POLYCHLORINATED BIPHENYL (PCB) CONTAMINATION ON UNALASKA ISLAND IN THE ALEUTIAN ARCHIPELAGO

By Elise M. Adams

A Thesis

Submitted in Partial Fulfillment

of the Requirements for the Degree of

Master of Science

in Biological Sciences

Northern Arizona University

August 2018

Approved:

Frank A. von Hippel, Ph.D., Chair

C. Loren Buck, Ph.D.

Bruce Hungate, Ph.D.

ABSTRACT

POLYCHLORINATED BIPHENYL (PCB) CONTAMINATION ON UNALASKA ISLAND IN THE ALEUTIAN ARCHIPELAGO

ELISE M. ADAMS

Polychlorinated biphenyls (PCBs) are a group of man-made, hydrophobic organochlorines that persist at highly toxic levels in the environment and biomagnify within food webs. Although banned, their continued release from pre-banned products and persistence in the environment impact human and wildlife health. PCBs are transported to the Arctic via global distillation and biomagnify to high levels in the lipid-rich food web. Thus the long-range transportation capacity of PCBs can affect food webs far from the area of release. In addition, the Arctic contains thousands of World War II and Cold War formerly used defense (FUD) sites, many of which are also a local source of PCB contamination. PCBs have the ability to modify or suppress thyroid, reproductive and immune function. Exposure can reduce cognitive function and greatly increase the risk of developing cancer, hypothyroidism and a host of other negative health effects. Human and animal exposure occurs via ingestion of contaminated food. PCB concentrations were analyzed in threespine stickleback (*Gasterosteus aculeatus*) and subsistence foods important to the Qawalangin Tribe of Unalaska (i.e., salmonid species and blue mussels (*Mytilus edulis*)). PCBs were extracted from samples using a modified QuEChERS method. Mean PCB concentrations were quantified in target species to assess potential risks associated with subsistence foods and to detect a difference between global and local sources of PCB contamination. Two FUD sites showed elevated levels of PCBs that exceed safe consumption guidelines. These results support the need to remediate the FUD sites of “Building 551/T Dock to Airport” and “Delta Western”. More generally, these results provide further evidence of the

continued problem of PCB contamination at FUD sites in the Arctic.

ACKOWLEDGEMENTS

Field work costs were supported by the Institute for Tribal Environmental Professionals (ITEP) with funding from the U.S. Environmental Protection Agency. We thank ITEP for supporting research and education with indigenous communities. Additional research funding was provided by the Aleutian and Bering Sea Islands (ABSI) Landscape Conservation Cooperative (LCC). Fish and mussel samples were collected under the Alaska Department of Fish and Game Permit SF2017-127. Conflicts of interest: none. We thank the Qawalangin Tribe and residents of Unalaska for their support and assistance with sample collection. We thank Thomas Robinson, Purevdulam Enkhbat and Carter Price for their assistance in the field. We also thank Chris Price of the Qawalangin Tribe for helping to arrange the project.

TABLE OF CONTENTS

List of Tables vi

List of Figures vii

Introduction 1

Materials and Methods 12

Results 15

Discussion 16

References 20

LIST OF TABLES

Table 1 23

LIST OF FIGURES

Figure 1 25

Figure 2 26

Figure 3 27

Figure 4 28

Figure 5 29

Figure 6 30

Introduction

Plants and naturally derived chemicals have long been used to improve the quality of life. The beginning of organic chemistry led to the creation of many synthetic compounds that are eventually released into the environment. The release of these chemicals into the environment has led to concerns about the physiological and toxicological effects of contaminants on singular, additive and synergistic bases (Briggs, 2003; Letcher et al., 2010). Today there are roughly 30,000 compounds used for everyday products and purposes of which only 1% have been put through detailed risk assessment (Briggs, 2003). The widespread toxic effects of contaminants from both local point sources (e.g., industrial discharge) and global sources (e.g., atmospheric pollution) are influenced by the physical, chemical and biological properties of the contaminants (EPA, 2000; Letcher et al., 2010). Despite current clean-up efforts, continual exposure to environmental pollution remains an issue of concern for both human and wildlife health (Briggs, 2003). Not only chemicals currently in use but also restricted or banned (legacy) contaminants contribute to these effects.

A small number of legacy and currently used contaminants are classified as persistent organic pollutants (POPs) because of their environmental persistence, bioaccumulative properties, long range atmospheric transport and adverse human and environmental health effects (Gioia et al., 2013). POPs, particularly those of relatively light molecular weight, undergo long-range transport and accumulate at high latitudes in a process known as global distillation (Bard, 1999; Muir et al., 1999; Wania and Mackay, 1993). Global distillation is a process by which persistent and relatively volatile compounds are transported from areas of lower latitude to areas of higher latitude (Bard, 1999). Contaminants volatize at higher ambient temperatures in tropical and temperate regions, are atmospherically transported and redeposited in colder regions where they can persist in high concentrations (Bard, 1999; Gioia et al., 2013). Henry's law constant (HLC) is the proportional concentration of a contaminant in liquid phase to the partial pressure of the gas phase (Bard, 1999). Atmospheric deposition occurs when the HLC lowers as contaminants reach high latitude regions (Bard, 1999). Global fractionation, the preferential transport of more volatile contaminants, is strongly influenced by ambient temperature (Bard, 1999; Wania and Mackay, 1993). Decreases in ambient temperature allow atmospheric contaminants to condense and be deposited in terrestrial and aquatic habitats and thereby to become incorporated into biota in colder regions (Blais, 2005; Wania and Mackay, 1993). Organic contaminants can be transported through the atmosphere in single or multiple jumps known as the 'grasshopper effect' (Semeena and Lammel, 2005), a singular or repeated cycle of volatilization and deposition that involves the seasonal cycle of temperature (Jurado and Dachs, 2008).

Having no natural sources, polychlorinated biphenyls (PCBs) are one of the pervasive anthropogenic contaminants classified as POPs (Bard, 1999; Blais, 2005). PCBs are a group of man-made, hydrophobic organochlorines that can persist at highly toxic levels in the environment and biomagnify within food webs (Bard, 1999). In the United States the commercial production of PCBs began in 1929 and continued until the late 1970's when it was determined that bioaccumulation and environmental persistence accompany their release (Garmash et al., 2013; Gioia et al., 2013). Due to their chemical and thermal stability, the primary use of PCBs was in dielectric fluid in transformers and capacitors (Hardell et al., 2010). Plasticizers, lubricating oils, hydraulic fluid, paint, floor and ceiling tiles, window caulking and ink were other common applications of PCBs (Carpenter, 2006; Gioia et al., 2013; Hardell et al., 2010). Although banned in the United States since 1979 (EPA, 1979) and internationally since

2001 (UNEP, 2001), the continued release of PCBs via both point and diffuse sources (e.g., landfills, transformers and capacitors, volatilization of previously released PCBs, abandoned military installations, byproducts of waste incineration) impact human and animal health (Blais, 2005; Garmash et al., 2013; Gioia et al., 2013).

PCBs have 209 congeners with unique physical and biological properties resulting from the differing number and placement of chlorine atoms on the biphenyl ring (Hardell et al., 2010). The extent of PCB transport is strongly influenced by the level of chlorination and ambient temperature (Bard, 1999). PCB congeners with lower chlorination levels are more volatile and can travel greater distances, while heavier, more highly chlorinated congeners are less volatile, staying in closer proximity to the area of release (Bard, 1999). Higher chlorinated congeners have a higher molecular mass, making them less mobile and less volatile than congeners with lower chlorination levels and molecular mass (Garmash et al., 2013). The more highly chlorinated congeners also demonstrate greater environmental persistence (Hardell et al., 2010). PCBs tend to have a high HLC in tropic or temperate regions allowing them to volatize and be transported to the Arctic (Bard, 1999). Given year-round low temperatures, the Arctic acts as a “cold trap” for POPs (Rahn and Heidam, 1981), including PCBs, leading to their incorporation into arctic food webs (Bard, 1999; Wania and Mackay, 1993).

The Arctic is characterized by low temperatures, low intensity sunlight and reduced productivity which reduce the photolysis and chemical degradation of PCBs (Garmash et al., 2013; Wania and Mackay, 1993). Due to the low concentrations of particulate matter in the water column, the Arctic Ocean has low rates of sedimentation, increasing the residency and bioavailability of PCBs (Bard, 1999). Resistance to degradation and the lipophilic nature of PCBs allow for prolonged environmental residency in the Arctic via incorporation into the adipose tissue of organisms, including many long lived fish and marine mammals (Ayotte et al., 1995; Bard, 1999; Wania and Mackay, 1993). PCBs and other hydrophobic contaminants adsorb to particulate organic material and biota (e.g., phytoplankton, algae) which are then consumed by filter feeding invertebrates, fish and other animals (Bard, 1999; Hardell et al., 2010). The primary route of PCB exposure for humans and animals is consumption of contaminated food (Carpenter, 2006). A large portion of the energy transfer within the arctic food web is through lipids (Muir et al., 1999). Many arctic predators take advantage of the short season of high summer productivity to accumulate ample fat stores for insulation and food reserves for the winter months (Bard, 1999; Wania and Mackay, 1993).

Once incorporated into the food web, PCBs bioaccumulate and biomagnify, especially in the lipid rich tissue of arctic animals (Bard, 1999; Hardell et al., 2010). The octanol-water partition coefficient (K_{ow}) helps predict the relative bioaccumulative properties of various contaminants (Bard, 1999). Due to PCBs low water solubility and high lipid solubility, they have a high K_{ow}, which correlates with a high potential for bioaccumulation in the arctic food web (Bard, 1999).

In addition to the bioaccumulation within an organism, PCBs biomagnify within food webs, resulting in an increasing concentration of PCBs as trophic level increases (Muir et al., 1999). PCBs with high chlorine content have a high biomagnification factor (BMF), the lipid weight ratio comparing the contaminant concentration of predator and prey (Muir et al., 1999). Comparing arctic high trophic level species (e.g., seals, birds) to water, PCBs show a difference in BMF of 10^9 (Bard, 1999). Salmon and salmon carcasses contaminated with PCBs act as a means of biotransport from the marine environment to freshwater habitats, which then increase the level of PCBs in the organisms that eat the contaminated eggs and spawning adults (e.g., fish, bears, eagles, humans) (Ewald et al., 1998). The high lipid content of anadromous salmon is depleted during migration, allowing PCBs to be transferred to the gonads or sequestered in lipid rich eggs prior to spawning, negatively affecting offspring viability and survivorship (Bard, 1999; Ewald et al., 1998).

Subsistence foods, including invertebrates, fish and long-lived, high trophic level species (e.g., seals, whales) are an integral part of arctic indigenous cultures (Hardell et al., 2010). High trophic level marine animals are at risk for exposure to high levels of PCBs, as are the subsistence communities that consume these animals (Ayotte et al., 1995; Wania and Mackay, 1993). In addition to trophic position, age, sex, and migratory patterns of an organism, the ability to metabolize and excrete PCBs influences accumulation and biomagnification (Bard, 1999). PCBs are among a group of hydrophobic contaminants that are not easily metabolized leading to their accumulation within an organism (Muir et al., 1999). The ecological relevance and risk of PCB exposure on organismal health is in part determined by an organism's ability to metabolize PCBs. The half-life of PCBs varies depending upon chlorination level and overall contaminant body burden (Carpenter, 2006), typically ranging from 2-6 years in the human body (Miodovnik, 2011). More highly chlorinated congeners often comprise a greater portion of the accumulated PCBs due to the preferential biotransformation of lower chlorinated congeners by means of detoxifying enzymes (Bard, 1999). When the adipose tissue is metabolized, PCBs are released and migrate to target tissues and organs, potentially initiating toxic effects (Bard, 1999; Wania and Mackay, 1993).

Due to the variation in physical properties, different PCB congeners can cause a variety of health effects including modification or suppression of thyroid, immune and reproductive functions as well as a reduced cognitive function and an increased risk of developing cancer (Carpenter, 2006; Hardell et al., 2010; Schell et al., 2008).

Thyroid hormones are essential for many bodily functions including growth, reproduction, metabolism and neural development (Cesh et al., 2010; Schell et al., 2008). Schell et al. (2008) showed that adolescents exposed to PCBs showed a reduction in thyroid function (hypothyroidism).

PCBs disrupt the sex steroid axis due to their ability to mimic or degrade estrogens (Carpenter, 2006). Congeners exhibiting a coplanar composition are anti-estrogenic and possess dioxin-like properties (Hardell et al., 2010). Most other congener configurations act as estrogen agonists (Hardell et al., 2010).

Cellular and organelle membranes contain lipids and various proteins that facilitate function (Steck, 1974). Membrane components and the incorporation of contaminants can affect the fluidity and resulting function of the cell or organelle (Tan et al., 2004). Ortho-substituted PCB congeners have chlorine atoms in position close to the biphenyl bond causing an increase in the angle between the rings (Tan et al., 2004). The resulting stereochemistry gives the ortho-substituted congeners a greater 3-dimentional structure potentially causing disturbance in the cell membrane structure and fluidity and thereby altering the function (Tan et al., 2004).

The ability of PCBs to suppress immune functions is a possible mechanism for the induction of cancer (Cogliano, 1998). Lower chlorinated congeners can more easily be metabolized into dihydroxy metabolites leading to oxidative DNA damage, and potentially carcinogenic effects including inhibition of tumor suppressor genes (Oakley et al., 1996). Additionally, evidence has shown hepatic carcinogenesis in rats associated with highly chlorinated congeners (Safe, 1989). PCB-contaminated fish collected downstream of formerly used defense (FUD) sites on St. Lawrence Island, Alaska showed differentially expressed genes important in the response of DNA to damage, DNA replication and cell signaling potentially leading to exacerbated carcinogenic effects (von Hippel et al., 2018).

The most notable health risks associated with consumption of PCBs are the effects on reproduction and development (Ayotte et al., 1995). PCBs cross the placental barrier and accumulate in the developing fetus as well as in breast milk (Bard, 1999; Dewailly et al., 1993). Reduction in IQ and cognitive function resulting from prenatal exposure to PCBs cannot be reversed (Carpenter, 2006). All of these potential health consequences of PCB exposure are particularly relevant to Arctic indigenous communities subsisting on high trophic level foods (Ayotte et al., 1995; Hardell et al., 2010).

The Aleutian Islands of Alaska are included in the circumarctic region within the arctic geographical coverage of the Arctic Monitoring and Assessment Programme (AMAP), placing the Aleutian Archipelago within the area of concern for arctic contamination. The Aleutian Islands contain over 300 volcanic islands that extend 1600km west from the Alaska Peninsula in the Northern Pacific Ocean. The Aleutian Islands are important habitat and breeding grounds for 10 million seabirds (Byrd et al., 2005). Some of the 26 species are of conservation concern including the red-throated loon (*Gavia stellata*) (Byrd et al., 2005). The daily foraging and migratory movement of seabirds can lead to the biotransport of contaminants on a local and widespread basis (Kenney et al., 2012). Additionally, drastic population declines in stellar sea lions (*Eumetopias jubatus*) residing in the Aleutian Islands resulted in their listed status as endangered (Beckmen et al., 2016). The adverse health and reproductive effects induced by PCBs and other anthropogenic contaminants are a potential contributing factor to this decline

(Beckmen et al., 2016). PCB concentrations found in free ranging stellar sea lions approach or exceed thresholds shown to induce deleterious health outcomes (Beckmen et al., 2016).

PCB contamination in arctic areas with a history of military use can stem from both atmospheric deposition and FUD sites. Due to their proximity to East Asia, the western-most islands of the Aleutians (the Near Islands) were invaded by the Japanese during World War II, which led to the militarization of much of the Aleutian Archipelago by U.S. forces. Subsequent to World War II, their proximity to the Soviet Union led to the creation of important Cold War military installations. Alaska contains approximately 600 FUD sites (Scrudato et al., 2012). For example, Adak Island, one of the most western islands in the Aleutians, contains many FUD sites, and fish and other marine organisms collected at Adak were found to have PCB concentrations at levels advised for minimal consumption (Hardell et al., 2010). Due to its long military history and associated contamination, Adak is on the National Priorities List for contaminant cleanup (Hardell et al., 2010).

Though no longer in use, contaminated sites including abandoned military sites can continue to leach chemicals such as PCBs into the environment and hence contaminate food webs (Blais, 2005). Many FUD sites are located near Alaska Native villages or on important subsistence land (Scrudato et al., 2012). For example, the island of Unalaska within the Aleutian Archipelago contains the largest town in the Aleutians, the city of Unalaska, as well as approximately 30 FUD sites (Blais, 2005; Scrudato et al., 2012).

Unalaska has a population of 5000 residents that increases during the summer commercial fishing season (USCB, 2018). Dutch Harbor on the island of Unalaska is a major commercial fishing area in the Aleutian/Bering Sea region (Fig. 1). Commercial fishing is an important source of income for many Aleut residents. Fish and other organisms harvested from this area are consumed by both the Qawalangin Tribe of Unalaska, who rely on subsistence animals, and recipient populations. Subsistence activities are important both culturally and for nutritional health, but may also lead to ingestion of unsafe levels of contaminants such as PCBs (Ayotte et al., 1995; Muir et al., 1999). Due to low population numbers and remote locations at many Alaskan FUD sites, remediation standards are often less strict (Scrudato et al., 2012). The FUD sites and abandoned military debris pose potential health risks for the Qawalangin Tribe, as well as other residents and visitors. Two FUD sites of great concern for the Tribe are Building 551, a former Dutch Harbor Naval Base Mess Hall located between the T Dock and airport landing strip, as well as the Delta Western Fuel Dock across from Building 551 (Fig. 2). During World War II, Japanese forces bombed a contract ship while it was docked adjacent to Building 551, which severely damaged PCB transformers and other electrical equipment in that area.

Similarly, the Midway Atoll, a group of tropical islands located in the central North Pacific Ocean, was contaminated with PCBs due to military activity. The Midway Atoll was under U.S. Naval control for nearly 100 years (Ge et al., 2013). During World War II, PCBs were released from capacitors and equipment contained in aircraft carriers and the aircraft that sunk near the islands (Ge et al., 2013). Similar to Unalaska, these tropical islands act as important habitat for seabirds and other marine life, where PCBs and other contaminants have been incorporated into the terrestrial and aquatic environment (Ge et al., 2013). Total PCB concentration found in the soil of Midway Atoll was dominated by congeners with four chlorine atoms (Ge et al., 2013) leading to PCB contamination in the black-footed albatross (*Phoebastria nigripes*) (Wang et al., 2015). Roughly 70% of total PCB concentration found in albatross plasma was contributed by congeners with five, six and seven chlorine atoms, indicating local contamination (Wang et al., 2015).

In the Aleutian/Bering Sea region of Alaska few studies have investigated the distinction between local sources of PCB contamination from FUD sites and atmospheric deposition. The fear of eating potentially contaminated foods and a lack of contaminant level data have led many subsistence communities to shy away from their traditional diet. Avoiding the traditional diet in favor of imported Western foods also carries health risks given the high nutritional value of the traditional marine diet vs. the often lower nutritional value of processed foods (Hardell et al., 2010). More information is needed to determine PCB levels in arctic subsistence foods and to distinguish between atmospheric deposition vs. point source contamination from FUD sites. Residents of Unalaska did not elect to host military installations and yet they may experience adverse effects from contaminants from these installations; hence, at the request of Unalaska residents, the PCB concentrations of select fish and invertebrates were quantified at both FUD sites and non-military sites on the island. The purpose of this study is to provide such information with an emphasis on the concentration of PCBs in important subsistence foods of the Qawalangin Tribe of Unalaska (i.e., blue mussels (*Mytilus edulis*), salmonid species). In addition to subsistence species, the threespine stickleback (*Gasterosteus aculeatus*) was also sampled because it is an important model organism in environmental toxicology (von Hippel et al., 2016).

These results will provide valuable information for land managers and risk assessors for their evaluation of level of risk associated with PCB exposure (or lack there-of) from traditional foods on Unalaska. The data collected will help the Qawalangin Tribe to make decisions about what subsistence foods are safe for consumption. The data in this study will also aid in prioritizing FUD site remediation on Unalaska. Additionally, the data will assist the U.S. Fish and Wildlife Service in their efforts to manage for contaminant exposure for wildlife within the Alaska Maritime National Wildlife Refuge. Determination of conservation priorities can be aided by monitoring the important prey species of seabirds, such as stickleback (Kenney et al., 2014; Kenney et al., 2012). PCB data from Unalaska are important not only to shed light on local issues of contamination but also to contribute to a global understanding of PCB contamination by sampling a poorly studied region.

Materials and Methods

Specimen Collection and Storage

Blue mussels, threespine stickleback, and various juveniles of salmonid species were collected from June 10-26, 2017 from both freshwater and marine sites on the island of Unalaska (Table 1, Fig. 3). Specimens were collected from multiple sites on the same day. During low tide mussels were collected by hand from intertidal zones. Mussels were collected from different substrates and positions within each site to be representative of the contamination level in the area. Mussels were selected based on size, with preference given to larger individuals, and those without broken shells. Two mussels were analyzed from each site with the exceptions of Wislow and Cascade Falls which had one analyzed per site. Fish were collected using unbaited 0.64cm wire-mesh minnow traps. Fish were euthanized with an overdose of pH neutral MS-222. Each fish family sampled (Salmonidae or Gasterosteidae) had n=10 collected at each site except Nateekin River which had n=3 for each family. Samples were labeled and held inside a cooler in the field for approximately 1 hour and then transferred to a -20°C freezer. At the completion of field work the samples were shipped frozen overnight and stored at -80°C until PCB extraction and analysis. All research protocols were approved by the Alaska Department of Fish and Game (collection permit SF2017-127) and the Northern Arizona University IACUC (protocol 17-003).

QuEChERS PCB Extraction

PCB extraction was conducted on whole body homogenate (fish) or soft tissue homogenate (mussels) using a modified QuEChERS (quick, easy, cheap, effective rugged, safe) method as outlined in Chamkasem et al. (2016). The increased solvent to sample ratio used in this method causes an associated increase in the efficiency of PCB congener extraction. Samples were removed from -80°C, kept on ice and allowed to thaw. Using a knife and dissection scissors sample tissue was thoroughly homogenized. Dissection tools were sterilized between each sample using Alconox Powdered Precision Cleaner (White Plains, NY, USA). All fish went through an additional homogenization using the Fisher Scientific PowerGen 1000 S1 homogenizer (Pittsburgh, PA, USA) and Fisher Scientific FB 505 sonicator (Pittsburgh, PA, USA) to optimize available surface area for PCB extraction.

A homogenate mass of 3 g wet weight was placed into a 50 mL centrifuge test tube. If the mass of an individual specimen did not meet the 3 g requirement, multiple individuals from the same site were used. All stickleback from Morris Cove Creek, Unalaska Lake, Matson Lake, and Nateekin River were composite samples as well as two salmonid samples from Nateekin River and one from Morris Cove Creek. All juvenile salmonid samples were unidentified to the genus and species level. Deionized water (5mL) and acetonitrile (30mL) were added to the sample tubes. Tubes were shaken on a Glas-Col large capacity mixer, speed set on 50 (Terre Haute, IN, USA) for 30 minutes. $MgSO_4$ (6g) and NaCl (1.5g) were added to the sample tubes, which were put on the shaker for another 10 minutes. Sample tubes were then centrifuged at 3000rpm for 10 minutes (Sorvall Legened XTR, Osterode am Harz, Germany). The centrifuge speed (3000rpm rather than 5000) is an alteration of the method outlined in Chamkasem et al. (2016) due to the rotor capabilities of our centrifuge. Acetonitrile extract (1mL) was pipetted into a 2mL prepared QuEChERS centrifuge tube (unitedchem.com, Bristol, PA, USA) containing 150mg of anhydrous $MgSO_4$, 150mg of PSA sorbent and 50mg of CEC18 sorbent. The tubes were capped and placed on the shaker for 1 minute. The tubes were then centrifuged at 2000rpm for 10 minutes. The final sample extract (~500-600µl) was transferred into a 1.5mL glass vial. The final extracts were kept at -80°C until shipment by overnight courier to the Arizona Laboratory for Emerging Contaminants (ALEC) in Tucson.

PCB Analysis by GC-MS

Prepared sample extracts were analyzed using an Agilent GC (7890 gas chromatograph with 7683 autosampler; Santa Clara, CA, USA) using a split-splitless inlet in splitless mode. With direct autosampler injection of 1µl of sample the analytes were separated using an SPB-Octyl column (Superlco; 30m x 0.25mm x 0.25µm) using He as the carrier gas at a flow rate of 1.1ml/min. The column temperature ran at 75°C for 2 minutes, increasing 15°C/min to 150°C, then increasing 2.5°C/min to 280°C.

The GC was coupled with electron impact mass spectrometry using a Waters Quattro micro triple quadrupole mass spectrometer (Milford, MA, USA) in selected ion recording (SIR) mode. The ion transfer line temperature was 280°C and the ion source was run at 220°C with trap current at 200 uA and electron energy of 70 eV. Presence and abundance of 20 PCB congeners was measured by comparison based on standard calibrant mixture C-SCA-06 (Accustandard Inc, New Haven, CT, USA). The standard mixture was diluted with nonane (Frontier Scientific, Logan, UT, USA) on an analytical balance to prepare the calibrants. Software used to acquire data from the GC-MS was MassLynx 4.1 and TargetLynx respectively.

Statistical Analysis

Statistical analyses were completed in R version 3.5.0. Resampling via bootstrapping was conducted to calculate the means and 95% confidence intervals for total PCB concentration contained in mussel and fish samples from each site. Additionally, bootstrapping was used to determine the detectable difference between the PCB concentration contained in mussel and fish samples at military vs. non-military sites. The minimum detection limit across all PCB congeners was used as the data point for samples in which all congeners tested below the detection limit.

Results

Out of 30 sites, PCBs were quantified at five using fish samples and at the other 25 using mussel samples. There are 21 sample sites considered military/FUD (18 mussel, 3 fish) sites while the other nine are non-military (7 mussel, 2 fish).

Bootstrapping for all mussel samples resulted in a mean PCB concentration of 4.7ppb with a lower confidence level of 1.7ppb and upper confidence level of 8.9ppb. The analysis comparing military vs. non-military mussel sites resulted in a mean PCB concentration of 5.7ppb for military sites, 1.8ppb for non-military sites, and a difference between the two of 3.9ppb (Fig. 4). The confidence interval for the difference has a lower confidence level of -0.62ppb and upper confidence level of 11.8ppb. The PCB concentrations found in blue mussels were especially elevated at two FUD sites. Mussels collected from "Building 551/T Dock to Airport" and "Delta Western" showed total PCB concentrations of 62.9ppb and 11.8ppb, respectively, compared to the mean of 5.7ppb for all mussel military sites.

Bootstrapping for all fish samples resulted in a mean total PCB concentration of 4.8ppb with a lower confidence level of 1.8ppb and an upper confidence level of 8.8ppb. The analysis comparing military vs. non-military fish sites resulted in a mean PCB concentration of 2.3ppb for military sites, 1ppb for non-military sites, and a difference between the two of 1.3ppb (Fig. 5). The confidence interval for the difference has a lower confidence level of 0.25ppb and upper confidence level of 2.5ppb.

Discussion

The U.S. Environmental Protection Agency (EPA) set risk assessment guidelines for consuming fish contaminated with PCBs (EPA, 1999). Consumption limits are far more stringent in reducing carcinogenic risks of PCBs than for non-cancer health endpoints. It is recommended that an upper limit of 16 fish-based meals a month not exceed a PCB concentration of 6ppb for non-cancer health outcomes and 1.5ppb for cancer endpoints (EPA, 1999). The monthly fish-based meal limit is reduced as the tissue concentration of PCBs increases. Data were resampled in order to determine if the concentration of PCBs found in mussel and fish samples is above or below consumption guidelines. Secondly, data were resampled to determine if there is a detectable difference between the PCB concentration found in military vs. non-military sites. The overall mean total PCB concentration was 4.7ppb for mussel samples, and 4.8ppb for fish samples. This concentration falls within the 8 meals/month for cancer related health endpoints and 16 meals/month for non-cancer health endpoints (EPA, 1999).

Bootstrapping results for differentiating PCB concentrations of mussels at military vs. non-military sites showed a difference of 3.9ppb, while the same analysis for fish showed a difference of 1.3ppb; in both cases the PCB concentrations were higher at military sites. The modified QuEChERS method showed a percent recovery of all tested PCB congeners to be within the range of 73 to 96 (Chamkasem et al., 2016). All congeners exceeded the acceptable 70 percent recovery level (Chamkasem et al., 2016). Therefore, the resulting PCB levels recovered from Unalaska samples are an underestimation of the total PCB concentrations present. Even with an underestimation, these results demonstrated higher concentrations at military sites and provide site-specific information for safe consumption levels based upon EPA criteria (EPA, 1999).

Highly elevated concentrations of PCBs were found in the mussel samples collected at two FUD sites: "Building 551/T Dock to Airport" and "Delta Western" (Fig 6). The PCB concentrations in blue mussels collected from Building 551/T dock to Airport exceed the EPA safe consumption guidelines within the 1-3 meals/month for noncancer health endpoints and 0-0.5 meals/month for cancer related health endpoints. The PCB concentrations in blue mussels collected from Delta Western exceed the safe consumption limits of 1-16 meals/month for non-cancer endpoints and 1-4 meals/month for cancer related health endpoints.

Filter feeding mussels are found to have a slightly elevated PCB body burden compared to surrounding sediments (Bard, 1999). In contrast, due to the lipophilic nature of PCBs coupled with bioaccumulation and biomagnification, high trophic level predators accumulate higher PCB body burdens during their relatively long life spans (Bard, 1999). Therefore, higher trophic level organisms at these sites would be expected to show even greater concentrations of PCBs in their tissues. Due to the World War II bombing at Building 551/T Dock to Airport and subsequent damage to transformers and electrical equipment in the surrounding area (which includes Delta Western), elevated PCB levels were expected. These results and associated consumption guidelines indicate that these FUD sites should be prioritized for PCB remediation.

Dewailly et al. (1989) observed that PCB concentrations in the breast milk of Inuit women were five times higher than concentrations in the breast milk of Caucasian women. The Inuit women in this study ate an average of between nine and 18 meals per month composed of fish and marine mammals. Prenatal PCB exposure is associated with low birthweight, slow growth and reduced intellectual and motor development (Ayotte et al., 2003). Early developmental PCB exposure occurs via diffusion across the placental barrier and after birth through breast feeding (Ayotte et al., 2003). Inuit breast-fed babies showed PCB plasma lipid concentrations of more than six times those of bottle fed infants (Ayotte et al., 2003) leading to elevated body burden through adulthood (Ayotte et al., 1996). Female mammals typically have a lower PCB concentration than males due to the partitioning of PCBs during reproduction and lactation (Bard, 1999). Lactation is an excretory route for the lower chlorinated congeners (Bard, 1999). Concentrations of PCBs found in the blood serum of many adult Inuits living a subsistence lifestyle reach close to the levels found to cause adverse health effects in laboratory studies (Ayotte et al., 1997). However, fish and other seafood rich in omega fatty acids provide beneficial protection against cardiovascular disease, so there are costs and benefits to the traditional marine-based diet (Harris, 1989).

In addition to potential adverse health effects for people, PCBs can cause detrimental effects in wildlife. For example, bald eagles (*Haliaeetus leucocephalus*) and other high trophic level birds rely on the near shore marine environment for most of their prey (Elliott et al., 2011). Eagles also forage in landfills exposing them to additional anthropogenic contaminants (Elliott et al., 2006). In response to PCB exposure, a decrease in thyroxine (T_4) levels is seen in nesting bald eagles potentially leading to negative physiological effects mediated by the endocrine system (Cesh et al., 2010). The Unalaska dump contains a PCB storage area around which eagles and other resident birds spend time foraging for food, potentially exposing them to increased levels of PCBs.

High trophic level organisms that rely on marine food sources, including many indigenous people of the Arctic, can be adversely affected by PCBs and other anthropogenic contaminants (Hardell et al., 2010; Letcher et al., 2010). The presence of PCB contamination in marine animals, particularly those associated with FUD sites, are of concern for safe consumption on Unalaska. In order to better understand the exposure risks for humans and wildlife, further work should investigate the food web dynamics of PCBs and other POPs in both marine and terrestrial habitats on Unalaska.

References

Ayotte, P., Carrier, G., Dewailly, E., 1996. Health risk assessment for Inuit newborns exposed to dioxin-like compounds through breast feeding. Chemosphere 32, 531-542.

Ayotte, P., Dewailly, E., Bruneau, S., Careau, H., Vezina, A., 1995. Arctic air pollution and human health: what effects should be expected? The Science of the Total Environment 160/161, 529-537.

Ayotte, P., Dewailly, E., Ryan, J.J., Bruneau, S., Lebel, G., 1997. PCBs and dioxin-like compounds in plasma of adult Inuit living in Nunavik (Arctic Quebec). Chemosphere 34, 1459-1468.

Ayotte, P., Muckle, G., Jacobson, J.L., Jacobson, S.W., Dewailly, É., 2003. Assessment of pre- and postnatal exposure to polychlorinated biphenyls: lessons from the Inuit cohort study. Environmental Health Perspectives 111, 1253-1258.

Bard, S.M., 1999. Global transport of anthropogenic contaminants and the consequences for the arctic marine ecosystem. Marine Pollution Bulletin 38, 356-379.

Beckmen, K.B., Keogh, M.J., Burek-Huntington, K.A., Ylitalo, G.M., Fadely, B.S., Pitcher, K.W., 2016. Organochlorine contaminant concentrations in multiple tissues of free-ranging steller sea lions (*Eumetopias jubatus*) in Alaska. The Science of the Total Environment 542, 441-452.

Blais, J.M., 2005. Biogeochemistry of persistent bioaccumulative toxicants: processes affecting the transport of contaminants to remote areas. Canadian Journal of Fisheries and Aquatic Sciences 62, 236-243.

Briggs, D., 2003. Environmental pollution and the global burden of disease. British Medical Bulletin 68, 1-24.

Byrd, G.V., Renner, H.M., Renner, M., 2005. Distribution patterns and population trends of breeding seabirds in the Aleutian Islands. Fisheries Oceanography 14, 139-159.

Carpenter, D.O., 2006. Polychlorinated biphenyls (PCBs): routes of exposure and effects on human health. Reviews on Environmental Health 21, 1-24.

Cesh, L.S., Elliott, K.H., Quade, S., McKinney, M.A., Maisoneuve, F., Garcelon, D.K., Sandau, C.D., Letcher, R.J., Williams, T.D., Elliott, J.E., 2010. Polyhalogenated aromatic hydrocarbons and metabolites: relation to circulating thyroid hormone and retinol in nestling bald eagles (*Haliaeetus leucocephalus*). Environmental Toxicology and Chemistry 29, 1301-1310.

Chamkasem, N., Lee, S., Harmon, T., 2016. Analysis of 19 PCB congeners in catfish tissue using a modified QuEChERS method with GC-MS/MS. Food Chemistry 192, 900-906.

Cogliano, V.J., 1998. Assessing the cancer risk from environmental PCBs. Environmental Health Perspectives 106, 317-323.

Dewailly, E., Ayotte, P., Bruneau, S., Laliberte, C., C.G.Muir, D., Norstrom, R.J., 1993. Inuit exposure to organochlorines through the aquatic food chain in arctic Quebec. Environmental Health Perspectives 101, 618-620.

Dewailly, E., Nantel, A., Weber, J.P., Meyer, F., 1989. High levels of PCBs in breast milk of Inuit women from arctic Quebec. Bulletin of Environmental Contamination and Toxicology 43, 641-646.

Elliott, K.H., Duffe, J., Lee, S.L., Mineau, P., Elliott, J.E., 2006. Foraging ecology of bald eagles at an urban landfill. The Wilson Journal of Ornithology 118, 380-390.

Elliott, K.H., Elliott, J.E., Wilson, L.K., Jones, I., Stenerson, K., 2011. Density-dependence in the survival and reproduction of bald eagles: linkages to chum salmon. The Journal of Wildlife Management 75, 1688-1699.

EPA, 1979. EPA bans PCB manufacture; phases out uses.

EPA, 1999. Polychlorinated biphenyls (PCBs) update: impact on fish advisories. United States Environmental Protection Agency.

EPA, 2000. EPA volume 2 risk assessment and fish consumption limits.

Ewald, G., Larsson, P., Linge, H., Okla, L., Szarzi, N., 1998. Biotransport of organic pollutants to an inland Alaska Lake by migrating sockeye salmon (*Oncorhynchus nerka*). Arctic 51, 40-47.

Garmash, O., Hermanson, M.H., Isaksson, E., Schwikowski, M., Divine, D., Teixeira, C., Muir, D.C., 2013. Deposition history of polychlorinated biphenyls to the Lomonosovfonna Glacier, Svalbard: a 209 congener analysis. Environmental Science and Technology 47, 12064-12072.

Ge, J., Woodward, L.A., Li, Q.X., Wang, J., 2013. Distribution, sources and risk assessment of polychlorinated biphenyls in soils from the Midway Atoll, North Pacific Ocean. PLoS One 8, e71521.

Gioia, R., Dachs, J., Nizzetto, L., Lohmann, R., Jones, K.C., 2013. Atmospheric transport, cycling and dynamics of polychlorinated biphenyls (PCBs) from source regions to remote oceanic areas, Occurrence, Fate and Impact of Atmospheric Pollutants on Environmental and Human Health, pp. 3-18.

Hardell, S., Tilander, H., Welfinger-Smith, G., Burger, J., Carpenter, D.O., 2010. Levels of polychlorinated biphenyls (PCBs) and three organochlorine pesticides in fish from the Aleutian Islands of Alaska. PLoS One 5, e12396.

Harris, W.S., 1989. Fish oils and plasma lipid and lipoprotein metabolism in humans: a critical review. Journal of Lipis Research 30, 785-807.

Jurado, E., Dachs, J., 2008. Seasonality in the “grasshopping” and atmospheric residence times of persistent organic pollutants over the oceans. Geophysical Research Letters 35.

Kenney, L.A., Eagles-Smith, C.A., Ackerman, J.T., von Hippel, F.A., 2014. Temporal variation in fish mercury concentrations within lakes from the western Aleutian Archipelago, Alaska. PLoS One 9, e102244.

Kenney, L.A., von Hippel, F.A., Willacker, J.J., O'Hara, T.M., 2012. Mercury concentrations of a resident freshwater forage fish at Adak Island, Aleutian Archipelago, Alaska. Environmental Toxicology and Chemistry 31, 2647-2652.

Letcher, R.J., Bustnes, J.O., Dietz, R., Jenssen, B.M., Jorgensen, E.H., Sonne, C., Verreault, J., Vijayan, M.M., Gabrielsen, G.W., 2010. Exposure and effects assessment of persistent organohalogen contaminants in arctic wildlife and fish. Science of the Total Environment 408, 2995-3043.

Miodovnik, A., 2011. Environmental neurotoxicants and developing brain. Mount Sinai Journal of Medicine 78, 58-77.

Muir, D., Braune, B., DeMarch, B., Norstrom, R., Wagemann, R., Lockhart, L., Hargrave, B., Bright, D., Addison, R., Payne, J., Reimer, K., 1999. Spatial and temporal trends and effects of contaminants in the Canadian Arctic marine ecosystem: a review. The Science of the Total Environment 230, 83-144.

Oakley, G.G., Devanaboyina, U.-s., Robertson, L.W., Gupta, R.C., 1996. Oxidative DNA damage induced by activation of polychlorinated biphenyls (PCBs): implications for PCB-induced oxidative stress in breast cancer. Chemical Research in Toxicology 9, 1285-1292.

Rahn, K.A., Heidam, N.Z., 1981. Progress in arctic air chemistry, 1977-1980: a comparison of the first and second symposia. Atmospheric Environment 15, 1345-1348.

Safe, S., 1989. Polychlorinated biphenyls (PCBs): mutagenicity and carcinogenicity. Mutation Research 220, 31-47.

Schell, L.M., Gallo, M.V., Denham, M., Ravenscroft, J., DeCaprio, A.P., Carpenter, D.O., 2008. Relationship of thyroid hormone levels to levels of polychlorinated biphenyls, lead, p,p'-DDE, and other toxicants in Akwesasne Mohawk youth. Environmental Health Perspectives 116, 806-813.

Scrudato, R.J., Chiarenzelli, J.R., Miller, P.K., Alexander, C.R., Arnason, J., Zamzow, K., Zweifel, K., Gologergen, J., Kava, J., Waghiyi, V., Carpenter, D.O., 2012. Contaminants at arctic formerly used defense sites. Journal of Local and Global Health Science 2, 1-12.

Semeena, V.S., Lammel, G., 2005. The significance of the grasshopper effect on the atmospheric distribution of persistent organic substances. Geophysical Research Letters 32, n/a-n/a.

Steck, T.L., 1974. The organization of proteins in the human red blood cell membrane. The Journal of Cell Biology 62, 1-19.

Tan, Y., Chen, C.H., Lawrence, D., Carpenter, D.O., 2004. Ortho-substituted PCBs kill cells by altering membrane structure. Toxicological Sciences 80, 54-59.

UNEP, 2001. Stockholm Convention on persistent organic pollutants (POPs)

USCB, 2018. Annual estimates of the resident population.

von Hippel, F.A., Miller, P.K., Carpenter, D.O., Dillon, D., Smayda, L., Katsiadaki, I., Titus, T.A., Batzel, P., Postlethwait, J.H., Buck, C.L., 2018. Endocrine disruption and differential gene expression in sentinel fish on St. Lawrence Island, Alaska: health implications for indigenous residents. Environmental Pollution 234, 279-287.

von Hippel, F.A., Trammell, E.J., Merila, J., Sanders, M.B., Schwarz, T., Postlethwait, J.H., Titus, T.A., Buck, C.L., Katsiadaki, I., 2016. The ninespine stickleback as a model organism in arctic ecotoxicology. Evolutionary Ecology Research 17, 487-504.

Wang, J., Caccamise, S.A., Woodward, L.A., Li, Q.X., 2015. Polychlorinated biphenyls in the plasma and preen oil of black-footed albatross (*Diomedea nigripes*) chicks and adults on Midway Atoll, North Pacific Ocean. PLoS One 10, e0123041.

Wania, F., Mackay, D., 1993. Global fractionation and cold condensation of low volatility organochlorine compounds in polar regions. Ambio 22, 10-18.

Table 1:

Site Name	FUD	Species Collected	Latitude	Longitude
APL/Rocky Point	Military	Blue Mussel	53.888171	166.526738
Captains Bay - Rt side (Crowley) Dock	Military	Blue Mussel	53.852301	166.569865
Captains Bay - Lf side (Crowley) Dock	Military	Blue Mussel	53.847266	166.578966
Captains Bay - Port Levashef	Military	Blue Mussel	53.83701	166.61732
Delta Western	Military	Blue Mussel	53.889898	166.53374
Front Beach	Military	Blue Mussel	53.885466	166.555203
Inside Dutch harbor Spit	Military	Blue Mussel	53.904176	166.511461
Margarets Bay Entrance	Military	Blue Mussel	53.880543	166.548745
Royal Aleutian Dock #1	Military	Blue Mussel	53.881965	166. 544153
Royal Aleutian Dock #2	Military	Blue Mussel	53.88165	166.541325
S Curves	Military	Blue Mussel	53.87753	166.565441
Sub Base	Military	Blue Mussel	53.878035	166.552895
Sub Base (Waleshek) Dock	Military	Blue Mussel	53.877883	166.555591
Sub Base Entrance	Military	Blue Mussel	53.876236	166.549375
Sub Base Haul Out	Military	Blue Mussel	53.877901	166.556416
Building 551/T Dock to Airport	Military	Blue Mussel	53.893015	166.536995
Unalaska Dump	Military	Blue Mussel	53.881233	166.511613
Wide Bay	Military	Blue Mussel	53.95234	166.62553
Captains Bay - Fox Site	Non-Military	Blue Mussel	53.867926	166.546333
Captains Bay - Left side Cannery	Non-Military	Blue Mussel	53.85714	166.556358
Captains Bay - Rt side Cannery	Non-Military	Blue Mussel	53.858605	166.55186
Cascade Falls	Non-Military	Blue Mussel	53.93166	166.64273
Constantine Bay	Non-Military	Blue Mussel	53.95692	166.409855
Rat Islands	Non-Military	Blue Mussel	53.84848	166.50273
Wislow	Non-Military	Blue Mussel	53.99942	166.726433
Morris Cove Creek	Military	Salmonids and Threespine Stickleback	53.916703	166.429546
Unalaska Lake	Military	Salmonids and Threespine Stickleback	53.86418	166.520698

Matson lake	Military	Threespine Stickleback	53.887175	166.540236
Nateekin River	Non-Military	Salmonids and Threespine Stickleback	53.8718	166.63892
Shaishnikoff River	Non-Military	Salmonids	53.824665	166.610461

Figure 1

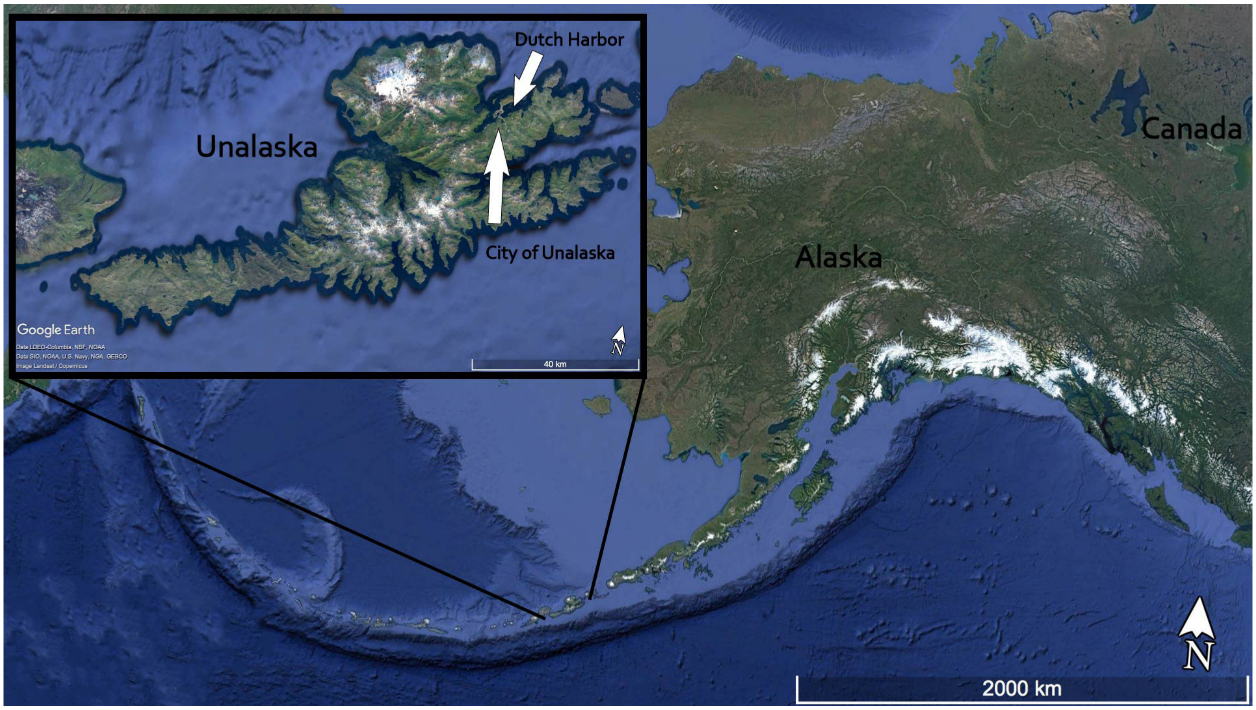

Dutch Harbor
Unalaska
City of Unalaska
Google Earth
40 km
N
Canada
Alaska
N
2000 km

Figure 2

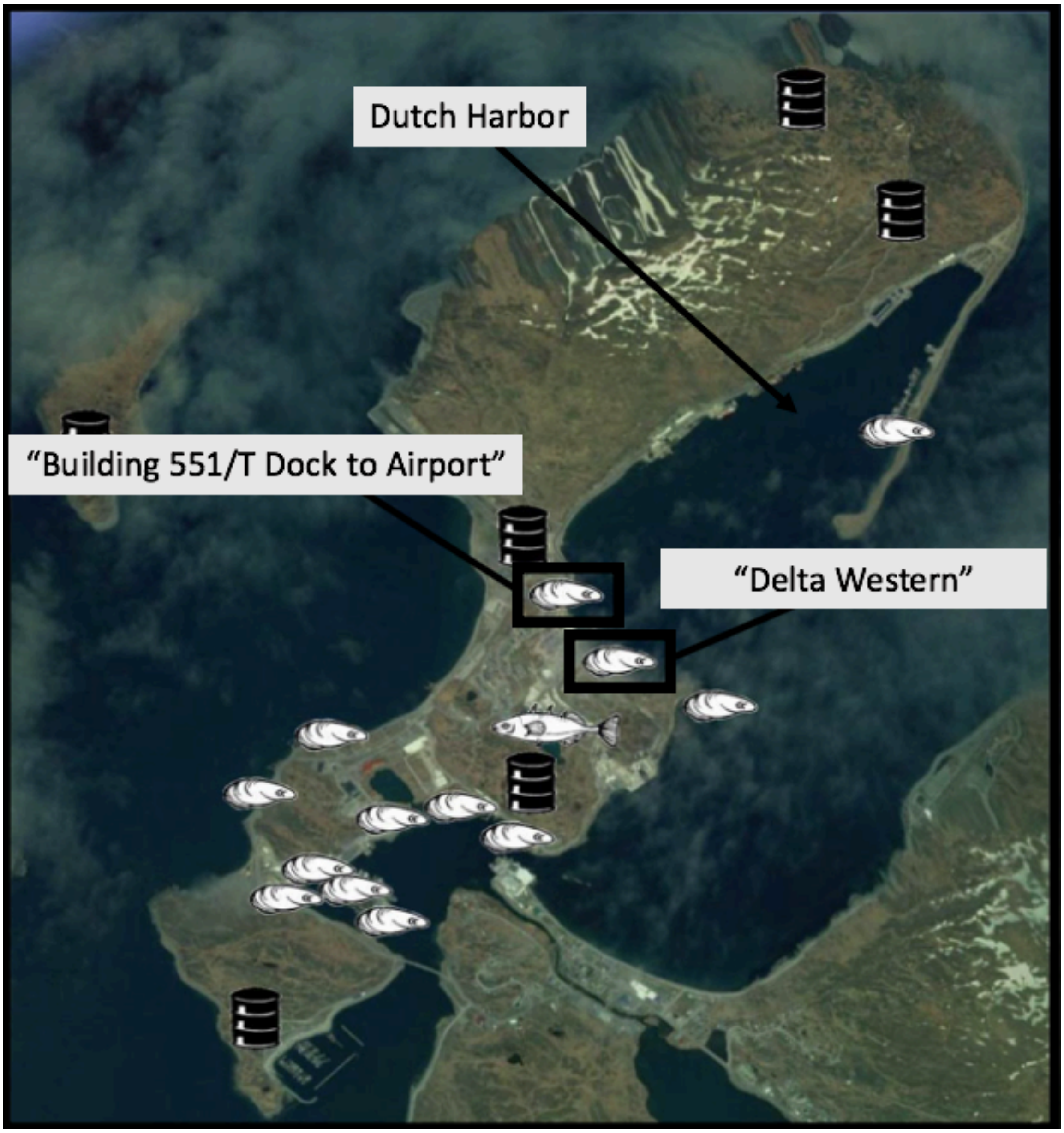


Figure 3

Google Earth
Data SIO, NOAA, U.S. Navy, NGA, GEBCO
Image © 2018 DigitalGlobe
Image Landsat / Copernicus
N
9 km

Figure 4

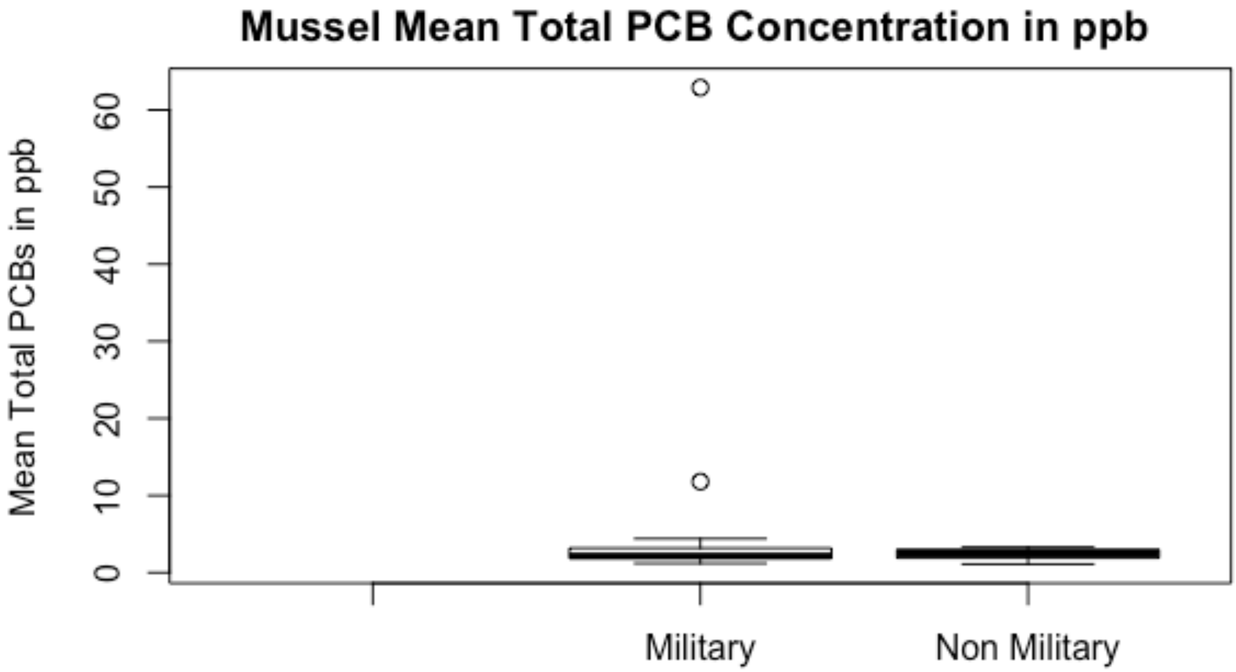

Mussel Mean Total PCB Concentration in ppb
Mean Total PCBs in ppb
0
10
20
30
40
50
60
Military
Non Military

Figure 5

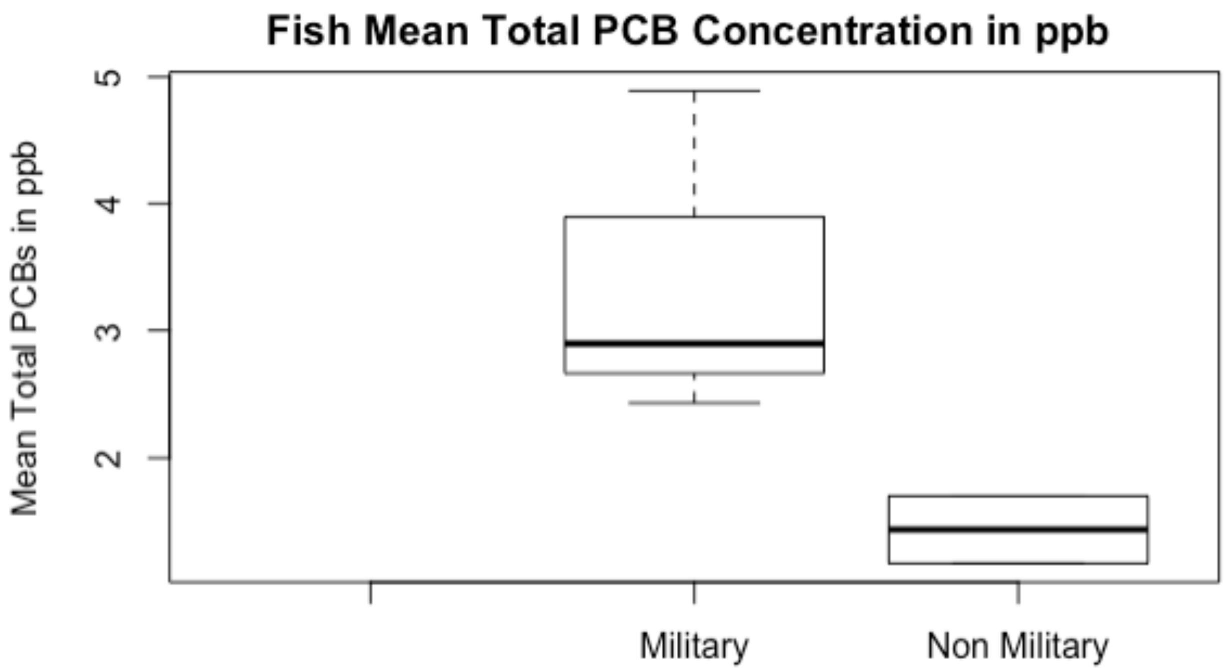
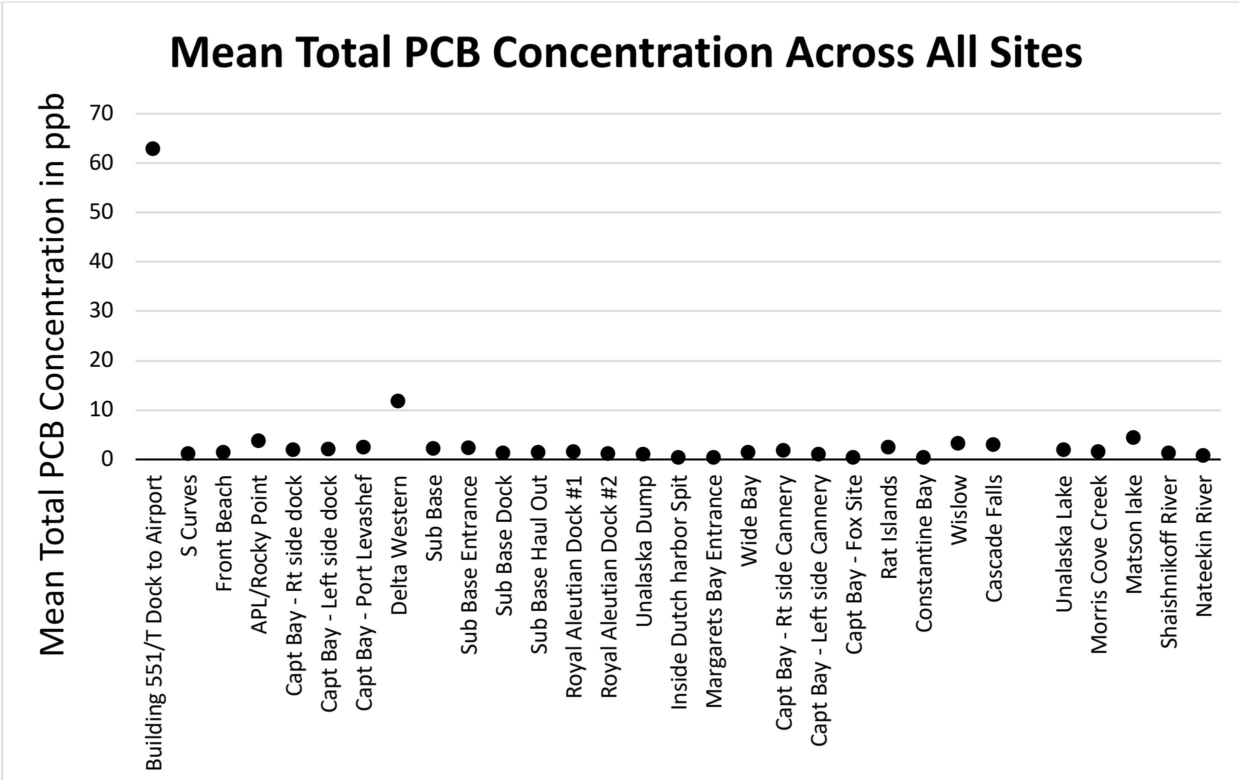

Fish Mean Total PCB Concentration in ppb
Mean Total PCBs in ppb
5
4
3
2
Military
Non Military

Figure 6


Mean Total PCB Concentration Across All Sites
Mean Total PCB Concentration in ppb
70
60
50
40
30
20
10
0
Building 551/T Dock to Airport
S Curves
Front Beach
APL/Rocky Point
Capt Bay - Rt side dock
Capt Bay - Left side dock
Capt Bay - Port Levashef
Delta Western
Sub Base
Sub Base Entrance
Sub Base Dock
Sub Base Haul Out
Royal Aleutian Dock #1
Royal Aleutian Dock #2
Unalaska Dump
Inside Dutch harbor Spit
Margarets Bay Entrance
Wide Bay
Capt Bay - Rt side Cannery
Capt Bay - Left side Cannery
Capt Bay - Fox Site
Rat Islands
Constantine Bay
Wislow
Cascade Falls
Unalaska Lake
Morris Cove Creek
Matson lake
Shaishnikoff River
Nateekin River