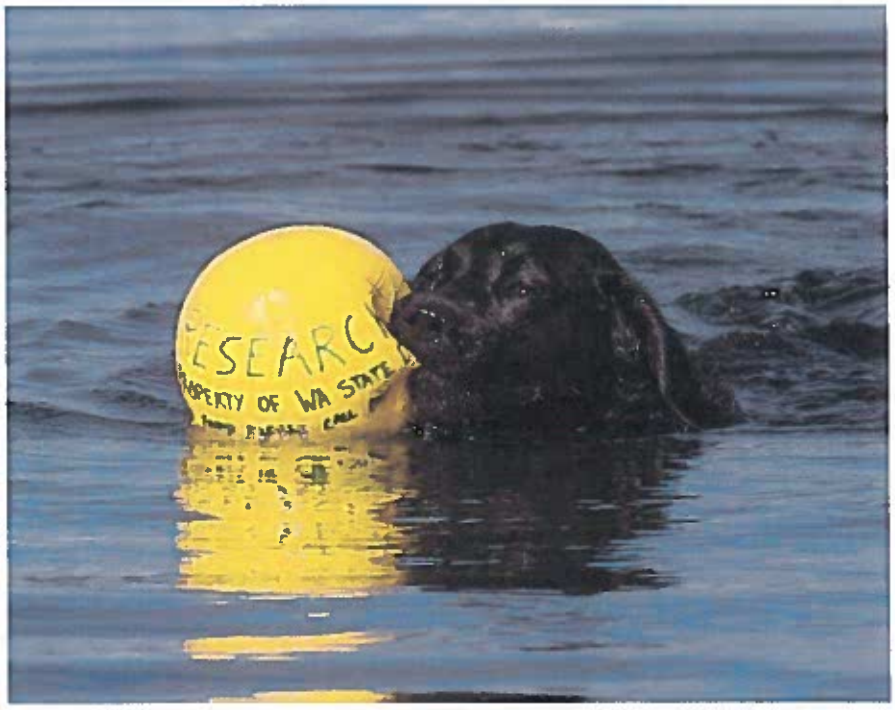
NATURAL RESOURCES

Technical Report

Chemicals of Emerging Concern in the Nearshore: POCIS at Shellfish Resource Sites

June 2017



WASHINGTON STATE DEPT OF
NATURAL RESOURCES
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Chemicals of Emerging Concern in the Nearshore: POCIS at Shellfish Resource Sites

June 2017

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1 Introduction

Recent study results show increased levels of pharmaceuticals being discharged to Washington's marine waters at potentially destructively high rates (Ankley et al., 2007, Kolpin et al., 2002). Among these human sourced chemicals of emerging concerns (CECs) are prescribed pharmaceuticals that have been shown in laboratory studies to cause sublethal and lethal effects to commercially valuable nearshore species (Bringolf et al., 2010, Meredith-Williams et al., 2012, Zhou et al. 2009). Controlled laboratory experiments exposing organisms to known concentrations of some of these CECs, particularly antidepressants and anti-seizure medications containing serotonin, epinephrine and dopamine regulators, demonstrate broad effects to benthic species, including shellfish (Silva et al. 2015, Bringolf et al. 2010, Hird et al. 2016, Fong and Ford 2014). What remains largely uninvestigated is the magnitude, spatial extent, and timing of *in situ* exposure of these CECs to shellfish species of interest.

This project was designed to sample from the water column near the sediment bed at prohibited or restricted shellfish harvest areas, and wildstock geoduck areas, using passive sampling devices that collect CECs of interest over time on a specially treated membrane. The membrane was then collected and analyzed for CECs in a laboratory. These CECs do not occur naturally. Their presence indicates an anthropogenic source. It is likely that high fecal coliform counts that coincide with high concentrations of these CECs are also from human sources (Kostich 2014, Anderson 2012, Dutch 2011). This could provide critical scientific evidence necessary in identifying wastewater outfalls that input effluent that is deleterious to marine aquatic life.

The Washington State Department of Natural Resources Aquatic Assessment and Monitoring Team (AAMT) selected sampling locations in the Puget Sound, Hood Canal and Straits of Juan de Fuca, and deployed sampling devices to ascertain the potential exposure shellfish resource sites have to these CECs. The University of Washington Tacoma at the Center for Marine Waters Laboratories partnered with AAMT and completed the sample processing component of the research. The following technical document outlines the research completed within this partnership; summarizes the results; and includes some discussion points related to shellfish resource sites on state owned aquatic lands.

2 Field Sampling

2.1 Passive Polar Organic Chemical Integrative Samplers (POCIS)

POCIS and deployment equipment were needed for passive sampling of CECs in various locations of nearshore waters in Puget Sound. The passive sampler has no mechanical or moving parts and samples chemicals in the water column from the dissolved phase, mimicking the respiratory exposure of aquatic organisms. The sampler has membranes that allow water and dissolved chemicals to pass through to the sorbent, where chemicals are then trapped and later extracted in a laboratory. For the purposes of this research design, three components were used to make one complete POCIS: 1) POCIS-HLB 2) POCIS holders 3) Canisters. A description of each of these is included below.

1) **POCIS-HLB:** Six (6) POCIS-HLB discs were used at each sample location. The POCIS-HLB consists of a solid material (sorbent) contained between two microporous polyethersulfone membranes. The membranes allow water and dissolved chemicals to pass through to the sorbent where chemicals are trapped. Larger materials such as sediment and particulate matter are excluded. The membrane typically resists biofouling. The POCIS disc is composed of two sheets of microporous (0.1µm pore size) polyethersulfone membrane encasing a solid phase sorbent (Oasis HLB). The Oasis HLB is a universal solid-phase extraction sorbent widely used for sampling a large range of hydrophilic to lipophilic organic chemicals from water. The surface area of each sheet measured 47.5cm each, which retains the CECs of interest. The sheets were enclosed with an upper and lower stainless steel support ring used to seal the POCIS microporous membranes and prevent any loss of the sorbent. (Figure 1)

2) **POCIS Holders:** Two (2) POCIS holders were used at each sample location to hold six (6) POCIS-HLB discs. The POCIS holders are made of stainless steel and have three locations for attachment of three (3) POCIS-HLB discs as the specifications describe above. The holders fit inside a pre-made protective canister that can house two (2) POCIS holders for water deployment by stacking one on top of the other (Figure 1).

3) **Canisters for Deployment:** One (1) canister was used at each sample location. The POCIS canisters are constructed of stainless steel and have the ability to hold (2) POCIS holders for a total of six (6) POCIS-HLB discs as the specifications describe above. The size of the canister was 30cm high by 16cm wide (Figure 2).

Figure 1(left). POCIS-HLB discs are the white material encased in the stainless steel POCIS holders. **Figure 2** (right). Canisters for deployment protect the POCIS holders and discs while at the sample location.

2.2 Site Selection

AAMT selected fourteen (14) locations for field sampling: 11 in Puget Sound, 1 in Hood Canal, and 2 in the Straits of Juan de Fuca (Figure 3). Sites were selected using criteria considered important in the management of prohibited and restricted shellfish harvest areas, and wildstock geoduck areas. The following geospatial data was gathered from multiple agencies and evaluated for site selection of POCIS sampling, especially when a confluence of conditions was surrounding a shellfish resource site.

***DOH 2020 Growing Area Goals*:** The Washington State Department of Health (DOH) has selected shellfish tracts it would like to restore and upgrade in status so that by 2020 the tract can be open for shellfish harvest without limitations.

Total Organic Carbon (TOC): The Washington State Department of Ecology (DOE) manages the EIM Database (Environmental Information Management). This database contains data on sediment sampling. High levels of TOC can imply lower benthic diversity and lower benthic health (Hyland 2005). Areas within the EIM database where TOC has been reported as 75%-100% was evaluated for site selection.

Sediment Quality Standard (SQS) Exceedances: SQS exceedances are available from the DOE. Areas where SQS exceedances have been reported were evaluated for site selection as exceedances can be indicative of an area with high anthropogenic inputs.

303(d) Listings for Dissolved Oxygen (DO): Areas with low levels of DO indicate poor water quality which can suggest lower benthic diversity and lower benthic health. Areas listed on the state 303(d) list for impaired waters for DO were evaluated for site selection.

Bathymetry: Sampling locations needed to be no deeper than approximately -16feet. This bathymetric level is consistent with where wildstock geoduck areas begin and also insured the POCIS stay submerged at extreme low tides.

Outfalls: Certain outfall locations were considered for site selection. WDNR manages state owned aquatic land that receive inputs from outfalls. Those inputs can include CECs of interest to this research.

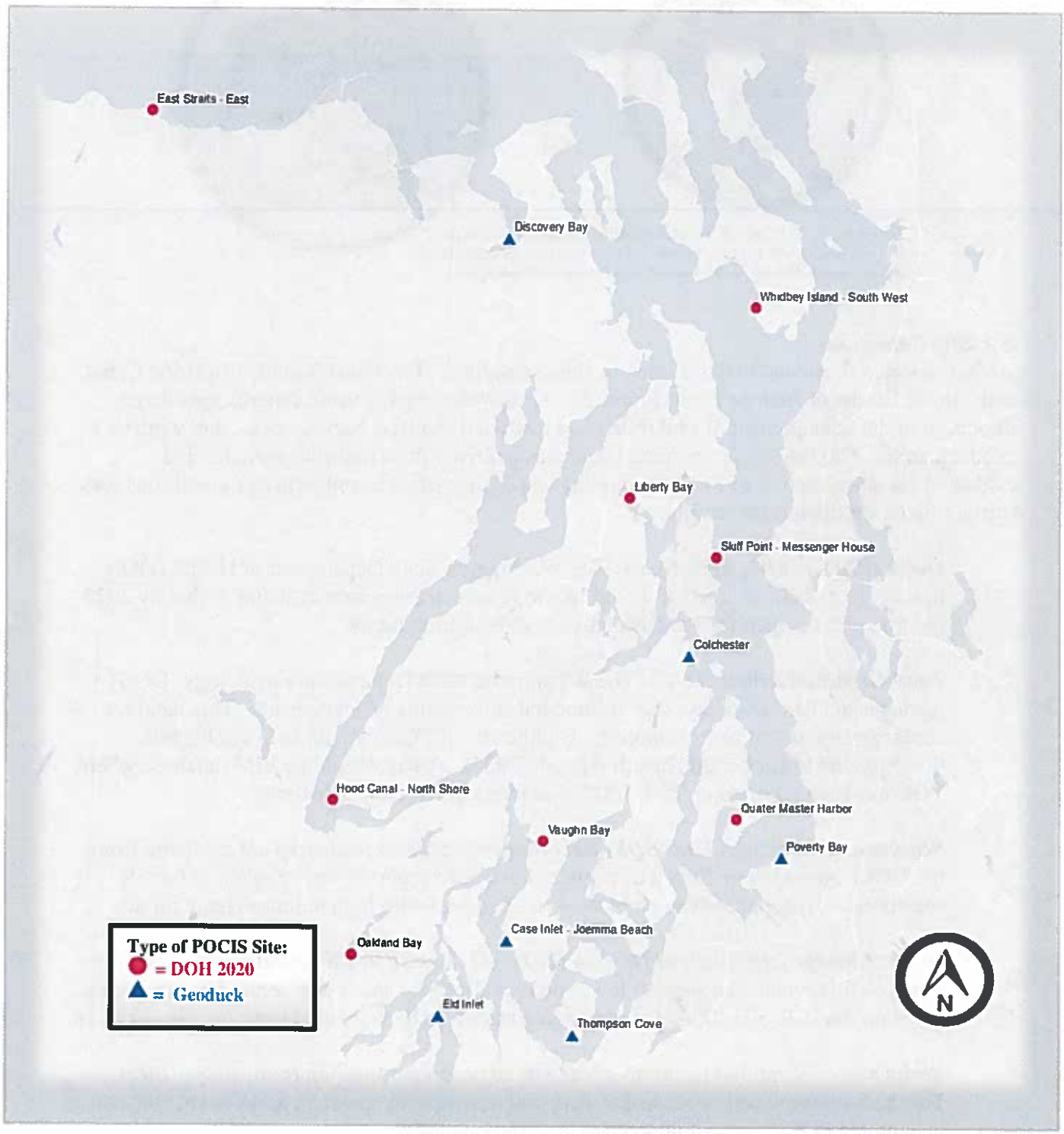

Figure 3. Using geospatial data 14 sites were selected for POCIS deployment. Sampling occurred either at a DOH 2020 Restoration Goal area or at a Wildstock Geoduck location.

2.3 Deployment

The deployment of fourteen (14) POCISs was completed by AAMT staff May-July 2016. In order for the POCIS to be deployed in the nearshore to sample for the desired list of CECs (Table 2), the POCIS needed to be attached to an anchor buoy system for a deployment period of 21- 30 days (Alvarez, USGS, per comm. 2016). The anchor buoy system was constructed of simple parts. The anchor included a cinder block attached to a 25-30ft nylon rope (anchor line), with a buoy attached to the end of the anchor line so the sample location could be identified easily at the water surface (Figure 4). For each sample location, one (1) POCIS deployment canister was used to protect six (6) POCIS-HLB discs placed on two (2) POCIS holders. The canister was attached to the anchor line using carabineers to sample one (1) meter above the sea floor.

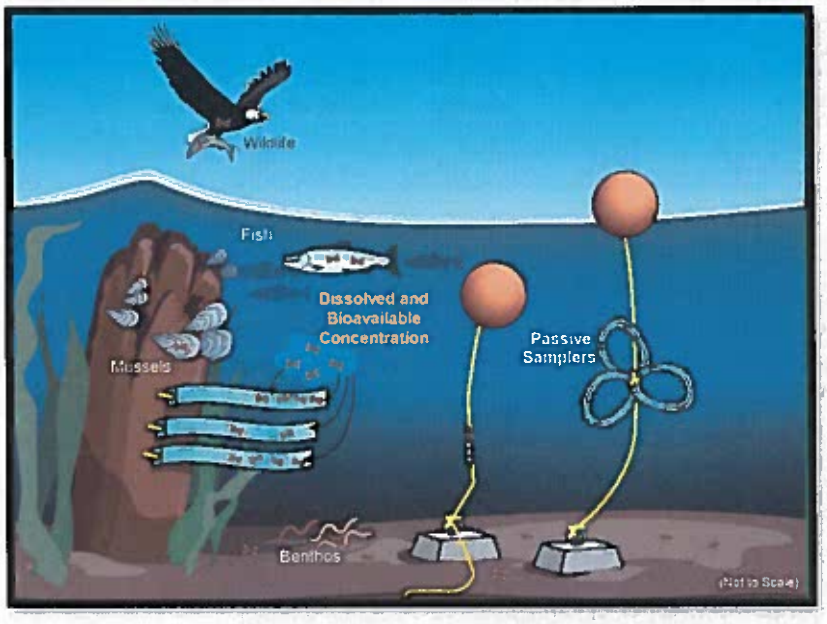

Figure 4. Passive sampling devices can be constructed in multiple ways. This image shows a buoy anchor system similar to what was used by AAMT for this research. AMMT created a version of this system with a cinder block-like anchor and a POCIS attached to the anchor line one (1) meter above the seafloor. Image provided by EPA (EPA 2012).

Deployment locations were identified in the office using the geospatial data described for site selection (section 2.2). Aerial orthophotos were used to determine land markers to help navigate the boat to the POCIS deployment sites. A motorized boat was used to transit to each sample location and the boat sonar was used to determine bathymetric depth of -16ft to -14ft. Deployment occurred at low tide to assure the POCIS was always submerged during the deployment period. Once the POCIS was deployed, GPS coordinates were taken (Table 1). Deployment occurred in three (3) phases due to tidal access and the geographic range of the sites.

POCIS samplers were received in sealed, nitrogen-filled containers and remained sealed until arrival on site for deployment (Environmental Sampling Technologies, St. Joseph, MO). Field staff wore nitrile powder free latex gloves while handling all POCIS parts and limited air exposure of the POCIS discs once opening the sealed container. POCIS discs can absorb air contaminants so two (2) field blanks were used during deployment, one in the month of June and one in the month of August. These field blanks were opened on the boat during the same amount

of time it took field staff to put together one complete POCIS and deploy it into the water column.

Table 1. Sampling locations, deployment date, and retrieval date for field investigation listed in order by date of deployment.

Site Name	General Region	Longitude	Latitude	Deployment Date	Retrieval Date	TotalDays Deployed
Eld Inlet	Outer Eld Inlet, Cooper Point	-122.9308666	47.14236102	5/23/2016	6/13/2016	21
Vaughn Bay	Case Inlet North East	-122.7823975	47.33953701	5/23/2016	6/13/2016	21
Case Inlet - Joemma Beach	Case Inlet South	-122.820741	47.22786788	5/23/2016	6/13/2016	21
Thompson Cove	Anderson Island	-122.7107972	47.12663501	5/23/2016	6/13/2016	21
Quarter Master Harbor	Dockton	-122.4525628	47.3732002	6/15/2016	7/13/2016	28
Poverty Bay	Dumas Bay	-122.3862404	47.33048935	6/15/2016	7/13/2016	28
Colchester	Manchester	-122.5410237	47.55277134	6/15/2016	7/13/2016	28
Skiff Point	Bainbridge Island East Side	-122.4963578	47.66086527	6/15/2016	7/13/2016	28
Liberty Bay	Poulsbo, Liberty Bay Northern End	-122.6415493	47.72621784	6/15/2016	7/13/2016	28
East Straits - East	Ediz Hook, Port Angeles	-123.4553703	48.1381025	7/14/2016	8/4/2016	21
Discovery Bay	Discovery Bay Southern End	-122.8631659	48.00576443	7/14/2016	8/4/2016	21
Whidbey Island - South West	Useless Bay	-122.4475587	47.94030174	7/14/2016	8/4/2016	21
Hood Canal - North Shore	Hood Canal South, Ayres Point	-123.1132784	47.38040523	7/14/2016	8/4/2016	21
Oakland Bay	Shelton	-123.0712339	47.20749703	7/19/2016	8/15/2016	27

2.4 Retrieval

POCIS were at each sample location for a deployment period of 21- 30 days (Table 1). Retrieval occurred on the same vessel that was used for deployment. Samples were collected from each location by pulling the buoy anchor system up from the seafloor and onto the boat. Power free nitrile gloves were used when handling all POCIS parts. Once the POCIS holder was removed from the container, the POCIS-HLB discs and holders were rinsed with deionized water (DI). The metal portion of the discs and holders were evaluated for biofouling and cleaned if deemed necessary. It was important to not alter the disc portion of the POCIS. A gloved finger or anything soft was used to clean metal parts such as a paper towel, q-tips and tooth brushes. Once rinsed, the POCIS discs and holders were covered with foil; placed in a labeled plastic zip lock bag; and placed in an ice cooler. Once done in the field, the samples were stored in a freezer and then transported on ice in a cooler to the laboratory for processing.

3 Laboratory

3.1 Processing POCIS Samples

Sample extraction, processing, and analysis were performed at the University of Washington Tacoma laboratories at the Center for Urban Waters. A set of thirteen (13) CEC compounds were selected for laboratory extraction from the POCIS discs (Table 2) consisting primarily of a suite of CECs and selected metabolites. POCIS sample materials were placed in individual chromatography columns containing a glass fiber layer and approximately 3 g anhydrous sodium sulfate. 10 µL of labeled surrogate mixture was added to the sorbent and then eluted with 50mL methanol. Methanol was reduced to 1 mL under nitrogen gas at 40°C, and transferred to clean autosampler vial, and held at 4°C until analysis (Alvarez 2010, Carlson et al. 2013, Li et al. 2010).

Two analytical platforms were used during analysis: 1) an Agilent 6530 quadrupole time of flight (QToF) mass spectrometer (MS) coupled with an Agilent 1260 liquid chromatograph (LC), and 2) an Agilent 6460 triple quadrupole (QqQ) dual mass spectrometer coupled with an Agilent 1260 LC system. The QToF allows for the non-targeted analysis of samples to investigate the presence of a wide range of compounds without preselecting compounds of interest. The QqQ is utilized during targeted and quantitative investigation of a known set of analytes, often to a higher degree of sensitivity than can be obtained with the QToF. All samples were analyzed with both systems during this work.

Laboratory SOPs describing sample extraction and analysis via LC-QToF-MS/MS and LC-QqQ-MS/MS are included in the appendix.

Table 2. List of CEC compounds of interest at all sample locations

	CAS	Formula	Notes
Citalopram	59729-33-8	C20H21FN2O	Trade names: Celexa, Cipramil
Escitalopram	128196-01-0	C20H21FN2O	S-enantiomer of citalopram
N-desmethylcetalopram	62498-67-3	C19H19FN2O	Active metabolite of citalopram
R-(-)-Fluoxetine	54910-89-3	C17H18F3NO	Trade names: Prozac, Sarafem
Norfluoxetine	126924-38-7	C16H16F3NO	Active metabolite of fluoxetine
Venlafaxine	93413-69-5	C17H27NO2	Trade names: Effexor, Lanvexin, Viepax, Trevilor
(+) O-desmethylvenlafaxine (desvenlafaxine)	93413-62-8	C16H25NO2	Active metabolite of venlafaxine
Sertraline	79617-96-2	C17H17Cl2N	Trade name: Zoloft Metabolizes to norsertraline via N-demethylation
Norsertraline	87857-41-8	C16H15Cl2N	Major metabolite of sertraline
Paroxetine	61869-08-7	C19H20FNO3	Trade name: Paxil, Pexeva, Brisdelle
Duloxetine	116539-59-4	C18H19NOS	Trade name: Cymbalta
Bupropion	34911-55-2	C13H18ClNO	Trade names: Wellbutrin, Zyban, Aplenzin, Buproban, and Budeprion
(+) Hydroxybupropion	357399-43-0	C13H18ClNO2	Active metabolite of bupropion
(+) Bupropion-D9			Isotopically labeled bupropion
Carbamazepine	298-46-4	C15H12N2O	Trade name: Tegretol Anti-seizure – not SSRI

4 Results

4.1 QA/QC

All samples were initially analyzed with the LC-QToF-MS/MS system and compared against the analytical standards of a selection of CECs for positive identification (Table 2). Initial QA/QC runs were performed to verify the efficacy of the method. A set of laboratory blanks were processed and analyzed according to the laboratory SOP to ensure there was no source of contamination. No analytes were detected in any of the blanks analyzed. In addition, a set of field blanks were analyzed to ensure there was no contamination during sample handling in the field. No analytes were detected in any of the field blanks.

A set of spike-and-recovery samples were prepared to 1) verify the ability to positively identify each of the analytes of interest, and 2) estimate the efficiency of the sample processing procedures. A fraction of contaminant loss is expected during each processing step and it is important to understand the magnitude of those losses. The spike-and-recovery experiments were performed by first, adding a known mass of the analytes to a set of POCIS samplers in the laboratory, processing and analyzing the samples, and then comparing the measured value to that initially added. All analytes were detected and confirmed using both MS-only, and MS/MS analysis, verifying that the method and instrumentation settings are appropriate to measure the CECs of interest. Results indicate that most compounds had recoveries ranging from 40-60% (Table 3). With the exception of bupropion, which had an average recovery of 3%, other values were within those reported in EPA 1694 (Pharmaceuticals and Personal Care Products in Water, Soil, Sediment, and Biosolids by HPLC/MS/MS). Results are shown in Table 3.

	Bupropion	Hydroxybupropion	Carbamazepine	Duloxetine	Citalopram	N-Desmethyl citalopram	Escitalopram	Fluoxetine	Norfluoxetine	Paroxetine	Sertraline	Venlafaxine	Desvenlafaxine
Average Percent Recovery	3%	37%	37%	44%	33%	41%	55%	55%	133%	40%	61%	25%	35%

Table 3. Summary of estimated recovery values for each of the CECs evaluated in this project. Percent recovery estimates the losses during the laboratory sample extraction and processing methods.

4.2 Key Laboratory Findings

All field samples were collected and analyzed via LC-QToF-MS/MS; no CECs were detected during this evaluation, likely due to the instrument sensitivity. In order to investigate the possible occurrence of the CECs at lower levels, a semi-quantitative method was developed for the LC-QqQ-MS/MS system (Parikh et al. 2014). Results are shown in Table 4.

The data support the notion that shellfish in the Puget Sound are exposed to a variety of anthropogenic compounds, including a suite of CECs. Carbamazepine (detection frequency = 97%) and at least one other CEC was detected at nearly all locations. There was a wide range of

detection frequency of the CECs. Venlafaxine, desvenalfaxine (a metabolite of venlafaxine), and desmethyl citalopram (a metabolite of citalopram/escitalopram) were detected in roughly 75% of the samples analyzed. Bupropion, paroxetine, and fluoxetine were not detected at any location. The instrument response to bupropion was low resulting in a relatively high detection limit, approximately 10-100x higher than the other compounds. In addition, the spike-and-recovery results in Table 3 indicates that bupropion is poorly recovered during sample processing. As such it is not possible to determine whether or not it was present at level similar to the other compounds. Much higher concentrations would be necessary before it would be detected.

The results are only semi-quantitative but suggest that CECs are generally present at levels well below those reported to cause environmental harm (Bringolf et al. 2010, Fong and Ford 2014, Hird et al. 2016, Silva et al. 2015)

4.3 Semi-Quantitative Results

POCIS have been utilized in similar monitoring campaigns elsewhere (Alvarez et al. 2014, Li et al. 2010, Tertuliani 2008) and are suitable for the detection of compounds such as CECs (Irv Schultz, personal communication). POCIS samplers sorb contaminants over a period of time resulting in an integrated measure. In order to determine environmental concentrations, however, the analyte uptake rate (the net rate at which a given compound sorbs onto the sampler matrix) must be known. Uptake rates have been published for a wide variety of compounds (Bartelt-Hunt et al. 2011, Miller et al. 2016) though there is some question about transferability of measurements across sites with varying environmental conditions. As such, the derived concentration measurements should be considered semi-quantitative and will be reported and discussed in this document.

Water concentration can be estimated from the extracted mass of POCIS samples by the following equation (Alvarez 2010):

$$C_w = \frac{N}{R_t t}$$

where:

C_w = water concentration (ng/L)
N = extracted mass (ng)
R_t = compound uptake rate (L/d)
t = exposure time (d)

As a general check of efficacy, this approach was used to estimate the aquatic concentration of carbamazepine based on extracted mass. The results indicate that carbamazepine is present from 0.2 – 2 ng/L in water, which is in agreement to measured concentrations reported from grab samples collected from throughout the sound (Miller-Schulze et al. 2016). Although this supports the validity of this approach, it is again important to realize that the concentrations are only estimates and should be treated as such. Determining the uptake rate in a field setting is complicated; rates are affected by temperature, salinity, biofouling, current, tidal movement, sampler placement, etc. (Alvarez 2010, Bartelt-Hunt et al. 2011, Harman et al. 2012, Miller et al. 2016). Uptake rates reported in the literature should be applied with caution and, again, only as estimates. Even considering the uncertainties, large differences in uptake rate may explain some

of the differences in the extracted mass reported for 6 discs at each sample location (Table 4) as there are differences in affinity for the various compounds and the POCIS sample sorbent.

4.4 Comparisons of Extraction Between Sites

The extracted mass varied by approximately an order of magnitude between sites. Since the same field and laboratory methods were applied for all sites, these differences may reflect differences in potential exposure between sites. Based on the CECs evaluated, Thompson Cove and Oakland Bay had the highest average extracted mass per sampler while Discovery Bay, Joemma Beach, Poverty Bay, and Quartermaster Harbor all had the lowest extracted mass. This may suggest that Thompson Cove and Oakland Bay have the highest exposure potential, while the other sites the lowest amongst the sites evaluated.

4.5 Non-Targeted Analysis

Lab results indicate there is a wide variety of CECs such as pharmaceuticals and personal care products, agricultural antibiotics, food additives, present in the Puget Sound (Keil et al. 2011, Miller-Schulze et al. 2016). This is an initial exploratory analysis, meant to gage the potential effectiveness in improving understanding of exposure patterns and risk. In order to get an idea of range of exposures, the data sets from the non-targeted LC-QToF-MS/MS analysis were evaluated to: 1) determine if there are general differences between sample sites, 2) investigate the occurrence of a wider range of compounds at selected sites, and 3) investigate the presence of biotoxins at selected sites.

4.6 Biotoxins

Sample data sets were screened for the presence of marine and freshwater biotoxins such as domoic acid, azaspiracid, and microcystin. Work in the UW Tacoma lab and elsewhere have demonstrated the presence of biotoxins in shellfish and in shellfish growing areas (Preece et al. 2015, Trainer and Hardy 2015). Sample data was compared with an accurate mass database containing a suite of marine and freshwater biotoxins to perform a preliminary screening of presence at the sampling locations. At least one biotoxin was identified at every site with the exception of Hood Canal where none were detected. Pectenotoxin 2 was identified at nearly every site. UW Tacoma has previously identified this compound in several similar passive samplers elsewhere in the Puget Sound. Pectenotoxin 2 secoacid (a metabolite of Pectenoacid 2) was also commonly identified. The two sites with the highest number of putative biotoxins were Discovery Bay where dinophysis toxin 1 and nodularin were likely present, and Quartermaster Harbor where domoic acid was detected.

5 Discussion

5.1 Summary

As stated in the introduction, recent study results show increased levels of pharmaceuticals being discharged to Washington's marine waters at potentially destructively high rates (Ankley et al., 2007, Kolpin et al., 2002). Among these human sourced CECs are prescribed pharmaceuticals that have been shown in laboratory studies to cause sublethal and lethal effects to commercially valuable nearshore species (Bringolf et al., 2010, Meredith-Williams et al., 2012, Zhou et al. 2009). Controlled laboratory experiments exposing organisms to known concentrations of some of these CECs, particularly antidepressants and anti-seizure medications containing serotonin, epinephrine and dopamine regulators, demonstrate broad effects to benthic species, including shellfish (Silva et al. 2015, Bringolf et al. 2010, Hird et al. 2016, Fong and Ford 2014). What remains largely uninvestigated is the magnitude, spatial extent, and timing of *in situ* exposure of these CECs to shellfish species of interest. AAMT selected sampling locations in Puget Sound, Hood Canal and Straits of Juan de Fuca, and deployed sampling devices to ascertain the potential exposure shellfish resource sites have to these CECs.

Lab results from the deployed sampling devices suggest the following, which will be discussed in detail later in this section:

- Shellfish are being exposed to CECs that can be ingested in dissolved form from the water column.
- Three compounds were found at comparably higher levels. These compounds include venlafaxine, desvenlafaxine and carbamazepine.
- The diversity of compounds found at all sample locations was not as complex as originally thought. However, the type of compounds found indicate that other CEC compounds of similar use are entering the water column.
- The reported values of each analyte can be difficult to extrapolate for field conditions at each sample location. The analyte values are an average for a given mass (sampling discs) over the period of time of deployment at a given sample location. Translating these values to apply to exposure of a bivalve or other benthic invertebrate over time is not interchangeable. In that regard, results from this research are semi-quantitative and qualitative, and infer what is occurring at shellfish resource sites from an evaluation of sample results.
- The biotoxins identified to occur at the sample locations, during the deployment period, are inconsequential and will not be discussed further.
- Further investigation using POCIS and other sampling methods is worth considering at a few select sites.

5.2 Compounds – Analytes from POCIS

Table 4 reports all CEC analytes extracted from the six (6) POCIS discs at each sample location. Averages were calculated by WDNR AAMT and assessed for application to the management of shellfish resources on state owned aquatic lands. Four analytes were detected at levels in order of magnitude of approximately 4 times (or greater), greater than the field and lab blanks (sample = ~4x> field/lab blanks). These analytes were considered important to include in this discussion:

1. Carbamazepine
2. Venlafaxine
3. Desvenlafaxine
4. Duloxetine

Of the 11 targeted compounds that could have been detected, only 3 were detected with consistency. Duloxetine was only found at one location, Port Angeles. Despite this lack of diversity, the types of use identified for each compound found, such as treatment of depression, anxiety disorder, panic disorder and social anxiety disorder, anti-seizure medication, etc. implies other CECs of similar use types are entering the water column, even though undetected by AAMT passive sampling devices. It is still likely other CECs are entering the water column and interacting with state owned aquatic land resources, such as shellfish.

Exposure to CECs can result in biological impacts to marine organisms, including bivalves (Silva et al. 2015). For example, a laboratory study demonstrated that exposure to fluoxetine at 300 – 3000 µg/L resulted in reproductive impairments to freshwater mussels (Bringolf et al. 2010). Marine worms exposed to fluoxetine concentrations from 10 – 500 µg/L resulted in weight loss, decreased feeding rate, and altered metabolism compared to the controls (Hird et al. 2016). Other reproductive related effects have been noted (Fong and Ford 2014).

A summary of each compound detected with consistency and any applicable research is described below. Current research is suggestive of the importance of understanding marine invertebrate exposure of these CECs compounds as it relates to natural resource management of shellfish in the nearshore.

Carbamazepine

Carbamazepine is an anticonvulsant (or anti-epileptic), nerve pain reducer, and a bipolar prescribed medication. Carbamazepine has shown to inhibit the growth rate of unicellular marine algal species and has the ability to induce oxidative effects on cells of non-target species, such as mussels, affecting their overall health status (Tsiaka 2013). Carbamezepine is one of the most commonly found CECs in freshwater and saltwater sampling for CECs. Most research on the compound has been to evaluate impacts to freshwater biota. Compounds respond differently in saltwater however, the prevalence of this compound, and the known impacts in freshwater systems could be indicative of the potential stress the exposure to the compound would have on marine bivalve species.

Venlafaxine

Venlafaxine is a nerve pain, antidepressant, and anti-anxiety prescribed medication. Venlafaxine was exposed to marine snails at environmentally relevant concentrations and caused foot detachment from the substrate. Snails were not able to reattach while exposed to the compound and movement was accelerated (Fong 2016, Fong 2015, Fong 2012). In addition, molluscan reproductive and locomotory systems are affected by antidepressants at environmentally relevant concentrations. In particular, antidepressants can induce spawning and larval release in bivalves and disrupt locomotion and reduce fecundity in snails (Fong 2016).

Desvenlafaxine

Desvenlafaxine is an antidepressant prescribed medication. It is used to treat depression, anxiety and panic attacks.

No current research was identified for this compound however it is a metabolite of venlafaxine.

Duloxetine

Duloxetine is a nerve pain and antidepressant prescribed medication. Duloxetine has shown it can alter normal embryo-larval development and metamorphphosis success of the pacific oyster *Crassostrea gigas* at a wide range of levels of exposure (0.1 – 400 ugL^{-1}). Four types of abnormalities have been observed including 1) a D-shaped shelling exhibiting shell and/or hinge abnormalities, 2) a D-shaped larvae exhibiting a hypertrophied mantle (increase in volume/size) 3) D-shaped larva exhibiting both shell and mantle abnormalities and 4) arrested development at the "old embryo" stage.(Di Poi et al. 2013). Although the exposure values were thousands of magnitude greater than known environmental exposure values, the results indicate the potential damage duloxetine can have on normal embryo-larval development and metamorphosis success.

5.3 Analytes that Co-occur

Of the compounds detected and reported in Table 4, three analytes were found to co-occur at 71% of the sites. The following sites are where venlafaxine, desvenlafaxine and carbamazepine were detected to co-occur during the deployment period of each POCIS. They are listed as they occur geographically in Washington marine waters from north to south.

1. Whidbey Island – South West*
2. Liberty Bay
3. Skiff Point*
4. Colchester
5. Hood Canal – North Shore*
6. Vaughn Bay*
7. Quarter Master Harbor*
8. Oakland Bay*
9. Eld Inlet
10. Thompson Cove

*Indicates a DOH 2020 growing area, restoration goal. No asterisk indicates a wildstock geoduck harvest area (Figure 3).

5.4 Site Conditions – Analytes with High Detection Rates

Of the 10 sites where the three compounds co-occur, 60% (6 sites) were within DOH 2020 growing area restoration areas and 40% (4) were wild geoduck harvest sites. The results of this analysis show that CECs occur at these locations at levels below those reported in the literature to cause environmental impacts (James 2017). As a result, four locations have been identified with the highest detection rate of the three CEC compounds that co-occur. Exposure studies generally do not evaluate chronic exposures to mixtures, which appears to be the conditions in the Puget Sound and at the sample locations.

Eld Inlet (Carbamazepine)
Carbamezepine was detected with an average value of **0.92 ng/mass** at the Eld Inlet sample site. This is approximately 18 times greater than lab and field blanks. Cooper Point is a wild stock geoduck harvest area and site conditions include SQS exceedances and the water body is listed as 303 (d) for DO.

Oakland Bay (Venlafaxine)
Venlafaxne was detected with an average value of **0.76 ng/mass** at the Oakland Bay sample site. This is approximately 14 times greater than lab/field blanks. Oakland Bay is a DOH 2020 shellfish restoration area and site conditions include various outfalls in the vicinity and multiple SQS exceedances.

Skiff Point (Carbamazepine)
Carbamezepine was detected with an average value of **0.92 ng/mass** at the Skiff Point sample site. This is approximately 18 times greater than lab and field blanks. Skiff Point is a wild stock geoduck harvest area and site conditions include an inputs from an identified outfall, and a potential CSO input further away.

Thompson Cove (Desvenlafaxine)
Desvenlafaxine was detected with an average value of **5.33 ng/mass** at the Thompson Cove sample site. This is approximately 106 times greater than lab and field blanks. Thompson Cove is an approved shellfish harvest area, near a wild stock geoduck harvest area and site conditions include multiple SQS exceedances and the water body is listed as 303 (d) for DO. Carbamezepine was also detected at Thompson Cove with an average value of **0.92 ng/mass**. This is approximately 18 times greater than lab and field blanks.

5.5 Future Investigation and Other Considerations

Examine the four locations with the highest values of analytes found during the POCIS deployment period for further sampling. Additional research could occur at Eld Inlet, Oakland Bay, Skiff Point, and Thompson Cove. A more detailed inquiry into the inputs into each area should occur prior to developing a sampling plan. PSEMP (Puget Sound Ecosystem Monitoring Program) sampling results from the multi-parameter sampling (sediment, water, and oyster tissue) completed summer 2016 should be considered at each site. POCIS should be deployed in the dry months of July or August. A more detailed literature review of the CECs of interest should be completed if research in these areas is proposed.

Consider using samplers with a method that allows a better way to measure what the extraction values represent. Using the POCIS extraction values independently do not provide a numeric value relatable to thresholds or values documented in literature. A couple options exist for fresh water and salt water systems. POCIS devices can be used in river systems where flow is strictly monitored. Temperature gauges can be attached to POCIS devices and can track an element of water flow by measuring water and air temperature. If the device is exposed to air, the temperature reading is different. Some POCIS discs can be embedded with a known measurable substance (compound) with a known dilution rate. Calculations can be made to determine a rate of dilution for that compound, which can be extrapolated and applied to all compounds being sampled. Tidal areas are complex because all calculations of data need to account for the ebb and flow of the tide, currents, and inputs. One consideration to account for this difficulty is to deploy a POCIS in a marine system and expose something else for an equal amount of time. For example, bivalves could be placed near a POCIS sampling device for the same amount of time. Results would then be comparable because of exposure over time. This is difficult because most labs do not accept tissue samples and methods have not been created to extract the targeted CECs of interest from tissue samples. Washington State Department of Fish and Wildlife has recently submitted tissue samples to a laboratory for processing. WDNR will remain in communication with WDFW staff and evaluate the results from their samples to determine if a similar tissue sample and submittal process could be used by DNR.

CECs might be traveling far distances from the source. There is evidence indicating compounds can travel considerable distances from the input site to where the contaminants are detected. Outfall effluent can get trapped in the water column and travel various distances. For each site selected for sampling, a more thorough investigation of larger scale inputs should be considered and discussed prior to POCIS site selection and deployment.

The DNR Outfall Program should not require CEC sampling from applicants. Sampling for CECs is experimental and applying the data is challenging. The DNR Outfall Program has considered sampling water, sediment, and shellfish tissues for a multitude of CECs. This type of sampling is experimental and should only be considered for research purposes rather than a requirement of the leasee.

CEC sampling results should be discussed with other experts and researchers. Many experts are figuring out the best way to approach research with CECs and passive samplers. Evaluating the data and applying it to resource management practices is a newer science. Discussing research design and results with other experts and researchers is key to gaining the optimal understanding of this developing science.

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Table 4. POCIS CEC analyte extraction results as reported by University of Washington Tacoma as mass (ng), mass being the sample disc as described in this report. Averages were calculated by WDNR AAMT. NAV means "no average value". NAV was applied to any average being equal to or less than the field/lab blank reported value. It was also applied to any analyte where more than half of the discs were equal to the field/lab blanks. For those compounds where field/lab blanks were included in the average, a zero (0) value was used in place of a greater than numeric value. An "x" in any field indicates no value reported.

Site Sample Name	Venlafaxine Mass (ng)	Desvenlafaxine Mass (ng)	Bupropion Mass (ng)	Hydroxybupropion Mass (ng)	Citalopram Mass (ng)	Escitalopram Mass (ng)	N-Desmethyl citalopram Mass (ng)	Duloxetine Mass (ng)	Paroxetine Mass (ng)	Fluoxetine Mass (ng)	Carbamazepine Mass (ng)
Skiff Point											
3.16 SPD_1	0.5	2	<5	<0.8	0.1	0.1	0.1	<0.5	<0.05	<0.5	1
3.16 SPD_2	1	3	<5	<0.8	0.1	0.1	0.1	<0.5	<0.05	<0.5	1
3.16 SPD_3	0.4	3	<5	<0.8	0.1	0.1	0.1	<0.5	<0.05	<0.5	0.5
3.16 SPU_4	0.3	3	<5	1	0.1	0.1	0.1	<0.5	<0.05	<0.5	1
3.16 SPU_5	1	4	<5	<0.8	0.1	0.1	0.1	<0.5	<0.05	<0.5	1
3.16 SPU_6	0.4	3	<5	<0.8	0.1	0.1	0.1	<0.5	<0.05	<0.5	1
Average	**0.6**	**3**	**NAV**	**NAV**	**0.1**	**0.1**	**0.1**	**NAV**	**NAV**	**NAV**	**0.92**
Thompson Cove											
6.13 TC_1	0.2	7	<5	1	0.1	0.1	0.2	<0.5	<0.05	<0.5	1
6.13 TC_2	0.2	6	<5	<0.8	0.1	0.1	0.2	<0.5	<0.05	<0.5	1
6.13 TC_3	0.4	5	<5	1	0.1	0.1	0.2	<0.5	<0.05	<0.5	1
6.13 TC_4	0.1	7	<5	1	0.1	0.1	0.1	<0.5	<0.05	<0.5	1
6.13 TC_5	0.3	4	<5	<0.8	0.1	0.1	0.2	<0.5	<0.05	<0.5	0.5
6.13 TC_6	0.1	3	<5	1	0.1	0.1	0.1	<0.5	<0.05	<0.5	1
Average	**0.22**	**5.33**	**NAV**	**0.67**	**0.1**	**0.1**	**0.17**	**NAV**	**NAV**	**NAV**	**0.92**
Vaughn Bay											
6.13 VB_1	0.1	<0.05	<5	<0.8	<0.05	<0.05	0.1	<0.5	<0.05	<0.5	0.3
6.13 VB_2	<0.05	<0.05	<5	<0.8	<0.05	<0.05	0.1	<0.5	<0.05	<0.5	0.1
6.13 VB_3	0.2	<0.05	<5	<0.8	<0.05	<0.05	0.1	<0.5	<0.05	<0.5	0.2
6.13 VB_4	0.2	2	<5	<0.8	<0.05	<0.05	0.1	<0.5	<0.05	<0.5	0.4
6.13 VB_5	0.5	3	<5	<0.8	<0.05	<0.05	0.1	<0.5	<0.05	<0.5	1
6.13 VB_6	0.3	3	<5	<0.8	<0.05	0.1	0.1	<0.5	<0.05	<0.5	0.4
Average	**0.22**	**1.33**	**NAV**	**NAV**	**NAV**	**NAV**	**0.1**	**NAV**	**NAV**	**NAV**	**0.4**

Site Sample Name Case Inlet - Joemma	Venlafaxine Mass (ng)	Desvenlafaxine Mass (ng)	Bupropion Mass (ng)	Hydroxybupropion Mass (ng)	Citalopram Mass (ng)	Escitalopram Mass (ng)	N-Desmethyl citalopram Mass (ng)	Duloxetine Mass (ng)	Paroxetine Mass (ng)	Fluoxetine Mass (ng)	Carbamazepine Mass (ng)
6.13 JB_1	0.1	<0.05	<5	<0.8	<0.05	<0.05	0.1	<0.5	<0.05	<0.5	0.2
6.13 JB_2	0.1	<0.05	<5	<0.8	<0.05	<0.05	0.1	<0.5	<0.05	<0.5	0.1
6.13 JB_3	0.1	0.3	<5	<0.8	<0.05	<0.05	0.1	<0.5	<0.05	<0.5	0.4
6.17 JB_4	<0.05	<0.05	<5	3	<0.05	<0.05	<0.05	<0.5	<0.05	<0.5	0.1
6.17 JB_5	<0.05	<0.05	<5	<0.8	<0.05	<0.05	<0.05	<0.5	<0.05	<0.5	<0.05
6.17 JB_6	<0.05	<0.05	<5	<0.8	<0.05	<0.05	<0.05	<0.5	<0.05	<0.5	0.1
Average	**0.05**	**NAV**	**NAV**	**NAV**	**NAV**	**NAV**	**0.05**	**NAV**	**NAV**	**NAV**	**0.15**
Eld Inlet											
6.13 CP_1	0.3	4	<5	1	0.1	0.1	0.2	<0.5	<0.05	<0.5	1
6.13 CP_2	0.2	3	<5	1	0.1	0.1	0.1	<0.5	<0.05	<0.5	1
6.13 CP_3	0.2	3	<5	1	0.1	<0.05	0.1	<0.5	<0.05	<0.5	1
6.13 CP_4	0.2	2	<5	<0.8	0.1	0.1	0.1	<0.5	<0.05	<0.5	1
6.13 CP_5	0.2	2	<5	<0.8	<0.05	<0.05	0.1	<0.5	<0.05	<0.5	0.5
6.13 CP_6	0.2	2	<5	<0.8	<0.05	<0.05	0.1	<0.5	<0.05	<0.5	1
Average	**0.22**	**2.67**	**NAV**	**0.5**	**0.06**	**0.05**	**0.12**	**NAV**	**NAV**	**NAV**	**0.92**
Quarter Master Harbor											
7.13 QM_1	0.4	1	<5	<0.8	<0.05	<0.05	0.1	<0.5	<0.05	<0.5	0.4
7.13 QM_2	0.2	0.1	<5	<0.8	0.1	0.1	0.1	<0.5	<0.05	<0.5	0.1
7.13 QM_3	0.2	<0.05	<5	<0.8	<0.05	<0.05	<0.05	<0.5	<0.05	<0.5	<0.05
7.13 QM_4	0.4	0.1	<5	<0.8	<0.05	<0.05	0.1	<0.5	<0.05	<0.5	0.3
7.13 QM_5	0.3	0.2	<5	<0.8	<0.05	<0.05	0.1	<0.5	<0.05	<0.5	0.3
7.13 QM_6	0.2	<0.05	<5	<0.8	<0.05	<0.05	0.1	<0.5	<0.05	<0.5	0.4
Average	**0.28**	**0.23**	**NAV**	**NAV**	**NAV**	**NAV**	**NAV**	**NAV**	**NAV**	**NAV**	**0.25**

Site Sample Name	Venlafaxine Mass (ng)	Desvenlafaxine Mass (ng)	Bupropion Mass (ng)	Hydroxybupropion Mass (ng)	Citalopram Mass (ng)	Escitalopram Mass (ng)	N-Desmethyl citalopram Mass (ng)	Duloxetine Mass (ng)	Paroxetine Mass (ng)	Fluoxetine Mass (ng)	Carbamazepine Mass (ng)
Colchester											
7.13 MA_1	0.4	3	<5	<0.8	0.1	0.1	0.1	<0.5	<0.05	<0.5	1
7.13 MA_2	1	6	<5	1	0.1	0.1	0.2	<0.5	<0.05	<0.5	1
7.13 MA_3	1	5	<5	1	0.1	0.1	0.2	<0.5	<0.05	<0.5	1
7.13 MA_4	0.1	<0.05	<5	<0.8	<0.05	<0.05	<0.05	1	<0.05	<0.5	0.1
7.13 MA_5	<0.05	<0.05	<5	<0.8	<0.05	<0.05	<0.05	<0.5	<0.05	<0.5	0.1
7.13 MA_6	x	x	x	x	x	x	x	x	x	x	x
Average	**0.42**	**2.33**	**NAV**	**NAV**	**0.05**	**0.05**	**0.08**	**NAV**	**NAV**	**NAV**	**0.53**
Liberty Bay											
7.13 POB_1	0.2	2	<5	<0.8	<0.05	<0.05	0.1	<0.5	<0.05	<0.5	0.5
7.13 POB_2	0.3	1	<5	<0.8	0.1	<0.05	0.1	<0.5	<0.05	<0.5	0.4
7.13 POB_3	0.3	2	<5	1	<0.05	<0.05	0.1	<0.5	<0.05	<0.5	0.4
7.13 POT_4	0.4	1	<5	<0.8	<0.05	<0.05	0.1	<0.5	<0.05	<0.5	0.4
7.13 POT_5	0.3	0.3	<5	<0.8	<0.05	<0.05	0.1	<0.5	<0.05	<0.5	0.4
7.13 POT_6	x	x	x	x	x	x	x	x	x	x	x
Average	**0.25**	**1.05**	**NAV**	**NAV**	**NAV**	**NAV**	**0.08**	**NAV**	**NAV**	**NAV**	**0.35**
Poverty Bay											
7.13 POVD_1	0.3	<0.05	<5	<0.8	<0.05	<0.05	0.1	<0.5	<0.05	<0.5	0.2
7.13 POVD_2	0.2	<0.05	<5	<0.8	<0.05	<0.05	0.1	<0.5	<0.05	<0.5	0.2
7.13 POVD_3	0.3	<0.05	<5	<0.8	<0.05	<0.05	0.1	<0.5	<0.05	<0.5	0.3
7.13 POVU_4	0.2	<0.05	<5	<0.8	<0.05	<0.05	0.1	<0.5	<0.05	x	0.2
7.13 POVU_5	0.2	<0.05	<5	<0.8	<0.05	<0.05	0.1	<0.5	<0.05	<0.5	0.2
7.13 POVU_6	0.1	<0.05	<5	<0.8	<0.05	<0.05	<0.05	<0.5	<0.05	<0.5	0.1
Average	**0.22**	**NAV**	**NAV**	**NAV**	**NAV**	**NAV**	**0.08**	**NAV**	**NAV**	**NAV**	**0.20**

Site Sample Name	Venlafaxine Mass (ng)	Desvenlafaxine Mass (ng)	Bupropion Mass (ng)	Hydroxybupropion Mass (ng)	Citalopram Mass (ng)	Escitalopram Mass (ng)	N-Desmethyl citalopram Mass (ng)	Duloxetine Mass (ng)	Paroxetine Mass (ng)	Fluoxetine Mass (ng)	Carbamazepine Mass (ng)
East Straits - East											
8.04 PA_1	0.1	<0.05	<5	<0.8	<0.05	<0.05	<0.05	1	<0.05	<0.5	0.1
8.04 PA_2	<0.05	<0.05	<5	<0.8	<0.05	<0.05	<0.05	<0.5	<0.05	<0.5	0.1
8.04 PA_3	<0.05	<0.05	<5	<0.8	<0.05	<0.05	<0.05	1	<0.05	<0.5	0.1
8.04 PA_4	<0.05	<0.05	<5	<0.8	<0.05	<0.05	<0.05	7	<0.05	<0.5	0.1
8.04 PA_5	<0.05	<0.05	<5	<0.8	<0.05	<0.05	<0.05	8	<0.05	<0.5	0.1
8.04 PA_6	<0.05	<0.05	<5	<0.8	<0.05	<0.05	<0.05	4	<0.05	<0.5	0.1
Average	**NAV**	**NAV**	**NAV**	**NAV**	**NAV**	**NAV**	**NAV**	**3.5**	**NAV**	**NAV**	**0.1**
Discovery Bay											
8.04 DB_1	0.3	<0.05	<5	<0.8	<0.05	<0.05	<0.05	<0.5	<0.05	<0.5	0.1
8.04 DB_2	0.2	<0.05	<5	<0.8	<0.05	<0.05	<0.05	<0.5	<0.05	<0.5	0.1
8.04 DB_3	0.2	<0.05	<5	<0.8	<0.05	<0.05	<0.05	<0.5	<0.05	<0.5	0.1
8.04 DB_4	0.1	<0.05	<5	<0.8	<0.05	<0.05	<0.05	<0.5	<0.05	<0.5	0.1
8.04 DB_5	0.1	<0.05	<5	<0.8	<0.05	<0.05	<0.05	<0.5	<0.05	<0.5	0.1
8.04 DB_6	<0.05	<0.05	<5	<0.8	<0.05	<0.05	<0.05	<0.5	<0.05	<0.5	<0.05
Average	**0.15**	**NAV**	**NAV**	**NAV**	**NAV**	**NAV**	**NAV**	**NAV**	**NAV**	**NAV**	**0.08**
Whidbey Island SW											
8.04 WH_1	0.5	3	<5	<0.8	0.1	0.1	0.2	<0.5	<0.05	<0.5	0.5
8.04 WH_2	0.4	3	<5	<0.8	0.1	0.1	0.2	<0.5	<0.05	<0.5	1
8.04 WH_3	1	3	<5	1	0.1	0.1	0.2	<0.5	<0.05	<0.5	0.5
8.04 WH_4	0.4	3	<5	<0.8	0.1	0.1	0.2	<0.5	<0.05	<0.5	0.4
8.04 WH_5	0.4	3	<5	1	0.1	0.1	0.2	<0.5	<0.05	<0.5	0.4
8.04 WH_6	1	2	<5	<0.8	0.1	0.1	0.1	<0.5	<0.05	<0.5	0.5
Average	**0.62**	**2.83**	**NAV**	**NAV**	**0.1**	**0.1**	**0.18**	**NAV**	**NAV**	**NAV**	**0.55**

Site Sample Name	Venlafaxine Mass (ng)	Desvenlafaxine Mass (ng)	Bupropion Mass (ng)	Hydroxybupropion Mass (ng)	Citalopram Mass (ng)	Escitalopram Mass (ng)	N-Desmethyl citalopram Mass (ng)	Duloxetine Mass (ng)	Paroxetine Mass (ng)	Fluoxetine Mass (ng)	Carbamazepine Mass (ng)
Oakland Bay											
8.15 SOB_1	0.4	4	<5	<0.8	0.1	0.1	0.1	<0.5	<0.05	<0.5	0.5
8.15 SOB_2	0.3	1	<5	<0.8	<0.05	0.1	0.1	<0.5	<0.05	<0.5	0.3
8.15 SOB_3	1	6	<5	1	0.1	0.1	0.2	<0.5	<0.05	<0.5	1
8.15 SOB_4	1	8	<5	<0.8	0.1	0.1	0.2	<0.5	<0.05	<0.5	1
8.15 SOB_5	1	7	<5	1	0.1	0.1	0.2	<0.5	<0.05	<0.5	1
8.15 SOB_6	0.5	5	<5	<0.8	0.1	0.1	0.1	<0.5	<0.05	<0.5	1
Average	**0.7**	**5.17**	**NAV**	**NAV**	**0.08**	**0.1**	**0.15**	**NAV**	**NAV**	**NAV**	**0.80**
Hood Canal-N. Shore											
8.03 HC_1	0.2	0.2	<5	<0.8	<0.05	<0.05	0.1	<0.5	<0.05	<0.5	0.2
8.03 HC_2	0.2	2	<5	<0.8	<0.05	<0.05	0.1	<0.5	<0.05	<0.5	1
8.03 HC_3	0.2	2	<5	<0.8	0.1	0.1	0.1	<0.5	<0.05	<0.5	1
8.03 HC_4	<0.05	<0.05	<5	<0.8	<0.05	<0.05	<0.05	<0.5	<0.05	<0.5	0.2
8.03 HC_5	0.4	0.4	<5	<0.8	<0.05	<0.05	0.1	<0.5	<0.05	<0.5	0.2
8.03 HC_6	0.1	<0.05	<5	<0.8	<0.05	<0.05	0.1	<0.5	<0.05	<0.5	0.3
Average	**0.18**	**0.76**	**NAV**	**NAV**	**NAV**	**NAV**	**0.08**	**NAV**	**NAV**	**NAV**	**0.48**
Field Blanks											
6.15 FB 1	0	<0.05	<5	<0.8	<0.05	<0.05	<0.05	<0.5	<0.05	<0.5	<0.05
7.14 FB 2	<0.05	<0.05	<5	<0.8	<0.05	<0.05	<0.05	<0.5	<0.05	<0.5	<0.05