

POLYCHLORINATED BIPHENYL CONCENTRATIONS IN ADULT CHINOOK  
SALMON (*Oncorhynchus tshawytscha*) RETURNING TO COASTAL AND PUGET  
SOUND HATCHERIES

by

Brian Missildine

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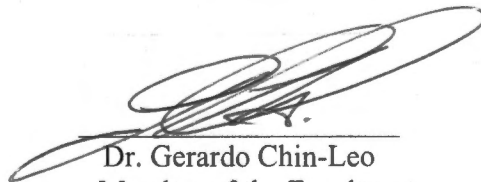
by

Brian Missildine

has been approved for

The Evergreen State College

by



Dr. Gerardo Chin-Leo  
Member of the Faculty



Dr. John Perkins  
Member of the Faculty



Dr. Roger Peters  
Fisheries Scientist  
U. S. Fish and Wildlife Service

\_\_\_\_\_  
Date

## ABSTRACT

Polychlorinated Biphenyl Concentrations in Adult Chinook Salmon (*Oncorhynchus tshawytscha*) Returning to Coastal and Puget Sound Hatcheries.

Brian Missildine

Polychlorinated biphenyl (PCBs) concentrations were evaluated in the muscle tissue of 4-year old Puget Sound and coastal hatchery Chinook salmon. Ten muscle tissue samples per location were taken from two Puget Sound hatcheries and two Washington coast hatcheries to determine PCB concentrations. Two trade name PCBs, Aroclor 1254 and Aroclor 1260, were found. Aroclor 1254 was detected in all samples, while Aroclor 1260 was detected in 16 out of 40 samples. Generalized linear modeling (GLM) was used to evaluate the influence of several variables on PCB concentrations. Twenty different GLMs, representing multiple null hypotheses, were ranked using Akaike Information Criterion (AIC). PCB concentrations were explained by region and lipids, followed by region, and location (hatchery) and lipids. Region appears to be the variable that explains most of the variation in PCB concentrations in Chinook salmon in the northwest. PCB concentrations in Chinook salmon muscle tissue from Puget Sound hatcheries were significantly greater (mean 49.26  $\mu\text{g}/\text{kg}$  wet weight; standard deviation 40.55  $\mu\text{g}/\text{kg}$ ) than those from coastal hatcheries (mean 17.41  $\mu\text{g}/\text{kg}$  wet weight; standard deviation 6.8  $\mu\text{g}/\text{kg}$ ). This suggests that the primary source of PCBs observed in Puget Sound Chinook salmon is contamination within Puget Sound. Four-227 gram (8oz) portions from Puget Sound Chinook salmon and sixteen-227 gram portions from coastal Chinook salmon can be consumed before the potential for adverse risk from PCB consumption becomes a concern, based on EPA's fish consumption guidelines and mean PCB concentrations observed during this study. In addition, salmon carcass supplementation may need to be reevaluated to determine if adding fish contaminated with PCBs to increase marine derived nutrients is worth the environmental risk.

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## INTRODUCTION

Chinook salmon (*Oncorhynchus tshawytscha*), also called king salmon, is distinguishable from all other Pacific salmon by its large size. Chinook salmon have been a mainstay of indigenous people of the Puget Sound and Washington coast for several thousand years (Morishima and Henry 1999). Recently, studies have indicated that Puget Sound Chinook salmon are contaminated with PCBs (PSAMP 2001; O'Neill et al. 1998). Human health issues have been associated with exposure to PCBs from fish consumption (Jacobson and Jacobson 1996; Korrick and Altshul 1998; EPA 2002). Humans have also been exposed to PCBs through occupational exposure (Kilburn et al. 1989; Ojajarvi et al. 2000), and in utero and breast milk (Jacobson and Jacobson 1996; Craan and Haines 1998). Although cancerous and non-cancerous health hazards such as developmental neurotoxic effects, reduced birth weights and immunotoxic effects have been associated with exposure to PCBs (EPA 2002); the overall influence of PCBs on human health are mixed.

There are two potential sources of PCB contamination for Puget Sound Chinook salmon: Puget Sound and the North Pacific Ocean. Present and past urban and industrial practices have lead to environmental degradations in the Puget Sound region. Pollution and contamination have become widespread in urban bays, estuaries, and rivers to the point it is causing the decline of a variety of organisms within the Puget Sound (PSAMP 2001).

There are currently two Superfund sites in critical estuaries of the Puget Sound, the Lower Duwamish Waterway in Seattle and Commencement Bay in Tacoma. Some locations within Elliot Bay and Commencement Bay that were listed as Superfund sites have since been remediated; however, there are still areas of concern. Furthermore, all of



these locations have been, and are currently subjected to heavy industrial uses that commonly contributed to PCB contamination.

There is a potential that the North Pacific Ocean is a sink for contaminants (Bailey et al. 2000; McCain et al. 2000). There is speculation that contaminants are originating from Asian countries and are being transferred by ocean and air currents into the North Pacific Ocean where conditions are conducive for PCBs to settle. The north-migrating Chinook salmon are potentially being exposed either through food sources or the water during their migration through the North Pacific Ocean.

### *Objective*

The primary objective of this research was to determine if PCB concentrations in Chinook salmon differed between coastal and Puget Sound stocks. This information will be useful for determining if the source of PCBs is occurring in the Puget Sound or the North Pacific Ocean. The secondary objective was to determine if other variables such as sex, weight or percent lipids influence PCB concentrations in Chinook salmon. These objectives were met by testing multiple hypotheses for my thesis including: There is no difference in PCB levels in Puget Sound versus coastal Chinook salmon; there is no difference in PCB levels between sexes; and, PCB concentrations are not related to size of fish. The results of this research will also be used to determine if PCB concentrations in Chinook salmon returning to Washington are high enough to pose a risk to human health based on EPA fish advisory guidelines.

## **PCB LITERATURE REVIEW**

PCBs are one of the most touted chemical classes of concern in the Puget Sound. PCBs are distributed by oceanic and atmospheric currents (Kanan et al. 1989; Bailey et al. 2000; Jaffe et al. 1999) and can be found in even the most remote locations throughout the world (Hidaka et al. 1983). Some of the more common uses of PCBs were in electrical transformers used in industrial and residential utilities, fire retardants, paint additives, and immersion oils. Monsanto Corporation was the major manufacturer of PCBs in the United States from the 1930's until 1976 when PCB use was banned (Davis 1993). Although PCBs are no longer produced in the United States, they are still used in developing countries. PCB concentrations in muscle tissue of fishes have declined at long-term monitoring stations in the Puget Sound since their use was banned in the U. S. (PSAMP 2001). However, PCB concentrations appear to be increasing in pinnipeds and cetaceans around the Northwest (PSAMP 2001).

Current research on PCB concentrations in Chinook salmon in the Pacific Northwest has been limited to Puget Sound stocks (Arkoosk et al. 1998 a and b; O'Neill et al. 1998; Stein et al. 1995). O'Neill et al. (1998) found that average concentrations in adult Chinook salmon in the Puget Sound marine environment were 74.2 µg/kg wet weight. O'Neill et al. (1998) estimated that only 1.1 percent of the adult Chinook salmon PCB burden came from exposure as juveniles; the other 99 percent was accumulated during saltwater migration. It is unclear whether Chinook salmon are accumulating PCBs in the Puget Sound or throughout their migratory pathways in the Pacific Ocean.

Relatively little research has been conducted on exposure of juvenile Chinook salmon to contaminants in estuaries or to determine if exposure could result in increased mortality rates during their ocean life phase (Arkoosh et al. 1998b). Factors that negatively affect the fitness of juvenile Chinook salmon may not only increase early life mortality, but could create biological responses in the adult that may inhibit growth, impair locomotion, or reduce fecundity.

Existing research suggests that juvenile Chinook salmon exposed to PCBs experience adverse biological effects. Arkoosh et al. (1998a) found mean PCB concentration levels in juvenile Chinook salmon ranged from 37 ng/g wet weight from the Kalama Creek Hatchery (non-urban), to 270 ng/g wet weight in the Duwamish Waterway (urban), in 1993. Arkoosh et al. (1998a) also found juvenile Chinook salmon sampled from the Duwamish Waterway were more susceptible to mortality induced by the marine pathogen *Vibrio anguillarum* after four days than fish taken from a hatchery in a nearby river basin (the control group). *Vibrio anguillarum* is a common bacterial pathogen of marine and brackish water fishes and has caused severe losses in marine and estuarine aquaculture (Post 1987). In addition, Arkoosh et al. (1998a) found an increase in mortality in juvenile Chinook salmon that had greater levels of PCBs than the test group three months after the fish were removed from their prospective estuary. This suggests that Chinook salmon exposed to PCB contamination as juveniles would remain immunosuppressed, potentially throughout their lives, even after they left the estuary. Stein et al. (1995) found mean concentration levels of PCBs in juvenile Chinook salmon ranged from 22 ng/g wet weight in the Nisqually River (non urban), to 300 ng/g wet weight in the Duwamish

Waterway (urban), in 1989. Stein et al. (1995) found that juvenile Chinook salmon exposed to PCBs had an increased activity of the enzyme P450 that plays a central role in the metabolism of toxins. Stein et al. (1995) also found higher levels of DNA damage in juvenile Chinook salmon from urban estuaries than juvenile Chinook salmon from non-urban estuaries.

PCB mobility within the Chinook salmon is influenced by the amount of lipids within the fish; hence, the higher the lipid content, the more the chemical is sequestered within the fatty muscle reserves and is not readily available to the organs of the fish (Meador 2000). Salmonid lipid content varies during their life cycle with the low points occurring during the fry, smolt, and spawning stage, increasing the probability of the toxin mobilizing into critical organs during crucial points in their life history. Jorgensen et al. (1999) found a 10-fold increase in PCB levels in the liver of an arctic char that was not fed for a significant time period (starved). Jorgensen et al. (1999) observed that the decrease in total body lipids due to starvation caused the PCB to mobilize to other lipid containing organs such as the liver, kidney, and brain.

### **Physical and Chemical properties of PCBs**

PCB is the generic name given to the entire group or a subset of the 209 different chemical compounds having the formula of  $C_{12}H_{10-n}Cl_n$ , where  $n = 1-10$  (Erickson 1997). These 209 compounds occur in 11 groups or “homologs” based on the number of chlorine atoms in the molecule. In turn, each homolog has from 1 to 46 isomers, based

on the position of the chlorine atoms (Tables 1 and 2) (Figure 1) (Erickson 1997).

PCBs are also defined as non-planar and planar. Non-planar PCBs are identified as having at least one chlorine atom on the 2,2' or 6,6' position(s) on the biphenyl ring. PCBs having the non-planar configuration are less stable than planar PCBs because the chlorine atoms are too large to fit adjacent to each other. This configuration causes the bond between the biphenyl rings to twist, causing the molecule to form at right angles which weakens the bond. Planar PCBs form in the same plane, which creates a very strong bond between the biphenyl rings. Planar PCBs are not easily broken down in the environment due to the strength of their bond. Planar PCBs are less common than non-planar PCBs with only 20 of the 209 congener's able form the planar configuration.

PCBs used in manufacturing were a clear viscous liquid typically used for insulation, especially in high voltage equipment such as capacitors and transformers. PCBs have low water solubility and low vapor pressure (Erickson 1997). However, physical properties such as boiling point, melting point, vapor pressure, bioconcentration factors in fish and evaporation rate change throughout the homologous series. PCBs are lipophilic (fat loving) and are soluble in organic solvents and in biological lipids.

Only half of the total 209 PCB congeners account for most of the environmental contamination. Even fewer are prevalent and toxic. According to McFarland and Clarke (1989), the number of congeners of concern is reduced to 36 if potential toxicity, environmental prevalence, and relative abundance are used as criteria. Approximately 25

Table 1. The number of individual compounds in each of the different PCB categories (Adopted from Erickson 1997).

Category	No. of individual compounds
Congener	209
Homolog	11
Isomers/homolog	1-46

Table 2. Composition of Chlorinated biphenyls by Homolog (Adopted from Erickson 1997).

Homologs	Chlorine %, by weight	No. of isomers
C <sub>12</sub> H <sub>9</sub> Cl	19	3
C <sub>12</sub> H <sub>8</sub> Cl <sub>2</sub>	32	12
C <sub>12</sub> H <sub>7</sub> Cl <sub>3</sub>	41	24
C <sub>12</sub> H <sub>6</sub> Cl <sub>4</sub>	49	42
C <sub>12</sub> H <sub>5</sub> Cl <sub>5</sub>	54	46
C <sub>12</sub> H <sub>4</sub> Cl <sub>6</sub>	59	42
C <sub>12</sub> H <sub>3</sub> Cl <sub>7</sub>	63	24
C <sub>12</sub> H <sub>2</sub> Cl <sub>8</sub>	66	12
C <sub>12</sub> HCl <sub>9</sub>	69	3
C <sub>12</sub> Cl <sub>10</sub>	71	1

of the remaining 36 congeners account for 50-75 percent of the total PCBs in tissue samples of fish, invertebrates, birds, and mammals.

PCBs in the United States are typically known by their trade name Aroclor. They are further identified by a specific number. For example, Aroclor 1254 contains 12 carbon atoms bound together within the biphenyl rings and contains 54 percent chlorine by weight. Aroclor analysis is the method currently recognized by EPA. The Aroclor analysis has been the most common method for measuring total PCBs, in part due to cost, equipment availability, time, and technology. Congener specific data is much more

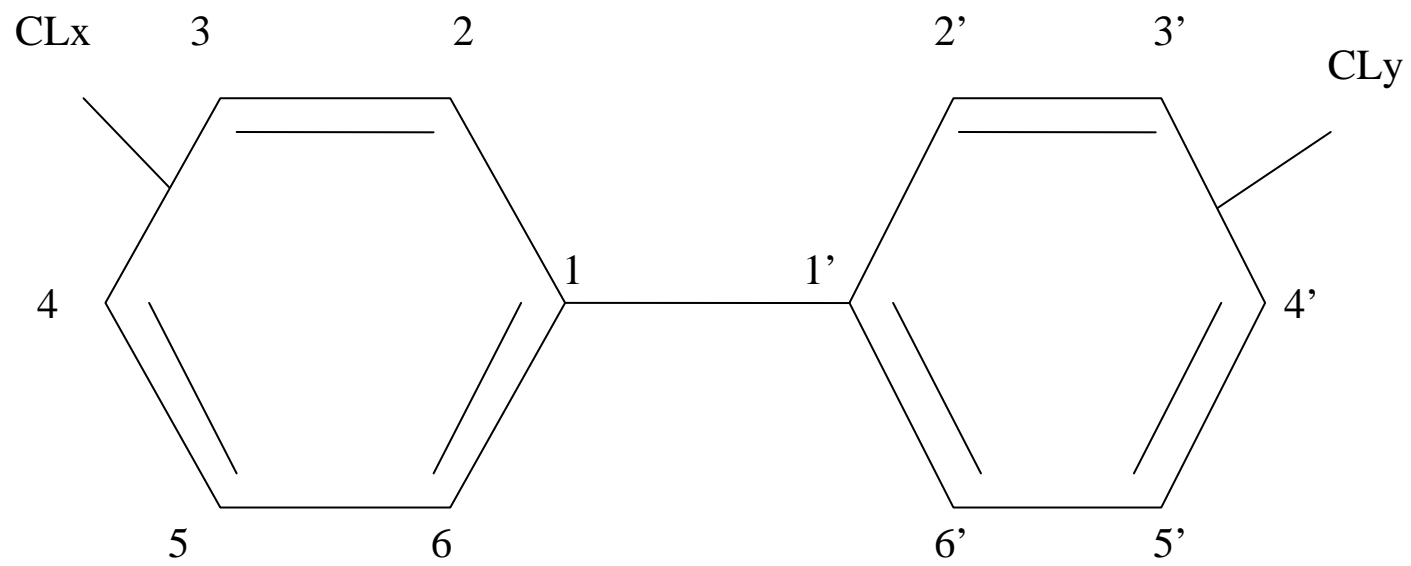


Figure 1. Structure and numbering pattern of PCB's in biphenyl ring system.

expensive and there is no single standard methodology for conducting congener specific analysis. However, congener specific analysis is more detailed than Aroclor analysis and may be more important in determining toxicity.

### **Transportation mechanisms**

One of the main pathways PCBs entered into the environment was through disposal. No literature searched indicated, or even attempted to predict the amount of PCBs that were dumped into oceans, lakes and rivers. PCBs bind tightly to organic matter once dumped into the environment. PCBs favor moist organic solids and will avoid the aqueous portion of sediment. PCBs tend to mobilize toward the organic component where sorption takes place by the carbon components of the sediment (Paya-Perez et al. 1991). Movement (mobility) within the sediment can take years depending on the soil and PCB characteristics (Girvin et al. 1993). PCBs, especially planar PCBs, are extremely stable and may remain within the environment for many years and can be readily resuspended during dredging, pile driving or other activities that disturb sediments (Eisler 1986).

Larsson and Okla (1987) theorized that oceanic suspended particles may provide transportation mechanism for PCBs. Phytoplankton can readily absorb PCBs into their lipid rich membranes and then into cellular material within the plankton (Broman et al. 1992). These lower order consumers store PCBs, are consumed by their predators where they are again stored until eaten by yet another predator. EPA (1999) noted that PCB concentrations could be 2,000 to more than a million times higher in an organism than the



concentrations found in surrounding waters, with the highest concentrations found at the top of the food chain. The rate of PCB bioaccumulation varies among environmental constituents. Factors such as age, species, gender, and lipid contents influence bioaccumulation within organisms.

The circulatory system provides the primary transportation pathway for PCBs within an organism (Duinkers et al. 1989). PCBs transported through the circulatory system are dispersed into fatty tissue and critical organs which are lipid rich. Goldstein and Safe (1989) indicated that one characteristic effect of planar PCBs is the induction of the cytochrome P450 enzymes. This class of enzymes is primarily found in the liver, and to a certain extent, the extrahepatic tissues in the lungs, kidney, intestines, spleen, and testes. The P450 enzymes and its monooxygenase activities are responsible for the metabolism of carcinogens, drugs, pesticides and other foreign compounds.

PCBs accumulate in the fatty tissue of organisms, including Chinook salmon, due to their lipophilic nature. The inability of Chinook salmon to properly metabolize toxic substances could lead to an increase in mortality in adult Chinook salmon, especially during spawning migration. Adult salmonids stop feeding during their spawning migration and depend on their fat reserves as a fuel source. The amount of fat reserve used increases with the length of time without feeding. This results in PCBs moving to other fatty tissues such as the internal organs and gametes (Hendry 1998). Jorgensen et al. (1999) found an increase in PCB concentrations in the liver and kidneys of salmonids that were starved. Miller (1993) found that concentrations of organochlorine compounds,

such as PCBs, found in the muscle tissue of adult Chinook salmon and lake trout (*Salvelinus namaycush*) were significantly correlated to concentrations found in the eggs. Miller's research may indicate that PCBs are readily available in the gametes during all the life stages of salmonids.

PCB transport in mammals is similar to fish with the main pathway through ingestion then through the circulatory system to fat cells. However, not all PCBs ingested are retained. This explains the difficulty of determining the impacts of PCB ingestion by people since they are getting unknown and different concentrations from eating the same contaminated food. Recent studies suggest that not all PCBs are absorbed once ingested into the body. Juan et al. (2002) studied volunteers who were consuming a diet that had some PCB contamination and found that congener type and body fat index influenced PCB absorption. Fourteen of the congeners were absorbed by all volunteers. Other congeners were absorbed in some volunteers but excreted by others. Volunteers tended to excrete congeners with higher chlorination more often while those with lower chlorination were more readily absorbed within the body. Volunteers with a higher body fat index tended to absorb more of the congeners studied while volunteers with a lower body fat index tended to excrete the congeners. This, along with exposures to other potential toxic substances, makes it difficult to determine PCB impacts on humans.

PCBs can be transferred maternally in humans. Lanting (1999) found newborns that were strictly breast fed for six weeks contained 4½ times the amounts of PCBs than infants fed formula. Patadin et al. (1999) discovered that 12 to 14 percent of long-term

dietary exposure to PCBs is partly due to breast feeding. Korrik and Altshul (1998) found that breast milk from four women contained PCB levels that were significantly higher than the rest of the study group of 122 women. The greater PCB concentrations in the four women were attributed mainly to fish consumption, and one was attributed to occupational exposure. All four women also lived near a contaminated site. The infants from these four women were delivered full term and healthy.

### **Impacts to humans**

PCBs are ubiquitous and have been associated with human health issues ranging from neurological impacts to cancer. Most humans in industrialized countries contain measurable levels of PCBs. Contamination has occurred mainly from the consumption of fatty sports fish (Jacobson and Jacobson 1996; Jensen 1984); however, occupational exposure (Kilburn et al. 1989), and maternal transfer have also occurred.

The scientific community currently debates the human health impacts of PCB exposure. Linking human health issues to PCB exposure is very difficult because humans are subjected to many other environmental factors such as nutrition, pesticides, chemicals and other pollutants, in addition to socio-economic stressors. Furthermore, results are often difficult to interpret because of study design limitations or inconclusive results for the most extreme health concerns (Shirai 1995). However, there are a numerous studies that associate PCB exposure to human health issues. Jacobson and Jacobson (1996) found an association between elevated PCB concentrations in children and poor verbal IQ

scores. Osius et al. (1999) found an association between PCB blood serum levels and thyroid hormone levels. This association may be important because some toxins such as PCBs share a similar molecular structure with thyroid hormones and may interfere with endocrine function by imitating natural hormones (McKinney and Waller. 1998; Brouwer et al. 1998). In addition, EPA (2002) has identified reduced birth weight, learning disabilities, immunosuppression, and other non-cancerous health effects to be associated with exposure to PCBs. Although it is difficult to determine the influence of chronic exposure to human health, case studies associate negative health effect from high episodic exposure to PCB concentrations.

Ingestion of food contaminated with high concentrations of PCBs has been linked to human health impacts. The most notorious exposure of PCBs occurred in Yucheng, Taiwan in 1979 where approximately 2,000 people were exposed to rice oil contaminated with PCBs during manufacturing. This exposure resulted in increased abnormal menstrual bleeding and still births in exposed women compared to unexposed women (Yu et al. 2000). Guo et al. (1999) also found that there was an increase in chloracne, hyperkeratosis, abnormal nails, gum swelling and gum pigmentation, as well as an increase in mortality from nonmalignant liver disease in those exposed at Yucheng compared to an unexposed control unit. Similar effects were observed in a population exposed to PCB contaminated rice oil during a similar incident in Yusho, Japan, 11 years earlier (Guo et al. 1999).

The Michigan Maternal Infant Cohort Study (Fein et al. 1984; Jacobson et al. 1985, 1990a, b) found developmental deficiencies in the offspring of women who consumed PCB contaminated fish from Lake Michigan in comparison to women who did not eat fish from Lake Michigan. Developmental deficiencies such as head circumference, gestational period, and birth-weight, as well as cognitive deficiencies were statistically significant in the study. Most of these cognitive and developmental deficiencies were still present up to age 4. These findings were subsequently subjected to scrutiny due to the sampling and testing methodology. However, Swain (1991), using the epidemiologic criteria of Susser (1986), found that there was strong epidemiological evidence to support the initial findings.

Individuals who have worked in electrical component manufacturing such as capacitor, light ballast, or transformer assembly in the past have likely been exposed to PCBs. However, other occupational exposure has also occurred. Kilburn et al. (1989) discovered 14 firefighters exposed to PCB fumes while fighting a fire in a transformer room (the transformer contained PCBs) showed symptoms of extreme fatigue, headaches, muscle weakness, and aching joints two days to three months after the fire. Several of the firefighters had memory loss, impaired concentration, irritability and other psychological impairments.

PCBs have also been implicated in breast cancer; however, the results are inconclusive. Aronson et al. (2000) found a correlation between PCB concentrations in breast tissue and cancer. Moysich et al. (1999) found an association between PCB concentrations and

cytochrome P4501A1 polymorphism and breast cancer risk. However, several authors found no significant differences in blood serum PCB levels and breast cancer (Stellman et al. 2000; Wolff et al. 2000; Laden et al. 2001). It is important to note that methodologies differed between the studies that found correlations and those that didn't. Most of the studies that found no association between PCBs and breast cancer analyzed PCBs in the blood serum while those studies that found an association analyzed adipose tissue or breast tissue. Lipid concentrations in blood serum are much lower than lipid levels in adipose or breast tissue. Aronson et al. (2000) suggests that the concentrations measured in serum are not representative of the concentrations found in adipose tissue. Aronson et al. (2000) also notes that PCB concentrations in serum and PCB concentrations in adipose tissue vary. Furthermore, measurements in breast adipose tissue may provide a better means for determining if PCBs cause breast cancer due to tissues proximity and the cancer (Aronson et al. 2000).

Overall, PCBs probably impact human health; however, the exposure level impacting human health and the human health impact are uncertain. Other variables such as genetics and environment make it difficult to directly implicate PCBs to human health.

## **METHODS**

### *Field Sampling*

Salmon tissue samples for PCB analysis were collected from four western Washington hatcheries, which represented two separate regions of western Washington; coastal and Puget Sound. The coastal region was represented by salmon collected at the Quinault

Lake Tribal Hatchery (Quinault) and the Makah National Fish Hatchery (Makah). The Puget Sound region was represented by salmon collected at Issaquah Creek (Issaquah) and Deschutes River State Fish Hatcheries (Deschutes) (Figure 2). Tissue samples used for PCB analysis were collected from fish that had been spawned (eggs removed and fertilized) by hatchery personnel on the dates listed below. Samples were collected on September 26 and October 8, 2003, at Deschutes; October 7 and 13, 2003 at Issaquah; October 28, 2003, at Makah; and November 5, 2003, at Quinault.

Tissue samples were collected from ten 4-year-old ocean type (salmon that leave fresh water for salt water as sub-yearlings) Chinook salmon at each hatchery for a total of forty tissue samples during the spawning season. Samples were collected from five males and five females from each hatchery. All fish were measured for fork length to the nearest centimeter and weight to the nearest gram. All females were weighed without eggs, since they had been spawned by hatchery staff. Scale samples were collected and read on-site to verify that the fish were 4-year-old ocean type Chinook salmon. This was conducted by placing the scales on a slide and viewing them with a microfiche reader (typically used for reading film). Determining the age of the fish by reading the scale is similar to counting the rings of a tree stump. Scale ring patterns can be close together or far apart, depending on growth rate. Freshwater and saltwater growth can also be determined by the ring spacing. In addition, the scales were taken to the Washington Department of Fish and Wildlife for age verification.

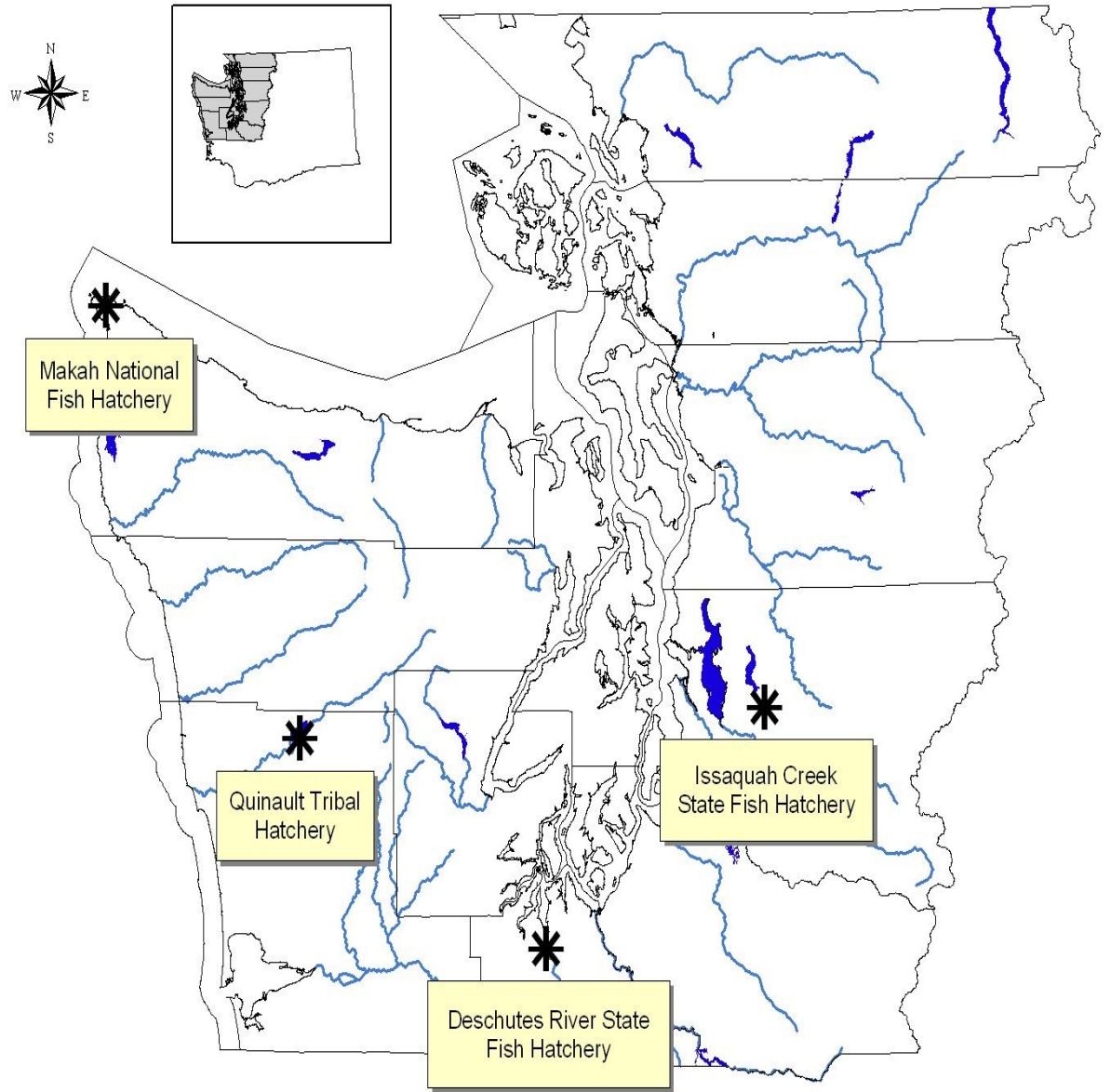


Figure 2. Sampling locations where Chinook salmon tissue was collected.



Tissue samples were collected using sterilized stainless steel scalpels and forceps. Tools were rinsed and stored in isopropyl alcohol (reagent-residue analysis grade) between samples. Instruments were cleaned with soap and water and isopropyl alcohol after sampling was complete. Two sets of instruments were used for sampling to minimize any chance of contamination. One set was used for skin removal (outside set) and the other set was for internal tissue collection (inside set).

The skin was removed by first making an incision just aft of the fish's head. The incision continued along the back of the fish just below the dorsal fin, to just in front of the tail. The incision then continued down and back along the ventral side just above the pelvic fin and back up to the origin of the incision using the outside scalpel. The skin was peeled from the flesh and was discarded using the outside forceps.

Tissue samples were collected from each fish after the skin was removed. An incision was made using the sterile inside scalpel at a minimum of ½ inch inside the cut that was left by the skin removal procedure to ensure no outside to inside contamination occurred. Tissue samples were collected by removing a fillet from the side of the fish. The tissue was weighed to insure that a minimum of 250 grams of tissue was collected and was then placed in a labeled, certified EPA clean, glass jar and stored at -0°C or below until analyzed for PCBs. Isopropyl alcohol rinsed aluminum foil was used on the scale and was changed between each tissue sample to eliminate contamination between samples.

### *Laboratory Analysis*

King County Environmental Laboratory analyzed tissue samples for PCB concentrations and percent lipids. King County Environmental Laboratory standard operating procedures were used to analyze the samples. All of the individual samples were homogenized using a commercial bar mixer. A sample was considered homogenized when it was blended to a uniform consistency as determined by the analyst.

Homogenized samples were then extracted from the mixer and placed into labeled specimen cups. Homogenized samples were mixed with methylene chloride and acetone for extraction. Samples were then analyzed for PCB Aroclor 1016, 1221, 1232, 1242, 1248, 1254, and 1260 using a gas chromatograph equipped with electron capture detectors (GC-ECD). A 1 to 2  $\mu\text{L}$  aliquot was introduced into the GC via an autosampler. A temperature program was used to separate the compounds as they moved through two dissimilar phased capillary columns used to retard the elution of the individual compounds. The compounds entered the separate ECDs as discrete compounds. The detector response was transferred to a data system where the voltages were charted and analyzed. Compounds were identified by their retention time matches with standards and their presence on both the primary and confirmatory columns.

Quantitation was accomplished by integrating each compound to baseline and comparing their responses with the responses of standards of known concentrations.

Percent lipid in each tissue sample was estimated using the following lipid extraction method. A 15 to 30 gram portion of tissue was mixed to a sandy texture with anhydrous sodium sulfate. A 1:1 mixture of methylene chloride ( $\text{MeCl}_2$ )/acetone was added. The

samples were sonicated for four minutes with approximately 100 mLs of 1:1 (MeCl<sub>2</sub>)/acetone. The resulting extract was transferred to a specimen cup and dried. The residue (the lipids) that was left over was weighed and percent lipids were calculated by dividing the residue weight by the sample weight.

#### *Data Analysis*

PCB concentrations reported by the King County Environmental Laboratory were reported in dry weight. Most PCB data in the literature is reported as wet weight. The data was converted to wet weight using the formula:

$$\text{PCB wet weight} = (\mu\text{g/kg PCB concentrations dry weight}) * (\text{percent total solids}/100)$$

The method used to determine PCB concentrations in these samples has a minimum detection limit ranging between 16 $\mu\text{g}/\text{kg}$  to 22  $\mu\text{g}/\text{kg}$ . The detection limit variation was due to the difference in the percent total solids between the samples. Concentrations falling below these detection limits may range from zero to just below the detection limit. Concentrations falling below the detection limit were given a concentration of  $\frac{1}{2}$  the detection limit. This was conducted to minimize the under estimation of PCB concentrations that would occur if all samples falling below the detection limit had been given a value of zero. The concentrations of all the Aroclor detected in the sample were summed to calculate total PCBs.

The influence of region, location (hatchery), sex, fish length, and percent lipids on PCB concentrations were evaluated using Generalized Linear Modeling (GLM). GLM was used since it can model categorical (region, location, sex) and continuous variables (length, percent lipids) simultaneously. GLM is a predictor that is based on combinations of measurements that are free to vary in response to other variables (Dobson 2002). GLM is based on the formula  $y=B_0+B_1X_1+B_2X_2\dots B_nX_n$ , where  $y$  is the predicted (or probable) outcome (dependent variable),  $B_0$  is the  $y$  intercept,  $B_x$  is the slope of the regression, which indicates the change in the mean of the probability distribution of  $Y$  per unit increase in  $X$ .  $X_i$  represents the independent variables that were evaluated. All data used in GLM was log transformed using the formula  $\text{LOG}(y+1)$ .

The multiple hypotheses testing method proposed by Anderson et al. (2000) was used to determine which variables had the greatest influence on PCB concentrations in the salmon tissue evaluated. This method is based on Kullback-Leibler (1951) Information Theory, Akaike Information Criteria, along with likelihood-based inference for data analysis (Anderson et al. 2000). This method relates the structure of relationships, estimates of model parameters and components of variance in order to make appropriate inferences about the data while separating out the noise (Anderson et al. 2000).

The following 20 models were evaluated to determine which variables influenced PCB concentrations:

a)  $y = B_0 + B_1(\text{SEX})$

b)  $y = B_0 + B_1(\text{SEX}) + B_2(\text{PERCENT LIPIDS})$

c)  $y = B_0 + B_1(\text{PERCENT LIPIDS})$

d)  $y = B_0 + B_1(\text{PERCENT LIPIDS}) + B_2(\text{LOCATION})$

e)  $y = B_0 + B_1(\text{REGION})$

f)  $y = B_0 + B_1(\text{REGION}) + B_2(\text{PERCENT LIPIDS})$

g)  $y = B_0 + B_1(\text{REGION}) + B_2(\text{SEX})$

h)  $y = B_0 + B_1(\text{REGION}) + B_2(\text{LENGTH})$

i)  $y = B_0 + B_1(\text{LENGTH})$

j)  $y = B_0 + B_1(\text{LOCATION})$

k)  $y = B_0 + B_1(\text{LOCATION}) + B_2(\text{REGION})$

l)  $y = B_0 + B_1(\text{LOCATION}) + B_2(\text{LENGTH})$

m)  $y = B_0 + B_1(\text{LOCATION}) + B_2(\text{SEX})$

n)  $y = B_0 + B_1(\text{LOCATION}) + B_2(\text{PERCENT LIPIDS})$

o)  $y = B_0 + B_1(\text{LENGTH}) + B_2(\text{SEX})$

p)  $y = B_0 + B_1(\text{REGION}) + B_2(\text{LENGTH}) + B_3(\text{LIPID})$

q)  $y = B_0 + B_1(\text{REGION}) + B_2(\text{LENGTH}) + B_3(\text{LIPID}) + B_4(\text{LENGTH} * \text{LIPID})$

r)  $y = B_0 + B_1(\text{REGION}) + B_2(\text{LENGTH}) + B_3(\text{LIPID}) + B_4(\text{LENGTH} * \text{REGION})$

s)  $y = B_0 + B_1(\text{REGION}) + B_2(\text{LENGTH}) + B_3(\text{LIPID}) + B_4(\text{LIPID} * \text{REGION})$

t)  $y = B_0 + B_1(\text{LENGTH}) + B_2(\text{LIPID}) + B_3(\text{LENGTH} * \text{LIPID})$

The number of models evaluated was limited due to sample size (n=40). There needs to be a minimum of 10 samples per variable when using GLM (Harrell 2001). Data analyses were conducted using PROC GENMOD (version 8.02) with gamma distribution and log link functions using SAS statistical package (SAS Institute, Inc. 1999).

The resulting models were ranked using Akaike's information criterion (AIC) (Burnham and Anderson 2002). The  $AIC_C$  ( $AIC_c$ =corrected for small sample size) and scaled Akaike weights ( $w_i$ ) were calculated for each model. Scaled Akaike weights range from 0 to 1, with values closer to one representing models with more support for being the best model. For example, a model with a scaled Akaike weight of 0.85 has more support for being the best model in the set evaluated than a model with a scaled Akaike weight of 0.60. A set of models in which no model has a scaled Akaike weight of 0.9 or greater suggests a high level of model uncertainty (Burnham and Anderson 2002). Parameter estimates were model averaged because no models possessed a scaled Akaike weight of 0.90 or greater. This process weights the predicted value by the scaled Akaike weight (Burnham and Anderson 2002). The influence of variables on PCB concentrations was determined by using model averaged 95 percent confidence intervals developed for each parameter estimate. Important differences between the variables existed if the 95 percent confidence intervals did not overlap for categorical variables and if the confidence interval did not include zero for continuous variables.

Model fit was evaluated using deviance residual plots. Plots were visually analyzed for patterns which may suggest that assumptions were violated (e.g. Increasing variance with

increases in the independent variable). Data that was precise around the y axis but was scattered was considered a good fit. Data that was spurious or showed specific patterns was either over fit or under fit by the model, or the assumptions (e.g. equality of variances) were violated.

The proportion of Aroclor 1254 was compared to the total PCB load to determine if the source of PCBs differed by region. This was conducted by dividing Aroclor 1254 by the total PCB concentration. The data was non-normal ( $P < 0.01$ ) and would not normalize using the arcsine transformation. Therefore, the data was analyzed using the Kruskal-Wallis test.

In addition to evaluating factors that may influence PCB concentrations in Chinook salmon, I also determined the amount of salmon individuals could consume without increasing health risks. This was determined by using the Environmental Protection Agency (EPA 1999) criterion, which is based on both non-cancer health endpoints and cancer health endpoints. Mean PCB concentrations for each hatchery were converted from  $\mu\text{g}/\text{kg}$  (parts per billion or ppb) to  $\text{mg}/\text{kg}$  (ppm) by dividing by 1000. The mean PCB concentrations were then divided by two based on Anderson et al. (1993) who states that a 50 percent reduction in PCB concentrations is reasonable based on proper preparation and cooking. Half the mean PCB concentrations were compared to EPA's recommended fish consumption limits (EPA 1999) which were based on a 227 gram (8 oz) portion over a month time frame for both non-cancerous and cancerous health endpoints.

## RESULTS

Aroclor 1254 and 1260 were the only Aroclors detected. Aroclor 1254 was detected in all 40 samples, while Aroclor 1260 was detected in 16 out of 40 samples. Mean Aroclor concentrations were similar in salmon tissue samples from the Deschutes and Issaquah hatcheries, which were both greater than those from the coastal samples. Mean Aroclor concentrations at Makah were slightly greater than the concentration levels at Quinault (Table 3 and Table 6).

Tissue samples from adult Chinook salmon collected at Deschutes had less lipid concentrations than samples collected at all other locations. Tissue samples collected at Quinault had more lipid concentrations than all other locations. Overall, Chinook salmon tissue samples collected from Puget Sound had less lipids than those collected from the coast (Table 4).

Table 3. Sample size (N), mean, standard deviation and range of total Aroclor concentrations observed in Chinook salmon sampled at each of the hatcheries. Aroclor concentrations were determined using ½ the detection limit for Aroclor 1260<sup>1,2,3</sup>.

Hatchery	N	Mean µg/kg	Std deviation	Range µg/kg
Puget Sound				
Deschutes	10	48.67	38.57	14-135
Issaquah	10	49.85	44.53	20-170
Coastal				
Makah	10	18.97	5.14	10-27
Quinault	10	15.86	8.10	9-38

<sup>1</sup>Data was not lipid normalized.

<sup>2</sup>Raw data is contained in Appendix 1.

<sup>3</sup>Data reported as wet weight.



Table 4. Sample size (N), mean, standard deviation and range of percent lipids observed in Chinook salmon sampled at each of the hatcheries.

Hatchery	N	Mean %	Std deviation	Range %
Puget Sound				
Deschutes	10	0.097	0.037	0.4-1.49
Issaquah	10	0.57	0.30	0.27-1.28
Coastal				
Makah	10	1.5	0.54	0.86-2.45
Quinault	10	1.8	1.07	0.76-3.81

*Factors influencing PCB concentrations*

Six of 20 models evaluated had substantial support for being the best model (Table 5).

No single model had enough support to be considered the single best model to describe PCB concentrations in Chinook salmon. The models containing REGION and LIPIDS; REGION; LOCATION and LIPIDS; REGION and SEX; REGION, LENGTH AND LIPIDS; and REGION and LENGTH, were the only models to have substantial support (i.e., scaled AICc <3) as the best model of those evaluated. The models with the variables REGION LENGTH/LENGTH\*LIPIDS; LOCATION and LENGTH; LOCATION; REGION LENGTH/LIPID\*REGION; LOCATION and SEX; and LOCATION and REGION; had moderate support as the best model in the set evaluated. The remaining models had very little support as the best model in the set evaluated.

The parameters estimates were model averaged using all models since no one model had enough support for being the best model. Region and location were the only two variables that influenced PCB concentrations in Chinook salmon (Table 6). PCB concentrations were greater in Chinook salmon tissue from the Puget Sound than from

Table 5. Number of estimated parameters (k), log likelihood, AIC<sub>c</sub>, scaled AIC<sub>c</sub>, Akaike weights, and the scaled Akaike weights for the generalized linear models evaluated for determining the influence of different variables on PCB concentrations in Chinook Salmon tissue (n = 40). Models with scaled AIC<sub>c</sub> less than 3 have substantial support for being the best model. Those with scaled AIC<sub>c</sub> values greater than 3 and less than 7 have moderate support. Those with scaled AIC<sub>c</sub> values greater than 7 have no support for being the best model of those evaluated (Burnham and Anderson 2002).

Model Variables				k	loglikelihood	AIC <sub>c</sub>	Scale AIC <sub>c</sub>	Akaike weights	Scaled Akaike weights
Intercept	REGION	LIPIDS		3	10.53	-14.38	0.00	1.00	0.26
Intercept	REGION			2	8.88	-13.44	0.94	0.62	0.16
Intercept	LOCATION	LIPIDS		5	12.21	-12.66	1.73	0.42	0.11
Intercept	REGION	SEX		3	9.66	-12.65	1.73	0.42	0.11
Intercept	REGION	LENGTH	LIPIDS	4	10.59	-12.05	2.34	0.31	0.08
Intercept	REGION	LENGTH		3	9.13	-11.60	2.78	0.25	0.07
Intercept	REGION	LENGTH	LENGTH*LIPIDS	5	11.48	-11.19	3.20	0.20	0.05
Intercept	LOCATION	LENGTH		5	10.96	-10.15	4.23	0.12	0.03
Intercept	LOCATION			4	9.63	-10.12	4.26	0.12	0.03
Intercept	REGION	LENGTH	LIPID*REGION	5	10.93	-10.09	4.29	0.12	0.03
Intercept	REGION	LENGTH	LENGTH*REGION	5	10.82	-9.87	4.52	0.10	0.03
Intercept	LOCATION	SEX		5	10.46	-9.16	5.23	0.07	0.02
Intercept	LOCATION	REGION		5	9.63	-7.50	6.88	0.03	0.01
Intercept	LENGTH	LIPIDS	LENGTH*LIPIDS	4	0.37	8.41	22.79	0.00	0.00
Intercept	LENGTH			2	-2.54	9.40	23.78	0.00	0.00
Intercept	LIPIDS			2	-2.67	9.67	24.05	0.00	0.00
Intercept	LENGTH	SEX		3	-1.84	10.35	24.73	0.00	0.00
Intercept	LIPIDS	SEX		3	-1.93	10.53	24.92	0.00	0.00
Intercept	LENGTH	LIPIDS		3	-2.13	10.92	25.31	0.00	0.00
Intercept	SEX			2	-3.40	11.12	25.51	0.00	0.00

Table 6. Model averaged parameter estimates, standard errors, confidence limits for parameters from the all-models generalized linear model evaluation of variables influencing PCBs in adult Chinook salmon.

Estimated Model Variable	Class	Parameter Estimate	Standard Error	95 % Confidence Interval		Effect
				Lower	Upper	
REGION	PUGET SOUND	0.27	0.07	0.13	0.42	Yes
REGION	COAST <sup>1</sup>	-	-	-	-	No
LOCATION	DESCHUTES	0.34	0.06	0.22	0.47	Yes
LOCATION	ISSAQUAH	0.36	0.07	0.23	0.49	Yes
LOCATION	MAKAH	0.10	0.06	-0.02	0.22	No
LOCATION	QUINULT <sup>1</sup>	-	-	-	-	No
REGION LENGTH LIPID	LENGTH*REGION	<0.01	<0.01	<-0.01	0.01	No
REGION LENGTH LIPID	LIPID*REGION	-0.07	0.08	-0.24	0.09	No
LENGTH LIPIDS	LENGTH*LIPIDS	<0.01	<0.01	<-0.01	<0.01	No
LIPIDS		0.03	0.06	-0.09	0.14	No
LENGTH		<0.01	<0.01	<-0.01	<0.01	No
SEX	FEMALE	-0.05	0.04	-0.14	0.13	No
SEX	MALE <sup>1</sup>	-	-	-	-	No

<sup>1</sup>Reference variables

the coast. PCB concentrations in Chinook salmon tissue from Deschutes and Issaquah were greater than Quinault and Makah, but were not different from each other.

### *Evaluating Model Fit*

Residual plots for the top six models are shown in Figure 3. These plots show that the variation in PCB concentrations was greater for the Puget Sound than the coast. Thus, estimates of mean PCB concentrations for the coast are likely precise, while estimated for the Puget Sound are likely imprecise. For the model REGION and LIPIDS (Figure 3A), LOCATION and LIPIDS (Figure 3C), and REGION LENGTH and LIPIDS (Figure 3E), the data fit the model fairly well up to a predicted value of 1.5. However, above a predicted value of 1.5, the variance increases as the predicted data increased indicating a poor fit and greater variability of data from the Puget Sound. However, these models fit the data substantially better than the other models with substantial support. The residual plots for the model REGION and SEX (Figure 3D) shows that much of the increase in variability in PCB concentrations in Chinook salmon from Puget Sound samples was due to males. There was a slight increase in variance from female Chinook salmon from the Puget Sound samples relative to coastal samples, but a large increase in variance for male Chinook salmon from the Puget Sound samples. Male and female Chinook salmon from coastal samples had similar variation in PCB concentrations. The models with the variable LOCATION and LIPIDS (Figure 3C) in the model were a good fit for the coastal data but showed greater variability for Puget Sound data. However, the fit was consistent for the hatcheries in each region. Similarly, the model REGION and LIPIDS (Figure 3A), and REGION LENGTH and LIPIDS (Figure 3E) fit the coast but showed greater variability for Puget Sound.

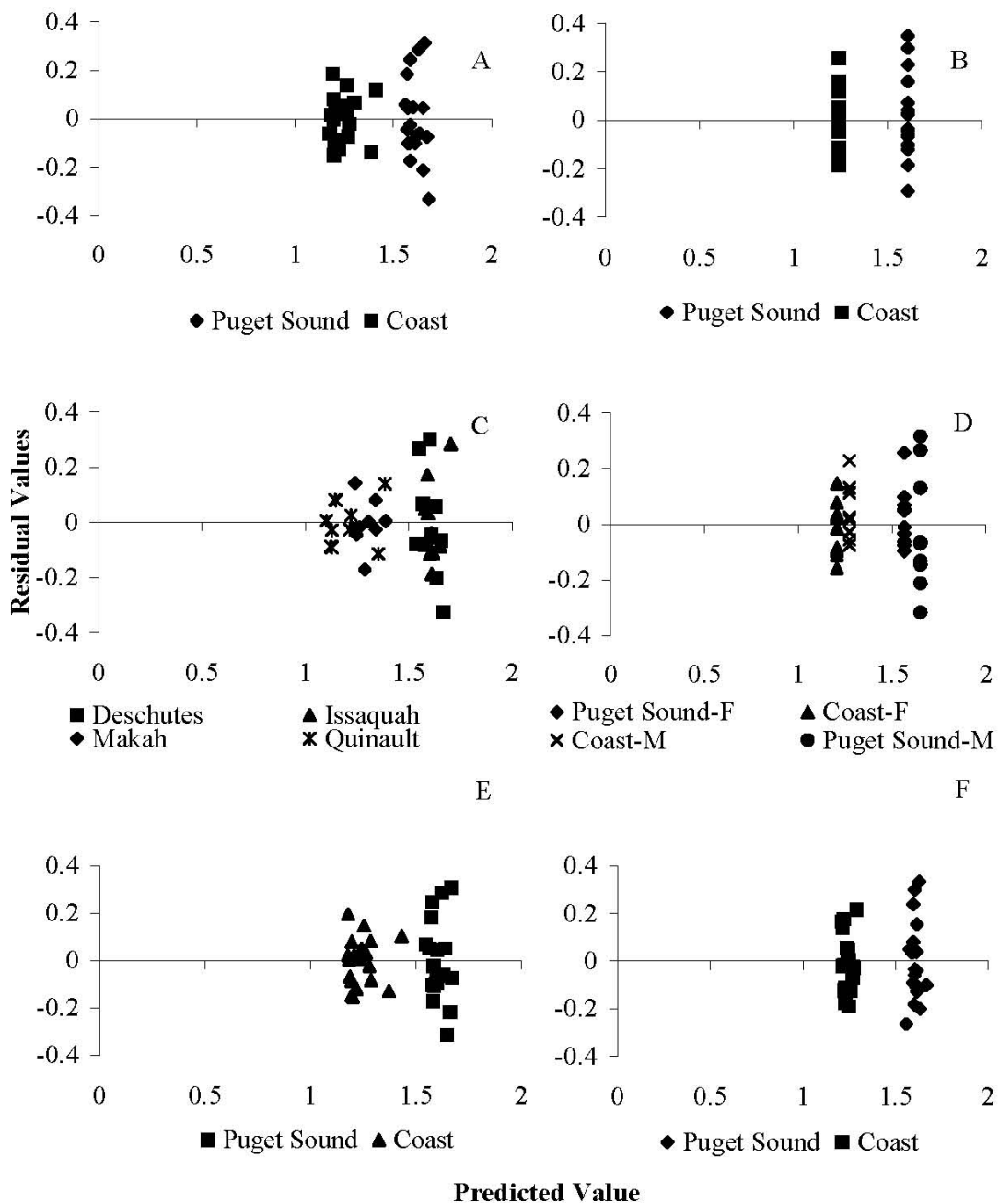


Figure 3. Residual plots for the model REGION and LIPIDS (A), REGION (B), LOCATION and LIPIDS (C), REGION and SEX (D), REGION, LENGTH and LIPIDS (E), and REGION and LENGTH (F).

Aroclor 1254 is more prominent in Chinook salmon tissue; representing more than 60 percent of the total PCB burden in all samples. Aroclor 1254 contribution significantly more to the overall PCB burden (Kruskall-Wallis  $P < 0.002$ ) in tissue samples from the coast compared to Puget Sound tissue samples (Table 7).

Table 7. Sample size (N), mean, standard deviation and range for the proportion of Aroclor 1254 in the total PCB concentrations for each region.

Hatchery	N	Mean	Std deviation	Range
Puget Sound	20	0.75	0.1	0.6-0.93
Coast	20	0.86	0.047	0.75-0.93

The recommended amount of Chinook salmon to be consumed by humans varied by region but was consistent within region (Table 8). In general, more coastal Chinook salmon could be consumed compared to Puget Sound for both cancerous and non-cancerous end points.

## DISCUSSION

Mean PCB concentrations in Chinook salmon from the Puget Sound hatcheries were almost 2.5 times greater than those in Chinook salmon from coastal hatcheries. PCB concentrations observed in Puget Sound hatchery Chinook salmon in the current study were similar to concentrations observed in samples collected by the Puget Sound Ambient Monitoring Program (PSAMP) (O' Neill WDFW, pers. comm. 2004).

Table 8. The recommended upper limit for the number of 8 oz (227g) meals of Chinook salmon which can be consumed per month from each hatchery before non-cancerous and cancerous health concerns arise. Estimates are based on EPA's recommendation using a reference dose of  $2.5 \times 10^{-5}$  mg/kg per day and half of the mean concentration of PCBs found in this study.

Hatchery	Half of the mean concentration of PCBs in ppm determined from this study (mg/kg)	Allowable consumption per month	
		Non-cancer health end point	Cancer health endpoint
		Puget Sound	
Deschutes	0.025 <sup>1</sup>	4	1
Issaquah	0.025 <sup>1</sup>	4	1
		Coast	
Makah	0.01 <sup>1</sup>	16	4
Quinault	0.01 <sup>1</sup>	16	4

<sup>1</sup> Numbers were rounded up

The mean PCB concentrations in Puget Sound Chinook salmon collected in-river during PSAMPs 10 year (1989-1999) monitoring program were slightly greater (53.88 µg/kg; average of 4 sample rivers) than I observed (49.26 µg/kg; average of 2 sample rivers). These data are relatively similar even though I sampled only four-year old fish, while PSAMP sampled fish from two-to-five year olds. The PSAMP data showed greater PCB concentrations in Chinook salmon from southern Puget Sound (south of the Tacoma Narrows Bridge) than from northern Puget Sound (north of Admiralty Inlet) (PSAMP 2001). In addition, PSAMP (2001) in-river Puget Sound Chinook salmon had higher lipid content (3.34 percent; average 4 sample rivers) than my samples (0.33 percent; average of 2 sample rivers). The difference in the lipid content could be the result of how long the fish had not been feeding (time in fresh water) compared to the time the samples were collected.

These results suggest that regional differences exist in PCB concentrations in Chinook salmon tissues. However, the residual plots suggest the fit of the models used to evaluate these differences were poor. The models were fairly robust when describing the coastal data, but tended to overestimate predicted values for the Puget Sound. The spuriousness of the Puget Sound predicted values reflects a large difference in the variances which could be due to the rearing, feeding or migration patterns of Puget Sound Chinook salmon. Even though the predicted values for Puget Sound were spurious, four out of the five best models have region as one of the variables, which indicates that Puget Sound is the primary driver of PCB concentrations in this study. In addition, even though the residual plots showed large variances, other data suggest that PCB concentrations decrease in northern Puget Sound (PSAMP 2001). Data from this study, along with PSAMP (2001) data indicates that Puget Sound Chinook salmon are more contaminated with PCBs than coastal hatchery Chinook salmon.

There are several estuaries within Puget Sound that have been targeted as Superfund sites where juvenile Chinook salmon tend to reside before migrating to sea. Juvenile Chinook salmon migrating out of the estuarine environment and through Puget Sound must also travel up to 100 miles (from the Deschutes sample site) through several urbanized and industrial sites that could lead to additional exposure. Several authors have shown that juvenile Chinook salmon are accumulating PCBs through their prey base while they are in the estuarine environment in Puget Sound (Arkoosh et al. 1998b; McCain et al. 1990; Meador 2000; Stein et al. 1995). PSAMP (2001) found a decrease in PCB concentration in Chinook salmon in northern Puget Sound compared to southern Puget Sound.



However, this does not fully explain why returning adult Chinook salmon have a greater body burden in comparison to the juvenile life stage upon returning to Puget Sound to spawn (O'Neill, pers. comm. 2004). Variation in residency time in Puget Sound and/or migration routes may explain the large variance observed in the Puget Sound data.

Puget Sound Chinook salmon have diverse ocean migratory patterns. In general, most Puget Sound Chinook salmon travel through the inland waterway between the Canadian mainland and Vancouver Island. Earlier returning adult Chinook salmon travel farther up the west coast of Canada and into southeast Alaska than later returning adult Chinook salmon (Figure 4) (NOAA 2002; Pacific Salmon Council 2003). There is also a segment of the Chinook salmon that may reside in Puget Sound throughout their life. These groups of fish obviously have different food sources which may have lead to the increased variance in PCB concentrations observed in the current study. The Puget Sound fish I sampled returned several weeks earlier than the coast fish which suggests a longer migratory route and potentially additional exposure to PCBs in the ocean. Currently, there is no way of determining whether or not the fish in my samples resided in Puget Sound or migrated to the Pacific Ocean. Coded wire tag data recoveries of sub-adults are limited and biased by fishing and sampling effort.

PCB concentrations in coastal Chinook salmon were less variable and were explained well by the models. Less variation may be the result of a less contaminated migratory route or a more consistent migratory route. The estuarine environment on the coast is

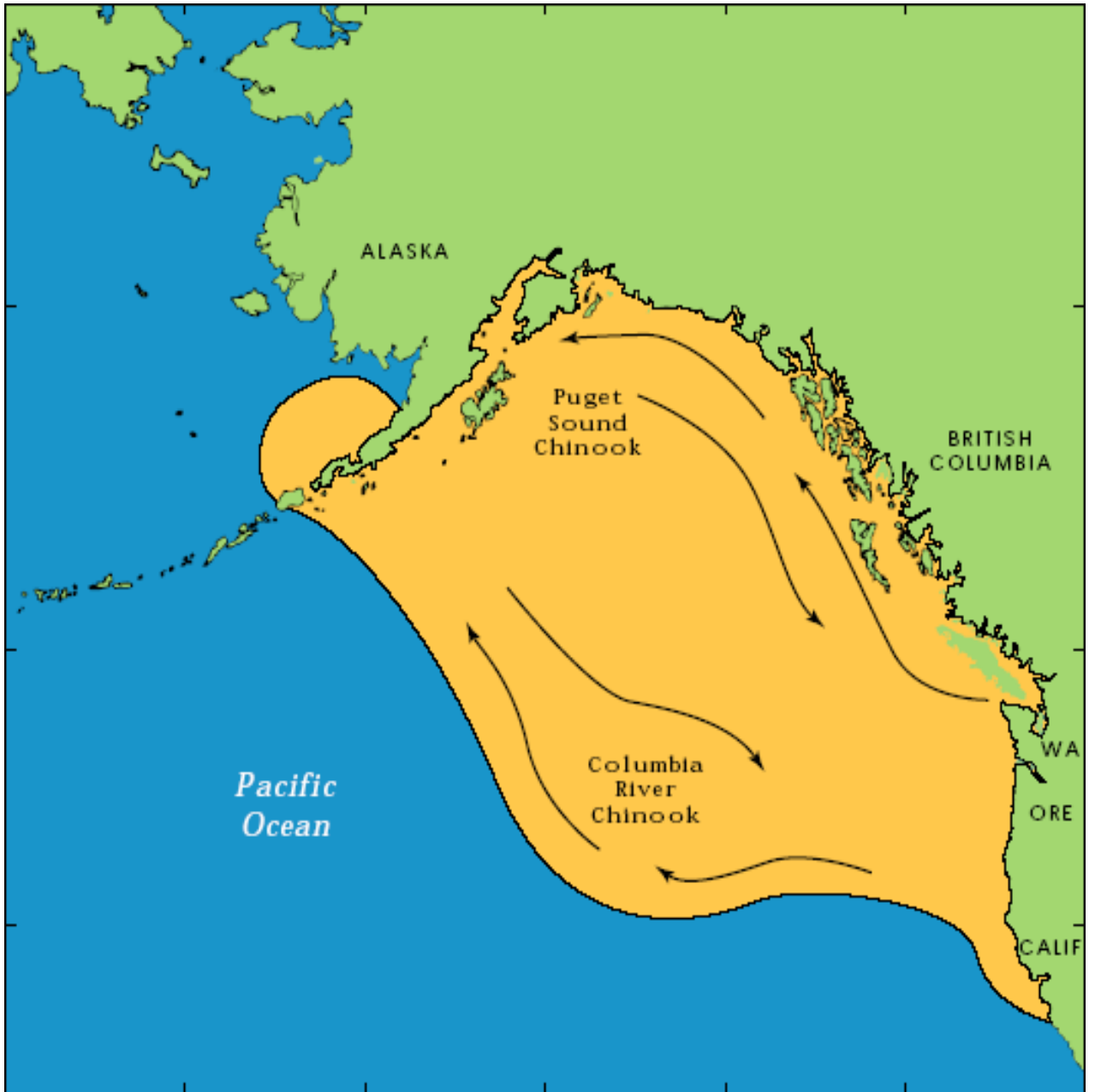


Figure 4. Puget Sound and coastal Chinook salmon migration pattern (Courtesy of Dave Galvin).

more pristine than that of the Puget Sound. The coastal environment in Washington State is very rural and has a very limited industrial/urbanized component. Migration patterns of coastal Chinook salmon also differ slightly from the Puget Sound Chinook salmon. Coded wire tag data indicate that coastal Chinook salmon migrate on the west side of Vancouver Island sometimes migrating to southeast Alaska (Pacific Salmon Council 2003; USFWS, unpublished data).

The observations of greater PCB concentrations in adult Puget Sound Chinook salmon raises concerns that PCBs may be contributing to the declining population in the Puget Sound, but it is unclear if PCBs impact Chinook salmon survival. Arkoosh et al. (1998a) suggests that pollutants (which include PCBs) adversely affect the environment and may affect certain life stages of salmonids. Arkoosh et al. (1998b) also suggests that exposure to PCBs suppresses the immune system in juvenile Chinook salmon. The juvenile and sub-adult life stage would be the most affected because it typically occurs within an urbanized setting of the Puget Sound region.

PCBs are maternally transferred to the eggs (Missildine, unpublished data; Miller and Amrhein 1995; Miller 1993) and most likely impact egg to fry survival (Walker et al. 1991; Ankley et al. 1991). Ankley et al. (1991) found that egg-to-fry survival was inversely related to PCB concentrations in Lake Michigan Chinook salmon. Miller (1993) found a direct correlation between PCB concentrations in muscle tissue ( $4.3 \pm 0.4$  mg/kg) and PCB concentrations in eggs ( $8.3 \pm 0.9$  mg/kg) of salmonids. Miller's (1993) PCB concentration levels were higher than the PCB concentrations found in the current

study. It is important to note that Miller's (1993) samples were processed with the skin intact. It would be extremely difficult to determine how much of an impact PCBs are having on egg-to-fry survival in the wild. Healy (1991), found egg-to-fry survival for Chinook salmon in the wild is approximately 30 percent, but can vary due to riverine conditions. Variables such as floods, scour, entombment, and superimposition of redds by other salmon contribute to egg mortality.

Research on salmon and other animals indicates the abnormal presence of genetic and hormonal markers that would typically be seen in the opposite gender (Carlson et al. 2000; Nagler et al. 2001). Researchers suggest that endocrine disrupting chemicals such as PCBs may be having this affect on salmon and other species. It is unknown whether or not the presence of these markers is having an impact on reproductive success.

Carlson et al. (2000) suggest that embryos were more susceptible to toxicants than older adult fish but that maternal transfer, especially in fish that live in heavily contaminated areas, could affect embryo survival and local fish populations. Carlson et al. (2000) also found that embryos injected with contaminants caused the embryo to hatch early, which could be detrimental to survival, especially in the Northwest where freshets are common during winter when most salmonid eggs are still in the gravel incubating.

### *Management Implications*

The observation of PCBs in Chinook salmon returning to Washington hatcheries may impact the way hatchery fish are managed after they have been spawned. Currently, fish from the Makah and Quinault hatcheries are distributed to tribal members for

consumption. The State hatcheries sell their fish to a buyer, who typically processes the fish for a variety of animal feeds and fertilizers, and/or they give the fish away to conservation groups that place the carcasses into streams to increase the input of marine derived nutrients. All of these methods reintroduce PCBs into the environment with little knowledge of the overall impact.

Washington State's salmon carcass distribution program is supported by local, state, and federal agencies. Carcass planting has become an important component in salmonid habitat restoration. Several authors have shown the benefits of carcass planting into streams (Helfield and Naiman 2001; Cederholm et al. unpublished data; Cederholm et al. 1999; Bilby et al. 1995). Cederholm et al. (1999) stated that salmonids supply a major source of marine derived nutrients to the aquatic and terrestrial landscape. Bilby et al. (1995) found that many aquatic invertebrates and streamside plants are enriched with marine derived nutrients from coho salmon (*Oncorhynchus kisutch*) carcasses. However, none of the authors discuss the potential of recontamination of the environment with PCBs or other persistent organic pollutants from planting salmon carcasses.

I could not find any published reports which evaluate the influence of salmon carcass deployment on pollutant reintroduction to stream ecosystems. However, PCB concentrations in the water column (O'Toole et al. In preparation), and in the sediment (Krummel et al. 2003), have increased following natural spawning. O'Toole et al. (In preparation) found an increase in PCB concentrations in the water column of the Credit River from post-spawned Chinook salmon. Krummel et al. (2003) found that PCBs

levels in the sediment of Alaskan lakes increased seven-fold upon the return of adult sockeye salmon. PSAMP (2001) data shows that coho salmon, a prevalent salmon species in Washington State, are also contaminated with PCBs, although at lower levels than Chinook salmon. These data indicate that PCB concentrations in rivers and streams in Washington State may increase following carcass deployment.

Carcass deployment may also distribute PCBs to the terrestrial ecosystem. Decaying salmon carcasses are an important component of the terrestrial food web. Willson and Halupka (1995) indicate that over 20 mammalian and avian species combined are direct consumers of salmonid carcasses. The consumption of salmon carcasses contaminated with PCBs may impact the survival rate of species that feed on salmon carcasses.

Bowerman et al. (1995) indicated that bald eagle (*Haliaeetus leucocephalus*) birth rates and adult mortality in the Great Lakes region may still be impacted by consuming post-spawned salmon carcasses contaminated with PCBs. Buck et al. (1999) indicated that bald eagles from the lower Columbia River were also experiencing lower birth rates than bald eagles in other northwest locations in association with PCB contaminated prey sources.

Currently, fish in the carcass distribution program are tested for bacteria and viruses but not contaminants. No research to date is occurring to determine if the PCBs in salmon carcasses are having an impact on other fish and wildlife species in Washington State that feed on these carcasses. Testing the hundreds of carcasses that are planted into streams and rivers each year would be cost prohibitive and time consuming for the entities

involved in the carcass distribution program and the labs that would be needed to analyzed the samples. Other alternatives such as pellets or seeding the streams and rivers could be an effective means of delivering marine derived nutrients to the ecosystem, but may not be as efficient as carcass deployment. Either way, we need to stop the cycle of PCBs, whether that means stopping the carcass deployment program or spending the money to test each fish. Others may argue that the naturally spawning fish are already contaminated so what difference does it make. I would argue that many of the salmon deployment projects are located in streams with very few returning salmon and therefore would have extremely low concentrations of PCBs.

Managers and scientists need to determine whether the benefits of marine derived nutrients outweigh the detrimental PCB impacts. Other toxic chemicals are also being transported by salmon including polybrominated diphenyl ether (PBDE; fire retardants) and dichlorodiphenyltrichloroethane (DDT) (O'Neill, pers. comm.2004). Planting carcasses will most likely result in the introduction of these toxic chemicals (or an increase, for streams that already have naturally spawning salmon), along with PCBs, into the environment where toxins might not currently be found. I would surmise that contamination would still occur by the die-off of natural spawners if the carcass deployment programs were discontinued. Furthermore, natural spawners will also provide the marine derived nutrients to the ecosystem, albeit not at historic levels. Technological advances may be able to duplicate the marine derived nutrients without the increased risk of contamination, or the use of less contaminated stocks such as those from the coast of Washington could be used for carcass deployment.

### *Human health implications*

PCBs are usually associated but not specifically implicated with human health issues because humans are subject to many other variables that can impact health. Schechter et al. (1994) noted that there is a variance in toxicity between the 209 specific congeners and that failure to report specific congeners can lead to two problems. Firstly, not all PCBs are toxic and determining if the PCB is causing toxic response is problematic. Secondly, total PCB levels within an exposed person may be within “normal” limits, but toxic congeners may be masked within the total PCB concentrations reported.

Even though PCBs are difficult to implicate with human health issues, several authors have found associated health issues. Brown (1987) found an increase in cancer in the biliary tract, liver, and gall bladder from workers exposed to PCBs compared to the national rates. Loomis et al. (1997) found an increase in malignant melanoma and brain cancer from workers exposed to PCBs. In the Michigan Maternal Infant Cohort Study, children of women who consumed PCB contaminated fish experienced developmental deficiencies compared to children whose mothers did not consume contaminated fish (Fein et al. 1984; Jacobson et al. 1985, 1990a, b).

The consumption of fish is one of the main vectors of human exposure to PCBs. Greater exposure to PCBs may occur to some cultures in the Puget Sound because salmon are a main staple in their diets. A literature search was conducted but did not find any information relative to PCB levels in northwest indigenous people. However, Schell et al. (2003) found increased PCB concentrations in blood serum from Akwesasne Mohawk



youth that consumed fish from the St. Lawrence River in New York. Schell et al. (2003) also discovered that breast fed Akwesasne children had PCB concentrations 1.3 times higher than non-breastfed Akwesasne children. Dellinger et al. (1997) reported higher levels of PCBs in Ojibwa members who ate fish in comparison with the overall population. Dellinger et al. (1997) also notes that the Ojibwa members may also be at a higher risk for health effects.

The consumption of fatty fish, especially salmon, may decrease cardiovascular disease. The U. S. Food and Drug Administration and the American Heart Association currently emphasize the benefits of Omega-3 fatty acids typically found in salmon and other fish. Stone (1996) reported that Omega-3 fatty acids may reduce the risk of heart disease. The American Heart Association recommends eating two servings of fish a week. This is double the recommended consumption rate calculated using EPA guidelines for the consumption of Puget Sound salmon; however, it is within the consumption rate calculated for coastal Chinook salmon.

Most individuals will have to weigh the benefits/risks for themselves. Those that have low risks of cardiovascular disease may choose not to eat as much fish as recommended where those at risk of cardiovascular disease may choose to eat more fish. There are several recommendations for cooking salmon that will reduce the amount of PCBs in the fish; 1) remove the skin; 2) trim away excess fat from the lateral line, ventral side and backbone (Figure 5); and 3) grill, bake or broil the fish (WDOH 2004). Anderson et al. (1993) states that a 50 percent reduction in PCBs occurs during cooking and preparation.

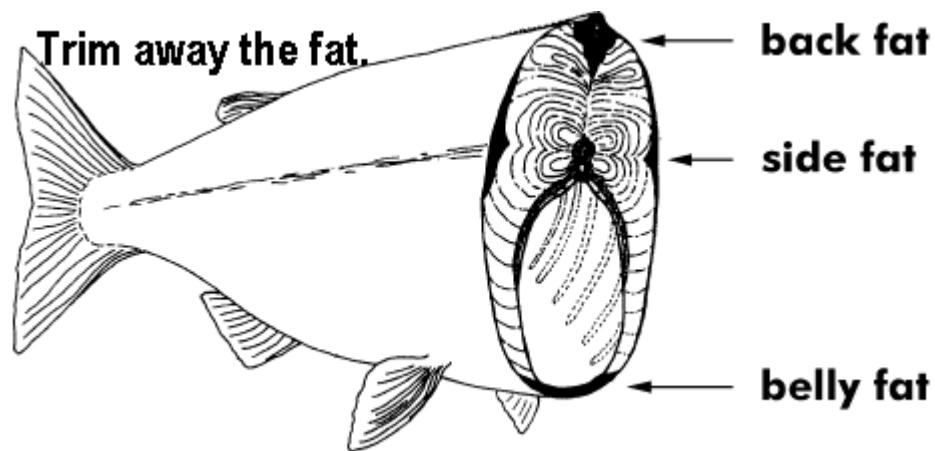


Figure 5. Proper trimming preparation to remove fat from salmon before cooking (Courtesy of Washington State Department of Health).

## CONCLUSION

Region appeared to be the primary factor influencing PCB concentrations in Chinook salmon from western Washington, with greater concentrations observed in Puget Sound Chinook salmon than coastal Chinook salmon. My data shows that inhabitants of the Puget Sound and coast who are eating Chinook salmon are still being exposed to PCBs long after they were outlawed in the United States. Many other species, such as bears and eagles, which rely on Chinook and other salmon, may be experiencing health risks associated with PCB exposure. Several Superfund clean ups within Puget Sound either have been completed or are going to be completed over the next several years which will help reduce exposure to PCBs. However, it appears that Chinook salmon may still be exposed to PCBs during their open ocean migration. Unfortunately, little can be done to minimize exposure without an international ban and clean up plan for PCBs worldwide.

### *Future Recommendations*

This research project is just one piece of the PCB puzzle. Research being conducted by PSAMP and USFWS will help develop a more complete picture of PCBs in salmon. Additional research needs to be conducted to determine if PCBs are affecting Chinook salmon survival throughout its life history. Evidence suggests that there is an inverse correlation between egg-to-fry survival and PCB concentrations (Ankley et al. 1991). Evidence also suggests that juvenile Chinook salmon contaminated with PCBs suffer immunosuppression, which could lead to increased disease susceptibility (Arkoosh et al. 1998b). We don't know if PCB concentrations in adult Chinook salmon are causing a decrease in survival in the open ocean due to immunosuppression. Research also needs to be conducted to determine if planting salmon carcasses in streams is recirculating PCBs into the environment, which could adversely impact other species that feed on salmon carcasses. We also need to determine the "hot spots" in the Pacific Ocean and Puget Sound where PCBs are accumulating.

In conclusion, PCBs are ubiquitous, but can be found in greater concentrations in urbanized areas such as the Puget Sound and Great Lakes. The chemical composition of PCBs that makes them stable in the environment may hinder its total elimination in the environment. International efforts must be put forth to eliminate the use and improper disposal of PCBs in the environment.

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APPENDIX 1. Combined lab results (All results in dry weight).

Location	Region	Fish length mm	Fish weight	Aroclor 1254 µg/kg	Aroclor 1260 µg/kg	total pcb µg/kg	Percent Lipids* %	Mercury, Total, CVAA mg/kg	Total Solids* %	Sex
Deschutes 1	Puget Sound	720	4.11	50	8	58	1.5	0.204	24	m
Deschutes 2	Puget Sound	880		80.1	9	89.1	1.2	0.235	23	m
Deschutes 7	Puget Sound	820		394	180	574	1	0.556	23	m
Deschutes 8	Puget Sound	830		103	41.8	145	1	0.563	23	m
Deschutes 10	Puget Sound	840		90.2	50.4	141	1.4	0.366	24	m
Deschutes 13	Puget Sound	800	5.8	320	154	474	0.5	0.695	21	f
Deschutes 14	Puget Sound	800	5.6	113	29	142	0.8	0.496	19	f
Deschutes 15	Puget Sound	845	5.5	79.5	40.5	120	0.4	0.533	21	f
Deschutes 16	Puget Sound	800	4.8	206	66.5	273	1.2	0.78	19	f
Deschutes 17	Puget Sound	840	4.6	163	66.3	229	0.7	0.559	20	f
Issaquah 30	Puget Sound	790	3.7	180	61.2	241	0.4	0.854	18	f
Issaquah 33	Puget Sound	820	4.7	98.6	53.1	152	0.9	0.483	21	f
Issaquah 34	Puget Sound	950	6.4	104	67	171	0.4	0.362	18	f
Issaquah 35	Puget Sound	760	5.1	169	80.7	250	0.3	0.727	18	f
Issaquah 36	Puget Sound	820	4.2	133	34	167	0.5	0.659	21	f
Issaquah 37	Puget Sound	810	4.7	92.8	10.5	103	0.5	0.307	20	m
Issaquah	Puget	845	4.8	274	120	394	0.4	0.633	19	m

39	Sound									
Issaquah 41	Puget Sound	830	5.6	128	10.5	139	0.6	0.315	19	m
Issaquah 47	Puget Sound	850	5	114	10	124	0.5	0.357	21	m
Makah 70	coast	880	7.3	69.3	10	79.3	1.2	0.327	21	f
Makah 72	coast	866	8.9	77	9	86	2	0.148	22	f
Makah 73	coast	833	6	43.4	9.5	52.9	1.5	0.167	21	f
Makah 75	coast	804	6.4	102	9.5	112	2.5	0.491	21	f
Makah 76	coast	800	6.9	112	9.5	122	1	0.397	21	m
Makah 77	coast	826	6.9	109	8.5	118	2	0.308	23	m
Makah 79	coast	834	6.1	62.8	9.5	72.3	0.9	0.171	21	m
Makah 80	coast	894	8.2	81.5	9.5	91	1.6	0.237	21	m
Makah 81	coast	810	5.4	63.1	10	73.1	1	0.263	20	f
Makah 82	coast	880	7.3	73.8	9	82.8	1	0.259	23	m
Quinalt 101	coast	898	7.9	64.1	8.5	72.6	2.1	0.182	23	f
Quinalt 102	coast	845	5.6	45	9.5	54.5	1.1	0.195	21	f
Quinalt 103	coast	925	7.9	45.3	9.5	54.8	0.8	0.094	22	f
Quinalt 104	coast	900	6.8	36	9.5	45.5	1	0.121	21	f
Quinalt 106	coast	851	5.5	36	9.5	45.5	1.1	0.135	21	f
Quinalt 107	coast	975	9.6	65.3	9	74.3	1.3	0.181	22	m
Quinalt 108	coast	845	6.6	50	8	58	3.5	0.184	26	m
Quinalt 109	coast	1010	13.2	106	34.8	141	3.8	0.206	27	m
Quinalt 111	coast	960	9.3	69.9	9.5	79.4	1.3	0.209	21	m
Quinalt 113	coast	960	10.7	56	9	65	2.1	0.107	22	m

