations were higher than the spiking level.

Seven-point calibration curves (concentration ranging from 0.5 to 100 ng μL^{-1}) were prepared by plotting the concentration dependent response factor of each target analyte (i.e. peak area of analytes divided by peak area of surrogates) versus the response dependent concentration factor (i.e. concentration of analytes divided by concentration of surrogates). However, $^{13}C_{12}$-

OCDD, OCDF, and $^{13}C_{12}$-OCDF calibration concentrations ranged from 1 to 200 ng μL^{-1}, and OCDD had five-point calibration curve ranging from 10 to 200 ng μL^{-1}. Response factor relative standard deviations (RSD) for surrogates were < 4.0% from equal weighting average of response factors, and the coefficients of determination (r^2) for analytes from equal weighting linear regression were ≥ 0.998.

Quality assurance and quality control protocols were followed to confirm analyte identification and quantification. Calibration curve verification (CCV) standards were run after every third sample to monitor instrument performance. If CCV recoveries were not within 25%, such observations provided an indication that instrument maintenance was required. Blanks were also run every third sample to check for carry over. Target analytes were identified based on their retention time, a quantitative ion, and two qualitative ions (±20).

3.1.2.7 Method validation and method detection limit

The entire analytical method was validated using triplicate recovery analysis of SRM 1944 and fortified sediment samples. Dioxins/furans, and PCBs were extracted from SRM 1944 using 5 and 0.5 g, respectively. SRM 1944 was spiked with isotopically-labeled surrogate standards and allowed to equilibrate for 1 hr. The samples were fortified with target analytes (middle calibration level concentration) and allowed to equilibrate for 1 hr. Isotopically-labeled surrogate standards and internal standard were spiked prior to chemical analysis. The overall method detection limits were determined using seven replicates of samples fortified with target analytes and surrogate standards. Target analytes and isotopically-labeled surrogate standards were spiked at the second and middle calibration level, respectively. SRM 1944, fortified

samples, and MDL samples were extracted as previously described. Recoveries were determined by HRGC–ECNI/MS and HRGC–HRMS both utilizing SIM.

3.2 Clams, Crab, and Fish

3.2.1 *Lipid Content*

Two-grams of tissue composites (clams, crab, and fish) were homogenized with ~20 g of anhydrous sodium sulfate. One cellulose filter (1.9 µm) was placed at the bottom of 100 mL ASE cell. Homogenate mass was transferred to the ASE cell. Lipids were extracted using 1 static extraction cycle DCM:HEX (1:1 v/v) followed by 1 static extraction cycle of TOL. Extractions were performed at 100 °C and 1,500 psi with 5 minutes of static extraction, 290s purge time, and 75% flush volume. DCM:HEX extracts and TOL extracts were combined and concentrated to 10 mL under nitrogen. The percentage lipid was calculated gravimetrically: 2 mL of concentrates were transferred to the pre-weighed aluminum boats, solvents were evaporated until the complete dryness, and boats containing lipid were weighed.

A summary of the percent lipid content for all biological matrices is provided in Appendix A.2.

3.2.2 *Analysis of Dioxins, Furans, and PCBs in Tissue*

3.2.2.1 Chemicals and materials

As noted above, the analytes included 7 dioxins, 10 furans, and 12 PCBs (**Table 2**) recommended by WHO/IPCS 2005 TEFs for human risk assessment. Surrogates include $^{13}C_{12}$-labelled analogues of 7 dioxin and 10 furan congeners, $^{13}C_{12}$-PCB 77, $^{13}C_{12}$-PCB 81, $^{13}C_{12}$-PCB 126, and $^{13}C_{12}$-PCB 169. $^{13}C_{12}$-PCB 189 was used as an internal standard. The native and $^{13}C_{12}$-

labeled dioxins, furans, and PCBs were purchased from Wellington Laboratories (Wellington Laboratories Inc., ON, Canada). Perfluorokerosene (PFK), Florisil®, Carbopack™ C (80–100 mesh size), activated acidic and basic aluminum oxide (Brockmann-I), and Celite® 545RG resin were purchased from Sigma Aldrich (Aldrich Chemical Company, MO, USA). Ultra resi-analyzed® granular sodium sulfate (12–60 mesh size), TOL, and DCM were purchased from J. T. Baker (Mallinckrodt Baker Inc., NJ, USA). High purity silica Gel® 60 Å and ultra resi-analyzed® HEX were purchased from VWR international (VWR International, PA, USA).

3.2.2.2 Contaminant analysis in tissue

The analytical instrumentation used for the analysis of dioxins, furans, and PCBs in solid matrices was previously outlined above in section 3.1.2.6.

3.2.2.3 Quality assurance and quality control

The peak identification of analytes was based on the retention time (±0.05 min) and qualitative to quantitative ion response ratio (±20%). Quality assurance and quality control during sample analysis were monitored by running a CCV standard solution at regular intervals (every 4th injection). CCV standard solution includes all analytes, surrogates, and internal standard at the middle point of the calibration curve. Analyte recoveries beyond ±25% range from CCV analyses required instrumental maintenance such as replacing the injection liner, cutting the head of the capillary column, and/or constructing a new matrix-match calibration curve. A matrix blank and a laboratory reagent blank were included during sample preparations and analyzed. The laboratory reagent blank analyzed prior to and after each sample batch showed no laboratory contamination of target analytes.

3.2.2.4 Method validation

A triplicate spike and recovery experiment was used to validate the entire analytical method. ~10 g Tilapia fish composites derived from farm-raised Tilapia fillets purchased from a retail store were homogenized with ~40 g of anhydrous Na_2SO_4, fortified with analytes (CCV level), extracted using ePLE, concentrated, spiked with surrogates and internal standards, and analyzed as described above. A matrix blank (~10 g fish composites) and a laboratory reagent blank were fortified with surrogates prior to the extraction.

Similarly, ~5 g crab or clam composites from SJRWP sampling or from farm-raised crab and clams purchased from a retail store; were homogenized with ~70 g of anhydrous Na_2SO_4, fortified with analytes, extracted using ePLE, concentrated, spiked with surrogates and internal standards, and analyzed as described above. A matrix blank (~5 g crab or clam composites) and a laboratory reagent blank were fortified with surrogates prior to the extraction. For crab composites, two extracts from DCM:HEX (1 cycle) were combined to increase the extracted tissue mass (up to ~10 g) before concentrating the extracts. Similarly, two extracts from TOL (2 cycles) were also combined before concentrating the extracts.

Mean recoveries (n = 3) of dioxins and furans in TOL extracts were 85.5 ± 3.0% **(Table 3**). Similarly, the mean recoveries (n = 3) for PCBs in DCM:HEX (1:1) extracts were 93.2 ± 2.4%. The individual surrogates recovery for both DCM:HEX (1:1) and TOL extracts were within ±25%. Target analytes were not measured in matrix blanks and laboratory reagent blanks. Repeatability of the developed method was calculated in terms of RSD of each analyte recovery (%) among the seven consecutive runs of one of the triplicate samples. Percent RSD of dioxins, furans, and PCBs were within ±9.0% and ±3.0%, respectively. These data illustrate the reproducibility and repeatability of the entire analytical method.

Mean recoveries (n = 3) of PCBs in DCM:HEX (1:1) extracts and dioxins and furans in TOL extracts in crab were 76 ± 5.4% and 93 ± 2.8%, respectively **(Table 3)**. Similarly, average recoveries (n = 3) of PCBs in DCM:HEX (1:1) extracts and dioxins and furans in TOL extracts in clams were 92 ± 2.5% and 87 ± 2.2%, respectively. The individual surrogate recovery for both DCM:HEX (1:1) and TOL extracts were within ±25%. Target analytes were not measured in matrix blanks and laboratory reagent blanks.

3.2.3 *Comparison to EPA Method 1316B*

The inherent cost associated with historic methods capable of simultaneously measuring both dioxins, furans, and PCBs in biological tissue, such as EPA 1613, includes time and labor, laboratory space, technical training, and solvent. Utilizing the ePLE technique, the sample preparation time was reduced by ~95% (ePLE: ~30 min; cleanup: ~0 min; and concentration: ~30 min) as compared to the EPA 1613B (Soxhlet: ~18 to 24 h; cleanup: ~4 h; and concentration: ~2 h). This was achieved by combining the extraction step and all the necessary cleanup steps into a single automated technique. In addition, the laboratory space and training required for the analysis was also reduced with ePLE, in contrast to conventional methods that require post-extraction cleanup steps. Similarly, extraction solvent volume were reduced by ~65% (ePLE: ~300 mL) as compared to the EPA 1613 (Soxhlet: ~400 mL; cleanup: ~500 mL).

Through automation and reduction of the number of steps, the ePLE technique also provided significant improvements in surrogate recoveries as compared to EPA method 1613B. Surrogate recoveries for the ePLE technique ranged from 75 to 125% as compared to the reported surrogates recovery of 17–197% by EPA method1613. This analytical method represents the first example of combining all substantial cleanup techniques (silica gel, florisil,

celite, carbopak, and alumina) required for the analysis of dioxins, furans, and PCBs in biological tissue within a single automated pressurized liquid extraction technique. In addition, overall improvements in accuracy and precision were achieved using this automated extraction technique.

3.2.4 *Isotopic Composition of C and N*

Fish, crab, and clam samples were dried to constant weight (for 24 h at 95 °C in a drying oven) and crushed to a fine powder using a mortar and pestle. Dried, crushed samples were weighted and wrapped in Sn capsules. Samples were analyzed for isotopic compositions of C and N using a Thermo-Electron Dual-Inlet Gas-Source Stable Isotope Mass Spectrometer at Baylor University.

A summary of the isotopic composition of C and N for all biological matrices is provided in Appendix A.4.

3.3 Data Summary for Sediment, Clams, Crab, and Fish

3.3.1 Summary Description of Data for Dioxins, Furans, and PCBs

A summary of the frequency of detection of dioxin, furan, and PCBs in sediment, clams, crab, and fish is provided in

Table 4. Sediment samples represented the matrix with the highest frequency of detects, and PCBs were the most frequently detected target analytes.

Table 4. Detection frequency for dioxins, furans, and PCBs in sediment, clams, crab, and fish

Congener abbreviation	Det. Freq. Sediment	Det. Freq. Clams	Det. Freq. Crabs	Det. Freq. Fish
Dioxins				
2378-TCDD	15/15	12/12	12/12	9/12
12378-PeCDD	0/15	0/12	0/12	0/12
123478-HxCDD	0/15	0/12	0/12	0/12
123678-HxCDD	0/15	0/12	0/12	1/12
123789-HxCDD	2/15	0/12	0/12	0/12
1234678-HpCDD	6/15	0/12	0/12	0/12
OCDD	15/15	8/12	9/12	3/12
Furans				
2378-TCDF	15/15	12/12	12/12	8/12
12378-PeCDF	14/15	0/12	3/12	0/12
23478-PeCDF	14/15	0/12	5/12	0/12
123478-HxCDF	14/15	0/12	3/12	1/12
123678-HxCDF	14/15	0/12	3/12	1/12
123789-HxCDF	12/15	0/12	2/12	1/12
234678-HxCDF	4/15	0/12	0/12	0/12
1234678-HpCDF	14/15	0/12	0/12	1/12
1234789-HpCDF	12/15	0/12	0/12	0/12
OCDF	11/15	0/12	0/12	0/12
PCBs				
PCB 77	14/15	0/12	12/12	11/12
PCB 81	3/15	11/12	12/12	0/12
PCB 105	15/15	12/12	12/12	12/12
PCB 114	15/15	12/12	12/12	11/12
PCB 118	15/15	12/12	12/12	12/12
PCB 123	15/15	12/12	12/12	12/12
PCB 126	14/15	12/12	12/12	11/12
PCB 156	15/15	12/12	12/12	12/12
PCB 157	15/15	12/12	12/12	12/12
PCB 167	15/15	12/12	12/12	9/12
PCB 169	4/15	2/12	0/12	0/12
PCB 189	15/15	12/12	12/12	11/12

Summaries of dioxin, furan, and PCB concentrations in sediment are provided in

Table 5 (dry weight normalized; pg/g ww), **Table 6** (wet weight normalized; pg/g ww), and **Table 7** (TOC normalized; pg/g TOC). The summary tables include the concentration

ranges, and arithmetic means and geometric means. Spatial distribution of 2,3,7,8-TCDD, 2,3,7,8-TCDF, and ΣPCBs dry weight concentrations in sediment are presented in **Figure 7**.

For the August 2010 sampling event, the concentration of dioxins in surficial sediment ranged from 3.7 to 17,000 pg/g dw. 2,3,7,8-TCDD demonstrated the greatest range in concentration in sediment among the dioxin analytes. TOC normalized 2,3,7,8-TCDD concentrations of surficial sediment ranged from 180 to 240,000 pg/g TOC with an mean concentration of 65,000 pg/g TOC. 2,3,7,8-TCDF also has the greatest range in concentration as compared to other furans measured in surficial sediment, from 36 to 50,000 pg/g dw. TOC normalized 2,3,7,8-TCDF concentrations of surficial sediment ranged from 1,700 to 560,000 pg/g TOC with an mean concentration of 220,000 pg/g TOC. The mean 2,3,7,8-TCDF concentrations measured in surficial sediment was ~3 times that of 2,3,7,8-TCDD.

Individual PCB concentrations measured in surficial sediment ranged from 0.9 to 230,000 pg/g dw. The highest measured PCB concentrations were for PCB-81, 105, 118, 123, and 156 with concentrations of 25,000, 94,000, 230,000, 35,000, and 30,000 pg/g dw, respectively. PCB-118 had the highest measured concentration of any target analyte. TOC-normalized PCB-118 concentrations of surficial sediment ranged from 14,000 to 2,600,000 pg/g TOC with a mean concentration of 800,000 pg/g TOC. The mean concentration of PCB-118 measured in surficial sediments were ~12 times that of 2,3,7,8-TCDD and ~3.6 times that of 2,3,7,8-TCDF. Overall, the ΣPCBs were greater than the Σdioxins and Σfurans.

Table 5. Summary statistics for dioxins, furans, and PCBs measured in sediment (dry weight normalized)

Target Analyte	Det. Freq. Sediment	All units pg/g Min	Max	Median	Mean	St. Dev.	Geomean
Dioxins							
2378-TCDD	15/15	3.7	17000	530	2700	5200	530
12378-PeCDD	0/15	--	--	--	--	--	--
123478-HxCDD	0/15	--	--	--	--	--	--
123678-HxCDD	0/15	--	--	--	--	--	--
123789-HxCDD	2/15	0.0	360	--	--	--	--
1234678-HpCDD	6/15	0.0	72	--	--	--	--
OCDD	15/15	79	1200	280	450	360	330
Furans							
2378-TCDF	15/15	36	50000	2000	8700	15000	2000
12378-PeCDF	14/15	0.0	1900	--	--	--	--
23478-PeCDF	14/15	0.0	1200	32	190	340	--
123478-HxCDF	14/15	0.0	2500	130	390	700	--
123678-HxCDF	14/15	0.0	570	30	90	160	--
123789-HxCDF	12/15	0.0	85	4.0	14	24	--
234678-HxCDF	4/15	0.0	23	--	--	--	--
1234678-HpCDF	14/15	0.0	670	33	100	190	--
1234789-HpCDF	12/15	0.0	240	13	39	69	--
OCDF	11/15	0.0	290	32	63	79	--
PCBs							
PCB 77	14/15	0.0	2400	87	390	700	--
PCB 81	3/15	0.0	25000	--	1700	6400	--
PCB 105	15/15	120	94000	2300	14000	28000	3000
PCB 114	15/15	8.2	5500	130	810	1600	170
PCB 118	15/15	290	230000	5700	34000	66000	7500
PCB 123	15/15	47	35000	880	5200	10000	1100
PCB 126	14/15	0.0	400	21	68	110	--
PCB 156	15/15	37	30000	1100	5100	8600	1400
PCB 157	15/15	6.9	5700	180	920	1600	240
PCB 167	15/15	9.2	9000	260	1400	2600	370
PCB 169	4/15	0.0	28	--	--	--	--
PCB 189	15/15	0.9	320	25	98	120	31

Table 6. Summary statistics for dioxins, furans, and PCBs measured in sediment (wet weight normalized)

Target Analyte	Det. Freq. Sediment	All units pg/g Min	Max	Median	Mean	St. Dev.	Geomean
Dioxins							
2378-TCDD	15/15	2.0	8700	220	1500	2900	290
12378-PeCDD	0/15	--	--	--	--	--	--
123478-HxCDD	0/15	--	--	--	--	--	
123678-HxCDD	0/15	--	--	--	--	--	--
123789-HxCDD	2/15	0.0	180	--	--	--	--
1234678-HpCDD	6/15	0.0	33	--	--	--	--
OCDD	15/15	65	640	170	230	160	180
Furans							
2378-TCDF	15/15	19	24000	890	4700	8000	1100
12378-PeCDF	14/15	0.0	940	--	--	--	--
23478-PeCDF	14/15	0.0	560	13	100	180	--
123478-HxCDF	14/15	0.0	1200	61	210	370	--
123678-HxCDF	14/15	0.0	270	15	49	84	--
123789-HxCDF	12/15	0.0	41	3.0	7.5	13	--
234678-HxCDF	4/15	0.0	11	--	--	--	--
1234678-HpCDF	14/15	0.0	330	14	56	100	--
1234789-HpCDF	12/15	0.0	120	6	21	37	--
OCDF	11/15	0.0	140	--	--	--	--
PCBs							
PCB 77	14/15	0.0	1100	51	210	380	--
PCB 81	3/15	0.0	12000	--	--	--	--
PCB 105	15/15	67	46000	1600	7700	15000	1700
PCB 114	15/15	4.5	2700	89	440	870	92
PCB 118	15/15	160	110000	4100	19000	36000	4200
PCB 123	15/15	26	17000	540	2800	5400	610
PCB 126	14/15	0.0	200	8.4	37	61	--
PCB 156	15/15	20	14000	860	2800	4600	770
PCB 157	15/15	3.7	2800	140	500	880	130
PCB 167	15/15	5.0	4400	190	780	1400	200
PCB 169	4/15	0.0	15	--	--	--	--
PCB 189	15/15	0.5	190	12	54	67	17

Table 7. Summary statistics for dioxins, furans, and PCBs measured in sediment (TOC normalized)

Target Analyte	Det. Freq. Sediment	All units pg/g Min	Max	Median	Mean	St. Dev.	Geomean
Dioxins							
2378-TCDD	15/15	180	240000	56000	65000	62000	34000
12378-PeCDD	0/15	--	--	--	--	--	--
123478-HxCDD	0/15	--	--	--	--	--	--
123678-HxCDD	0/15	--	--	--	--	--	--
123789-HxCDD	2/15	0.0	2800	--	--	--	--
1234678-HpCDD	6/15	0.0	4600	--	--	--	--
OCDD	15/15	1600	75000	30000	32000	22000	21000
Furans							
2378-TCDF	15/15	1700	560000	190000	220000	170000	130000
12378-PeCDF	14/15	0.0	17000	--	--	--	--
23478-PeCDF	14/15	0.0	13000	3800	4200	3600	--
123478-HxCDF	14/15	0.0	29000	9100	11000	8700	--
123678-HxCDF	14/15	0.0	7400	2200	2500	2100	--
123789-HxCDF	12/15	0.0	1400	400.0	440	390	--
234678-HxCDF	4/15	0.0	890	--	--	--	--
1234678-HpCDF	14/15	0.0	7900	2200	2700	2300	--
1234789-HpCDF	12/15	0.0	3300	940	1100	980	--
OCDF	11/15	0.0	6000	2100	2200	1700	--
PCBs							
PCB 77	14/15	0.0	42000	8300	11000	11000	--
PCB 81	3/15	0.0	200000	--	--	--	--
PCB 105	15/15	6000	1100000	180000	330000	310000	190000
PCB 114	15/15	400	63000	9600	18000	18000	11000
PCB 118	15/15	14000	2600000	450000	800000	730000	480000
PCB 123	15/15	2300	390000	63000	120000	110000	69000
PCB 126	14/15	0.0	4500	1800	2000	1500	--
PCB 156	15/15	1800	340000	150000	150000	100000	88000
PCB 157	15/15	330	64000	26000	25000	19000	15000
PCB 167	15/15	450	99000	40000	38000	28000	23000
PCB 169	4/15	0.0	390	--	--	--	--
PCB 189	15/15	45	13000	3200	4200	4200	2000

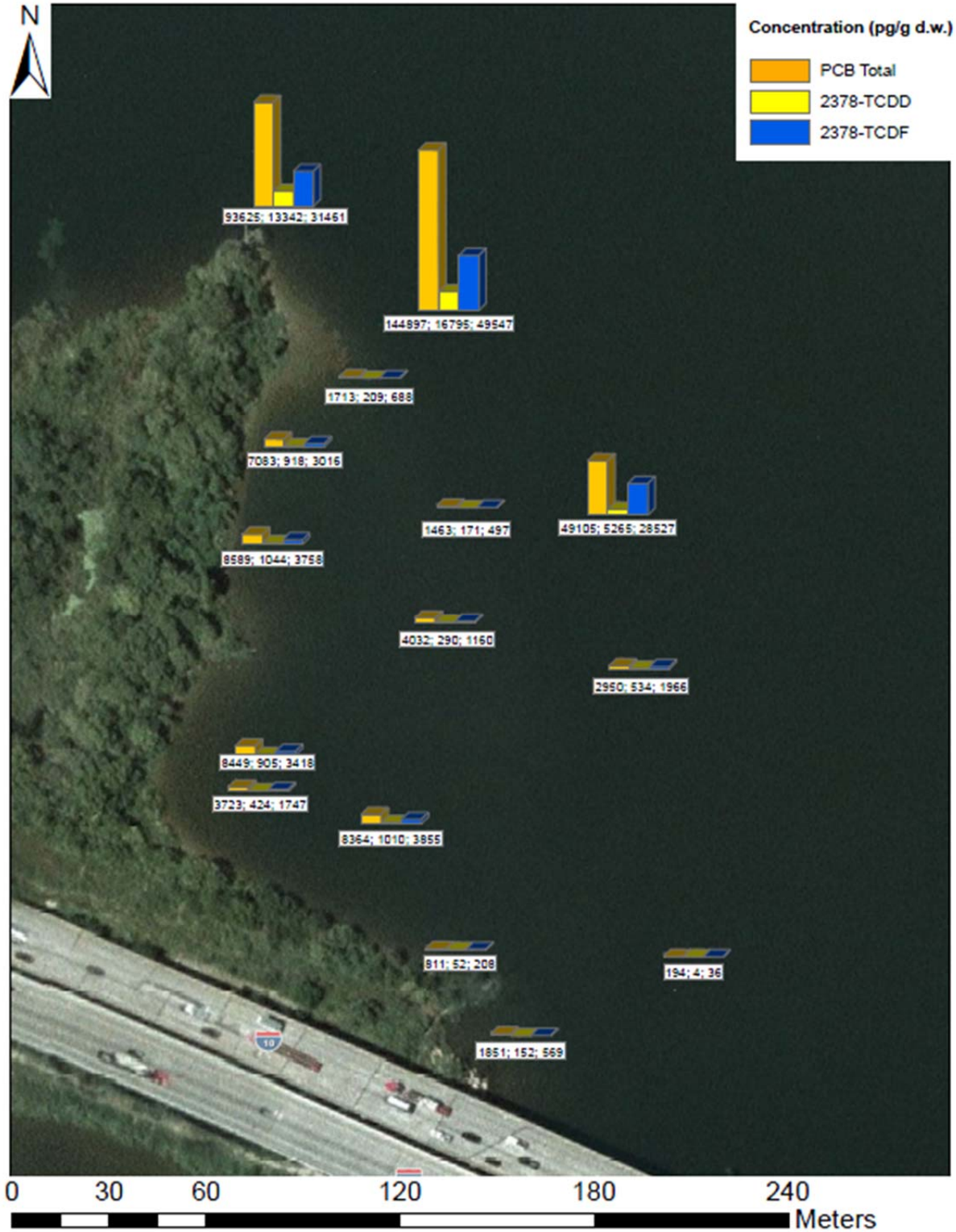

Figure 7. Surficial sediment concentrations (pg/g dw) of 2,3,7,8-TCDD, 2,3,7,8-TCDF, and ΣPCBs collected at the San Jacinto River Waste Pits, Texas, in August 2010.

Summaries of dioxin, furan, and PCBs concentrations in clams are provided in **Table 8** (wet weight normalized; pg/g ww) and **Table 9** (lipid normalized; pg/g lw). Summary tables include the concentration ranges, and the arithmetic means and geometric means.

2,3,7,8-TCDD concentrations of clams collected from the August 2010 sampling event at the SJRWPs ranged from 1,100 to 5,800 pg/g lw with a mean of 3,300 pg/g lw. 2,3,7,8-TCDD was detected in all samples (100%); of the other dioxin congeners, only OCDD was detected in clam tissue (67%). 2,3,7,8-TCDF was also detected in all 12 samples (100% detection frequency) with concentrations ranging from 2,700 to 14,000 pg/g lw with a mean of 9,000 pg/g lw. The mean 2,3,7,8-TCDF concentrations measured in clams was ~2.7 times that of 2,3,7,8-TCDD. No other furan congeners were detected in clam tissue.

Similar to observations in sediment samples, PCBs were the most frequently detected class of compounds in clams. PCBs concentrations measured in clams ranged from 3,300 to 510,000 pg/g lw. The highest measured PCB concentrations were for PCB-105, 118, 123, and 156 with concentrations of 190,000, 510,000, 110,000, and 140,000 pg/g lw, respectively. Also consistent with sediment observations, PCB-118 had the highest measured clam concentration of any target analyte with a mean concentration of 300,000 pg/g lw. The mean concentration of PCB-118 measured in clams was ~90 times that of 2,3,7,8-TCDD and ~33 times that of 2,3,7,8-TCDF. As in sediment, the ΣPCBs were greater than the Σdioxins and Σfurans.

Table 8. Summary statistics for dioxins, furans, and PCBs measured in clams (wet weight normalized)

Target Analyte	Det. Freq. Clams	All units pg/g Min	Max	Median	Mean	St. Dev.	Geomean
Dioxins							
2378-TCDD	12/12	12	59	21	28	15	25
12378-PeCDD	0/12	--	--	--	--	--	--
123478-HxCDD	0/12	--	--	--	--	--	--
123678-HxCDD	0/12	--	--	--	--	--	--
123789-HxCDD	0/12	--	--	--	--	--	--
1234678-HpCDD	0/12	--	--	--	--	--	--
OCDD	8/12	0.0	130	--	--	--	--
Furans							
2378-TCDF	12/12	31	139	68	74	33	68
12378-PeCDF	0/12	--	--	--	--	--	--
23478-PeCDF	0/12	--	--	--	--	--	--
123478-HxCDF	0/12	--	--	--	--	--	--
123678-HxCDF	0/12	--	--	--	--	--	--
123789-HxCDF	0/12	--	--	--	--	--	--
234678-HxCDF	0/12	--	--	--	--	--	--
1234678-HpCDF	0/12	--	--	--	--	--	--
1234789-HpCDF	0/12	--	--	--	--	--	--
OCDF	0/12	--	--	--	--	--	--
PCBs							
PCB 77	0/12	--	--	--	--	--	--
PCB 81	11/12	0.0	72	62	54	20	--
PCB 105	12/12	570	1600	1000	990	350	940
PCB 114	12/12	62	130	84	86	20	84
PCB 118	12/12	1400	4300	2500	2500	890	2300
PCB 123	12/12	330	930	590	570	190	540
PCB 126	12/12	49	60	55	55	3.6	55
PCB 156	12/12	320	1100	700	660	250	610
PCB 157	12/12	96	200	140	140	34	140
PCB 167	12/12	130	320	230	220	58	210
PCB 169	2/12	0.0	29	--	--	--	--
PCB 189	12/12	43	58	50	50	4.8	50

Table 9. Summary statistics for dioxins, furans, and PCBs measured in clams (lipid weight normalized)

Target Analyte	Det. Freq. Clams	All units pg/g Min	Max	Median	Mean	St. Dev.	Geomean
Dioxins							
2378-TCDD	12/12	1100	5800	3000	3300	1500	2900
12378-PeCDD	0/12	--	--	--	--	--	--
123478-HxCDD	0/12	--	--	--	--	--	--
123678-HxCDD	0/12	--	--	--	--	--	--
123789-HxCDD	0/12	--	--	--	--	--	--
1234678-HpCDD	0/12	--	--	--	--	--	--
OCDD	8/12	0.0	23000	--	--	--	--
Furans							
2378-TCDF	12/12	2700	139	68	74	33	68
12378-PeCDF	0/12	--	--	--	--	--	--
23478-PeCDF	0/12	--	--	--	--	--	--
123478-HxCDF	0/12	--	--	--	--	--	--
123678-HxCDF	0/12	--	--	--	--	--	--
123789-HxCDF	0/12	--	--	--	--	--	--
234678-HxCDF	0/12	--	--	--	--	--	--
1234678-HpCDF	0/12	--	--	--	--	--	--
1234789-HpCDF	0/12	--	--	--	--	--	--
OCDF	0/12	--	--	--	--	--	--
PCBs							
PCB 77	0/12	--	--	--	--	--	--
PCB 81	11/12	0.0	11000	6500	6500	3200	--
PCB 105	12/12	52000	190000	120000	120000	41000	110000
PCB 114	12/12	4800	15000	10000	11000	3200	10000
PCB 118	12/12	130000	510000	290000	300000	110000	280000
PCB 123	12/12	32000	110000	67000	69000	24000	65000
PCB 126	12/12	3800	11000	6400	6900	2100	6600
PCB 156	12/12	31000	140000	71000	80000	34000	73000
PCB 157	12/12	7800	26000	16000	18000	5800	17000
PCB 167	12/12	12000	39000	25000	27000	9100	26000
PCB 169	2/12	0.0	29	--	--	--	--
PCB 189	12/12	3300	10000	6300	6300	1900	6000

A summary of dioxin, furan, and PCB concentrations in crab are provided in **Table 10** (wet weight normalized; pg/g ww) and **Table 11** (lipid normalized; pg/g lw). Summary tables include the range of concentrations, and arithmetic and geometric mean values.

2,3,7,8-TCDD concentrations in crab collected from the SJRWP site ranged from 430 to 1,500 pg/g lw with a mean of 750 pg/g lw. 2,3,7,8-TCDD was detected in all crab samples (100%). 2,3,7,8-TCDF also 100% detection frequency with concentrations measured in crab ranging from 590 to 2600 pg/g lw with a mean of 1500 pg/g lw. The mean 2,3,7,8-TCDF concentrations measured in crab was 2 times that of 2,3,7,8-TCDD.

Similar to sediment and clam samples, PCBs were the most frequently detected class of compounds in crab. PCBs concentrations measured in crab ranged from 280 to 570,000 pg/g lw. The highest measured PCB concentrations were for PCB-105, 118, 123, and 156 with concentrations of 190,000, 570,000, 94,000, and 64,000 pg/g lw, respectively. Again, consistent with observations in sediments and clams, PCB-118 had the high measured crab concentration of any target analyte with a mean concentration of 180,000 pg/g lw. The mean concentration of PCB-118 measured in crab was 240 times that of 2,3,7,8-TCDD and 120 times that of 2,3,7,8-TCDF. Again, the ΣPCBs were greater than the Σdioxins and Σfurans in crab.

Table 10. Summary statistics for dioxins, furans, and PCBs measured in crab (wet weight normalized)

Target Analyte	Det. Freq. Crab	All units pg/g Min	Max	Median	Mean	St. Dev.	Geomean
Dioxins							
2378-TCDD	12/12	2.3	5.8	3.3	3.6	0.9	3.5
12378-PeCDD	0/12	--	--	--	--	--	--
123478-HxCDD	0/12	--	--	--	--	--	--
123678-HxCDD	0/12	--	--	--	--	--	--
123789-HxCDD	0/12	--	--	--	--	--	--
1234678-HpCDD	0/12	--	--	--	--	--	--
OCDD	9/12	0.0	23	--	--	--	--
Furans							
2378-TCDF	12/12	3	11	7.6	7.5	2.6	7.0
12378-PeCDF	3/12	0.0	1.7	--	--	--	--
23478-PeCDF	5/12	0.0	2.0	--	--	--	--
123478-HxCDF	3/12	0.0	2.0	--	--	--	--
123678-HxCDF	3/12	0.0	1.5	--	--	--	--
123789-HxCDF	2/12	0.0	1.7	--	--	--	--
234678-HxCDF	0/12	--	--	--	--	--	--
1234678-HpCDF	0/12	--	--	--	--	--	--
1234789-HpCDF	0/12	--	--	--	--	--	--
OCDF	0/12	--	--	--	--	--	--
PCBs							
PCB 77	12/12	22	47	33	33	7.5	32
PCB 81	12/12	12	35	18	20	7.8	19
PCB 105	12/12	150	620	240	280	120	260
PCB 114	12/12	12	38	18	19	7.4	18
PCB 118	12/12	400	1800	640	810	380	740
PCB 123	12/12	64	300	110	130	63	120
PCB 126	12/12	2.0	8.1	3.1	3.7	1.9	3.4
PCB 156	12/12	44	200	73	94	48	86
PCB 157	12/12	11	36	15	18	8	16
PCB 167	12/12	18	99	29	38	22	34
PCB 169	0/12	--	--	--	--	--	--
PCB 189	12/12	2.2	10	4.5	4.9	2.4	4.4

Table 11. Summary statistics for dioxins, furans, and PCBs measured in crab (lipid weight normalized)

	Det. Freq.	All units pg/g					
Target Analyte	Crab	Min	Max	Median	Mean	St. Dev.	Geomean
Dioxins							
2378-TCDD	12/12	430	1500	690	750	280	710
12378-PeCDD	0/12	--	--	--	--	--	--
123478-HxCDD	0/12	--	--	--	--	--	--
123678-HxCDD	0/12	--	--	--	--	--	--
123789-HxCDD	0/12	--	--	--	--	--	--
1234678-HpCDD	0/12	--	--	--	--	--	--
OCDD	9/12	0.0	6200	--	--	--	--
Furans							
2378-TCDF	12/12	590	2600	1600	1500	590	1400
12378-PeCDF	3/12	0.0	310	--	--	--	--
23478-PeCDF	5/12	0.0	360	--	--	--	--
123478-HxCDF	3/12	0.0	400	--	--	--	--
123678-HxCDF	3/12	0.0	270	--	--	--	--
123789-HxCDF	2/12	0.0	310	--	--	--	--
234678-HxCDF	0/12	--	--	--	--	--	--
1234678-HpCDF	0/12	--	--	--	--	--	--
1234789-HpCDF	0/12	--	--	--	--	--	--
OCDF	0/12	--	--	--	--	--	--
PCBs							
PCB 77	12/12	4000	15000	6100	7100	3200	6500
PCB 81	12/12	2600	6300	3600	4000	1400	3800
PCB 105	12/12	27000	190000	49000	62000	46000	53000
PCB 114	12/12	1600	12000	3400	4300	2800	3700
PCB 118	12/12	75000	570000	140000	180000	140000	150000
PCB 123	12/12	12000	94000	23000	30000	22000	25000
PCB 126	12/12	280	2600	600	810	610	680
PCB 156	12/12	8500	64000	15000	21000	16000	17000
PCB 157	12/12	1700	11000	3200	3800	2700	3300
PCB 167	12/12	3500	31000	6000	8600	7800	6900
PCB 169	0/12	--	--	--	--	--	--
PCB 189	12/12	430	3200	790	1100	830	900

Summaries of dioxin, furan, and PCB concentrations in black drum are provided in **Table 12** (wet weight normalized; pg/g ww) and **Table 13** (lipid normalized; pg/g lw). Summary tables include the range of concentrations, and the arithmetic and geometric means.

Dioxin and furan congeners were detected in relatively few samples. 2,3,7,8-TCDD was the most frequently detected dioxin congener (75%). 2,3,7,8-TCDD concentrations in fish collected from the SJRWP site ranged from <MDL to 1,700 pg/g lw with a mean of 710 pg/g lw. 2,3,7,8-TCDF had a detection frequency of 67% with concentrations measured in fish ranging from <MDL to 1,000 pg/g lw. Beyond 2,3,7,8-TCDD and 2,3,7,8-TCDF, no other congener was detected at a frequency greater than 10%.

Similar to observations from sediment, clam, and crab samples, PCBs were the most frequently detected class of compounds in fish. PCBs concentrations measured in clams ranged from 530 to 430,000 pg/g lw. The highest measured PCB concentrations were for PCB-105, 118, 123, and 156 with concentrations of 140,000, 430,000, 84,000, and 44,000 pg/g lw, respectively. Similar to sediment, clams, and crab, PCB-118 had the highest measured concentration of any target analyte in fish with a mean concentration of 130,000 pg/g lw. The mean concentration of PCB-118 measured in fish was ~180 times that of 2,3,7,8-TCDD. As in sediment, clams, and crab, the ΣPCBs concentrations were greater than the Σdioxins and Σfurans in fish.

Table 12. Summary statistics for dioxins, furans, and PCBs measured in fish (wet weight normalized)

	Det. Freq.	All units pg/g					
Target Analyte	Fish	Min	Max	Median	Mean	St. Dev.	Geomean
Dioxins							
2378-TCDD	9/12	0.0	4.4	2.6	2.3	1.5	--
12378-PeCDD	0/12	--	--	--	--	--	--
123478-HxCDD	0/12	--	--	--	--	--	--
123678-HxCDD	1/12	--	--	--	--	--	--
123789-HxCDD	0/12	--	--	--	--	--	--
1234678-HpCDD	0/12	--	--	--	--	--	--
OCDD	3/12	0.0	14	--	--	--	--
Furans							
2378-TCDF	8/12	0.0	4.4	--	--	--	--
12378-PeCDF	0/12	0.0	--	--	--	--	--
23478-PeCDF	0/12	0.0	--	--	--	--	--
123478-HxCDF	1/12	0.0	0.7	--	--	--	--
123678-HxCDF	1/12	0.0	0.5	--	--	--	--
123789-HxCDF	1/12	0.0	0.5	--	--	--	--
234678-HxCDF	0/12	--	--	--	--	--	--
1234678-HpCDF	1/12	--	--	--	--	--	--
1234789-HpCDF	0/12	--	--	--	--	--	--
OCDF	0/12	--	--	--	--	--	--
PCBs							
PCB 77	11/12	0.0	66	28	28	16	--
PCB 81	0/12	--	--	--	--	--	--
PCB 105	12/12	32	200	120	120	69	100
PCB 114	11/12	0.0	14	8.9	8.6	4.4	--
PCB 118	12/12	94	650	380	370	210	300
PCB 123	12/12	25	150	74	75	40	64
PCB 126	11/12	0.0	7.5	5.6	5.3	2.0	--
PCB 156	12/12	4.0	74	46	39	25	29
PCB 157	12/12	1.5	15	10	8.9	4.5	7.5
PCB 167	9/12	0.0	20	10	9.2	8.0	--
PCB 169	0/12	--	--	--	--	--	--
PCB 189	11/12	0.0	9.2	7.8	7.3	2.4	--

Table 13. Summary statistics for dioxins, furans, and PCBs measured in fish (lipid weight normalized)

	Det. Freq.	All units pg/g					
Target Analyte	Fish	Min	Max	Median	Mean	St. Dev.	Geomean
Dioxins							
2378-TCDD	9/12	0.0	1700	690	710	590	--
12378-PeCDD	0/12	--	--	--	--	--	--
123478-HxCDD	0/12	--	--	--	--	--	--
123678-HxCDD	1/12	--	--	--	--	--	--
123789-HxCDD	0/12	--	--	--	--	--	--
1234678-HpCDD	0/12	--	--	--	--	--	--
OCDD	3/12	0.0	7600	--	--	--	--
Furans							
2378-TCDF	8/12	0.0	1000	--	--	--	--
12378-PeCDF	0/12	--	--	--	--	--	--
23478-PeCDF	0/12	--	--	--	--	--	--
123478-HxCDF	1/12	0.0	200	--	--	--	--
123678-HxCDF	1/12	0.0	130	--	--	--	--
123789-HxCDF	1/12	0.0	130	--	--	--	--
234678-HxCDF	0/12	--	--	--	--	--	--
1234678-HpCDF	1/12	--	--	--	--	--	--
1234789-HpCDF	0/12	--	--	--	--	--	--
OCDF	0/12	--	--	--	--	--	--
PCBs							
PCB 77	11/12	0.0	25000	6900	8700	6200	--
PCB 81	0/12	--	--	--	--	--	--
PCB 105	12/12	12000	140000	28000	43000	38000	31000
PCB 114	11/12	0.0	9100	2000	2900	2500	--
PCB 118	12/12	34000	430000	87000	130000	110000	94000
PCB 123	12/12	8700	84000	19000	26000	21000	20000
PCB 126	11/12	0.0	4200	1600	1700	1000	--
PCB 156	12/12	1400	44000	9200	13000	12000	8900
PCB 157	12/12	530	10000	2400	3100	2600	2300
PCB 167	9/12	0.0	10000	2400	3100	3300	--
PCB 169	0/12	--	--	--	--	--	--
PCB 189	11/12	0.0	5800	2300	2400	1400	--

3.3.2 Quality Assurance Summary

Laboratory blanks (reagent blanks) were analyzed at a frequency of >15% and accompanied each batch. CCV (calibration curve verification) standards were also included in each batch of samples and were analyzed before and after every third sample. In addition, each batch included a matrix spike (MS) and matrix spike duplicate (MSD) sample. MS and MSD are utilized to measure the precision of the analytical method over the course of the project (**Table 14**). The overall percent relative standard deviation (%RSD) measured for all surrogates and matrices was 7.5%. The %RSD measured for dioxins, furans, and PCBs in all matrices were 9.2%, 6.6%, and 7.0%, respectively. The mean MS and MSD surrogate %RSD measured in surficial sediment was 8% with averages for dioxins, furans, and PCBs, of 12.2, 5.7, and 6.1%, respectively. The mean MS and MSD surrogate %RSD measured in fish tissue was 7.5% with averages for PCBs, dioxins, and furans of 7.5, 7.2, and 7.7%, respectively.

Table 14. Percent relative standard deviation of surrogates measured in matrix spikes and matrix spike duplicate samples for sediment, clams, crab, and fish.

	PCBs	Dioxins	Furans	All surrogates
Sediment	6.1	12.2	5.7	8.0
Clams	2.6	11.3	6.4	7.3
Crabs	11.7	6.2	6.4	7.3
Fish	7.5	7.2	7.7	7.5
All matrices	7.0	9.2	6.6	7.5

The percent surrogate recoveries for sediment, clams, crab, and fish is provided in **Table 15**. The overall average percent surrogate recoveries for all matrices and compounds was 78%. The mean surrogate recovery in surficial sediments was 72% with averages for PCBs, dioxins, and furans of 83, 74, and 67%, respectively. Mean surrogate recoveries for $^{13}C_{12}$ 2,3,7,8-TCDD in surficial sediment was 80%. The fraction of surrogate recoveries out of 50-120% (set

by the project Quality Assurance Project Plan (QAPP)) in surficial sediment samples was 20%. Within fish tissue, ~92% of PCBs surrogate recoveries were in compliance with the projects QAPP. In addition, 100% of the dioxin and furan surrogate recoveries were in compliance. Surrogate recoveries in compliance with the project QAPP measured in crab was ~96% (PCBs) and ~99% (dioxins and furans). Similarly, the surrogate recoveries measured in clams was ~100% (PCBs), ~93% (dioxins), and ~99% (furans). The mean surrogate recovery in fish tissue was 80% with mean values for PCBs, dioxins, and furans of 83, 74, and 67%, respectively.

Table 15. Percent surrogate recoveries for sediment, clams, crab, and fish.

	PCBs	Dioxins	Furans	All surrogates
Sediment	83	74	67	72
Clams	97	97	81	89
Crabs	70	81	78	78
Fish	82	75	71	74
All matrices	83	82	74	78

4.0 Food Web

4.1 Trophic Analysis

For site-specific applications of bioaccumulation models it can be useful to employ stable isotope measurements at various trophic position in an approach known as isotopic discrimination. During each trophic exchange, lighter isotopes (e.g., ^{14}N) are reduced in organism relative to heavier isotopes (e.g., ^{15}N; (Minagawa and Wada 1984). Higher trophic positions subsequently have higher levels of heavier isotopes, which can be extremely useful for determining site-specific trophic structure in aquatic communities (Post 2002). Further, quantification of trophic transfer of contaminants among various species within an aquatic

community is possible by relating tissue concentrations to $\delta^{15}N$ measurements (Cabana and Rasmussen 1994).

In the present research project, we utilized such isotopic discrimination to identify trophic position of the collected species from the SJRWP. In addition to following common methods (Cabana and Rasmussen 1994), we compared our findings to a recent study (Akin and Winemiller 2006) of Mad Island Marsh Preserve, an estuarine site located near Matagorda Bay, Texas. This was performed for two reasons: 1) it represented the most comprehensive similar study of its type in an estuary with a remarkably similar food web that was located near the present study site; and 2) it allowed for comparison of estuarine food webs from a contaminated and a non-contaminated site. Based on stable isotope methods described above, **Table 16** presents results from trophic position analysis of the organisms collected at the SJRWP in August 2010.

Table 16. Taxa for which stable isotope analysis was performed for trophic position after sampling of the San Jacinto River Waste Pits, Texas in August 2010.

Species or Taxa	N	Mean $\delta^{15}N$	Trophic Position
Fundulus grandis	6	15.86	1.98
Mugil cephalus - small	6	16.27	2.21
Menidia beryllina	6	17.33	2.28
Dorosoma cepedianum	4	17.5	2.31
Mercenaria spp	12	17.91	2.14
Mugil cephalus - large	6	18.23	2.74
Cynoscion nebulosus	4	18.23	3.26
Brevoortia patronus	10	18.6	2.61
Farfantepenaeus aztecus	6	18.66	2.52
Callinectes sapidus	6	18.89	2.54
Palaemonetes pugio	4	19.18	2.88
Pogonias cromis	12	19.42	2.9
Elops saurus	6	19.73	2.91
Arius felis	6	19.96	3.1
Lagodon rhomboides	5	20.29	2.97
Paralicthys lethostigma	6	20.74	3.34
Cyprinodon variegatus	1	22.74	3.92

A statistically significant relationship ($p < 0.05$) was observed for trophic positions between the San Jacinto River Pits and Mad Island Marsh Preserve (

Figure 8A) based on stable isotope analysis, when detritivorous outliers were excluded as previously recommended (Akin and Winemiller 2006). Akin and Winemiller (2006) further proposed trophic position based on stomach content and size relationships because a number of organisms display ontogenetic dietary feeding shifts. For example, in the present study, small mullet (mean weight = 10.3 g) occupied a trophic position of 2.21, whereas larger mullet (mean weight = 397.3 g) were observed at the higher trophic position of 2.74. Though it was outside the scope of the present study to examine gut contents and further refine trophic position based on size structure of the aquatic community at the SJRWP site, we also observed a statistically significant relationship ($p < 0.05$) between trophic levels at the SJRWP and Mad Island Marsh Preserve (

Figure 8B) when stomach content analysis was considered.

As noted above, based on consultation with the TEHI project coordinators and the August 2010 sampling information, we selected black drum for further analysis of bioaccumulation. Black drum (*P. cromis*) is a large bodied predatory fish common in the bays and estuaries of the Gulf of Mexico. It is primarily an estuarine species. Mark-recapture studies have shown it to have a small home range, typically moving less 5 miles (Sutter et al. 1986). In the coldest months of the year black drum may move to deeper water, but is not considered migratory. Juveniles feed on polychaetes, amphipods, and copepods while benthic invertebrates comprise most of adult diet with mollusks being the most prominent followed by crab and shrimp (Sutter et al. 1986). Black drum feed mostly on bivalves in autumn but include crab and a more diverse diet in spring due to seasonal variation in prey abundance (Brown et al. 2008). Thus, it was not

surprising that black drum was observed to occupy a higher trophic position of 2.9 in the present study (**Table 16**).

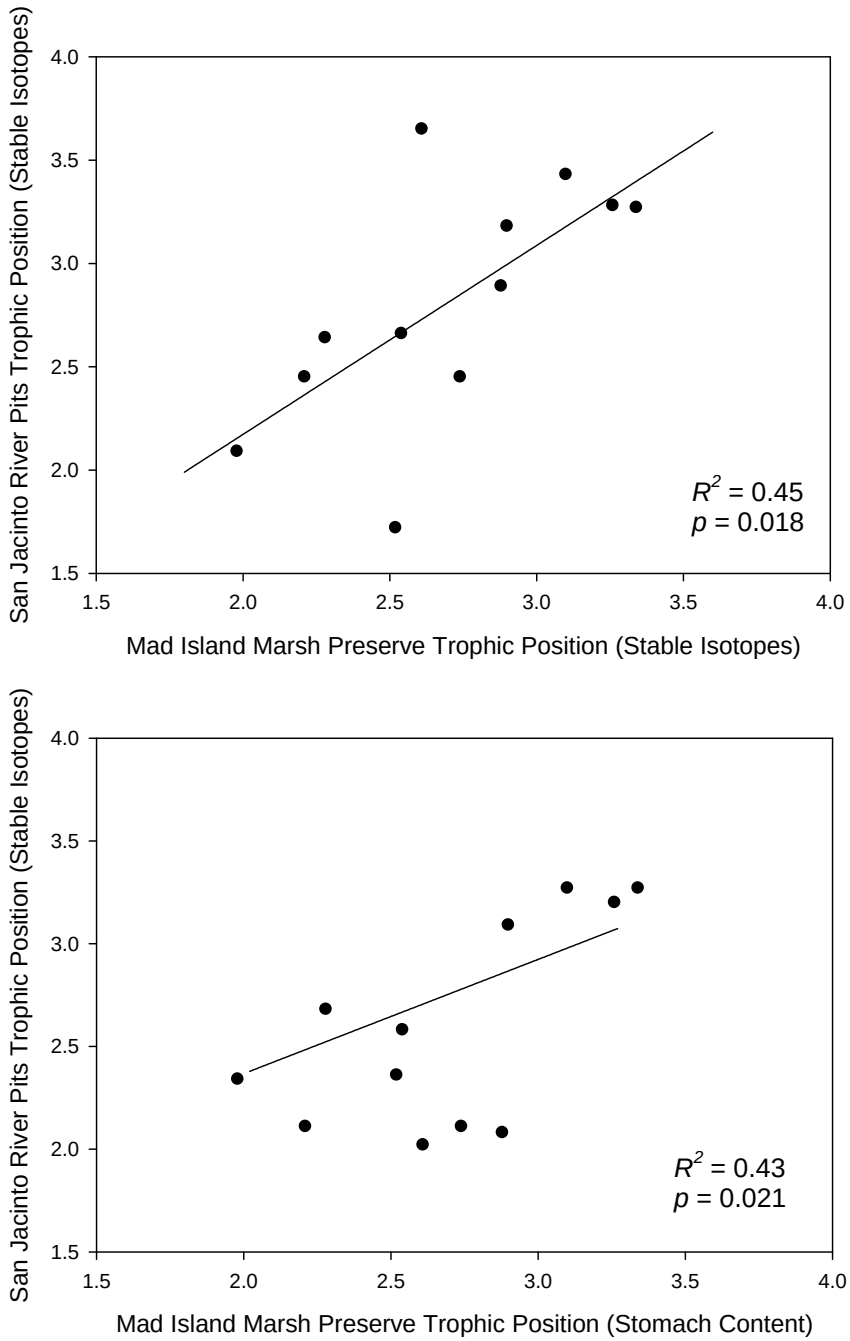

Figure 8. Relationship between trophic positions of the Mad Island March Preserve, Texas, based on stable isotope analysis (A) and stomach content analysis (B) (Akin and Winemiller 2006), and trophic positions of the San Jacinto River Waste Pits, Texas, based on stable isotope analysis.

Blue crab (*C. sapidus*) is a euryhaline species commonly found throughout every habitat in the Gulf of Mexico. It can be found in a variety of habitats but often prefers shallow, muddy bottom estuaries. It feeds on a variety of bivalves and scavenges on dead or dying organisms. Blue crab are also notorious for their cannibalistic nature, as other blue crab can comprise up to 13% of their diet (Laughlin 1979). Similar to black drum, this species will move to deeper water during cold periods, but is not generally considered migratory. In the present study, the trophic position of blue crab was observed to be 2.54 (**Table 16**).

Hard clams (*Mercenaria spp.)* are filter feeding bivalve mollusks with high site fidelity because they burrow in sediments usually 1 to 2 cm (Roberts et al. 1989). *M. campechiensis* closely resembles and has been known to hybridize *with M. mercenaria*, but is slightly larger and lack smooth areas around its umbo (Dillon and Manzi 1992). Hard clams prefer sand or mud substrates close to intertidal flats, where they filter feed on single celled algae and diatoms (Hibbert 1977). They have been known to live as long as 46 years and grow to a size of 150 mm (Stanley and Dewitt 1983) and thus provide robust indicators of contaminated sites. Hard clams were observed to occupy one of the lowest trophic levels (2.12) in the present study (**Table 16**).

In addition to hard clams collected during August 2010 at the SJRWP site, it may be reasonable to anticipate that black drum and blue crab were part of the local resident population at least for some unknown part of the year. These three species were selected for BSAF calculations because they are readily collected and consumed by local fisherman. During one of our sampling trips, for example, all three of these species were collected by resident fishers at the site with the intent of consumption (**Figure 9**). Clams and blue crab were also selected for trophic modeling because they represent prey of black drum.

Figure 9. Fishers in the San Jacinto River Waste Pits during site visit by TCEQ, TDSHS and Baylor personnel in the fall of 2009. Black drum, red drum, mullet, sheepshead and hard clams were observed in possession of fishers.

5.0 Bioaccumulation of Dioxins, Furans, and PCBs in the SJRWP

Bioaccumulation and biomagnification of hydrophobic chemicals are critical parameters that drive decision-making in risk and remediation associated with Superfund sites. The relationship between sediment and tissue concentrations is most often expressed as a "biota-sediment accumulation factor" or BSAF. This ratio is used in federal and state regulatory frameworks to provide technical support for the development of remedial goals in contaminated sediment sites, such as the SJRWP site. BSAFs are an important parameter in the calculation of protective concentration levels for sediment under the Texas Risk Reduction Program (TRRP).

$$Sed_{Fish}PCL = \frac{RBEL_{Fish}}{BSAF}$$

where the $RBEL_{fish}$ represents the risk-based exposure limit for human ingestion of fish or shellfish tissue.

Scientists serving with the United States EPA have provided extensive guidance on the development of BSAFs for Superfund sites (Burkhard 2009). The BSAF is a calculated ratio of paired observations of the concentrations of hydrophobic contaminants in sediment and in tissue. To account for the partitioning of hydrophobic chemicals, sediment concentrations are corrected on an organic carbon basis, and tissue concentrations are corrected on a lipid basis. The BSAF is calculated thusly:

$$BSAF = \frac{C_{Tissue-Lipid}}{C_{Sediment-TOC}}$$

where $C_{Tissue\text{-}Lipid}$ is the lipid-corrected chemical concentration in the organism, and $C_{Sediment\text{-}TOC}$ is the organic carbon-corrected chemical concentration in surficial sediment. While regression is a potential approach for understanding the relationship between these parameters, the USEPA recommends the use of an averaging approach, "whereby the BSAF is estimated by averaging the BSAFs from the paired observations across the site" (Burkhard 2009).

One weakness in the BSAF approach is the difficulty in addressing the variability in home range among assessed organisms. For example, black drum are believed to spend most of their time within a 5 mile range; however, the variability among individuals in these ranges, particularly in dynamic estuarine environments like the San Jacinto River adds inherent uncertainty to any BSAF estimate calculated for the species.

The paired approach, while unsatisfying from an ecological perspective, does provide an avenue to incorporate variability in sediment concentrations across a geographic range. In the

HSC complex, it would be difficult to overstate the importance of the SJRWP as a source of dioxins and furans; however, sampling stations adjacent to Patrick Bayou have high concentrations of PCBs most likely arising from industrial and municipal discharges, as well as runoff from nearby industrial and residential areas (http://www.epa.gov/superfund/sites/npl/nar1638.htm). This area has also been listed as a Superfund site since 2002. As noted above, it was not the objective of this research project to derive BSAF values in a manner consistent with EPA guidance for use in remedial decision making.

5.1 Gobas/Arnot Model of Bioaccumulation

A number of mathematical models are available to predict the tissue concentrations of hydrophobic contaminants. The best known of these is the Gobas and Gobas/Arnot models, first published in 1993 and updated in 2004 (Gobas 1993; Arnot and Gobas 2004) (www.rem.sfu.ca/toxicology/models). Several reviews have assessed the efficacy of these models in predicting tissue concentrations of hydrophobic contaminants (Imhoff, Clough et al. 2004; Gustavson, von Stackelberg et al. 2011). The Gobas model serves as the algorithmic basis for several software tools used to model bioaccumulation, including TrophicTrace, a software tool available through the US Army Corps of Engineers (http://el.erdc.usace.army.mil/trophictrace/) and developed in collaboration with USEPA, and the K_{OW} (based) Aquatic Bioaccumulation Model (KABAM), available through the USEPA (http://www.epa.gov/oppefed1/models/water/index.htm#KABAM). These models been applied to bioaccumulation of dioxins, furans, and PCBs at a number of Superfund sites, including the

Passaic River in New Jersey (Imhoff, Clough et al. 2004; Gustavson, von Stackelberg et al. 2011).

The Gobas and Gobas/Arnot models rely on an equation that the authors suggest describes the exchange of contaminants between an organism and its ambient environment (Gobas 1993; Arnot and Gobas 2004).

$$\frac{dM_B}{dt} = \{W_B * (k_1 * [m_O * \phi * C_{WT,O} + C_{WD,S}] + k_D * \Sigma (P_i * C_{D,i}))\} - (k_2 + k_E + k_M) * M_B$$

M_B: mass (g) of chemical in the organism

dM_B/dt: net flux of parent chemical being absorbed or depurated at any point in time t

W_B: weight of the organism (g)

k_1: clearance rate constant (L/Kg*d)

m_O: fraction of respiratory ventilation that involves sediment-associated pore water

ϕ: fraction of total chemical concentration in the overlying water that is freely dissolved and can be absorbed via membrane diffusion

$C_{WT,O}$: total chemical concentration in the water column above the sediments (g/L)

$C_{WD,S}$: freely dissolved chemical concentration in the sediment associated pore (or interstitial) water (g/L)

k_D: clearance rate constant (kg/kg*d) for chemical uptake via ingestion of food and water

P_i: fraction of the diet consisting of prey item i

$C_{D,i}$: concentration of the chemical (g/kg) in prey item i

k_2: rate constant (d^{-1}) for chemical elimination via the gills and skin

k_E: rate constant (d^{-1}) for chemical elimination via excretion into egested feces

k_M: rate constant (d^{-1}) for metabolic transformation of the chemical

However, the ability of environmental health scientists to adequately parameterize and utilize this model is inherently limited by a lack of data obtained and analyzed across temporal scales. Thus, a steady-state assumption is used to allow for simplification of the model (i.e., the concentration of the contaminants of potential concern (COPCs) in the organism is stable over time).

$$C_B = \{k_1 * (m_O * \phi * C_{WT,O} + k_P * C_{WD,S}) + k_D * \Sigma P_i * C_{D,i}\} / (k_2 + k_E + k_G + k_M)$$

C_B: Chemical concentration in the organism (g/kg ww)

k_1: clearance rate constant (L/Kg*d)

m_O: fraction of respiratory ventilation that involves sediment-associated pore water

ϕ: fraction of total chemical concentration in the overlying water that is freely dissolved and can be absorbed via membrane diffusion

$C_{WT,O}$: total chemical concentration in the water column above the sediments (g/L)

$C_{WD,S}$: freely dissolved chemical concentration in the sediment associated pore (or interstitial) water (g/L)

k_D: clearance rate constant (kg/kg*d) for chemical uptake via ingestion of food and water

P_i: fraction of the diet consisting of prey item i

$C_{D,i}$: concentration of the chemical (g/kg) in prey item i

k_2: rate constant (d-1) for chemical elimination gia the gills and skin

k_E: rate constant (d^{-1}) for chemical elimination via excretion into egested feces

k_M: rate constant (d^{-1}) for metabolic transformation of the chemical

k_G: growth rate constant ($dW_B/(W_B*dt)$)

The base Gobas/Arnot model requires relatively little parameterization before modeled biota concentrations can be obtained, as many of the parameters which underlie the algorithm are preset from known values. Parameters required for the model include the mean water temperature, organic content of water, organic carbon content of sediment, lipid content of tissue, the octanol-water partition coefficient, and total water and sediment concentrations (in ng/L and ng/g dry weight, respectively). A metabolic transformation rate can also be specified, but for the purposes of preliminary modeling, no transformation was assumed because this information is not available for all analytes examined within our study organisms. Data from the current effort were used to characterize the weight and lipid content of the four modeled species. Information about feeding behaviors of fish is also required, and this was obtained through a thorough examination of available resources on Gulf Coast food chains as described above. The base model is somewhat limited in the number of species that can be included, so a simplified food web was posited consisting of phytoplankton and zooplankton as primary producers, clams and blue crab as benthic primary consumers, and black drum as upper-level consumers.

As noted above, an objective for bioaccumulation modeling in this project phase was to determine the extent to which the Gobas/Arnot model could accurately predict tissue concentrations from a relatively small number of parameters. Dioxins, furans, and PCBs are highly hydrophobic contaminants, with log p values ranging from 6.34 (PCB-81) to 8.6 (OCDF). Thus, in preliminary applications of the Gobas/Arnot model to bioaccumulation of dioxins, furans, and PCBs in the SJRWP, dissolved water concentrations were disregarded as an important source of these COPCs in fish tissue. The lack of primary data on water concentrations obtained during the current effort was also a deciding factor in this assumption.

Though other investigators have observed statistically significant relationships between water concentrations and tissue concentrations of several dioxins, furans, and PCB congeners, in blue crab (Integral Consulting), application of available data from the previously conducted TMDL process for the HSC indicates that the contribution of dissolved water concentrations of 2,3,7,8-TCDD and TDCF is <1% of the final wet weight concentration in biota. Tissue concentrations were not modeled in biota for which measured concentrations were not detected at frequencies greater than 67%.

Modeled concentrations of 2,3,7,8-TCDD and 2,3,7,8-TCDF in clams, blue crab, and black drum were consistently higher than those observed (**Table 17**). Observed 2,3,7,8-TCDD concentrations in clams are 20 times lower than predicted by the model. As would be expected from the model, the concentrations of all dioxins, furans, and PCB congeners are predicted to be highest in black drum, the highest food chain member assessed. The predicted 2,3,7,8-TCDD concentrations are 653 fold higher than the mean 2,3,7,8-TCDD concentration observed in black drum, and 334 fold higher than the maximum observed 2,3,7,8-TCDD concentration. For 2,3,7,8-TCDF, predicted concentrations are 1,925 fold greater than observed. These discrepancies are at least partly a function of the disconnect between the conceptual framework of the model (which conceives a theoretical ecosystem in which the upper-level predators always feed on prey species that are impacted by contaminated sediments) and real-world aquatic ecosystems (Gustavson, von Stackelberg et al. 2011), particularly in systems that are tidally influenced like the San Jacinto River. This possibility is seemingly reinforced by the trend of higher discrepancies between modeled and observed concentrations for more mobile species.

Table 17. A comparison of modeled and observed concentrations of polychlorinated dioxins, furans, and biphenyls near the San Jacinto River Waste Pits

	Modeled concentrations (pg/g wet weight)			Observed concentrations (pg/g wet weight)			Modeled:observed ratio		
COPC	Clams	Blue Crab	Black drum	Clams	Blue Crab	Black drum	Clams	Blue Crab	Black drum
Dioxins									
2378-TCDD	563	188	1,470	27.6	3.6	2.3	20	52	653
12378-PeCDD	--	--	--	--	--	--	--	--	--
123478-HxCDD	--	--	--	--	--	--	--	--	--
123678-HxCDD	--	--	--	--	--	--	--	--	--
123789-HxCDD	--	--	--	--	--	--	--	--	--
1234678-HpCDD	--	--	--	--	--	--	--	--	--
OCDD	351	117	885	--	--	--	--	--	--
Furans									
2378-TCDF	2148	716	4870	74.2	7.5	2.5	5	16	1925
12378-PeCDF	74.5	24.8	202	--	--	--	--	--	--
23478-PeCDF	42.6	14.2	116	--	--	--	--	--	--
123478-HxCDF	106	35.5	310	--	--	--	--	--	--
123678-HxCDF	31.9	10.6	93.1	--	--	--	--	--	--
123789-HxCDF	10.6	3.55	31	--	--	--	--	--	--
234678-HxCDF	--	--	--	--	--	--	--	--	--
1234678-HpCDF	31.9	10.6	88.6	--	--	--	--	--	--
1234789-HpCDF	21.3	7.09	59.1	--	--	--	--	--	--
OCDF	42.6	14.2	81.2	--	--	--	--	--	--
PCBs									
PCB 77	128	42.6	307	--	33.2	28.2	--	3.9	11
PCB 81	--	--	--	--	--	--	--	--	--
PCB 105	3240	1080	8420	992.0	279.4	122.6	3.3	3.9	69
PCB 114	181	60.3	499	86.0	19.4	8.6	2.1	3.1	58
PCB 118	8020	2670	22800	2459.2	807.5	368.3	3.3	3.3	62
PCB 123	1170	390	3230	572.5	134.2	74.5	2.0	2.9	43
PCB 126	21.3	7.09	58.8	55.0	3.7	5.3	0.4	1.9	11
PCB 156	1470	489	4280	655.8	94.2	39.0	2.2	5.2	110
PCB 157	255	85.1	744	142.8	17.5	8.9	1.8	4.9	84
PCB 167	394	131	1150	220.2	38.3	9.2	1.8	3.4	125
PCB 169	--	--	--	--	--	--	--	--	--
PCB 189	31.9	10.6	77.8	50.4	4.9	7.3	0.6	6.5	11

This trend (i.e., increasing modeled:observed ratios in increasingly mobile species) is observed in all of the PCB congeners, though the differences from species to species are not as different as relationships between modeled and observed values for TCDD. For PCBs, the model does a reasonably accurate job of forecasting contaminant concentrations in clam tissue, with modeled:observed ratios ranging from 0.4 to 3.3, indicating a reasonably good performance of the model for low-mobility benthic organisms. Ratios are slightly higher in blue crab (1.9-6.5), indicating that the trend of increasing biota mobility being associated with higher ratios continued. The ratios in black drum further illustrate the trend.

However, the larger ratios observed with the PCDD/F congeners was not seen with PCBs. This may be a result of more widespread contamination of PCBs throughout the system, while PCDD/F contamination is mostly associated with the SJRWP site. Previous studies of PCB concentrations in sediment conducted during the TMDL process indicate that the highest concentrations of several PCB congeners are found at Patrick Bayou (Rifai and Palachek 2006) though our study indicates that some congeners are found at higher concentrations at the edge of the SJRWP site.

A limited understanding of COPC bioavailability has also been identified as a conceptual gap in bioaccumulation modeling systems that are currently in use (Imhoff, Clough et al. 2004; Barber 2008). Differing bioavailability among the dioxins, furans, and PCB analytes may impart additional variability to tissue concentrations throughout the food chain, and therefore also to calculated BSAFs.

An implicit assumption of the Gobas/Arnot model is that the structure of the food web and the contaminant concentrations in each member are constant across time (Gobas 1993; Arnot and Gobas 2004; Gustavson, von Stackelberg et al. 2011). A recent study has demonstrated that

trophic levels of organisms in an adjacent and very similar aquatic ecosystem do vary between winter and summer (Akin and Winemiller 2006), indicating changes in producer-consumer/predator-prey relationships; however, there is not currently an approach to account for such seasonal variations, nor was it within the scope of the present study to explore influences of such variability on uncertainties associated with modeling predictions. These slight changes may reflect differences in availability of prey during different seasons, which would likely be a function of changing forage ranges for many aquatic species. Increased inflow of fresh water during the expected rainier seasons in the winter and spring in Texas would affect these food webs by altering the salinity of estuarine habitats, among other parameters, which subsequently influence the distribution of organisms with site-specific reaches of the San Jacinto River. Temperature represents another important factor because blue crab are sometimes driven to behaviors akin to hibernation during periods of very low temperature, removing them as a prominent food source for predators including black drum and redfish. It is not difficult to envision a scenario of seasonal variation in which the food web delineated as described above (as characterized in August 2010) might only occur for 50% of the year or less, and thus the steady-state assumption in the Gobas/Arnot model must be expected to overstate tissue levels in biota, particularly in fish and crustaceans.

The output of Gobas/Arnot modeling as applied here is dependent on the concentration of the COPC in sediment. Though a mean concentration and standard error are entered into the model, the algorithm does not attempt to characterize the variability in biota concentrations that may result from variability in the sediment concentration. The relatively limited number of sediment samples collected and analyzed and the relatively limited geospatial scope of sampling make a sophisticated statistical analysis of COPC concentrations difficult. Geometric means

were important for the current work given the highly skewed distribution of data; within this study site, for example, multiple orders of magnitude differences in COPCs demonstrate high sediment heterogeneity.

It is also possible that overestimation of bioaccumulation in the modeled species may have occurred due to the assumption that biotransformation (and subsequent excretion) is not significant for these COPCs in these species. There are some indications that higher rates of metabolism and excretion could explain the higher ratios between modeled and observed concentrations among the individual congeners (Hu and Bunce 1999; Consulting 2010). Future efforts at understanding bioaccumulation of dioxins, furans, and PCBs near the SJRWP will include a number of strategies for characterizing the importance of metabolism in lower-than-expected concentrations of these COPCs in fish tissue.

The ability of bioaccumulation models to predict tissue concentrations may be limited by the large number of confounding factors that cannot always be accounted for in a chemical- and site-specific fashion (Gustavson et al. 2011; Integral Consulting). These include: residence time of mobile species in the exposure unit, realistic geospatial assessment of contaminant concentrations in sediment, bioavailability, rates of biotransformation and excretion, and stability and adaptation of site food webs in response to seasonal variations. Models based on the Gobas/Arnot paradigm have been shown to predict tissue concentrations within 2-10 fold, as seen in this exercise. However, a comprehensive analysis of model application to Superfund sites has indicated that model accuracy is essentially (Gustavson et al. 2011). The models generally provide point estimates for concentrations that are expected to vary significantly among individuals in the real-world environment, and thus provide the illusion of accuracy. Further, when models are modified to account for this phenomenon, the variability in outputs often

outweighs that seen in measured concentrations (Gustavson et al. 2011). Due to these issues, increasing complexity of the bioaccumulation model beyond the baseline may not be appropriate, nor may it provide a more realistic prediction of tissue concentrations (Gustavson, von Stackelberg et al. 2011).

5.2 Calculation of Site-Specific Biota-Sediment Accumulation Factors in the SJRWP

Using COPC concentrations for the 29 dioxin, furan, and PCB analytes in tissue and sediment as described above, we calculated preliminary BSAFs for clams (*Mercenaria* spp.), blue crab (*Callinectes sapidus*), and black drum (*Pogonias cromis*) caught on or adjacent to the SJRWP site (**Table 18**). BSAFs were calculated for COPCs that were detected in greater than 67% of biota and sediment samples (i.e., 11/15 in sediment and 9/12 in biota). Censored values were substituted using ½ of the MDL, as recommended by Superfund site guidance (Burkhard 2009). Due to low detection frequencies, BSAFs were only calculated for 2,3,7,8-TCDD and 2,3,7,8-TCDF, and for 10 PCB congeners (excluding PCB-81 or PCB-169). Interestingly, analysis of data developed during the TMDL project for dioxins and furans in the HSC determined that statistically significant relationships between sediment concentrations and tissue concentrations were observed only for 2,3,7,8-TCDD and 2,3,7,8-TCDF, even though this project failed to normalize sediment data by TOC or include lipid normalization for tissue values of COPCs (Consulting 2010).

Clams were sampled from the SJRWP site adjacent to sediment samples -8 and -13, and the BSAFs were calculated on the basis of the sediment concentrations available from these respective locations at the SJRWP site. However, as crab and black drum are more mobile, their BSAFs were calculated using a measure of central tendency for sediment COPC concentrations from the site (i.e., geometric mean). This approach is a slight departure from that espoused by

the USEPA, which recommends the collection of paired samples of sediment and biota across the home range of the organisms of interest. However, this was beyond the scope of the current investigation; to the extent possible, future interpretation of our dataset may incorporate data from historical and ongoing monitoring of the HSC complex. The limited scope of the approach employed here may inherently lead to somewhat lower BSAFs than may have been observed elsewhere, and thus it must be noted that the BSAF values contained in this document are not appropriate for setting of remedial goals or for risk assessment.

Table 18. Site-specific preliminary biota sediment accumulation factors for dioxins, furans, and PCBs at the San Jacinto River Waste Pits

	Baylor SJRWP		
COPC	Clams	Crabs	Fish
Dioxins			
2378-TCDD	0.070	0.022	0.044*
12378-PeCDD	--	--	--
123478-HxCDD	--	--	--
123678-HxCDD	--	--	--
123789-HxCDD	--	--	--
1234678-HpCDD	--	--	--
OCDD	0.226*	0.243*	--
Furans			
2378-TCDF	0.049	0.012	0.007*
12378-PeCDF	--	--	--
23478-PeCDF	--	--	--
123478-HxCDF	--	--	--
123678-HxCDF	--	--	--
123789-HxCDF	--	--	--
234678-HxCDF	--	--	--
1234678-HpCDF	--	--	--
1234789-HpCDF	--	--	--
OCDF	--	--	--
PCBs			
PCB 77	--	0.980	1.226
PCB 81	--	--	--
PCB 105	0.495	0.319	0.221
PCB 114	0.849	0.404	0.293
PCB 118	0.510	0.373	0.266
PCB 123	0.843	0.426	0.367
PCB 126	5.584	0.565	1.321
PCB 156	0.854	0.238	0.151
PCB 157	1.044	0.252	0.202
PCB 167	1.028	0.372	0.164*
PCB 169	--	--	--
PCB 189	4.624	0.546	1.301

*Detection frequency were between 67% and 75%.
Note: All other BSAFs were calculated with detection frequencies greater than 92%

For COPCs that are expected to biomagnify, one would expect also that BSAFs will tend to increase as the organism moves up the food chain; that is, BSAFs for clams would be expected to be lower than those observed in blue crab, and the BSAFs for black drum should be higher still. This was not the case in this study. In the present study, magnification was not observed from clams or blue crab to black drum, though both benthic species are known to be important prey (Sutter, Waller et al. 1986). This trend is consistent with the SJRWP site's relatively small area of impact in the HSC, leading to availability of uncontaminated food sources for mobile organisms like blue crab and black drum. Furthermore, dioxins and furans may not biomagnify as readily as PCBs (Wan, Hu et al. 2005).

BSAFs have been developed previously for dioxins and furans in catfish and blue crab in the HSC (Rifai, Palachek et al. 2005). However, it is worth noting that these BSAFs were not based on lipid-normalized tissue concentrations, as the authors observed that "tissue lipid normalizations decrease the strength of the relationship between tissue dioxin concentrations and organic carbon-normalized sediment dioxin concentrations" (p. 262). This exercise, conducted by scientists at the University of Houston and at Parson's Water & Infrastructure, may have encountered uncertainty in this relationship that was related to the variable range of catfish and blue crab. The authors further note that "the lipid measurement method used was imprecise" (p. 262). Regardless, this shift in methodology makes it difficult if not impossible to robustly compare BSAFs from other sources and those calculated in this project (which correctly use lipid-corrected COPC concentrations, as dioxins, furans, and PCBs are strongly hydrophobic). Overall, the approach used by the University of Houston and Parson's Water & Infrastructure would be expected to result in lower than expected BSAFs.

The US Army Corps of Engineers maintains a database of BSAFs derived from published articles and so-called "gray literature" that is judged to be of sufficient scientific quality. BSAFs for clams (*Mercenaria*), blue crab, and black drum from the USACE database are summarized in **Table 19**. All BSAFs for black drum are greater than 1 and are generally higher than calculated BSAF values in the present study (range from 0.007 to 01.226). Though not included in the USACE database, BSAFs for TCDD have been published for blue crab on the order of 0.09 near a paper mill to 0.28 in the Passaic River (Schell, Campbell et al. 1993; Iannuzzi, Butcher et al. 2011). BSAF values for dioxins and furans into upper level predators in Lake Ontario ranged from 0.00065 to 0.095 (USEPA 2004).

A growing number of studies have noted that BSAF models, as applied, are poor predictors of bioaccumulation of hydrophobic substances from sediment to edible fish species (Wong, Capel et al. 2001; Gustavson, von Stackelberg et al. 2011; Iannuzzi, Butcher et al. 2011). The depth and breadth of the data underlying these calculations are critical to the calculation of a robust BSAF, but the variability of bioaccumulated tissue and spatially-distributed sediment concentrations impart uncertainty to any estimate of BSAF. Certainly, variability of BSAFs between sites is difficult to account for, and the data available to evaluate such relationships is limited. It has also been recognized that the influence of different methods for measuring lipid content in tissue can have significant impacts on reported BSAFs (Melwani, Greenfield et al. 2009).

Conder et al. (2012) suggest the development of trophic-level normalized BSAFs through the following equation.

$$BSAF_{TL} = 10^{(\log BSAF)/TL}$$

$BSAF_{TL}$s were developed based on data availability (**Table 20**). As this is a relatively new approach to BSAF methodology, no other values are available for comparison.

Table 19. A comparison of biota sediment accumulation factors for the San Jacinto River Waste Pits and the US Army Corps of Engineers database

	Baylor SJRWP			USACE Database		
COPC	Clams	Crabs	Fish	Clams	Crabs	Fish
Dioxins						
2378-TCDD	0.070	0.022	0.044*	--	--	--
12378-PeCDD	--	--	--	--	--	--
123478-HxCDD	--	--	--	--	--	--
123678-HxCDD	--	--	--	--	--	--
123789-HxCDD	--	--	--	--	--	--
1234678-HpCDD	--	--	--	--	0.003	--
OCDD	0.226*	0.243*	--	--	0.001	--
Furans						
2378-TCDF	0.049	0.012	0.007*	--	1.401	--
12378-PeCDF	--	--	--	--	0.063	1.187
23478-PeCDF	--	--	--	--	0.099	1.930
123478-HxCDF	--	--	--	--	0.009	--
123678-HxCDF	--	--	--	--	0.008	--
123789-HxCDF	--	--	--	--	--	--
234678-HxCDF	--	--	--	--	0.029	--
1234678-HpCDF	--	--	--	--	0.003	2.639
1234789-HpCDF	--	--	--	--	--	--
OCDF	--	--	--	--	0.003	--
PCBs						
PCB 77	--	0.980	1.226	--	1.513	2.377
PCB 81	--	--	--	--	0.348	--
PCB 105	0.495	0.319	0.221	3.720	3.408	7.498
PCB 114	0.849	0.404	0.293	--	2.268	10.568
PCB 118	0.510	0.373	0.266	--	--	--
PCB 123	0.843	0.426	0.367	--	--	--
PCB 126	5.584	0.565	1.321	19.664	2.055	--
PCB 156	0.854	0.238	0.151	--	4.932	9.293
PCB 157	1.044	0.252	0.202	--	3.317	12.244
PCB 167	1.028	0.372	0.164*	--	4.262	13.176
PCB 169	--	--	--	--	--	--
PCB 189	4.624	0.546	1.301	--	2.781	--

*Detection frequency was between 67% and 75%.
Note: All other BSAFs were calculated with detection frequencies greater than 92%

Table 20. Trophic level-normalized biota sediment accumulation factor values for the San Jacinto River Waste Pits

	BSAF-$_{TL}$		
COPC	Clams	Crabs	Fish
Dioxins			
2378-TCDD	0.097	0.081	0.154*
12378-PeCDD	--	--	--
123478-HxCDD	--	--	--
123678-HxCDD	--	--	--
123789-HxCDD	--	--	--
1234678-HpCDD	--	--	--
OCDD	0.271*	0.394*	--
Furans			
2378-TCDF	0.071	0.054	0.051*
12378-PeCDF	--	--	--
23478-PeCDF	--	--	--
123478-HxCDF	--	--	--
123678-HxCDF	--	--	--
123789-HxCDF	--	--	--
234678-HxCDF	--	--	--
1234678-HpCDF	--	--	--
1234789-HpCDF	--	--	--
OCDF	--	--	--
PCBs			
PCB 77	--	0.987	1.130
PCB 81	--	--	--
PCB 105	0.540	0.472	0.405
PCB 114	0.866	0.551	0.479
PCB 118	0.554	0.522	0.453
PCB 123	0.861	0.571	0.549
PCB 126	4.521	0.687	1.181
PCB 156	0.871	0.389	0.323
PCB 157	1.038	0.404	0.384
PCB 167	1.024	0.522	0.339*
PCB 169	--	--	--
PCB 189	3.832	0.672	1.171

*Detection frequency were between 67% and 75%.

Recent workshops and publications have highlighted and recommended the use of trophic magnification factors (TMFs) and biomagnification factors (BMFs) (Gobas, de Wolf et al. 2009; Conder, Gobas et al. 2012). TMFs are calculated from the slope of a regression line between log-concentrations of COPCs vs. the trophic level of organisms in the food chain. BMFs are a ratio of the concentration of COPCs in predators and in their prey (Borga, Kidd et al. 2012). The TMF relies on the assumptions that diet is the primary route of exposure to the COPC (as expected with dioxins, furans, and PCBs) and that biotransformation is relatively similar among species (Borga, Kidd et al. 2012). TMFs are believed to correlate well with $BSAF_{TL}$ values (Conder, Gobas et al. 2012).

The apparent absence of biomagnification for either dioxins, furans, or PCBs in this data set would create TMFs that are less than one, indicating a net trophic dilution of these hydrophobic contaminants; this is in stark contrast to what is understood about bioaccumulation patterns for PCBs from other sites. However, there is evidence to indicate that dioxins and furans do not always biomagnify (Wan, Hu et al. 2005; Consulting 2010). The application of TMFs in this context is further complicated by the low number of available data points for tissue concentrations, and the lack of a predator-prey relationship between the two benthic organisms in the model (clams and blue crab). Previous TMF exercises have demonstrated that the results are strongly dependent on the number of available data points (Borga, Kidd et al. 2012). Furthermore, TMFs may render an inaccurate estimate of bioaccumulation for migratory species (Borga, Kidd et al. 2012). This seems particularly relevant for the SJRWP, where the dioxins and furans contaminant concentrations are elevated in a relatively small area of the feeding range of black drum, in particular. Future research should seek to expand the availability of paired

COPC concentrations and trophic level analysis to provide sufficient foundation of data for the generation of TMFs.

6.0 QSAR Modeling of BSAF Values

Quantitative structure activity relationships (QSARs) are multi-variant statistical correlations between an expressed chemical property and the key geometric or chemical characteristics of a molecular system. QSARs are constructed by analyzing known and/or calculated property information and a set of descriptors that represents the system attribute (Gombar 1998). There are thousands of descriptors that can be used to describe the biological activity of a compound; spatial, electronic, topological, information-content, thermodynamic, conformational, quantum mechanical, and shape descriptors are a select few that were considered in this study (AccelrysSoftwareInc. 2005). The descriptors are analyzed for correlation and uniformity from which appropriate dependent and independent variables are identified and used in the model development.

Building reliable QSARs from experimental data requires constraints in development. For the QSARs develop in this study, there are some important constraints to mention. First, these models are site specific as well as species specific. The model parameters were chosen based on specific characteristics of the species and the site and therefore should not be extended for predictions in other species or at other sites. Comparisons of this data with that at other sites should be done with caution, being mindful of data normalization techniques (i.e.,TOC and %lipid) used in this study that may or may not have been used in other studies. Additionally, uniformity in experimental protocols (i.e., chemical analyses techniques, sampling techniques) should be considered during comparisons of this data with that of other studies. It is important

that the criteria of uniformity in the experimental protocols be strictly adhered to so that the developed database from which to derive the QSAR is valid and consistent for that scenario and for similar chemicals in the same experiment (Debnath 2001).

QSARs are developed after a careful process of selecting experimental parameters and constraints as well as choosing information-rich descriptors. Building a robust, relevant model begins with identifying a training set of chemicals to be analyzed. The individual models are representative of the activity of similar compounds: for example, the family of PCB compounds. A model developed for PAHs would not be valid for estimating properties of PCBs. Next, for each of the molecules to be analyzed, their observed biological data is entered and the appropriate descriptors calculated. For this study, the previously mentioned data for each species was collected, analyzed and incorporated into the models developed (Figures 10, 11, and 12).

Several statistical methods are available for generating a QSAR equation including multiple linear regressions, partial least squares (PLS), simple linear regression, stepwise multiple linear regression, principal components regression (PCR), or genetic function approximations (GFA). This study utilizes the GFA method for model development. Next, validation and analysis of the QSAR equation, by applying techniques to identify outliers and leverage points, is performed. Characterizing the robustness of the QSAR is accomplished using graphical analysis and cross-validation. Finally, the calculated QSAR equation is ready to predict biological activity of compounds similar in structure to the training set of compounds.

The objective of this research was to build QSARs for dioxins, furans, and PCBs that are species and site-specific predictions of BSAFs using COPC data from sediment and tissues that were TOC and lipid normalized, respectively. These models incorporate any available chemical, physical, and biological, data to increase the predicative capabilities of the model. Models are

built individually for each class of compounds and each species (Figures 10, 11, and 12). Each species-specific model will be predictive over the family of compounds (i.e. dioxins, furans, or PCBs). The models were built with no less than 8-12 compounds and include as many compounds (from that family of compounds) as was available. Critical data that was included in these models are: developed BSAFs (from this study), log K_{ow}, f_{OC}, % lipid, and concentration. The model descriptors also begin to shed light on the important parameters in predicting species-specific BSAFs. In this study, the mean of atomic composition of each congener and the covalent atomic radii were dominant independent variables that were in each model derivation.

The Discovery Studio 2.1 (DS 2.1) simulation package (Accelrys Inc., San Diego, CA) was used for QSAR development. Each of the molecules (e.g. each dioxin, furan, and PCB congener) was imported into DS 2.1 as a structure SD file or SMILES string for use in the training set. The energy of each molecule was minimized using a CFF91 open forcefield (Maple, Hwang et al. 1994). The training set of molecules and its corresponding data was then used to create a study table. Descriptors were added to the study table using QSAR+, a module of the DS 2.1 package. The descriptors were calculated using MOPAC7 due to the accuracy of this method as compared to other methods in DS 2.1. The final training set contained descriptors from several categories (i.e. conformational, electronic. spatial, among others.). To develop a QSAR for each of the family of compounds (i.e., dioxins, furans, and PCBs), the corresponding parameter (e.g. BSAF) was set as the dependent variable and the descriptors as the independent variables.

QSARs were created using a genetic function approximation (GFA) algorithm built in the QSAR+ module of DS 2.1 (Rogers and Hopfinger 1994). The GFA algorithm is preferred over other traditional regression methods for two reasons: the GFA algorithm generates better quality

equations and it provides, through study of the evolving models, additional information not available from standard regression analysis (Rogers and Hopfinger 1994; Hansch and Leo 1995; Debnath 2001). The algorithm generated 100 parent equations that included randomly selected descriptors. Regressions were performed on the generated equations and equations were ordered according to their lack-of-fit (LOF) score (Friedman 1991). LOF scores are a measure of the statistical fit and an indicator of overfitting. Crossover operations (repeated 20,000 times) used random pairs of parent equations and randomly mixed descriptors to produce offspring equations. The offspring equations were substituted for the equation with the highest LOF, otherwise it was discarded. The crossover procedures and recombination of equations improved the final population of equations that were ordered according to the individual LOF scores. Statistical analysis was performed on the final 100 equations generated by the GFA for each BSAF. The relevant statistical parameters calculated were the correlation coefficient, R, the coefficient of determination, R^2, and the adjusted value, R^2-adj, the lack-of-fit-score, LOF, and the least squares error, LSE. The significance of the regression equations was tested using the F-test. If the F-value is greater than a standard tabulated value, the equation was considered significant. The significance level for the F-test was set at 0.05.

Validation testing was used to evaluate the generated QSAR for its uncertainties and predictive power using a bootstrap validation test and a randomization test. The bootstrap test calculates the coefficient of determination ($R^2_{bootstrap}$) and the uncertainty associated with it. Repetitive analysis of random samples of the dataset with resampling was also used. Fisher's randomization method was used to test the assumption that adequate random regressions exist for a parameter thus testing the validity of the model and data that was used to generate it. Each BSAF value predicted by the QSAR was randomly reassigned 19 times (0.05 significance level)

and statistical parameters recalculated each time. These parameters were then compared to the non-random QSAR for each congener. There are four additional statistics along with the previously mentioned bootstrap coefficient of determination ($R^2_{bootstrap}$) reported from the validation testing. These include the sum of squared deviations of the dependent variable values from their mean (SD), the predicted sum of squares (PRESS), the cross-validated R^2, and the outliers (Appendix F).

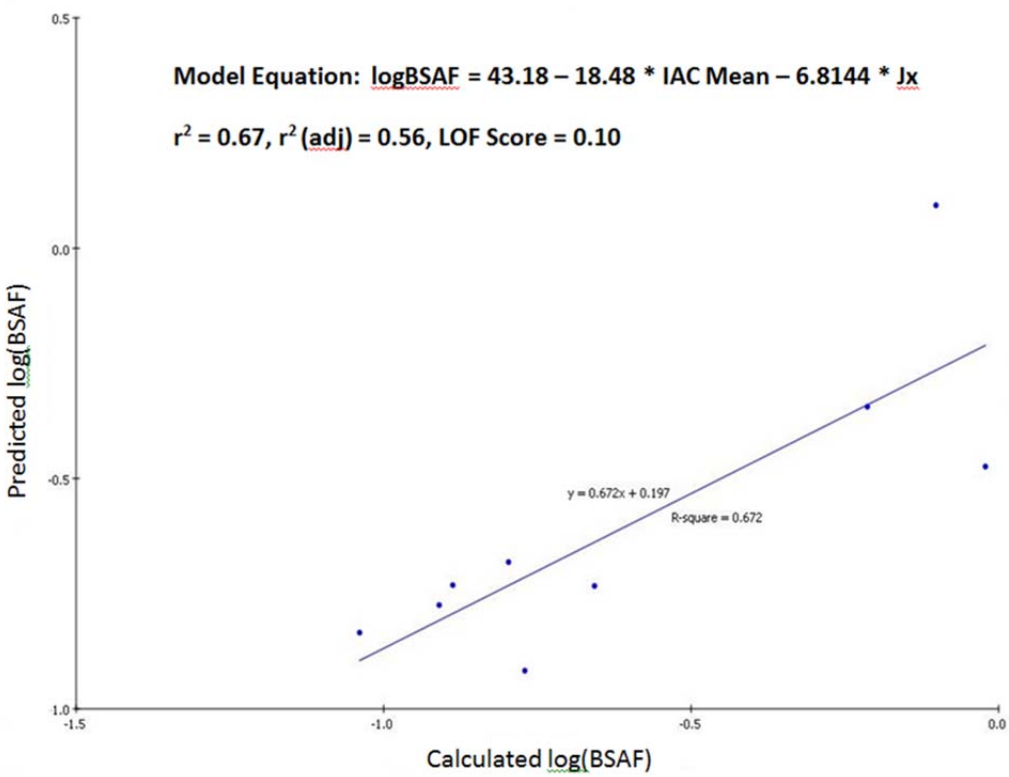

Figure 10. Quantitative structure activity relationship model of biota-sediment accumulation factors for PCBs in black drum at the San Jacinto River Waste Pits Site.

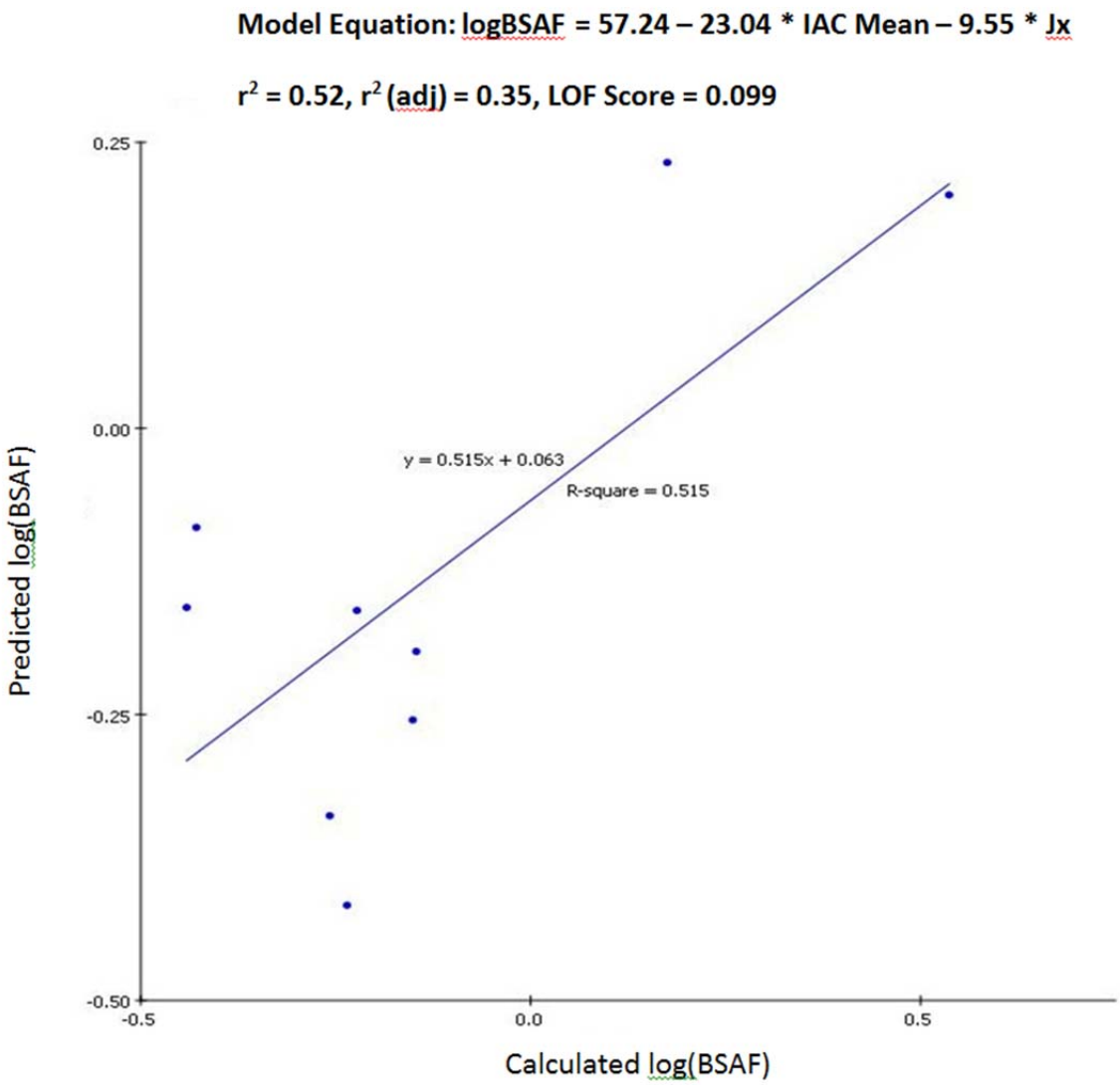

Figure 11. Quantitative structure activity relationship model of biota-sediment accumulation factors for PCBs in clams at the San Jacinto River Waste Pits Site.

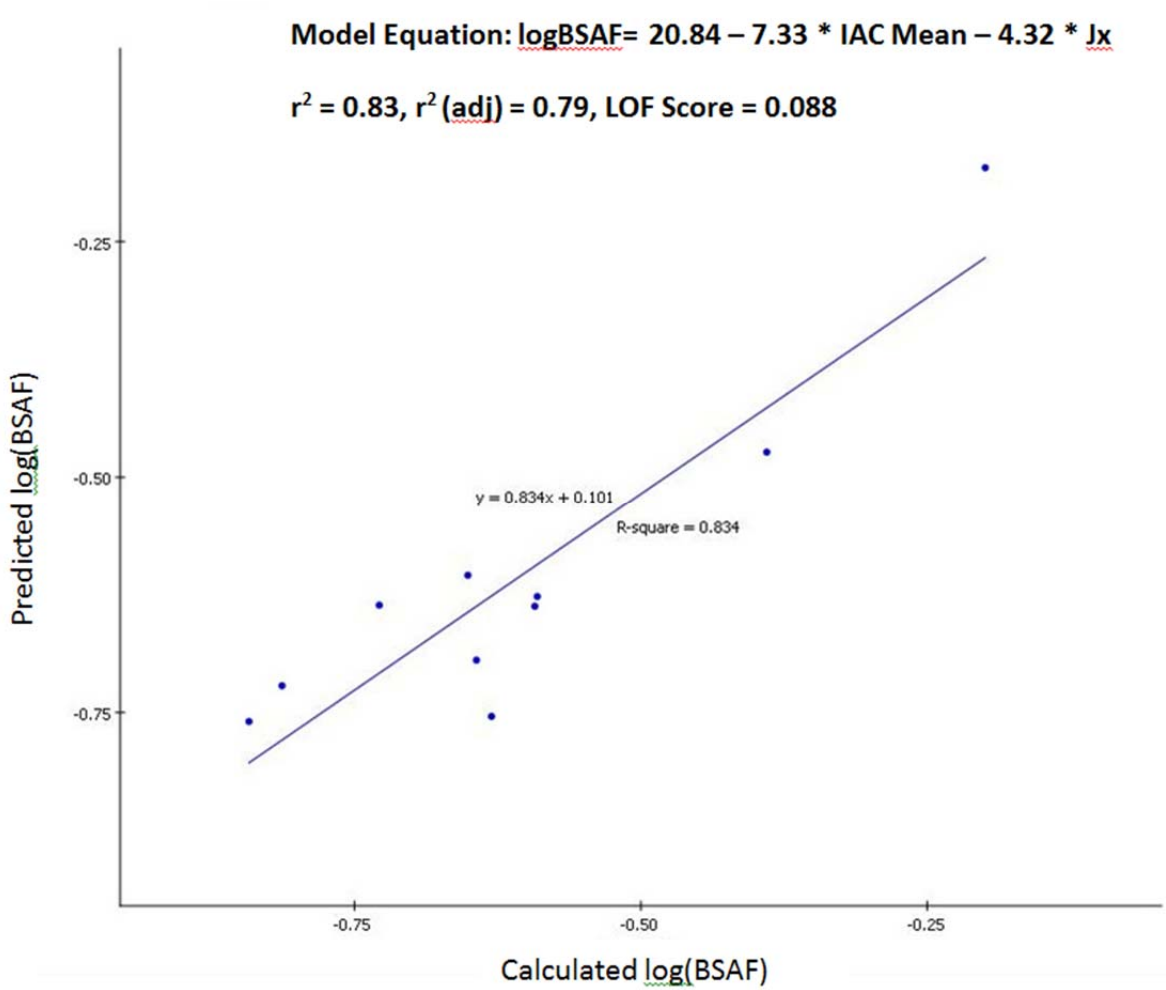

Figure 12. Quantitative structure activity relationship model of biota-sediment accumulation factors for PCBs in blue crab at the San Jacinto River Waste Pits Site.

7.0 Conclusions

The objectives of this research project delve into a dynamic field: understanding the bioaccumulation of environmental contaminants into ecological receptors. Since initiation of the project, an international workshop on bioaccumulation has produced several peer-reviewed publications that modify the standard operating procedures for understanding the movement of hydrophobic chemicals at Superfund sites such as the SJRWP. The outcomes of this project

incorporate techniques that have been long used while using updated processes, and future efforts using the data generated in this project will further the development of the field of bioaccumulation. Below, we briefly summarize research findings from this project.

Development and application of a novel extraction method for dioxins, furans, and PCBs

This enhanced pressurized liquid extraction technique eliminated an additional post-extraction cleanup(s) by combining all cleanup adsorbents with PLE. ePLE expands on historical PLE techniques by layering known sample preparative adsorbent(s) (silica gel, florisil, alumina, and carbopack/celite) beneath the sample homogenates within the ASE cell. During ePLE, analytes and interferences were extracted from the sample and subsequently partition between the pressurized extraction solvent(s) and the adsorbents within a commercially available high-capacity 100 mL ASE cell at high pressure and high temperature. Significant analytical improvements were achieved by combining historical extraction solvents and cleanup adsorbents based on EPA 1613 with PLE, namely a reduction in the intrinsic costs associated with the sample preparation protocol: time (~95%), solvent (65%), labor, laboratory space and training, and potential loss of analytes. As a result of these analytical improvements, laboratory capacity and preparedness was also increased for the analysis of dioxins, furans, and PCBs in fish tissue.

Analysis of dioxin, furan, and PCB congeners at the San Jacinto River Waste Pits site revealed significant contamination with a highly heterogeneous distribution.

The sediment concentrations of dioxins, furans, and PCB congeners were highly variable within the site. The highest concentration of 2,3,7,8-TCDD reported (~22,000 pg/g dry weight) during the TMDL process was at sampling site 15, within the SJRWP itself. The TOC of this

sample was on the order of 10.5%, indicating that sampling had most likely captured paper process sludge (as samples from nearby areas were closer to 1% TOC). Sediment obtained during a sampling expedition in August 2010 contained 2,3,7,8-TCDD concentrations between 3.7 to 16,800 pg/g dry weight, spanning four orders of magnitude. The samples containing the highest concentrations also had higher levels of TOC (6-13%).

The Patrick Bayou Superfund site has traditionally been identified as the major site of PCB contamination in the HSC complex. Our data demonstrate that the concentration of some PCB congeners (PCB-105, 126) is higher in selected samples at the SJRWP than samples taken near Patrick Bayou.

The variability in concentrations across a known site of contamination is intriguing. It may reflect the result of depositional processes from tidal action and natural flow of the San Jacinto River from Lake Houston. The pattern of contamination, however, does complicate the conceptual framework that underlies bioaccumulation modeling for the site.

Trophic level analysis of eighteen species collected at or near the San Jacinto River Waste Pits site indicates that the food web functioning at the site on August 2010 is very similar to an ecosystem at another uncontaminated estuary along the Texas coast.

Top level predators in the vicinity of the SJRWP were determined to be redfish, flounder, speckled trout, and catfish (TL > 3), followed by black drum, ladyfish, and pinfish. The findings of our trophic level analysis were not significantly different with findings from a previous study conducted in Matagorda Bay. The scope of this study did not permit normalization of trophic level by the length or weight of individual organisms or examining gut contents; however, most

organisms captured and analyzed were within one or two size classes per TCEQ guidelines. This area represents an important topic for future research at contaminated sites.

Patterns of bioaccumulation of dioxins, furans, and PCBs at the San Jacinto River Waste Pits site did not indicate significant biomagnification in the species studied.

The outputs of bioaccumulation modeling using the traditional Gobas/Arnot framework suggested that biomagnification of dioxins, furans, and PCBs would be expected in the food web that surrounds the SJRWP. This is consistent with numerous studies which have demonstrated that dioxins, furans, and PCBs tend to biomagnify in aquatic food webs. However, this phenomenon was not consistently observed in the food web of the SJRWP. This is perhaps less surprising for 2,3,7,8-TCDD and 2,3,7,8-TCDF, as research indicates that higher-trophic-level organisms may have more effective biotransformation and excretion rates than benthic organisms. Previous studies have documented trophic dilution of dioxins and furans in marine harbor environments, though the causes are unclear. Also, the geographic extent of contamination by dioxins and furans in the HSC complex may be relatively small, and thus the more mobile organisms (i.e., blue crab and black drum) have opportunity to feed in areas that do not have high concentrations of dioxins and furans in either sediment or lower level prey species. The SJRWP is also in an estuarine system which undergoes season- and event-dependent changes in salinity and temperature which can alter the food web markedly. Gobas/Arnot-based bioaccumulation modeling was reasonably accurate for PCBs in clams (i.e., ratios of modeled to observed concentrations ranged from 0.4-5), which were the least mobile organisms examined.

Development of preliminary QSAR models for BSAFs at the SJRWP site may provide tools which deepen understanding of bioaccumulation of chlorinated organic compounds in aquatic environments.

Preliminary QSAR models were developed for predicting BSAF for clams, crab and fish at the SJRWP. The QSAR model developed of BSAF for dl-PCBs in clams has the least predictability of all the models developed. Due to the observed diversity in sediment concentrations determined in this study, these results are not surprising. The highest predictive capability of any of the models developed was BSAF for dl-PCB in crab. The low LOF score indicates that the model is statistically fit while not being overfit. The developed model of BSAF for dl-PCBs in fish also showed reasonable predictive capabilities and a low LOF score. Improving the predictive capabilities of these models, for this particular site, should include an extended sampling campaign (sediment and organisms) that would allow for additional data to be included in the training set of congeners. Extending these models to other sites for comparison purposes would benefit from additional sampling, and normalization of sampling and analysis techniques for making this type of predictions. QSAR models have the capability of providing a novel prediction method for BSAF. Careful analysis of the data inputs and the extent to which the final model can be applied is critical in their ultimate use.

Calculated site-specific preliminary biota sediment accumulation factors for dioxins, furans, and **PCBS the San Jacinto River Waste Pits were lower than expected.**

A comparison of site-specific BSAF values for PCBs from the SJRWP to the BSAF database maintained by the US Army Corps of Engineers reveals a marked discrepancy between the values. BSAF values >1 were observed in clam tissue from the SJRWP site for PCBs -126, -

156, -167, and -189. All BSAF values for PCBs in blue crab were <1, and only PCBs -77, -126, and -189 are >1 in black drum. However, values from the USACE database were all >1, with the sole exception of PCB-81 in blue crab (0.348). Previously calculated BSAFs were somewhat more scarce; however, available data indicates that BSAFs for 2,3,7,8-TCDD were in the lower end of values calculated in other areas. These departures are most likely attributable to the sharper focus of our study on smaller area of a contaminated site; most studies used to calculate BSAFs include sediment in uncontaminated areas in their calculations, though this was outside the scope of the present research project. Thus, the preliminary BSAF values calculated in this project should not be used for risk assessment or remedial decision-making.

The results of the present study offer a number of avenues for future research to further clarify the occurrence and bioaccumulation of dioxins, furans, and PCBs in the HSC complex and other sites with similar patterns of contamination.

Alternative parameterization of Gobas/Arnot bioaccumulation models: This well-established model of bioaccumulation has been modified in a number of ways to allow for additional complexity of the model. While such efforts do not always provide better model accuracy, they do take into account the variability in the available parameters of the site. Alternative parameterization of the model, and use of probabilistic approaches (i.e., parameter distributions) may be explored for this or other sites, with the goal of developing an understanding of critical determinants of model performance (e.g., variability in sediment and biota contaminant concentrations, residence time of upper trophic level organisms, food web stability, seasonal and climatic fluctuations, etc.).

Additional sampling and analysis to permit robust estimates of trophic magnification factors for dioxins and furans congeners: Experts in the field have recently identified that trophic magnification factors (TMFs) provide a more robust understanding of bioaccumulation than traditional biota-sediment accumulation factors. This development occurred during the execution of the present study. The scope of the effort did not allow for the development of TMFs for this study; however, future endeavors may attempt to characterize dioxins, furans, and PCB concentrations in the tissues of existing biota samples throughout the food web to allow for TMF derivation.

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