2016 Squid Contaminant Data Report

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King County

Department of Natural Resources and Parks
Water and Land Resources Division
Science and Technical Support Section
King Street Center, KSC-NR-0600
201 South Jackson Street, Suite 600
Seattle, WA 98104
206-477-4800 TTY Relay: 711
www.kingcounty.gov/EnvironmentalScience

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Submitted by:

Rory O'Rourke and Jenée Colton
King County Water and Land Resources Division
Department of Natural Resources and Parks

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Table of Contents

Figures

Tables

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EXECUTIVE SUMMARY

Background

Contaminants in squid are of interest in Puget Sound because of its high importance in recreational and tribal fisheries. Because a boat is not needed and jigging equipment is affordable to many, squid-jigging is one of the most inexpensive and popular ways to catch squid, providing access to a low-cost food for a diversity of anglers. Market squid may become an even more important fishery in Puget Sound as climate change facilitates their migration northward.

What is this report about?

This report presents results of the first effort since 1997 by King County Water and Land Resources Division (WLRD) to monitor contaminants in market squid (*Doryteuthis [Loligo] opalescens*). The following contaminants were measured in squid: butyltins, metals, polybrominated diphenyl ethers (PBDEs), polychlorinated biphenyls (PCBs), chlorinated pesticides, and other semivolatile organic chemicals. In 2016, King County scientists collected squid from two piers in King County that are popular destinations for recreational squid anglers: Pier 86 and Redondo Pier. The objective was to implement King County's Tissue Monitoring Program Work Plan goals which are to understand how fish and shellfish health may be harmed by exposure to chemicals, to measure chemical contaminants in species consumed by local fishers, and to evaluate changes in chemical body burdens in fish and shellfish over time as water quality improvements are made.

Is a consumption advisory warranted?

With the exception of PCBs, none of the chemical concentrations measured in 2016 exceeded the Washington State Department of Health (WDOH) high consumer or general population screening levels for metals or organics. PCB concentrations exceeded the high consumer screening level, which at a consumption rate of 14 or more ounces per week, may be a concern for human health.

Which contaminants were detected in squid?

Eight metals (arsenic, cadmium, chromium, copper, lead, mercury, nickel, silver, and zinc), PCBs, PBDEs, benzoic acid and bis(2-ethylhexyl)phthalate (BEHP) were detected in one or more squid samples.

Which Pier has the cleanest squid?

For most contaminants, levels in squid from Seattle Pier 86 were higher than in squid from Redondo Pier in 2016. However, mercury, total PBDE and BEHP concentrations were higher in squid collected from Redondo Pier compared to those collected from Seattle Pier 86. This pattern is consistent with squid north-to-south Sound migration route because accumulation of contaminants would increase, not decrease, as squid move through Elliott

Bay south to Redondo. However, cadmium, chromium, copper, nickel, and silver concentrations were higher at Seattle Pier 86. The higher concentrations of metals may reflect sources of these metals in Elliott Bay and the surrounding area. The specific cause of the differences in metals concentrations between these two locations is unknown. Concentrations of PCBs, arsenic, lead zinc and benzoic acid were similar in squid from Redondo Pier and Seattle Pier 86. Pesticides and butyltins were not detected in squid at either location.

Have contamination levels declined since 1997?

Overall, arsenic, chromium, mercury, isophorone and tributyltin (TBT) concentrations were lower in 2016 than 1997, while copper and zinc concentrations were higher in 2016. Total PCB levels were comparable. Samples collected in 1997 were not analyzed for pesticides and PBDEs. Isophorone concentrations decreased, but BEHP and benzoic acid increased between 1997 and 2016.

What are the implications of this monitoring information?

Given the low contaminant concentrations measured in squid and results of the comparison to WDOH human health screening levels, we conclude that market squid from the sampled locations contain relatively low levels of contamination. As a result, we recommend that WDOH evaluate these findings to issue a determination on whether squid are a safe choice for consumers to eat. Inferences based on this monitoring effort are limited to the sampling sites, as they are not necessarily representative of squid at other locations in Puget Sound.

1.0 INTRODUCTION

1.1 Background

This report presents the chemistry results for market squid (*Doryteuthis [Loligo] opalescens*) collected in 2016 from Elliott Bay and Redondo Pier. The overall objective of this sampling effort is to advance the goals outlined in King County's Tissue Monitoring Program Work Plan (King County 2016a) (Work Plan). The findings reported here contribute to advancing two of the tissue monitoring program goals: (1) understand the type and concentration of chemicals in shellfish that could be consumed by local fishers, and (2) understand the impact of chemical exposures on the health of marine invertebrates in local King County waters.

The Tissue Monitoring Program includes annual collection and analysis of marine species from Elliott Bay and the Central Basin of Puget Sound within King County boundaries. This report presents results of the first effort by King County Water and Land Resources Division (WLRD) to monitor contaminants in squid tissue since 1997. Squid tissue chemistry data from 1997 (King County and Parametrix 1999) provide an opportunity to determine how contaminant levels have changed in nearly 20 years.

1.2 Why Did We Sample Squid?

Contaminants in squid are of particular interest in Puget Sound because of squid's high importance in recreational and tribal fisheries. Washington Department of Fish and Wildlife (WDFW) regulations allow for daily collection of up to 10 pounds of squid per harvester (WDFW 2017). Because a boat isn't needed and cost of equipment is minimal, jigging is one of the most inexpensive ways to catch squid (WDFW 2019), thereby providing access to an economic food source for low-income communities. Because low-income communities are often exposed to relatively higher health risks from environmental pollution, we believe it is important to investigate this potential food source. Subsistence tribal fishery regulations exist for market squid as well (Point No Point Treaty Council 2017).

In addition to serving King County, findings from this monitoring effort also serve the needs of state and regional organizations, such as the Washington State Department of Health (WDOH), WDFW, and the Puget Sound Partnership. For example, these data can be used by public health agencies to update consumption advisories, as necessary. These data also indicate how concentrations of measured contaminants in Elliott Bay squid have changed since 1997 (King County and Parametrix 1999).

1.3 Squid Life History

Market squid, also known as opalescent and Pacific squid, is a species commonly caught in Puget Sound in the fall/winter. Squid are mollusks with eight arms and two feeding tentacles. The range for market squid spans from southeastern Alaska to Baja California, Mexico. Commercial fishery data indicate that market squid can grow to 7.9 inches (20 centimeters) in mantle length and weigh up to 5 ounces (144 grams [g]) (CDFW 2008). Adult market squid found in Puget Sound waters average about eight inches total length (mantle plus tentacles) (WDFW website). Their lifespan is short, spanning six to nine months (CDFW 2008), limiting their potential for bioaccumulation of contaminants. Squid are a nearshore species found within 200 miles (322 kilometers) of shore (CDFW 2008). Although they are generally considered pelagic (open water-dwelling), market squid are found over the continental shelf from the surface to depths of 2,300 feet [ft] (700 meters [m]) (CDFW 2008). Adult market squid move into deeper water during the day, but return to surface waters at night within the upper 295 ft (90 m) of the water column to feed (CDFW 2008). Market squid occupy the middle trophic level as active predators of copepods, euphausiids, and fish, and are a principal forage species preyed on by many fishes, birds and marine mammals (CDFW 2008). Adult market squid only spawn at the end of their life cycle (CDFW 2008).

1.4 Study Locations

This monitoring event included collection of market squid from two public fishing piers: Pier 86 in Elliott Bay and Redondo Pier near Des Moines (Table 1, Figure 1). Two recent *Seattle Times* articles indicate that these piers are popular public squid jigging locations in King County (Yuasa 2015 and 2016). The two piers are also located in urban areas near potential contaminant sources.

Table 1. Locator ID and general coordinates for each sampling location.

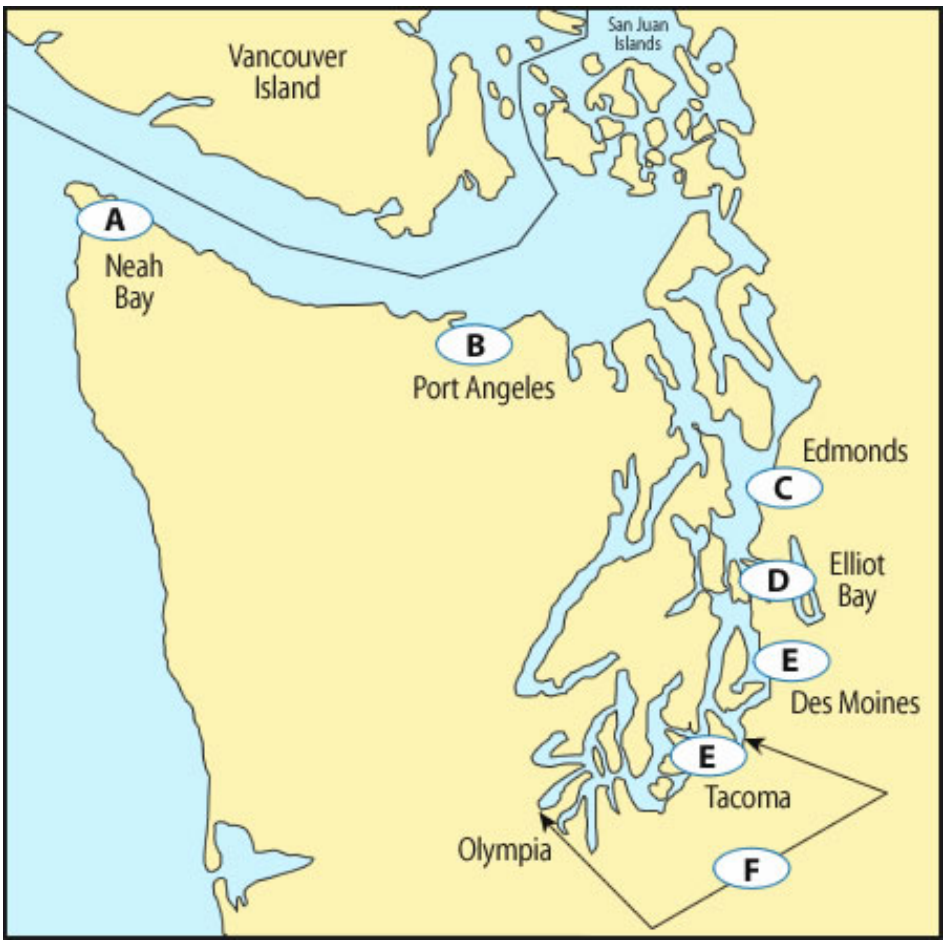
Location	Locator ID	Description	Coordinates in State Plane North NAD83 (US Survey Feet)	
			X	Y
Seattle Pier 86	SEA_PIER86	Elliott Bay fishing pier at Terminal 86	1260471	232125
Redondo Pier	REDONDO_PIER	Poverty Bay fishing pier in Redondo	1270511	130755

1.5 How Can Squid Become Contaminated?

Market squid are migratory, and as adults, travel south through the Strait of Juan de Fuca to southern Puget Sound (Figure 2). Unlike more sedentary marine animals, chemical concentrations in squid are expected to reflect contaminant exposure beyond just the collection site. However, squid that move through highly 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 have potentially also been exposed to higher chemical concentrations there.

Figure 1. 2016 squid sampling stations.



A Squid are usually first seen in Neah Bay in late May

B Squid present at City Pier and surrounding area from late June to the end of August

C Squid appear near Edmonds waterfront starting about September

D Squid in Elliott Bay and surrounding Seattle shoreline

E Squid appear in Des Moines and Tacoma in late November and December

F Squid likely throughout South Puget Sound in December and January

Figure 2. Migration pattern of squid in the Strait of Juan de Fuca and Puget Sound (figure and description courtesy of WDFW).

This report presents a summary of the field sampling methods, sample processing, and analytical procedures (Section 2.0). Subsequent sections present results (Section 3.0), including a comparison to data from a previous King County study (King County and Parametrix 1999) in 1997 (Section 3.8) as well as conclusions (Section 4.0).

2.0 SAMPLING AND ANALYSIS METHODS

2.1 Field Sampling Methods

2.1.1 Sampling Collection

Squid were collected from Seattle Pier 86 on October 11, 2016, and from Redondo Pier on November 1. All squid were collected under a standard recreational shellfish harvesting license, with permission for scientific collection obtained by WDFW (Velasquez Pers. Comm. 2016).

Sampling took place on the piers after dark, around the high tide. A light-weight rod and reel equipped with up to two squid jigs was lowered into the water. A 1,000-watt halogen light powered by a portable 2,000-watt Honda generator was used to attract squid to the sampling location at Redondo Pier. A light was not needed at Seattle Pier 86 since there was enough illumination from other nearby jiggers at the sampling location.

Squid grabbed the jig with their tentacles, and when one was felt on the line, a quick upward jerk of the rod was used to hook the squid. Once hooked, the squid were quickly reeled into the dock. Squid were not weighed in the field, but the field biologist assessed whether they were approximately adult size and should be retained for analysis. None of the squid collected had noticeable lesions, tumors or other visible health problems. Each squid was securely wrapped in aluminum foil and placed in a large resealable plastic bag. The bagged squid were kept in a cooler on ice for the duration of the sampling event.

At the end of each sampling night, the specimens were transported to the sample freezer at King Street Center for overnight storage. The next day, samples were put into coolers on ice for transport to the King County Environmental Lab (KCEL), where they were stored frozen at -20˚C until processing and homogenization.

In total, 34 individual squid were collected from Seattle Pier 86 and 33 individual squid were collected from Redondo Pier (Table 2). Collected squid had a mean total length of 169 mm and a mean total whole-body mass of 43.8 g.

Table 2. Mean total mass and total length by sample location.

Sample Location	# of Individual Squid per station	Mean standard length[1] (mm)	Mean total whole-body mass (pre-cleaned) (g)
Seattle Pier 86	34	161	39.5
Redondo Pier	33	177	48.4
Total	67	169	43.8

1 = Standard length was measured from the tip of the tail down to the unstretched length where the majority of arms rested.

2.2 Sampling Processing and Homogenization

Processing was conducted by WLRD personnel within a few weeks after squid collection according to procedures outlined in the Sampling and Analysis Plan (SAP) (King County 2016b). Each squid was partially thawed, removed from the aluminum foil, and total length and weight recorded on the sample processing sheet. Standard length was measured from the tip of the tail down to the unstretched length where the majority of arms rested. In future years, mantle length should also be recorded since this measure has been used frequently in stock and growth assessments (CDFW 2008, Yang et al. 1986). Mantle length is a more reliable and replicable measure of size used by cephalopod scientists since it is less susceptible to stretching of the arms and tentacles. After measuring length, squid were placed one at a time on a decontaminated, aluminum foil-wrapped cutting board with tentacles grouped together away from the mantle. Using a decontaminated knife, the tentacles were cut from the mantle, just below the eye (Figure 3). The beak was removed by squeezing gently. The mantle was cut length-wise, then the quill and viscera were removed by gently scraping. The mantle was then placed in the foil with the tentacles. The tools were decontaminated before the next squid was cleaned. The cleaning procedures uses here are based on the method recommended by WDFW (https://wdfw.wa.gov/fishing/shellfish/squid/clean_prepare.html) for use by recreational anglers. However, these cleaning procedures may differ from other anglers, especially those from ethnic communities.

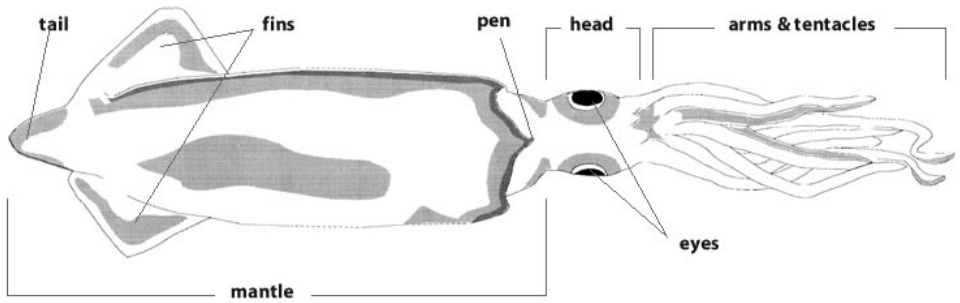


Figure 3. Squid anatomy (from WDFW website).

After cleaning, the squid was re-weighed, and the "cleaned" mass recorded on the sample processing sheet. The squid was then assigned to a composite sample based on the mass requirements. Each composite sample was given a unique sample number. All composite samples were stored at -20°C until homogenization.

Squid "cleaned mass" of mantle and tentacles were cut into cubes. Squid were cut into quarters lengthwise and then cut widthwise across the mantle and arms and tentacles. Cubes of cleaned mass from individual squid were combined and added to the sample jar until the individual target mass was reached. This step was repeated for each individual squid until the target composite mass was achieved and each individual squid had equal mass representation in the composite. Once sufficient composite sample mass was achieved, the tissue was homogenized using the handheld Tissumizer®. The sample jar was given a sample ID label and placed into storage at -20°C. The Tissumizer® was

decontaminated with laboratory detergent and rinsed with copious amounts of DI water after each composite sample was processed.

Four composite samples of 7-10 squid from each location were created (Table 3). The smaller individual squid required more squid per composite, while larger squid included fewer squid per composite.

Table 3. Compositing scheme by sample location.

Sample Location	# of Individual Squid per station	# of Individual Squid per Composite	Total Composite Samples
Seattle Pier 86	34	7-10	4
Redondo Pier	33	7-10	4
Total	67		8

2.3 Laboratory Methods

Squid samples were analyzed by KCEL for conventional parameters (lipids and total solids), as well as metals (arsenic, beryllium, cadmium, chromium, copper, lead, nickel, selenium, silver, thallium, and zinc), mercury, polychlorinated biphenyls (PCBs) (measured as homologs and Aroclors), polybrominated diphenyl ethers (PBDEs), pesticides, butyltins, and Base/Neutral/Acid extractable compounds (BNAs). The following section summarizes the laboratory methods; more detail is presented in the SAP (King County 2016b).

2.3.1 Conventional Parameters

Total solids were analyzed according to Standard Method 2540-G and followed KCEL Standard Operating Procedure (SOP) #307. Lipid analysis was conducted following KCEL draft SOP #740. Samples for lipid analysis were extracted by the same method as was used for PCBs.

2.3.2 Metals and Mercury

Total mercury was analyzed according to KCEL SOP #604 using cold vapor atomic absorption spectrometry (CVAA). This method retains elements of EPA 245.1 revision 3, SW-846 7470, 7471B and PSEP (1997). Tissue samples require acid digestion before analysis.

Other metals were analyzed by inductively coupled plasma mass spectrometer (ICP-MS) according to KCEL SOP #623. This method generally follows PSEP (1997) protocols.

2.3.3 PCBs

PCBs were analyzed by two methods: the Aroclor method and a low resolution homolog method. PCB Aroclor analysis followed KCEL SOP #757, which is a modification of EPA

Method SW846-8082A. Sample preparation is described in SOP #705. The preparation method was a modified Soxhlet extraction with 100% methylene chloride as the solvent following EPA Method SW846-3540C.

The PCB homolog analysis followed KCEL SOP #782. The PCB homolog extraction method was based on EPA Method 1668C using a Soxhlet extraction and 100% methylene chloride as the solvent (KCEL SOP #705). Two cleanups were also performed: Gel Permeation Chromatography (GPC) (KCEL SOP #718) followed by an Anthropogenic Isolation Column cleanup (which is also from EPA Method 1668C) (KCEL SOP #783).

The PCB homolog analysis uses EPA Method 680 and 1668C as guidelines. PCB homologs are analyzed by a low-resolution method gas chromatograph/mass spectroscopy—selective ion monitoring (GC/MS-SIM) that generates PCB concentrations based upon each of the 9 homolog groups—mono- through nona-chlorobiphenyls (KCEL SOP #782).

2.3.4 PBDEs

PBDE analysis was 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 followed KCEL SOP #705 for soils, tissue, and sediments. The preparation method was a Soxhlet technique following EPA Method SW846-3540C using methylene chloride as the extraction solvent. PBDEs were co-extracted with chlorinated pesticides and lipids (see Sections 2.3.1 and 2.3.5),

2.3.5 Pesticides

Pesticides were 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 lipids, PBDEs, and pesticides are processed in one extraction).

2.3.6 Butyltins

Butyltins were analyzed according to KCEP SOP #714 and #742, which is a modification of Krone et al. 1989 and EPA Method 8270D SIM. This method employs solvent extraction with tumbling and analysis by GC/MS in SIM mode.

2.3.7 BNAs

BNA compound analysis were 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.

2.4 Deviations from SAP

The SAP called for the cleaned and pre-cut squid to be processed in a meat grinder hopper. However, the meat grinder was not used to homogenize the squid. The squid "cleaned mass" of mantles and tentacles were cut into cubes. Squid were cut into quarters lengthwise and then cut widthwise across the mantle and arms and tentacles. Cubes of cleaned mass from individual squid were combined in the composite sample jar until individual target mass was reached. This process was repeated for each individual squid until the target composite mass was achieved and each individual squid had equal mass in the composite. The tissue mass was then homogenized using the handheld Tissumizer® to minimize tissue mass loss. The meat grinder would have been more challenging to use for such a small tissue mass and would have resulted in more sample loss within the grinder's parts. This deviation is not expected to substantially affect the analytical results.

The method detection limits (MDLs) for six samples were raised for HeptaBDE-183 and DecaBDE-209 due to sample dilution and matrix inferences. This is not expected to impact the results because these PBDE congeners are not substantial components of the total PBDE concentration. The MDLs and reporting detection limits (RDLs) for many BNAs were also increased due to sample dilution and matrix interferences. This may have resulted in non-detects at concentrations of BNAs that were higher than in 1997.

3.0 RESULTS

Squid tissue chemistry results are presented by location and presented as wet weight concentrations. Associated quality assurance/ quality control (QA/QC) information is summarized in each section. Section 3.1 to 3.7 sequentially describe results for lipids, PCB concentrations by both the Aroclor and homolog methods, PBDEs, metals and mercury, pesticides, butyltins, and BNA compounds. Section 3.8 compares 2016 results to results from a 1997 King County study (King County and Parametrix 1999).

3.1 Lipids

The analytical results for lipids met all lab QA/QC limits. Lipid content (Table 4) ranged from 2.83 to 3.27% (mean = 3.01%) across all squid composite samples and locations.

Table 4. Lipids by sample location.

Sample Location	FOD	Min	Max	Mean	Std. Dev.
Seattle Pier 86	4/4	2.83	3.27	3.03	0.19
Redondo Pier	4/4	2.93	3.09	3.00	0.08
All Locations	8/4	2.83	3.27	3.01	0.13

FOD = frequency of detection
Std. Dev. = Standard deviation on the mean

3.2 Metals and Mercury

The analytical results for metals and mercury met all acceptable lab QA/QC limits except for the following exceptions. Samples L66682-1 through -8 were held at -20c approximately eight weeks past the suggested hold time of 28 days. It is unknown what effect exceeding the suggested hold time had on the reported mercury results. The method blank for all samples had a copper concentration greater than the MDL. Since all copper results were greater than ten times the observed method blank result, no corrective actions were taken. Laboratory control samples exceeded the lab acceptance criteria for chromium; therefore, chromium results may be biased low.

All targeted metals except lead were detected in all squid tissue composites samples. Lead was only detected in five of eight samples, two from Seattle Pier 86 and three from Redondo Pier.

Based on visual inspection, cadmium, copper and silver concentrations were higher at Seattle Pier 86 than at Redondo Pier. The specific cause of these differences is unknown. Mercury concentrations were higher at the Redondo Pier than the Seattle Pier 86 (Table 5). This finding would be consistent with the exposure resulting from the migratory path and lifespan described by WDFW, since it suggests that squid collected from Redondo Pier are older and, therefore, have greater potential to bioaccumulate mercury.

Table 5. Summary statistics for metal and mercury concentrations (mg/kg) detected in squid composite samples by location.

Metal	Sample Location	FOD	Min	Max	Mean[a]	Std. Dev.
Arsenic	Seattle Pier 86	4/4	1.17	1.30	1.22	0.05
	Redondo Pier	4/4	1.17	1.61	1.45	0.19
	All Sites	8/8	1.17	1.61	1.34	0.18
Cadmium	Seattle Pier 86	4/4	0.0096	0.0204	0.0132	0.0050
	Redondo Pier	4/4	0.0039	0.0070	0.0060	0.0015
	All Sites	8/8	0.0039	0.0204	0.0096	0.0051
Chromium	Seattle Pier 86	4/4	0.014	0.0629	0.0337	0.022
	Redondo Pier	4/4	0.018	0.028	0.024	0.005
	All Sites	8/8	0.014	0.0629	0.0287	0.016
Copper	Seattle Pier 86	4/4	8.45	11.2	9.66	1.36
	Redondo Pier	4/4	6.23	8.63	7.80	1.12
	All Sites	8/8	6.23	11.2	8.73	1.52
Lead	Seattle Pier 86	2/4	(0.0041)	0.0056	0.0048	0.00079
	Redondo Pier	3/4	(0.0040)	0.0061	0.0050	0.00094
	All Sites	5/8	(0.0040)	0.0061	0.0049	0.00079
Mercury	Seattle Pier 86	4/4	0.0123	0.0132	0.0128	0.0004
	Redondo Pier	4/4	0.0148	0.0190	0.0171	0.0019
	All Sites	8/8	0.0123	0.0190	0.0149	0.0026
Nickel	Seattle Pier 86	4/4	0.0120	0.0326	0.0247	0.0098
	Redondo Pier	4/4	0.0140	0.0170	0.0153	0.0015
	All Sites	8/8	0.0120	0.0326	0.0200	0.0082
Silver	Seattle Pier 86	4/4	0.0135	0.0207	0.0157	0.0034
	Redondo Pier	4/4	0.0071	0.0114	0.0091	0.0019
	All Sites	8/8	0.0071	0.0207	0.0124	0.0043
Zinc	Seattle Pier 86	4/4	12.7	15.2	14.4	1.12
	Redondo Pier	4/4	13.9	15.4	14.8	0.64
	All Sites	8/8	12.7	15.4	14.6	0.88

FOD = Frequency of detection
MDL = Method Detection Limit
(MDL) = Not detected at MDL (MDL value in parentheses)
Std. Dev. = Standard deviation on the mean
[a] = Mean concentrations calculated with detected concentrations and MDL for non-detect results.

3.3 PCBs

The analytical results for PCBs met all acceptable lab QA/QC limits. Section 3.3.1 compares the Aroclor and homolog results by sample and location. Section 3.3.2 describes the composition of homologs detected in squid tissue composite samples.

3.3.1 Comparison of Total PCBs by Aroclor and Homolog Methods

PCBs were detected in all squid tissue composites as analyzed by both the Aroclor and homolog methods. In general, total PCBs concentrations based on these two methods were similar when compared on an individual sample basis. Total PCBs as measured by Aroclors tended to be higher than those measured by homologs (Table 6; Figure 4). Relative percent differences (RPDs) were calculated for each sample to compare total PCBs by the two methods. RPDs for all samples (all species and tissue types) were within the acceptable QA/QC precision limit for lab duplicates (RPD < 35%). This suggests the two analytical methods performed similarly for quantifying total PCBs in these tissues.

Mean PCB concentrations at Seattle Pier 86 were lower at Redondo Pier which is inconsistent with the increased accumulations expected for squid traveling southward. However, the difference in mean concentrations (for Aroclors or homologs) is less than would be expected from analytical variability alone (RPD < 35%). Therefore, PCB concentrations in squid at these locations are considered comparable.

Table 6. Total PCBs (µg/kg) by both Aroclors and homologs with relative percent difference.

Sample Location	Sample ID	Total Aroclors	Total Homologs	RPD
Seattle Pier 86	L66682-1	17.43	15.266	13.2
	L66682-2	13.75	11.67	16.4
	L66682-3	14.09	12.403	12.7
	L66682-4	11.83	10.68	10.2
	Location Mean	**14.3**	**12.5**	**13.2**
Redondo Pier	L66682-5	15.04	13.086	13.9
	L66682-6	11.51	9.54	18.7
	L66682-7	11.69	9.335	22.4
	L66682-8	9.47	7.334	25.4
	Location Mean	**11.9**	**9.82**	**19.3**

RPD = Relative Percent Difference

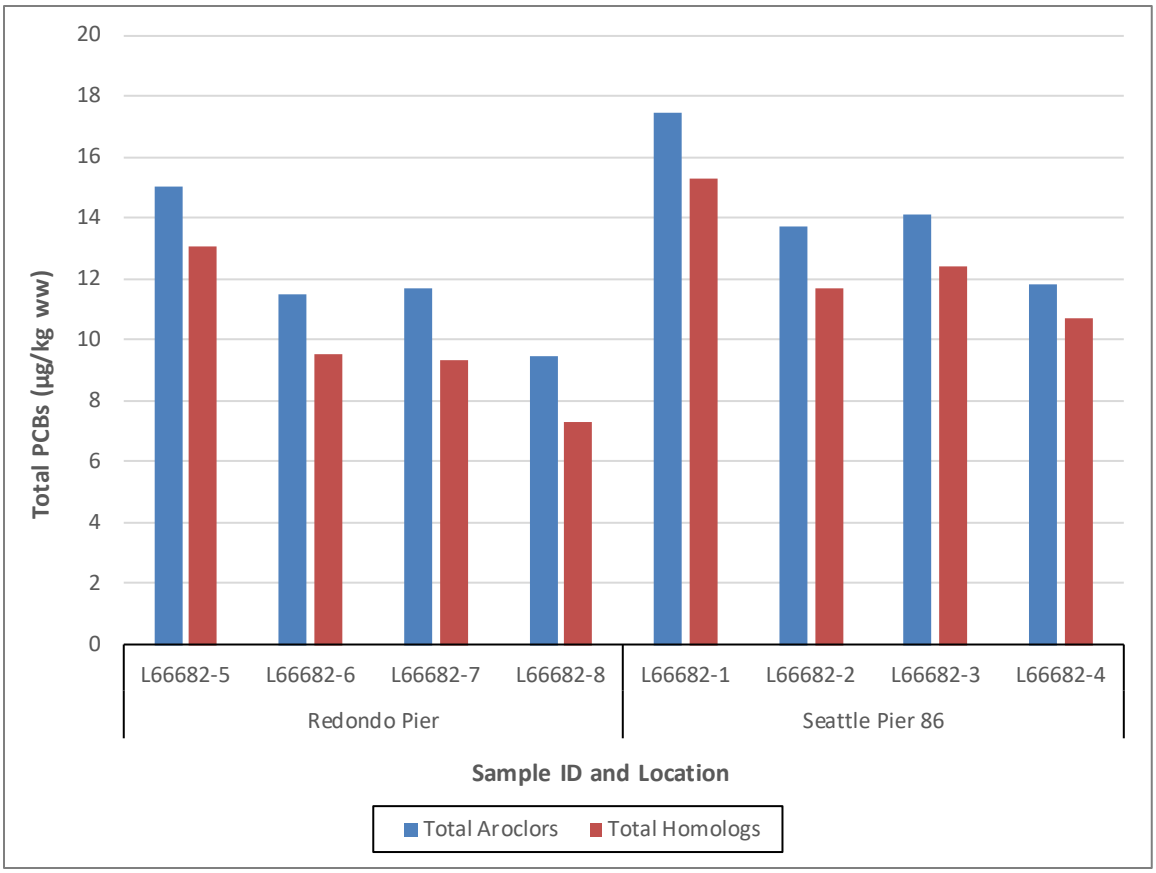


Figure 4. Total PCBs (µg/kg) by composite sample as measured by Aroclors and homologs.

3.3.2 PCB Homolog Composition

PCB homologs detected in squid tissue were dominated by the penta- and hexachlorobiphenyls (Figure 5). Hexachlorobiphenyls represented the greatest contribution to the total PCB sum at 41% followed by pentachlorobiphenyls at 35%, and heptachlorobiphenyls at 14%. This PCB homolog distribution was comparable to the pattern observed in Dungeness crab muscle and hepatopancreas and Red rock crab hepatopancreas tissues (King County 2016c).

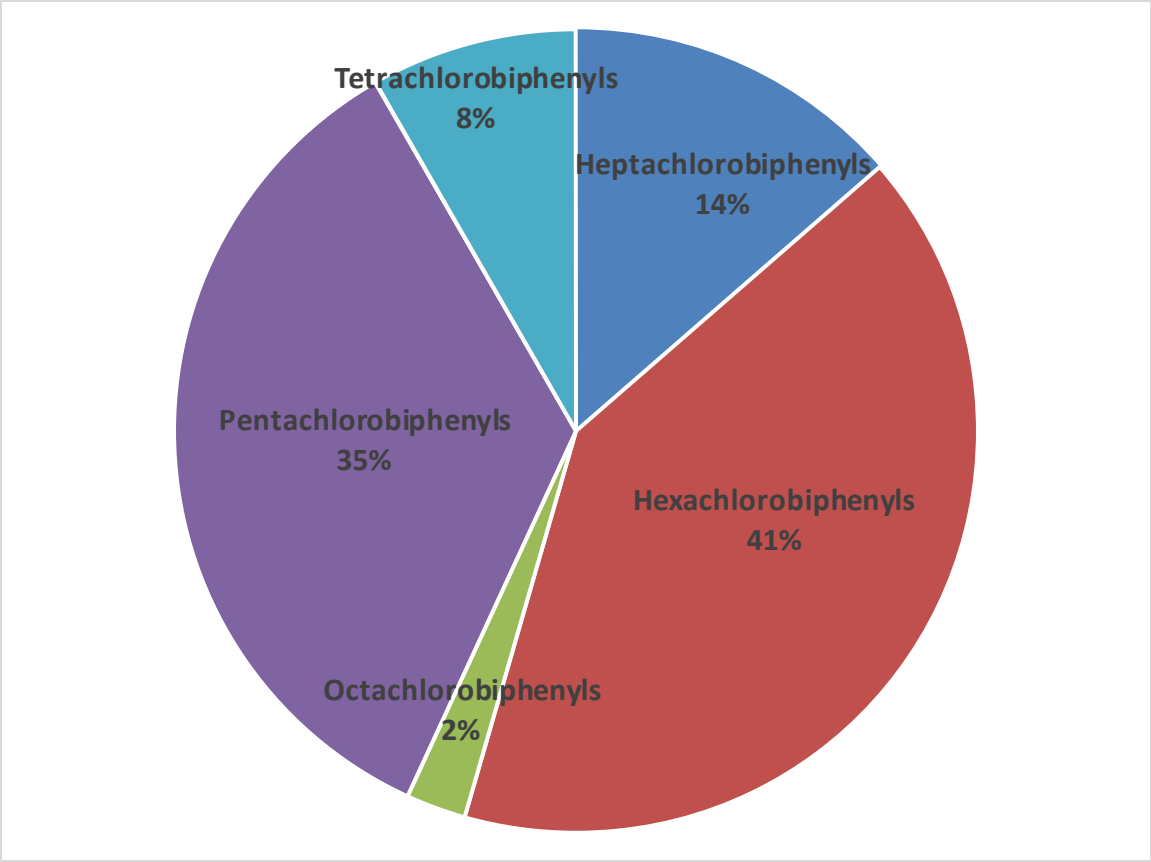


Figure 5. Composition of mean detected PCB homologs as a percent of total detected PCBs in squid tissue from all locations.

3.4 PBDEs

The PBDE analytical results met all acceptable lab QA/QC limits with the following exception. TetraBDE-47 exceeded the lab limits in the standard reference material (SRM) duplicate. The spike blank, SRM recoveries and the RPD between the SRM/SRM duplicate for TetraBDE-47 were within lab limits; therefore, data were used without qualification.

PBDEs were frequently detected in squid tissue; PBDEs were not detected in only two of eight composite samples, one from each stations (Table 7). PBDEs were slightly higher on average in squid from the Redondo Pier compared to Elliott Bay. This may be attributable to the squid's migratory path. Squid collected from Seattle Pier 86 would just be entering Elliott Bay, while those collected from at the Redondo Pier would have already migrated south through Elliott Bay and been exposed to other contaminant sources in the Seattle metro area.

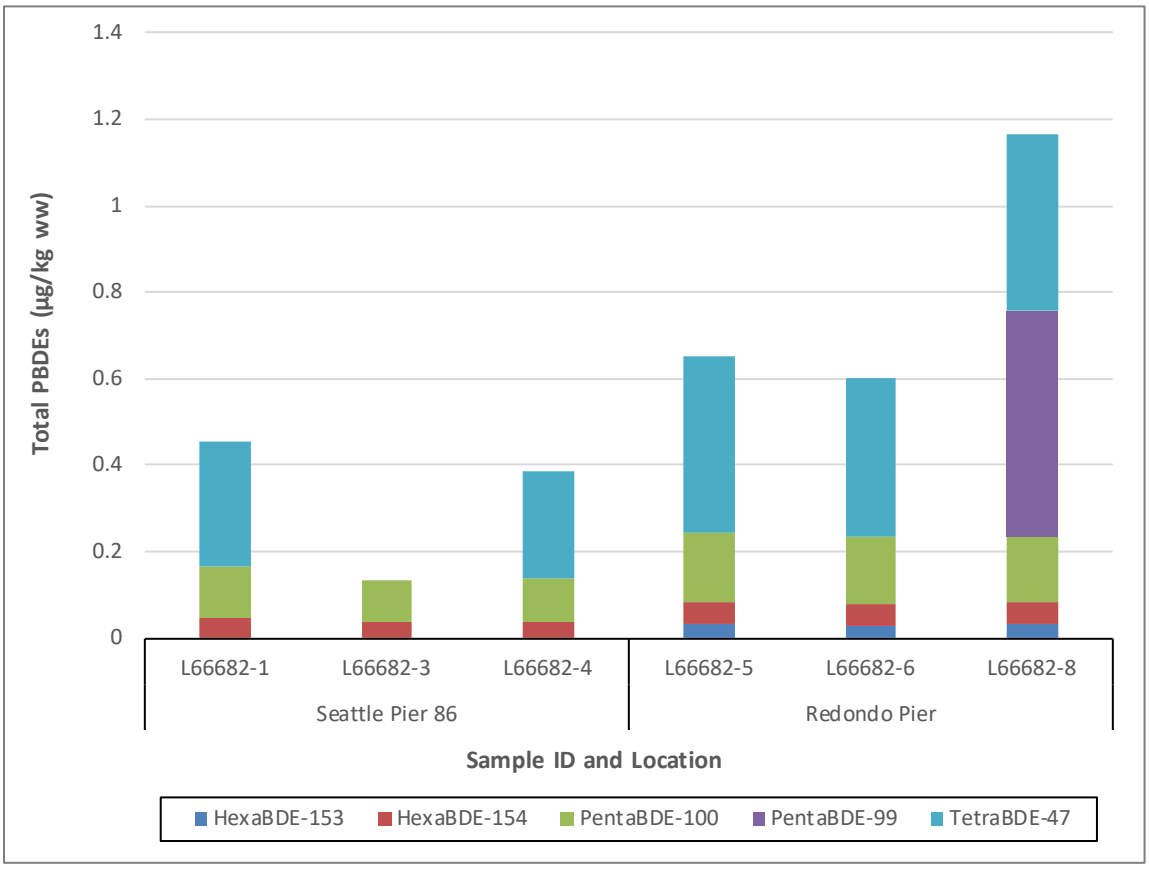
PentaBDE-100 and HexaBDE-154 were the most frequently detected PBDE congeners, although TetraBDE-47 represented the greatest contribution to the total PBDE sum (Figure 6). HexaBDE-153 was more frequently detected in squid from the Redondo Pier (three of four samples); it was not detected in squid from Seattle Pier 86. However, all

PBDE concentrations were low relative to detection limits, only one sample exceeding 1 µg/kg total PBDEs across both locations sampled.

Table 7. Congener and Total PBDE concentrations by sample ID and location.

Sample Location	Sample ID or Mean	TetraBDE-47	PentaBDE-99	PentaBDE-100	HexaBDE-153	HexaBDE-154	Total PBDEs
Seattle Pier 86	**Site Mean**	**0.32**	**(0.57)**	**0.120**	**(0.34)**	**0.045**	**0.472**
	L66682-1	0.29	(0.45)	0.120	(0.027)	0.045	0.455
	L66682-2	(0.48)	(0.91)	(0.15)	(0.053)	(0.059)	(0.91)
	L66682-3	(0.24)	(0.45)	0.097	(0.027)	0.037	0.134
	L66682-4	0.25	(0.45)	0.100	(0.027)	0.037	0.387
Redondo Pier	**Site Mean**	**0.60**	**0.93**	**0.209**	**0.056**	**0.076**	**1.18**
	L66682-5	0.41	(0.45)	0.157	0.032	0.053	0.652
	L66682-6	0.37	(0.45)	0.154	0.030	0.049	0.603
	L66682-7	(1.2)	(2.3)	(0.37)	(0.13)	(0.15)	(2.3)
	L66682-8	0.41	0.52	0.153	0.033	0.050	1.166

Congeners not included in table were not detected in any sample.
(MDL) = Not detected at MDL (MDL value in parentheses)
Site means were calculated using the MDL value for non-detects.
Total PBDEs include the sum of detected congeners. For samples with all non-detects, the value of the highest MDL is listed.



Note: L66682-7 and L66682-2 not included since all PBDE congeners were less than MDL

Figure 6. Total detected PBDEs (µg/kg) in squid tissue by congener and location.

3.5 Pesticides

The analytical results for pesticides met all acceptable lab QA/QC limits except for the following. Alpha-BHC, delta-BHC, gamma-BHC, endrin aldehyde, endosulfan sulfate, 4,4'-DDD, and 4,4'-DDT concentrations may be biased low for all samples and the matrix spike/ matrix spike duplicate (MS/MSD) have been flagged with a "JG." Heptachlor epoxide, trans-chlordane, and 4, 4'-DDT are flagged with a "J" due to the surrogate recovery exceeding QC limits.

Squid tissue was analyzed for twenty pesticides and their metabolites/degradation products (4,4'-DDT; 4,4'-DDE; 4,4'-DDD; Aldrin; α, β, γ, and δ-BHC; α and trans-chlordane; dieldrin; endosulfan I, II, and sulfate; endrin and endrin aldehyde; heptachlor and heptachlor epoxide; methoxychlor; and toxaphene). No pesticides were detected in squid tissue composite samples. Detection limits ranged from 0.67 to 13 µg/kg.

3.6 Butyltins

The analytical results for butyltins met all acceptable lab QA/QC limits. Butyltins were not detected in any squid tissue composite samples. Detection limits ranged from 3 µg/kg for tributyltin to 10 µg/kg for monobutyltin.

3.7 BNAs

The analytical results for BNAs met all acceptable lab QA/QC limits with the following exceptions. Bis(2-ethylhexyl)phthalate (BEHP) values less than five times the method blank value were "B" flagged because the method blank had a reportable level of this compound. Due to low/no recoveries in the MS/MSD, benzyl alcohol, indeno(1,2,3-Cd)pyrene, dibenzo(a,h)anthracene, and benzo(g,h,i)perylene were "JG" flagged for the data set and should be considered to have a low bias. Due to recoveries above the upper recovery limit, benzoic acid results for the data set were flagged with a "JL" and may have a high bias.

Squid tissue composite samples were analyzed for a variety of BNA compounds, including polycyclic aromatic hydrocarbons, phthalates, among others. Only two BNA compounds were detected in squid tissue: benzoic acid and BEHP (Table 8). Benzoic acid was detected in all composite samples, while BEHP was only detected in three of four samples from the Redondo Pier, and not detected in samples from Seattle Pier 86.

Table 8. Summary statistics for BNA concentrations (µg/kw) detected in squid composite samples by location.

Analyte	Sampling Location	FOD	Min	Max	Mean[a]
Benzoic Acid	Seattle Pier 86	4/4	2730	4120	3690
	Redondo Pier	4/4	3210	3660	3468
	All Sites	8/8	2730	4120	3579
BEHP	Seattle Pier 86	0/4	(130)	(130)	130
	Redondo Pier	3/4	(130)	615	386
	All Sites	3/8	(130)	615	258

BEHP = Bis(2-Ethylhexyl)Phthalate
FOD = Frequency of detection
MDL = Method Detection Limit
(MDL) = Not detected at MDL (MDL value in parentheses)
a = Mean concentrations calculated with detected concentrations and MDL for non-detect results.

3.8 Comparison to Historical Data

Three samples of cleaned and three samples of whole body squid were analyzed as part of a King County study in 1997 (King County and Parametrix 1999). Squid were collected from the Seattle Pier 86 location. Each sample was a composite of 10 market squid. The cleaned squid samples were prepared by removing the quill, beak, and viscera, comparable to the sample preparation used in the monitoring effort described here.

In 1997, BNAs were analyzed by EPA Method 8270, PCB Aroclors by EPA Method 8080, and metals by PSEP (1996). Mercury was analyzed by CVAA, chromium and zinc by ICP, and the remaining metals by ICP-MS. Pesticides, PBDEs and PCB homologs were not analyzed in the 1997 samples.

3.8.1 Methodological Differences between 1997 and 2016 Studies

Comparisons between 1997 and 2016 lipid content and organic chemicals are limited due to differences in solvents, extraction, and calibration methods that may have affected reported results. The biggest difference is likely attributed to changes in extraction methods and solvents. The 1997 analyses used a 50/50 methylene chloride/acetone solvent and sonication, while the 2016 analyses used a 100% methylene chloride and Soxhlet extraction. Calibration procedures also changed between the two sampling events. The 1997 analyses typically used a four-point calibration curve, while the 2016 analyses typically used a five-point calibration curve with the RDL as a point on the calibration curve. The two analyses also had differing mass-to-volume ratios for the GPC. The 1997 analyses used 12.5 g to 0.5 mL GPC final volume extract, while the 2016 analyses used 15 g to 1 mL.

Lipid content was substantially higher in cleaned squid from 2016 (2.83 to 3.27%) than in 1997 (0.68% to 1.34%). Differences in extraction methods may explain some of these differences. In 1997, extraction was conducted by sonication with methylene chloride,

while in 2016, a Soxhlet extraction with methylene chloride was used. Due to these differences, lipid results are not comparable between the two monitoring events.

The 1997 MDLs and RDLs for butyltins were calculated much differently than those in the current study. In 1997, the butyltin MDLs and RDLs were established by a standard addition method similar to that used for the metal analyses. A series of standards were spiked into an extracted fish liver tissue and analyzed by GC/MS-SIM. This approach is atypical for analysis of organic compounds by the KCEL, but at the time was the best approach to perform butyltin analyses on tissues. The MDLs/RDLs are based on a 20 g to 1 mL final volume extract.

3.8.2 Comparison of 1997 and 2016 Results

All of the analytes detected in 1997 were also detected in 2016 squid samples except for the butyltins and isophorone (Table 9). Arsenic, cadmium, lead, nickel, benzoic acid and BEHP were not detected in cleaned squid 1997, but were detected in 2016. Shifts in detection limits over time may likely explain some of these patterns as discussed below. PBDEs were not analyzed in 1997 because the analytical method had not yet been developed at KCEL. Because whole-body squid were not analyzed in 2016, the comparison here will focus only on cleaned squid results.

Table 9. Detected compounds in cleaned squid tissue: 1997 compared to 2016.

Contaminant	1997	2016
METALS		
Arsenic	Not Detected	Detected
Cadmium	Not Detected	Detected
Chromium	Detected	Detected
Copper	Detected	Detected
Lead	Not detected	Detected
Mercury	Detected	Detected
Nickel	Not detected	Detected
Silver	Detected	Detected
Zinc	Detected	Detected
BUTYLTINS		
TBT	Detected	Not detected
DBT	Detected	Not detected
ORGANIC CHEMICALS		
PCBs	Detected	Detected
PBDEs	Not Analyzed	Detected
Benzoic acid	Not Detected	Detected
Bis(2-ethylhexyl)phthalate	Not Detected	Detected
Isophorone	Detected	Not detected

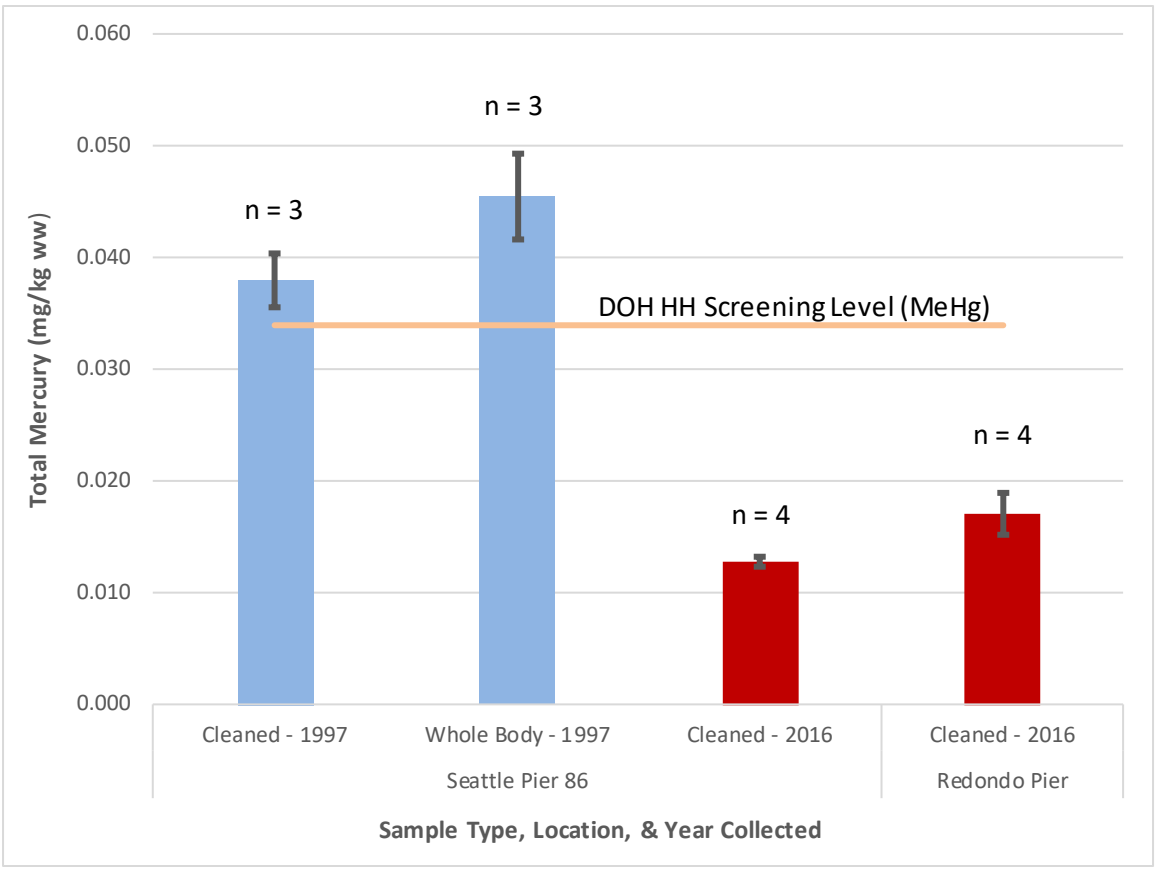
Mercury concentrations in cleaned squid collected from Elliott Bay were lower in 2016 (0.0123 to 0.0132 mg/kg) than in 1997 (0.0363 to 0.0408 mg/kg) (Figure 7).

The remaining eight metals were also detected in cleaned squid samples from Seattle Pier 86 in 1997. However, the MDLs for cadmium, lead and nickel were as much as ten times lower in 2016 than in 1997 and detected concentrations were below or near the 1997 MDLs; thus, levels of these metals are not likely much different despite the increased frequency of detection. Concentrations of arsenic and chromium in cleaned squid decreased between 1997 and 2016, while levels of copper and zinc increased. The mean arsenic level in cleaned squid was 3.0 mg/Kg in 1997 and 1.2 mg/Kg in 2016, while the mean chromium concentration dropped from 0.11 mg/Kg in 1997 to 0.034 mg/Kg in 2016. Mean copper concentrations in cleaned squid increased from 2.84 mg/kg in 1997 to 9.66 mg/kg in 2016. Zinc averaged 9.0 mg/kg in 1997 cleaned squid compared to 14.4 mg/kg in 2016. In 1997, mean silver concentrations in cleaned squid (0.015 mg/kg) were similar to those detected in 2016 (0.016 mg/kg).

Despite changes in analytical methods, PCB concentrations in cleaned squid from Elliott Bay were comparable to those detected in 2016. Only Aroclors were measured in 1997, but total PCBs concentrations were similar as measured by both Aroclors and homologs in 2016. Total Aroclor concentrations were generally higher than the sum of homologs; however, the difference is within that expected by analytical variability.

In 1997, both tributyltin and dibutyltin were detected in squid; tributyltins ranged from 17.8 to 33.5 µg/kg and dibutyltins ranged from 1.1 to 4.02 µg/kg. Tributyltin concentrations in squid tissue have decreased since 1997 based on the lack of detections in 2016 (MDL of 3 µg/kg). TBT use was partially banned in the United States in 1988 (through cancelled pesticide registrations) and effectively banned through international law by 2008 (AFS Convention; IMO 2001). Thus, decreases in TBT concentrations in squid seen between 1997 and 2016 are likely reflecting the decreased use of TBT over this period. No dibutyltins were detected in squid in 2016; however, the concentrations measured in 1997 were below the 2016 analytical MDL. Thus, it is uncertain if dibutyltin concentrations have decreased in squid.

Isophorone was detected in cleaned squid in 1997. Given the lack of isophorone detections in 2016 without a shift in detection limit, concentrations of this chemical appear to have decreased. Although the MDLs for both BEHP and benzoic acid increased between 1997 and 2016, these chemicals were still detected in 2016 above the MDL and appear to have increased.



Note: DOH HH Screening Level = Washington State Dept. of Health Screening Level for methylmercury (MeHg) based on 175 g/ day fish consumption

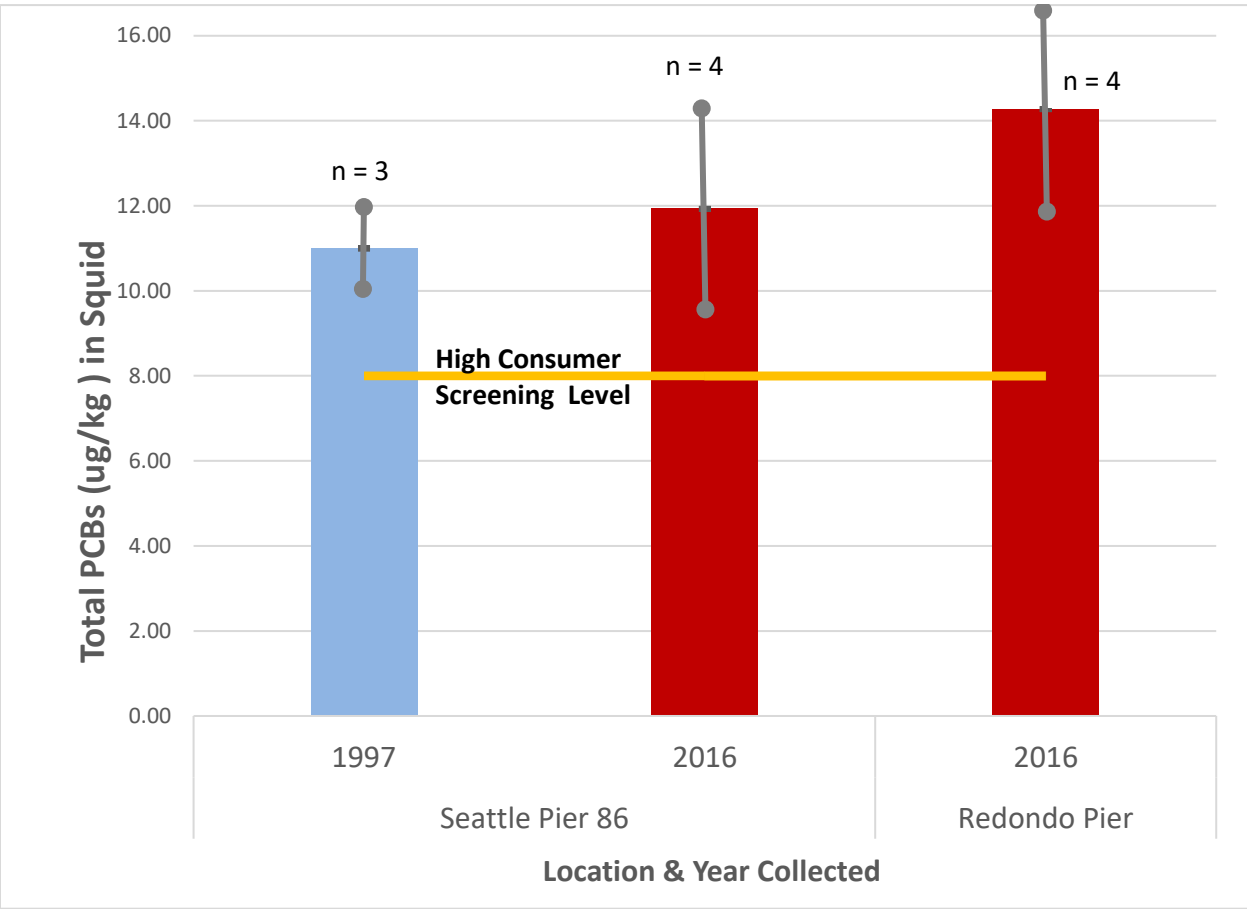
Figure 7. Comparison of mean mercury concentrations in 1997 and 2016.

3.8.3 Comparison of Results to Human Health Screening Levels

Detected mercury concentrations were compared to the WDOH high consumer screening level for fish consumption advisories (McBride Pers. Comm. 2018) for methylmercury based on a consumption rate of 175 gm (6 ounces) per day (0.034 mg/kg). Mercury is the cause of a general statewide fish consumption advisory in Washington for recreationally-caught freshwater and commercially-caught marine fish (WDOH advisory website). Although WDOH would evaluate other factors, including data sufficiency, before an advisory is issued, this screening level was used to provide context for potential human health impacts. Although only total mercury was measured, all mercury was assumed to be methylmercury as a conservative estimate. Mean mercury concentrations in cleaned squid from both sites were about half of this screening level. Mean metal concentrations were also below all available screening levels for other detected metals (cadmium, chromium [VI or III], nickel, silver and zinc) Therefore, metals concentrations in squid collected in the study are low relative to the human health screening levels for fish consumption.

High consumer human health screening levels are also available for select organic chemicals detected in squid in 2016 (total PCBs, total PBDEs and BEHP). Concentrations of total PBDEs and BEHP in all samples were below the high consumer screening levels;

however, concentrations of total PCBs were above the high consumer screening level(8 µg/Kg) (Figure 8) as measured by Aroclors or homologs, except for one sample from Redondo Pier measured as homologs. Total PCB concentrations in all samples were below the general population human health screening level based on 60 g (2.1 oz) of fish per day of 23 µg PCBs/Kg. Therefore, squid from Seattle Pier 86 or Redondo Pier are low in organic chemicals relative to WDOH human health screening levels, except perhaps for PCBs when consuming 14 or more ounces of squid per week. WDOH will consider if a fish consumption advisory is warranted for PCBs in squid.



Note: High Consumer Screening Level = Washington State Dept. of Health Screening Level for total PCBs based on 175 g/ day fish consumption.

Figure 8. Mean total PCB concentrations (as Aroclors) in squid collected in 1997 and 2016 .

4.0 CONCLUSIONS

Is a fish consumption advisory warranted for market squid?

WDOH would need to evaluate the sufficiency of this study and has the legal authority to determine whether a consumption advisory is warranted. However, this study demonstrates that concentrations of metals, including mercury, in cleaned squid collected in 2016 were low relative to WDOH human health screening levels. This conclusion also applies to organic chemicals with the possible exception of PCBs when squid are consumed at 14 or more ounces per week. The relatively low contaminant levels in squid can be attributed to their low lipid content and short lifespan; they do not live long enough or contain enough fat to accumulate significant levels of contaminants. Accordingly, the findings of this study confirm initial assumptions that market squid would tend to be low in contaminants.

Which sampling location had squid with the lowest contaminant levels?

Overall, squid from Seattle Pier 86 were more contaminated than squid from the Redondo Pier. In 2016, mercury, total PBDE and BEHP concentrations were higher in squid collected from the Redondo Pier compared to those collected from Seattle Pier 86. Cadmium, chromium, copper, nickel, and silver concentrations were higher in squid collected from Seattle Pier 86. Higher concentrations of contaminants may reflect local differences in contaminant sources or variability in squid migratory patterns and subsequently their exposure. The specific cause of the differences in metals concentrations between the two locations is unknown. Concentrations of PCBs, arsenic, lead zinc and benzoic acid were similar in squid from both piers. Pesticides and butyltins were not detected in squid from either location.

Which contaminants were detected in squid?

Eight metals (arsenic, cadmium, chromium, copper, lead, mercury, nickel, silver and zinc), PCBs, PBDEs, benzoic acid and BEHP were detected in one or more squid samples.

Have contamination levels in squid declined since 1997?

Overall, arsenic, chromium, mercury, isophorone, and TBT concentrations were lower in 2016 than in 1997. However, copper and zinc concentrations were higher in 2016. Total PCB levels appear to be comparable between years. Samples collected in 1997 were not analyzed for pesticides and PBDEs. Isophorone decreased since 1997, but levels of BEHP and benzoic acid increased between 1997 and 2016.

Recommendations for future studies

If squid are sampled in the future, both the mantle length and total length will be recorded for each individual squid. Mantle length appears to be the more reliable and replicable measure used by cephalopod scientists.

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