2016 Elliott Bay Market Squid Tissue Monitoring Sampling and Analysis Plan

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1.0. INTRODUCTION

This sampling and analysis plan (SAP) describes the objectives and study design of the 2016 Elliott Bay market squid tissue monitoring effort. The following sections outline the project scope, and sampling and analytical strategies.

1.1 Study Background and Objectives

In 2014, King County initiated a routine tissue monitoring program. A tissue monitoring program work plan was drafted in 2015 and included a compilation of local historical tissue data to help identify data gaps and opportunities. The lack of squid tissue chemistry was identified as one of these data gaps (King County 2016). There is an active squid fishery around Puget Sound, including Elliott Bay, but squid tissue has been analyzed for chemicals only once, in 1997 by King County. The purpose of the sampling and analysis effort described here is to assess potential human health risks from squid consumption.

The 1997 squid sampling effort included collection of six composite samples (about 10 squid per composite) from Elliott Bay over two consecutive days in December. The samples were analyzed for 15 metals (including mercury), butyltins, polychlorinated biphenyls (PCBs) as Aroclors, and a large list of semi-volatile organic chemicals (SVOCs) including polycyclic aromatic hydrocarbons (PAHs) and phthalates. Squid in three of the composite samples were "cleaned" (i.e., beak, quill, and viscera removed) before analysis, while organisms in the remaining three samples were analyzed whole. Few organic compounds were detected in these samples, but most metals were detected. Concentrations of cadmium, silver and total Aroclors were consistently higher in whole squid than in the cleaned squid samples, but otherwise, all results were comparable. The sampling effort described here will provide updated results to assess present-day human health risks from squid consumption.

1.2 Scope of Work

Market squid (*Loligo opalescens*) will be collected from two locations in Puget Sound with a goal of six composite samples at each location. The squid will be cleaned according to popular cooking preparations (as done for the subset in 1997) to represent squid tissues regularly consumed by humans. Tissue samples will be analyzed for select bioaccumulative chemicals including metals, mercury, chlorinated pesticides, SVOCs, butyltins, PCBs as Aroclors, a subset of samples analyzed for PCB homologs, polybrominated diphenyl ethers (PBDEs), and Base-neutral-acid Extractables (BNAs). Total solids and total lipids will also be analyzed. The details of chemical analyses are discussed in Section 4.0.

1.3 Schedule

Squid tissue samples will be collected between October and January. Exact scheduling will be dependent on King County Water and Land Resources Division (WLRD) personnel

availability and presence of squid in Puget Sound. The goal will be to collect all samples during one sampling event at each location.

King County Environmental Lab (KCEL) will analyze all squid tissue samples. With the exception of PCB homologs and butyltins, the turn-around time for all analytical data is approximately eight weeks from the completion date of all samples being cleaned and homogenized. The turn-around-time for PCB homologs and butyltins is approximately 14 weeks. Quality Assurance (QA)-approved data will be entered into the laboratory information management system (LIMS) following completion of analysis. A QA narrative memo will be completed 30 days after all data are entered into LIMS.

1.4 Project Team

The following King County staff members are responsible for project execution:

Jenée Colton, Science Section Program Manager..... 206-477-4075

Carly Greyell, Science Section Project Manager..... 206-477-4703

Rory O'Rourke, Science Section Technical Support..... 206-477-7769

Chris Gregersen, Science Section Fisheries Biologist..... 206-477-4699

Fritz Grothkopp, KCEL Project Manager..... 206-477-7114

Colin Elliott, KCEL QA Manager..... 206-477-7113

2.0. MONITORING DESIGN

The objective of the 2016 squid sampling effort is to help fill data gaps in tissue monitoring in marine waters of King County. Results from this sampling effort will help assess potential human health risks from local market squid consumption.

Two King County locations were selected based on their popularity for recreational squidding (Yuasa 2015) as well as proximity to potential pollution sources: Seattle Pier 86 and Redondo Pier. One sampling station is located in northern Elliott Bay near the Seattle waterfront, while the second is located in a less urbanized area near Des Moines, south of Elliott Bay. Market squid are migratory, and as adults, travel south through the Strait of Juan de Fuca to southern Puget Sound (WDFW 2016). Unlike more sedentary marine animals, chemical concentrations in squid are not expected to only reflect exposure to contaminants at the collection site. However, squid that move through urbanized areas (e.g., Elliott Bay) may be exposed to higher contaminant levels than those migrating through less urbanized areas of Puget Sound. Squid collected near Des Moines may have already migrated through Elliott Bay, and could potentially have also been exposed to higher chemical concentrations.

The following sections provide a description of the study design including data quality objectives (DQOs), sampling methods, and field and analytical parameters measured.

2.1 Data Quality Objectives

The DQOs are to collect data of known and sufficient quality to meet the monitoring objectives described above in Section 1.1. No DQOs mandated through federal or state regulations apply to this effort. The project manager will assess project data and evaluate whether the data collected are of sufficient quality to meet monitoring goals. The data quality issues of precision, accuracy, bias, representativeness, completeness, comparability, and sensitivity are described in the following sections, along with data assessment. Data not meeting objectives will be identified and potentially used to improve future monitoring efforts.

2.1.1 Precision, Accuracy, and Bias

Precision is the agreement of a set of results among themselves and is a measure of the ability to reproduce a result. Accuracy is an estimate of the difference between the true value and the measured value. The accuracy of a result is affected by both systematic and random errors. Bias is a measure of the difference, due to a systematic factor, between an analytical result and the true value of an analyte. Precision, accuracy, and bias for analytical chemistry may be measured by one or more analyses of various laboratory quality control (QC) samples such as blanks, surrogates, and replicates.

Chemical concentrations in field-collected tissue samples can be fairly variable, and influenced by age and growth rate. Laboratory precision will only be evaluated by the use of laboratory quality control samples, including spikes and spike duplicates. If precision is

considered too low for project needs, these data will be used to guide future sampling efforts.

Analytical bias cannot be quantified for tissue data due to the high natural variability in chemical concentrations. However, composite samples will target a more average concentration and reduce some of this variability.

2.1.2 Representativeness

Representativeness expresses the degree to which sample data accurately and precisely represent a characteristic of a population, parameter variations at the sampling point, or an environmental condition. The target species and sizes of squid were selected to help fill a data gap for an active local fishery. Market squid are consumed by recreational fisherpersons. The sampling methods used for this project (i.e., jigging, and cleaning) are commonly used by the general population.

2.1.3 Completeness

Completeness is defined as the total number of samples analyzed for which acceptable analytical data are generated, compared to the total number of samples submitted for analysis. Sampling with adherence to standardized sampling and testing protocols will aid in providing a complete set of data for this survey. The goal for completeness is at least six composite samples. If six composite samples are not collected, the project team will evaluate if the DQOs can still be met or if additional sample collection and analysis is necessary prior to data reporting and analysis.

2.1.4 Comparability

Comparability is a qualitative parameter expressing the confidence with which one data set can be compared with another. This goal is achieved through the use of standard techniques to collect and analyze representative samples, along with standardized data assessment and reporting procedures. By following the guidance of this SAP, the goal of comparability between this and future sampling events will be achieved. Historical squid tissue data may be compared with data generated from this program, although historical data is very limited.

2.1.5 Sensitivity

Sensitivity is a measure of the capability of analytical methods to meet the monitoring goals. The analytical method detection limits presented in Section 5 are sensitive enough to detect analytes at concentrations of interest to assess risk of adverse effects to humans.

2.1.6 Data Assessment

Chemical data and QC results will be assessed against requirements of the analytical methods as well as the requirements of this SAP.

2.2 Sampling and Analytical Strategy

Market squid will be collected from two stations in Puget Sound, one in Elliott Bay off Pier 86 in Seattle, Washington and one at Redondo Pier in Des Moines, Washington (Figure 1). A minimum of six composite samples will be collected total. These six samples could be collected either all at one site, or as three composites at each site (Table 1). The maximum tissue mass goal will be to collect six composite samples from each location (total of 12 composite samples). A composite sample mass of 330-370 grams is required for analysis. Adult market squid weigh about 40 grams; therefore, only squid greater than 35 grams will be targeted for collection (Table 2). The cleaning process removes about 40% or less of the whole body weight (Turner and Hebard), and some mass can be lost during homogenization. WDFW allows a maximum catch per day of 10 lbs. per person. If a maximum of 12 composite samples is collected by two staff, then the total whole body mass will be less than the 10-pound limit.

Table 1. Sample Number Goals and Distribution.

Goal	# Samples per station	# of Stations	Total Samples
Minimum (Option 1)	6	1	6
Minimum (Option 2)	3	2	6
Maximum (Goal)	6	2	12

Table 2. Market Squid Mass Needed for Each Sample.

Processing Step	Mass per squid (g)	Mass per sample (g)	Total squid per sample
Before cleaning	>35	330-370	7-10
After cleaning	20-25	200-240	

Length and weight measurements, cleaning (i.e., removal of beak, quill, and viscera), sorting into composite samples, packaging for storage, and sample labeling will be done at KCEL, led by a Science Section field biologists (See Section 3). Composite sample mass must be sufficient to meet or exceed minimum analytical requirements (Table 3). All samples will be stored frozen until homogenization.

Table 3. Minimum Analytical Mass Requirements for Tissue Samples

Analyte	Squid Tissue (g)	Extra mass for QC (g)
Total Solids	5	15
Total Lipids	0[a]	15 (LD)
PCB Aroclors	15	60 (LD, MS, MSD)
Mercury	10	10
Metals (ICP-MS)	10	10
Pesticides	15	30 (MS, MSD)
Butyltins	10	30 (LD, MS, MSD)
PCB Homologs	0[b]	30 (MS, MSD)
BNA	20	60 (LD, MS, MSD)
PBDE Homologs	0[a]	30 (MS, MSD)
Total Mass per Sample:	Between 200 and 240 grams [c]	

[a] Coextracted with pesticides
[b] Coextracted with PCB Aroclors (subset only)
[c] This total mass takes into account mass that may be lost by homogenization and extra mass needed for QC samples.
LD – Lab Duplicate
MS – Matrix Spike
MSD – Matrix Spike Duplicate
ICP-MS – inductively coupled plasma mass spectrometry
BNA – base/neutral/acid extractable analyte list
PBDE – polybrominated diphenyl ethers

2.2.1 Sampling Locations and Schedule

Sampling locations were selected based on popular locations for recreational squid jigging in King County (Yuasa 2015). The Seattle Pier 86 location is in Elliott Bay, whereas, the Redondo Pier location in Des Moines is south of Elliott Bay. The samples will be collected in winter 2016-2017.

Table 4. Locator ID and General Coordinates for Each Sampling Location

Location	Locator ID	Description	Coordinates in State Plane North NAD83 (US Survey Feet)	
			X	Y
Seattle Pier 86	SEA_PIER86	Elliott Bay fishing pier at Terminal 86	1260471	232125
Redondo Pier	REDONDO_PIER	Poverty Bay fishing pier in Redondo	1270511	130755

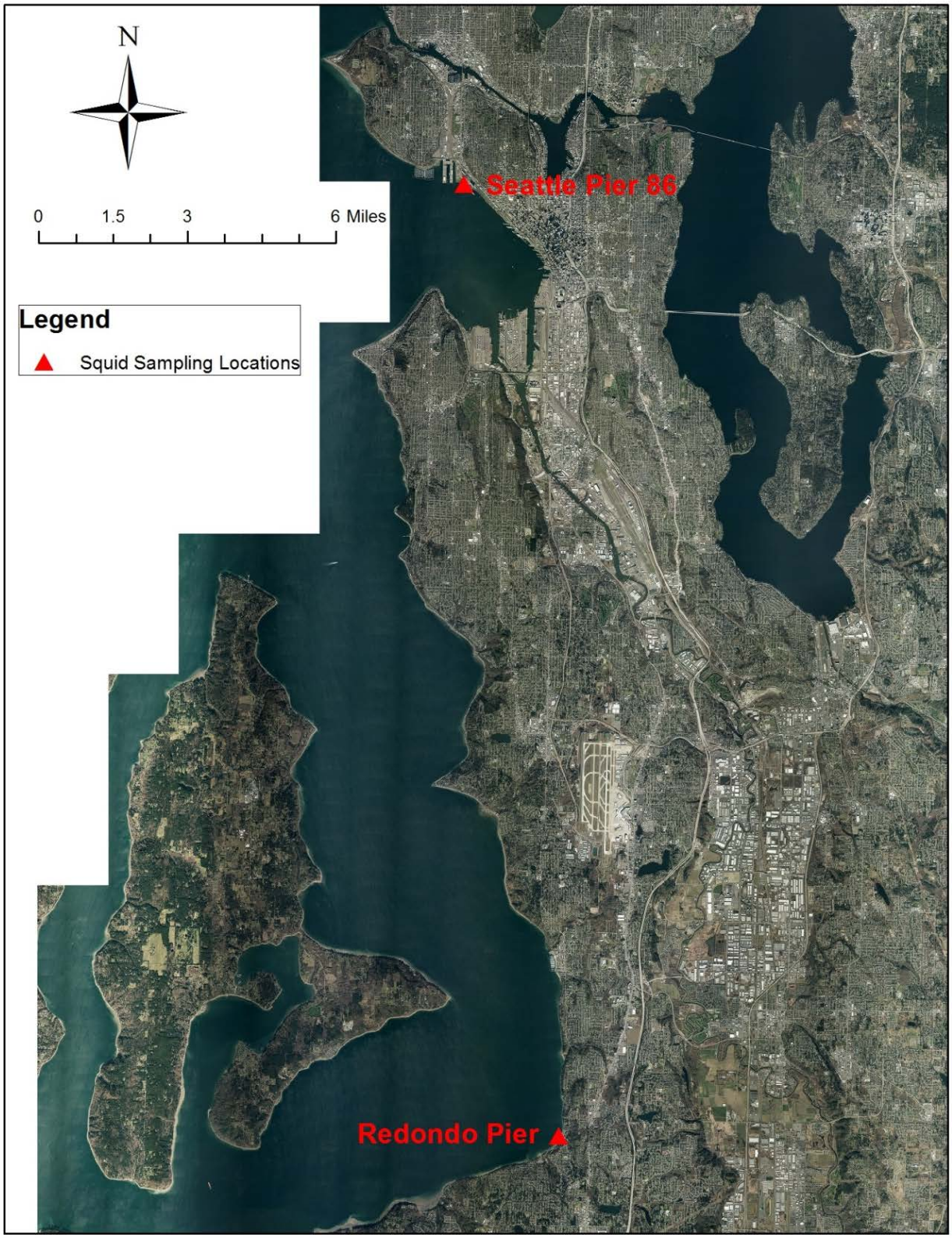

Figure 1. 2016 Squid Sampling Stations

2.2.2 Sample Acquisition and Analytical Parameters

Sampling will be conducted using a rod and reel method from each pier. Detailed sampling techniques are discussed in Section 3. Composite samples will be analyzed for total solids, total lipids, total metals, total mercury, PCB Aroclors, pesticides, BNAs, PBDEs and butyltins (Table 5). A subset of samples will also be analyzed for PCB homologs. Analyses for all chemical and conventional parameters will be conducted by the KCEL. If constraints, such as sample volume, limit analyses, the prioritization in Table 5 will be followed.

Table 5. Prioritized Analyte List and Methods

Analyte Group	Analyte List	KCEL References	Standard method
Total Solids	Solids	KCEL SOP# 307	SM 2540-G
Total Lipids	Lipids	KCEL SOP# 705/740	GRAVIMETRIC SOP 740
PCB Aroclors (GC/ECD)	Aroclor 1016 Aroclor 1221 Aroclor 1232 Aroclor 1242 Aroclor 1248 Aroclor 1254 Aroclor 1260	KCEL SOP# 705/757	SW 846-3540C/8082A;
Total Mercury (CVAA)	Mercury	KCEL SOP# 604; PSEP (1997)	PSEP (1997)
Total Metals (ICP-MS)	Arsenic Cadmium Chromium Copper Lead Nickel Silver Zinc	KCEL SOP# 623; PSEP(1997)	PSEP1997*SW846 6020A
Pesticides (GC/ECD)	Alpha-BHC Beta-BHC Delta-BHC Gamma-BHC (Lindane) Heptachlor Aldrin Heptachlor Epoxide Endosulfan I Dieldrin 4,4'-DDE Endrin Endrin Ketone Endosulfan II 4,4'-DDD Endrin Aldehyde Endosulfan Sulfate 4,4'-DDT Methoxychlor trans-Chlordane Alpha-Chlordane Toxaphene	KCEL SOP# 705/733	SW 846-3540C/8081B

Analyte Group	Analyte List	KCEL References	Standard method
Butyltins (GC/MS)	Mono-n-Butyltin Di-n-Butyltin Tri-n-Butyltin Tetra-n-Butyltin	KCEL SOP# 714/742	Krone et al.(1989)*8270D SIM
PCB Homologs (GC/MS)	Monochlorobiphenyls Dichlorobiphenyls Trichlorobiphenyls Tetrachlorobiphenyls Pentachlorobiphenyls Hexachlorobiphenyls Heptachlorobiphenyls Octachlorobiphenyls Nonachlorobiphenyls Decachlorobiphenyl Total PCB Homologs	KCEL SOP# 705/783	SW 846 3540C; EPA 680 SIM
BNA* (GC/MS)	1,2,4-Trichlorobenzene 1,2-Dichlorobenzene 1,4-Dichlorobenzene 1-Methylnaphthalene 2,4-Dimethylphenol 2-Methylnaphthalene 2-Methylphenol 3-,4-Methylphenol Acenaphthene Acenaphthylene Anthracene Benzo(a)anthracene Benzo(a)pyrene Benzo(b,j,k)fluoranthene Benzo(g,h,i)perylene Benzoic Acid Benzyl Alcohol Benzyl Butyl Phthalate Bis(2-Ethylhexyl)Phthalate Carbazole Chrysene Dibenzo(a,h)anthracene Dibenzofuran Diethyl Phthalate Dimethyl Phthalate Di-N-Butyl Phthalate Di-N-Octyl Phthalate Fluoranthene Fluorene Hexachlorobenzene Hexachlorobutadiene Indeno(1,2,3-Cd)Pyrene Naphthalene N-Nitrosodiphenylamine Pentachlorophenol Phenanthrene Phenol Pyrene		SW846 3540B*SW846 8270D

Analyte Group	Analyte List	KCEL References	Standard method
PBDEs (GC/MS)	TriBDE-17 TriBDE-28, 33 TetraBDE-47 TetraBDE-66 TetraBDE-71 PentaBDE-85 PentaBDE-99 PentaBDE-100 HexaBDE-138 HexaBDE-153 HexaBDE-154 HeptaBDE-183 HeptaBDE-190 DecaBDE-209	KCEL SOP# 781	NA

*The project manager will consult with the chemist to determine if reporting of tentatively identified compounds (TICs) is appropriate on a per sample basis.
CVAA – cold-vapor atomic absorption
ICP-MS – inductively coupled plasma mass spectrometry
GC/ECD – gas chromatography with electron capture detector
GC/MS – gas chromatography mass spectrometry

3.0. SAMPLING PROCEDURES

This section describes the sampling procedures to be followed over the course of all sampling events to meet the monitoring DQOs: representativeness, comparability, and completeness. Sample handling, storage, and preparation will generally follow EPA (2000) guidance.

3.1 Sample Collection and Permits

All squid will be collected using King County-owned equipment from public fishing piers. All squid will be collected under a standard recreational shellfish harvesting license, with permission for scientific collection obtained by WDFW (Velasquez pers comm. 2016). No endangered species will be harassed or harmed during these activities. The jigging procedures to be used and the roles for King County staff during sampling and processing are summarized below.

3.1.1 Jigging Procedures and Field Processing

King County fisheries biologist, Chris Gregersen, will lead the sampling effort. Sampling will take place on the pier after dark, preferably around high tide. A light-weight rod and reel equipped with one to four squid jigs will be lowered into the water, and light from the dock or from a 1,000-watt halogen light powered by a portable 2,000-watt Honda generator will be used to attract squid to the sampling location.

Squid grab the jig with their tentacles, and when one is felt on the line, a quick upward jerk of the rod will be used to hook the squid. Once hooked, the squid will be quickly reeled into the dock. Squid will not be weighed in the field, but the field biologist will assess whether they are adult size and should be retained for analysis. Squid with lesions, tumors or other visible health problems may be retained and their condition will be recorded in the field notes. Each squid will be securely wrapped in aluminum foil and placed in a large re-sealable plastic bag. The foil will be marked with an identifying number if any notes were recorded for the squid. The bagged squid will be kept in a cooler on ice for the duration of the sampling event.

3.2 Field Sampling Equipment

Field equipment necessary for sampling is identified below. The field lead will check that all equipment is included and in working order each day before sampling personnel go in the field.

1) Sampling supplies:
    a) Light-weight fishing rod
    b) Squid jigs
    c) Halogen light (optional)

d) Generator (optional)
e) Extension cord (optional)
f) Coolers
g) Ice
h) Re-sealable plastic bags
i) Nitrile gloves (optional)
j) Recreational shellfish harvesting license

2) Safety equipment:
   a) Rain gear
   b) Cellular phone
   c) First aid kit
   d) Headlamp
3) Documentation supplies:
   a) Field notebook and field sheets (with Chain-of-Custody stamp)
   b) Pencils, and markers with indelible ink
   c) Camera

3.3 Sample Delivery and Storage

After sufficient squid have been collected, the organisms will be transported to the sample freezer at King Street Center. The re-sealable plastic bag must be labelled with the field crew names, contents, and sampling date. This includes labelling the outside of the bag and inclusion of an internal label on write-in-the-rain paper. The following day, the squid will be transported on ice to the KCEL and stored frozen at -20°C. At a later date, the samples will be thawed and processed (Section 3.4). Processed samples will be stored frozen at -20°C until analysis.

3.4 Sample Processing and Decontamination

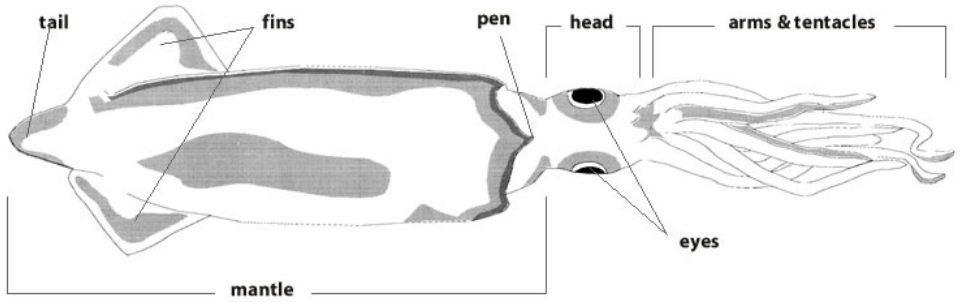

Figure 2. Squid Anatomy (Taken from WDFW website)

Processing will be conducted within 40 days of collection by WLRD personnel, led by Chris Gregersen. Each squid will be partially thawed, removed from the aluminum foil, and length and weight recorded on the sample processing sheet. Then, one at a time, squid will be placed on a decontaminated, aluminum foil-wrapped cutting board with tentacles grouped together away from the mantle (see decontamination steps below). Using a decontaminated knife, the tentacles will be cut from the mantle, just below the eye (Figure 3). The beak will likely remain with the tentacles, close to the cut, where it can be removed by squeezing gently (Figure 3). Then the tentacles will be placed on a new piece of aluminum foil. The mantle will be cut length-wise (Figure 4), then the quill and viscera will be removed by gently scraping. Once these are removed, the mantle will be placed in the foil with the tentacles. The tools will be decontaminated before the next squid is cleaned. These cleaning procedures follow the method provided on the WDFW website for use by recreational fisherpeople.
(http://wdfw.wa.gov/fishing/shellfish/squid/clean_prepare.html).

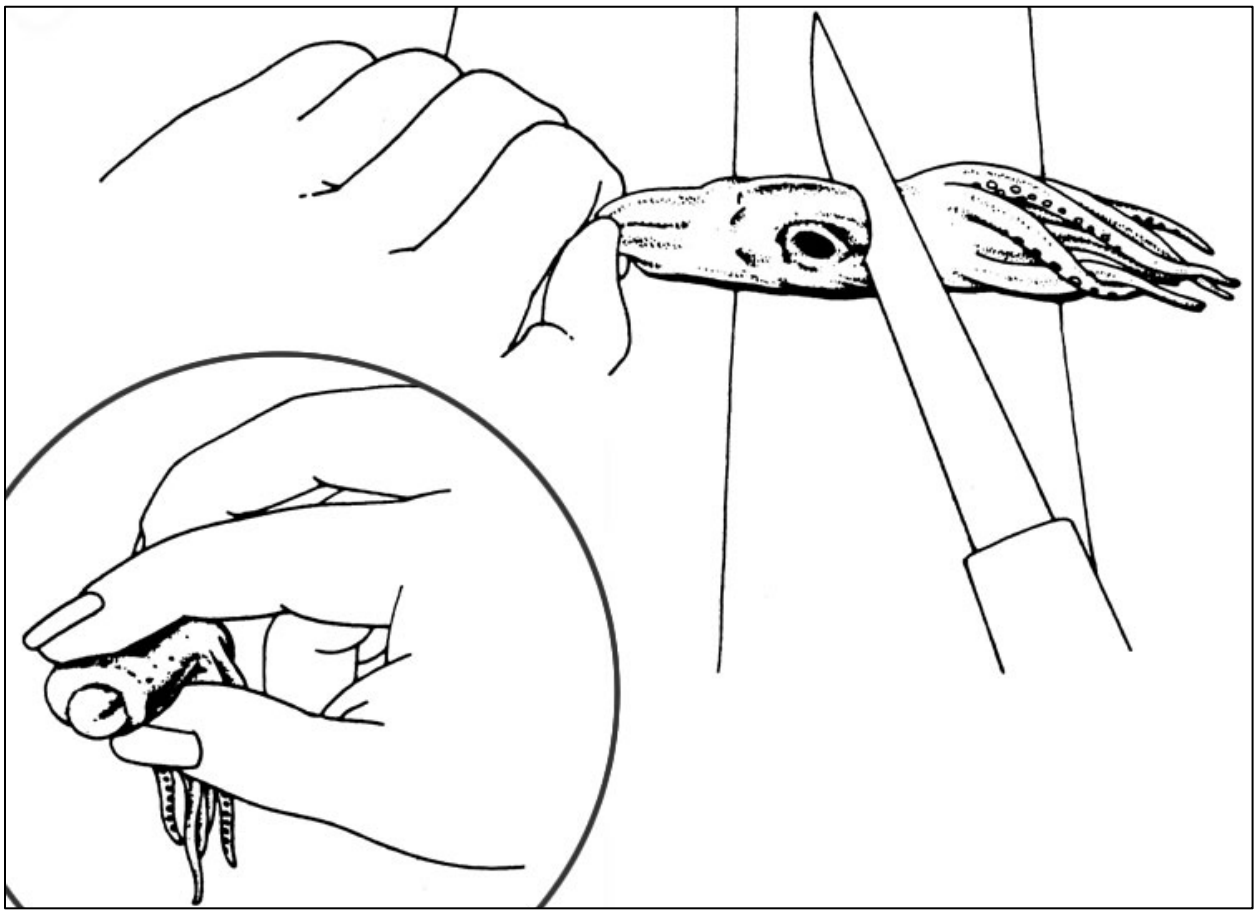

Figure 3. Squid Cleaning: Cutting Tentacles and Removing Beak (Taken from WDFW website).

The following steps will be followed to decontaminate all cutting utensils and surfaces used (e.g., cutting boards). Cutting utensils may be ceramic, stainless steel, or PTFE (Teflon).

1. Wash with a commercial laboratory detergent,
2. Rinse with acetone which should remove the water,
3. Air dry in hood (acetone evaporates quickly).

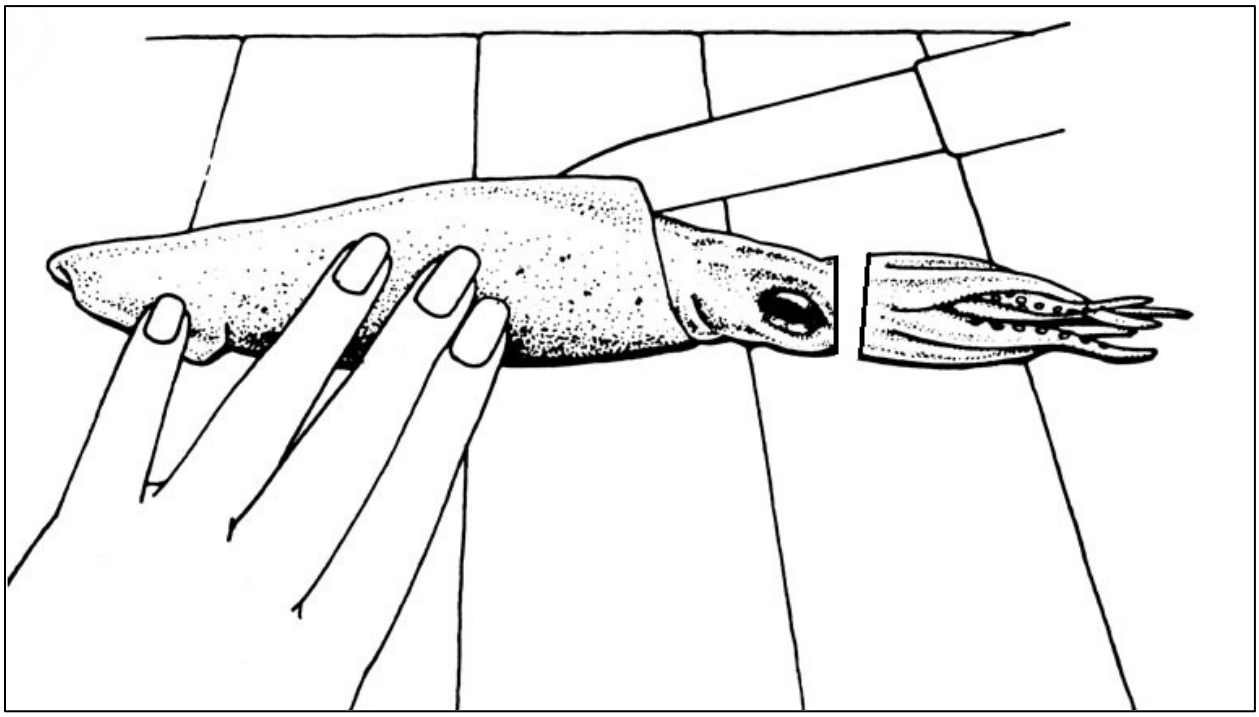

Figure 4. Squid Cleaning: Cutting Mantle (Taken from WDFW website).

After cleaning, the squid will be re-weighed, and the "cleaned" mass recorded on the sample processing sheet. The squid will then be returned to its original aluminum foil and assigned to a composite sample based on the mass requirements. Each composite sample will be held in a separate large plastic bag, and given a unique sample number. Individual squid that go into each composite sample number will be given a unique number and recorded on the sample processing sheet. All composite samples will be stored at -20°C until homogenization.

Homogenization procedures follow the KCEL SOP # 106v1 and recommendations in EPA (2000):

1) Knives should be detergent washed, acetone rinsed, and air dried.
2) The aluminum foil shall be removed from each cleaned squid.
3) The squid will be placed on an aluminum foil-wrapped plastic cutting board which is washed with Alconox® detergent and air dried.
4) The cleaned and pre-cut squid will be homogenized by the meat grinder hopper.

5) Place 1/7th (or 1/x with x being the total number of squid in the composite) of the total mass necessary in an aluminum foil-wrapped boat.
6) Repeat steps 3–5 for each squid.
7) Equal aliquots of squid tissue mass will be combined and reprocessed through the grinder to fully homogenize the sample. Additional homogenization equipment may be used as needed (e.g., hand mixer).
8) The mixture will be divided into appropriate sample containers.
9) Any remaining homogenate will be retained in the original container and archived in the freezer at -20°C.
10) The grinder will be washed with Alconox® detergent and rinsed with copious amounts of DI water after each sample is processed. Any excess water will be allowed to drip off before proceeding to the next sample.

3.5 Chain of Custody

Chain of custody (COC) will commence at the time that the first squid is collected during each sampling event. All samples will be under direct possession and control of King County WLRD personnel. All sample information will be recorded on a COC form, which is the same as the field record sheet (Appendix A). The date and time of sample transfer will be recorded and both parties will sign the COC form. When samples arrive at KCEL, they will be placed in a storage freezer at -20°C until processing. After processing occurs and sample IDs are assigned, samples will be logged into the KCEL LIMS and the COC form will be stamped and signed. After login, original COC forms will be archived in the project file.

3.6 Sample Documentation

Sampling information and sample metadata will be documented using the methods noted below.

- Field sheets will be used at all stations and will include the following information:
  - station name (locator)
  - date and time of sample collection
  - counts of squid taken and returned
  - notes on squid condition
  - initials of all sampling personnel
- Sample processing and compositing sheets (see Appendix B) will be completed for each sample recording station name (locator), LIMS sample ID, lengths and weights of individuals will all be recorded.
- LIMS-generated container labels will identify each container with a unique sample number, station and site names, collect date, analyses required, and preservation method.

- COC documentation will consist of KCEL's standard COC form, which is used to track release and receipt of each sample from collection to arrival at the lab.

3.7 Field Replicates

Field replicates are intended to show within station variability. However, a true field replicate for squid tissue can't be collected because each squid at a station is inherently different (e.g. age, size, diet) and no individuals would be expected to have exactly the same tissue chemistry. Collection and analysis of trip blanks or equipment blanks from jigging equipment is neither practical nor necessary for this project. The amount of contamination from equipment or sample exposure during transport to the laboratory is relatively insignificant compared to the concentrations measured in squid tissue.

3.8 Sample Holding Times

Immediately following collection, squid will be placed in foil and labeled plastic bags on ice in the field. Squid may be temporarily stored by WLRD field personnel, and will be frozen if kept more than 1 day. Once received, squid will be frozen at KCEL until processed. Squid will be processed within 40 days of collection. After processing, the holding times in Table 6 apply to the homogenized samples.

Table 6. Prioritized List of Sample Containers, Storage Conditions, and Analytical Hold Times

Analyte	Container	Preferred		Minimum	
		Preferred Storage Conditions	Hold Time if Stored Frozen	Acceptable Storage Conditions	Hold Time if Refrigerated
Total solids	8-oz. glass	freeze at ≤-18°C	6 months to analyze	refrigerate at 4°C	14 days to analyze
Lipids	16-oz. glass	freeze at ≤-18°C	1 year to extract Analyze immediately after extraction	refrigerate at 4°C	14 days to extract Analyze immediately after extraction
PCBs (Aroclors)	16-oz. glass	freeze at ≤-18°C	1 year to extract 1 year to analyze (if extracts stored at ≤-18°C)	refrigerate at 4°C	14 days to extract 40 days to analyze
Mercury (CVAA)	4-oz. CWM PP container	freeze at ≤-18°C	28 days to analyze	refrigerate at 4°C	28 days to analyze
Total metals (ICP-MS)	4-oz. CWM PP container	freeze at ≤-18 °C	2 years to analyze	refrigerate at 4 °C	180 days to analyze

Analyte	Container	Preferred		Minimum	
		Preferred Storage Conditions	Hold Time if Stored Frozen	Acceptable Storage Conditions	Hold Time if Refrigerated
Pesticides	16-oz. glass	freeze at ≤-18°C	1 year to extract 1 year to analyze (if extracts stored at ≤-18°C)	refrigerate at 4°C	14 days to extract 40 days to analyze
Butyltins	16-oz. glass	freeze at ≤-18°C	1 year to extract 1 year to analyze (if extracts stored at ≤-18°C)	refrigerate at 4°C	14 days to extract 40 days to analyze
PCB Homologs (subset only)	16-oz. glass	freeze at ≤-18°C	1 year to extract 1 year to analyze (if extracts stored at ≤-18°C)	refrigerate at 4°C	14 days to extract 40 days to analyze
BNA	8-oz. glass	freeze at ≤-18°C	1 year to extract 1 year to analyze (if extracts stored at ≤-18°C)	refrigerate at 4°C	14 days to extract 40 days to analyze
PBDEs	16-oz. glass	freeze at ≤-18°C	1 year to extract 1 year to analyze (if extracts stored at ≤-18°C)	refrigerate at 4°C	14 days to extract 40 days to analyze

CWM PP – Clear, wide-mouth polypropylene

4.0. ANALYTICAL METHODS, DETECTION LIMITS AND QUALITY CONTROL

Analytical methods for metals, mercury, PCBs, chlorinated pesticides, butyltins, SVOCs, and conventional parameters are presented in this section, along with analyte-specific detection limits. The method detection limit (MDL) is defined as the *minimum concentration of a chemical constituent that can be reliably determined with 99% confidence to be greater than zero* (40 CFR Part 136.2), while the reporting detection limit (RDL) is defined as *the minimum concentration of a chemical constituent that can be reliably quantified* (also known as the practical quantitation limit) *within specified limits of precision and accuracy during routine laboratory operating conditions* (50 FR 46906, November 13, 1985).

4.1 Conventional Parameters

Analysis of total solids will be conducted by the KCEL Conventionals Unit and will follow KCEL SOP # 307. The MDL is 0.005 % and the RDL is 0.010 % in non-aqueous samples. Total solids will be analyzed according to SM2540-G. Quality control samples for total solids will include method blanks and laboratory replicates.

Lipid analysis will be conducted following KCEL draft SOP # 740v1 by the KCEL Trace Organics Unit. Samples are extracted by the same method used for pesticide analysis. The MDL and RDL for lipids are 0.05 and 0.1 %, respectively.

Conventional QC samples will be analyzed at the frequency of one per QC batch, defined as up to 20 samples analyzed together.

4.2 Total PCBs

For all samples, total PCBs will be analyzed as PCB Aroclors, but a subset of three samples will also be analyzed for PCB Homologs. PCB Aroclor analysis will follow KCEL SOP #757, which is a modification of EPA Method SW846-8082A. Sample preparation is described in SOP#703 v5 for soils, tissues, and sediments. The PCB Aroclor MDLs and RDLs are presented on a wet-weight basis and are based on a 15 g extraction with gel permeation cleanup and concentration to a final volume of 0.5 mL (Table 7). The preparation method used is a soxhlet technique following EPA method SW846-3540C using methylene chloride as the extraction solvent. The extraction is followed up with GPC clean up and an Anthropogenic Column cleanup, KCEL SOP #718 and #783. The PCB Aroclors and homologs will be analyzed using the same extract. PCB Homolog analysis will follow KCEL draft SOP #782 which generally follows the guidelines of EPA methods 680 and 1668C. This method relies on the quantitation of congeners using gas chromatography/mass spectrometry-selected ion monitoring (GC/MS-SIM). This low-resolution method will generate PCB concentrations based on each of the 10 homolog groups. These are listed with their associated MDLs/RDLs for tissue samples in Table 8.

The MDL and RDL for analytes requiring dilution (e.g., exceedance of analyte calibration range or matrix interferences) will be increased to reflect the dilution.

Table 7. PCB Target Analytes and Detection Limit Goals for EPA Methods 3540C/8082A (SW 846) (µg/Kg ww)

Analyte	Wet weight MDL	Wet weight RDL
Aroclor 1016	0.83	3.33
Aroclor 1221	2.5	3.33
Aroclor 1232	2.5	3.33
Aroclor 1242	0.83	3.33
Aroclor 1248	0.83	3.33
Aroclor 1254	0.83	3.33
Aroclor 1260	0.83	3.33

Table 8. PCB Homolog Detection Limit Goals by GC/MS (µg/Kg)

Homolog group	Wet Weight MDL	Wet Weight RDL
Monochlorobiphenyls	0.04	0.083
Dichlorobiphenyls	0.04	0.083
Trichlorobiphenyls	0.04	0.083
Tetrachlorobiphenyls	0.04	0.167
Pentachlorobiphenyls	0.08	0.167
Hexachlorobiphenyls	0.09	0.167
Heptachlorobiphenyls	0.08	0.25
Octachlorobiphenyls	0.11	0.25
Nonachlorobiphenyls	0.11	0.25
Decachlorobiphenyls	0.12	0.25

4.3 Total Mercury

Total mercury will be analyzed according to KCEL SOP # 604v5 using cold vapor atomic absorption spectrometry (CVAA). The mid-range detection limits should be appropriate for this analysis based on historical data for this species. This method retains elements of EPA 245.1 revision 3, SW-846 7470, 7471B and PSEP (1997). Tissue samples require acid digestion before analysis. The following detection limits will be targeted and are calculated on a wet weight basis (Table 9).

Table 9. Mercury Detection Limits (mg/Kg) by CVAA

Analyte / Range	MDL	RDL
Mercury/Mid-Range	0.004	0.016

Every effort will be made to report detectable mercury values. It may be necessary to dilute samples to get them into calibration range, in which case the MDL and RDL will increase by the dilution factor.

4.4 Total Metals

Metals will be analyzed by inductively coupled plasma mass spectrometer (ICP-MS) according to KCEL SOP # 623v1. This method generally follows PSEP (1997) protocols. Tissue samples require acid digestion before analysis. The detection limits will be targeted and are calculated on a wet weight basis (Table 10).

Table 10. Target Metals and Detection Limits (mg/Kg ww) by ICP-MS

Analyte	MDL	RDL
Arsenic	0.004	0.02
Cadmium	0.002	0.01
Chromium	0.008	0.04
Copper	0.016	0.08
Lead	0.004	0.02
Nickel	0.004	0.02
Silver	0.002	0.01
Zinc	0.02	0.1

4.5 Pesticides

Pesticides will be analyzed under KCEL SOP #733 following EPA Solid Waste Method SW846-8081A by GC/ECD. Sample preparation is described SOP #705 for soils, tissue, and sediments. The preparation method uses a soxhlet technique following EPA method SW846-3540C and methylene chloride as the extraction solvent (samples analyzed for percent lipids, PBDEs, and pesticides are processed in in one extraction). Before cleanups are performed, half of the extract will be set aside for lipid analysis. Gel permeation cleanup (GPC) and Alumina cleanups will be performed on the remaining half per KCEL SOP #718 and 719 respectively. Half of this remaining extract will be used for pesticide analysis and the remaining half will have TBA and Acid cleanups performed per KCEL SOP #721 and 720 respectively for PBDEs. Following cleanup, each extract will be vialed at 0.5 mL for PBDEs and 0.5 mL for pesticides, respectively.

The detection limit goals are presented in Table 11. MDLs/RDLs are calculated on a wet weight basis using 15 g of sample to 2 mL final volume, not corrected for lipid content or total solids. The MDLs and RDLs for specific analytes requiring dilution (e.g., exceedance of analyte calibration range or matrix interferences) will be increased to reflect the dilution.

Table 11. Pesticide Target Analytes and Detection Limit Goals in µg/Kg ww.

Parameter	MDL	RDL
Alpha-BHC	0.7	1.33
Beta-BHC	0.7	1.33
Delta-BHC	0.7	1.33
Gamma-BHC (Lindane)	0.7	1.33
Heptachlor	0.7	1.33
Aldrin	0.7	1.33
Heptachlor Epoxide	0.7	1.33
Endosulfan I	0.7	1.33
Dieldrin	0.7	1.33
4,4'-DDE	0.7	1.33
Endrin	0.7	1.33
Endrin Ketone	0.7	1.33
Endosulfan II	0.7	1.33
4,4'-DDD	0.7	1.33
Endrin Aldehyde	0.7	1.33
Endosulfan Sulfate	0.7	1.33
4,4'-DDT	0.7	1.33
Methoxychlor	3.3	6.66
trans-Chlordane	0.7	1.33
Alpha-Chlordane	0.7	1.33
Toxaphene	13.33	66.65

4.6 Butyltins

Butyltin analysis will be performed according to Krone et al. 1989 and EPA method 8270D SIM (SW 846), which employs solvent extraction with tumbling and analysis by GC/MS in SIM mode. The detection limit goals for the target butyltin compounds are summarized in Table 12. The MDLs and RDLs are presented on a wet weight basis and are based on a 5 g extraction and a final volume of 1.0 mL for analysis.

The MDLs and RDLs for specific analytes requiring dilution (e.g., exceedance of analyte calibration range or matrix interferences) will be increased to reflect the dilution.

Table 12. Butyltin Compound Target Analytes and Detection Limit Goals (µg/Kg ww).

Parameter	MDL	RDL
Mono-n-Butyltin (as monobutyltin ion)	10	20
Di-n-Butyltin (as dibutyltin ion)	6.0	10
Tri-n-Butyltin (as tributyltin ion)	3.0	6.0
Tetra-n-Butyltin (as tetrabutyltin)	4.0	10

4.7 BNAs

BNA analysis will be performed according to EPA methods 3540B/8270D (SW 846), which employs solvent extraction with soxhlet and analysis by GC/MS in a full scan/SIM mode.

The detection limit goals for the target BNA compounds are summarized in Table 13. The MDLs and RDLs are presented on a wet weight basis and are based on a 30 g extraction with GPC and concentration to a final volume of 1.0 mL for analysis.

The MDL and RDL for specific analytes requiring dilution (e.g., exceedance of analyte calibration range or matrix interferences) will be increased to reflect the dilution.

Table 13. BNA Target Analytes and Detection Limit Goals for EPA Method 3540B/8270D(SW 846) (µg/Kg ww)

Parameter	MDL	RDL
1,2,4-Trichlorobenzene	0.33	0.667
1,2-Dichlorobenzene	3.3	3.33
1,4-Dichlorobenzene	5.0	5.00
1-Methylnaphthalene	3.3	6.67
2,4-Dimethylphenol	3.3	6.67
2-Methylnaphthalene	3.3	6.67
2-Methylphenol	3.3	6.67
3-,4-Methylphenol	16.7	33.3
Acenaphthene	3.3	6.67
Acenaphthylene	3.3	6.67
Anthracene	3.3	6.67
Benzo(a)anthracene	3.3	6.67
Benzo(a)pyrene	3.3	6.67
Benzo(b,j,k)fluoranthene	3.3	6.67
Benzo(g,h,i)perylene	3.3	6.67
Benzoic Acid	67	66.7
Benzyl Alcohol	8.3	8.33
Benzyl Butyl Phthalate	5.0	5.00
Bis(2-Ethylhexyl)Phthalate	6.7	13.3

Parameter	MDL	RDL
Carbazole	3.3	6.67
Chrysene	3.3	6.67
Dibenzo(a,h)anthracene	3.3	6.67
Dibenzofuran	3.3	6.67
Diethyl Phthalate	6.7	13.3
Dimethyl Phthalate	6.7	6.67
Di-N-Butyl Phthalate	6.7	13.3
Di-N-Octyl Phthalate	6.7	6.67
Fluoranthene	3.3	6.67
Fluorene	3.3	6.67
Hexachlorobenzene	0.33	0.67
Hexachlorobutadiene	1.7	3.33
Indeno(1,2,3-Cd)Pyrene	3.3	6.67
Naphthalene	3.3	6.67
N-Nitrosodiphenylamine	8.3	8.33
Pentachlorophenol	50	50.0
Phenanthrene	3.3	6.67
Phenol	17	50.0
Pyrene	3.3	6.67

4.8 PBDEs

PBDE analysis will be performed according to EPA method 8270D NCI (SW 846), which employs analysis by GC/MS in NCI (negative chemical ionization) SIM mode, KCEL SOP #781. Sample preparation is described in KCEL SOP# 705 for soils, tissue, and sediments. The preparation method used is a soxhlet technique following EPA method SW846-3540C using methylene chloride as the extraction solvent. PBDEs will be co-extracted with chlorinated pesticides and percent lipids (See Section 4.5 above).

The PBDE MDLs and RDLs are presented on a wet weight basis and are based on a 15 g extraction with GPC and concentration to a final volume of 2.0 mL (Table 14).

The MDL and RDL for specific analytes requiring dilution (e.g., exceedance of analyte calibration range or matrix interferences) will be increased to reflect the dilution.

Table 14. PBDE Target Analytes and Detection Limit Goals for EPA Methods 3540B/8270D NCI (SW 846) (µg/Kg ww)

Analyte	MDL	RDL	Analyte	MDL	RDL
PBDE-17	0.03	0.053	PBDE-100	0.07	0.15
PBDE-28/-33	0.03	0.053	PBDE-138	0.03	0.053
PBDE-47	0.24	0.48	PBDE-153	0.03	0.053

Analyte	MDL	RDL	Analyte	MDL	RDL
PBDE-66	0.04	0.077	PBDE-154	0.03	0.059
PBDE-71	0.03	0.053	PBDE-183	0.03	0.053
PBDE-85	0.03	0.053	PBDE-190	0.03	0.053
PBDE-99	0.45	0.91	PBDE-209	0.33	0.67

4.9 Laboratory Quality Control

All samples for this project will be run in one analytical batch (20 samples); therefore, only one set of QC samples will be run for each analysis. Laboratory QC samples include: method blanks, laboratory replicates, matrix spikes, spike blanks, and standard reference materials when available. The laboratory QC sample requirements for each analysis are summarized in Table 15. The recommended QC limits for tissue analysis are summarized in Table 16.

Table 15. Minimum QC Samples by Analysis

Parameter	Blank [a]	Duplicate [b]	Matrix Spike	SRM [c]	Surrogates	Spiked Blank
Total Solids	1 Per Batch	1 Per Batch	No	No	No	No
Total Lipids	1 Per Batch	1 Per 10 Samples	No	No	No	No
PCB Aroclors	1 Per Batch	No	2 Per Batch [d] (MS/MSD)	No	Yes [f]	1 Per Batch
Mercury	1 Per Batch	1 Per Batch [e]	1 Per Batch	Yes	No	1 Per Batch
Metals (other than mercury)	1 Per Batch	1 Per Batch	1 Per Batch	Yes	No	1 Per Batch
Pesticides	1 Per Batch	No	2 Per Batch [d] (MS/MSD)	Yes	Yes	1 Per Batch
Butyltins	1 Per Batch	No	2 Per Batch [d] (MS/MSD)	No	Yes	1 Per Batch
PCB Homologs	1 Per Batch	No	2 Per Batch [d] (MS/MSD)	No	Yes [f]	1 Per Batch
BNAs	1 Per Batch	No	2 Per Batch [d] (MS/MSD	No	Yes	1 Per Batch
PBDEs	1 Per Batch	No	2 Per Batch [d] (MS/MSD	Yes	Yes	1 Per Batch

[a] Batch – A group of samples analyzed together for QC purposes containing a maximum of 20 samples.
[b] Duplicate – Triplicate analysis for all conventional parameters, duplicate analysis for metal and organic parameters.
[c] SRM – Standard reference material (must be certified by NIST or NRCC).
[d] MS/MSD – matrix spike/matrix spike duplicate; analyzed if sample volume allows, otherwise a spiked blank duplicate will be analyzed.
[e] Mercury samples are analyzed with a MS/MSD.
[f] Only 1 surrogate (TCX) analyzed due to co-extraction of PCB Aroclor and PCB Homolog analysis. DCB is a target analyte for PCB Homologs.

Table 16. Recommended Chemistry QC Limits for Tissue Samples

Parameter	Blank [a]	RPD [b]	Matrix Spike [c]	SRM [d]	Surrogates	Spiked Blank
Total Solids	< MDL	≤ 20%	N/A	N/A	N/A	N/A
Total Lipids	< MDL	< 20%	N/A	N/A	N/A	N/A
PCB Aroclors	< MDL	≤ 35%	Lab Derived QC Limits	N/A	Lab Derived QC Limits	Lab Derived QC Limits
Mercury	< MDL	≤ 20%	75 - 125%	80 to 120%	N/A	85 to 115%
Metals (other than mercury)	< MDL	≤ 20%	75 - 125%	Limits to be derived on DORM3 before project start. Not all metals will have SRM limits.	N/A	85 to 115%
Pesticides	<MDL	≤ 35%	Lab Derived QC Limits	50 to 150%	Lab Derived QC Limits	Lab Derived QC Limits
Butyltins	<MDL	<100%	Lab Derived QC Limits	N/A	Lab Derived QC Limits	Lab Derived QC Limits
PCB Homologs	< MDL	< 35%	Lab Derived QC Limits	N/A	Lab Derived QC Limits	Lab Derived QC Limits
BNAs	< MDL	< 35%	Lab Derived QC Limits	N/A	Lab Derived QC Limits	Lab Derived QC Limits
PBDEs	<MDL	≤ 40%	Lab Derived QC Limits	50 to 150%	Lab Derived QC Limits	Lab Derived QC Limits

[a] Concentration of all analytes should be < MDL.
[b] Relative percent difference (RPD) for duplicate analysis and percent relative standard deviation (%RSD) for triplicate analysis.
[c] Percent recovery for matrix spike, standard reference material, and surrogates.
[d] If SRM is available.

5.0. DATA ASSESSMENT, REPORTING AND RECORD KEEPING

KCEL will conduct a standard data assessment. Full documentation will also be maintained on record should a more detailed review be necessary.

5.1 Data Assessment

Data assessment is critical to evaluate how well analytical data meet project DQOs. Data assessment is performed, at some level, during several steps in the process of sample analysis. Data assessment will also be performed by the KCEL QA Officer for this program by reviewing complete data packages supplied by the KCEL. Data assessment memoranda will be produced and maintained along with the analytical data as part of the project records.

5.2 Reporting

A final data report will be prepared that will include a presentation and interpretation of the squid tissue chemistry results. The results will be compared to fish tissue-equivalent concentrations of EPA-promulgated human health-based water quality criteria applicable to Washington (81 FR 85417-85436). All data and supporting information will be available for review upon request. Analytical data reports may be requested in electronic formats in Microsoft Excel spreadsheets or PDF format. Data assessment memoranda will be available electronically in Microsoft Word or PDF format. A data report will be produced that contains copies of field sheets/COC forms, sample processing and compositing data sheets, sample data results, and QC information. The data report will be available in electronic format.

5.3 Record Keeping

All hard-copy field sampling records, custody documents, raw lab data, and laboratory summaries and narratives will be archived according to KCEL policy for a minimum of 10 years from the date samples were collected. Analytical data produced by KCEL will be maintained on its LIMS database in perpetuity.

6.0. REFERENCES

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APPENDIX A: FIELD RECORD SHEET

Field Record for 2016 Squid Tissue Monitoring Event

Date of Collection: _____
Approximate Time: _____
Sampling Location: _____

Number of Squid Caught and Retained (>35 grams) _____

Number of Squid Released _____

Observations (Squid Conditions):

Notes:_____
_____
_____
_____

Field Personnel: _____

Chain of Custody:

Relinquished By: _____ _____ Date: _____
Print Name Signature

Accepted By: _____ _____ Date: _____
Print Name Signature

APPENDIX B: SAMPLE PROCESSING AND COMPOSITING SHEETS

Sample Processing for 2016 Squid Tissue Monitoring Event

Date of Collection: _____
Locator: _____

Individual # (sequential)	LIMS Sample ID	Total Length (mm)	Whole Body Mass (g)	"Cleaned" Mass (g)
1				
2				
3				
4				
5				
6				
7				
8				
9				
10				
11				
12				
13				
14				
15				
16				
17				
18				
19				
20				
21				
22				
23				
24				
25				
26				
27				
28				
29				
30				

Notes:_____
_____
_____
_____

Personnel: _____