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Polybrominated Diphenyl Ethers and Polychlorinated Biphenyls in Different Tissue Types from Chinook Salmon (*Oncorhynchus tshawytscha*)

D. Stone

Oregon Health Services, Portland, 800 NE Oregon Street, Portland, OR 97232, USA

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Polybrominated diphenyl ethers (PBDEs) and polychlorinated biphenyls (PCBs) are lipophilic compounds that may persist in the environment over many years. Aquatic organisms are particularly susceptible to bioaccumulation of these compounds. While the majority of PCBs detected in wildlife are the result of historical use and legacy contamination, rising levels of PBDEs have been documented in North America wildlife (Rayne et al. 2003, Luross et al. 2000). In human populations, PBDE levels in breast milk (Ohta et al. 2002) and PCBs in fetal cord blood (Stewart et al. 1999) were strongly correlated to the degree of fish intake. The differential accumulation of PBDEs and PCBs in various tissues of fish, such as the skin, whole body or fillet, is an important determinant of exposure for people that consume these fish.

PBDEs are widely used flame retardants that are added to plastics, foams and fabrics. Three commercial formulations are frequently used in the marketplace with varying degrees of bromination. The deca formulation is used in some textiles and plastics and consists mainly of brominated diphenyl ether (BDE) congener 209. The penta formulation is a mixture of tetra, penta and hexabromianted congeners and can be found in polyurethane foams and other products. The octa formulation consists of hepta and octabromintated congeners and is used in thermoplastics. PCBs are hydrophobic and stable compounds with differing levels of chlorination and were extensively used as coolants and lubricants. In contrast to the current widespread use of PBDEs, PCBs were banned from most applications in the United States in 1977. PBDEs share similar structure-toxicity relationships with PCBs, including limited binding to the Ah receptor (Chen et al. 2001) and the induction of hepatic microsomal monooxygenases (de Wit 2002). Both PBDEs and PCBs have 209 theoretically possible congeners that are numbered using the International Union of Pure and Applied Chemistry (IUPAC) system (Ballschmiter et al. 1993) (Figure 1).

In this study, chinook salmon (*Oncorhynchus tshawytscha)* were collected from the Clackamas River in Northwest Oregon while returning to spawn. The objectives of this study are to report PBDE and PCB congener profiles from three different types of tissue composites collected in salmon and to investigate the reduction of contaminants by preparation methods.

a) PBDEs b) PCBs

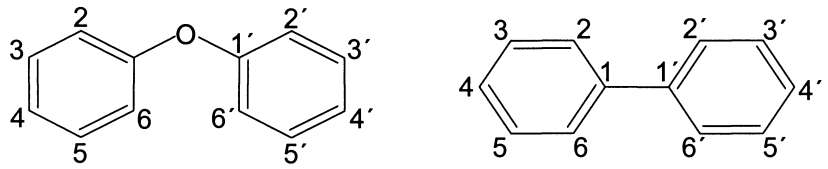

Figure 1. Generalized structure of a) PBDEs with ten possible positions for bromine and b) PCBs with ten possible positions for chlorine.

MATERIALS AND METHODS

Spring chinook salmon (*Oncorhynchus tshawytscha)* were collected with dip nets from the Clackamas River, near its confluence with the Willamette River. Field staff from the Oregon Department of Fish and Wildlife hand collected all of the salmon used for this study at a hatchery fish ladder. Four composites of whole-body tissue (WB), three composites of fillet with skin (FS) and three composites of fillet without skin (FNS) were collected. Each composite consisted of three salmon of similar length, weight and age-class. Composites sampled were collected such that the difference in average length of individuals within and between composites did not exceed 25%.

FS and FNS samples were filleted in the field by United States Environmental Protection Agency (EPA) staff. The skin was removed by cutting directly behind the gills for the FNS samples. The remaining skin and muscle tissue was separated using a stainless steel knife. Care was taken to avoid contaminating fillet tissues with material released from any inadvertent puncture of internal organs. Each sample was wrapped in aluminum foil and immediately packed on dry ice. Fish were kept frozen at -20 °C until homogenized upon receipt in the laboratory.

Samples were placed in a series of grinders and blenders, followed by homogenization for approximately 45 minutes using a Tempest Virtishear homogenizer. PBDEs were analyzed by AXYS Analytical Services, Ltd. (Sidney, British Columbia) using AXYS method MLA-025 "Analytical Method for the Determination of Polybrominated Diphenylethers by High Resolution GC/MS." This method is in accordance with USEPA Method 1614: Polybrominated diphenyl ethers in water, soil and tissue by high resolution gas chromatograph (Hewlett-Packard 6890) and high resolution mass spectrometry (Micromass Autospec Ultima). The analysis included a procedural blank, a known control sample and a duplicate analysis. Samples were Soxhlet extracted for 16 hours with dichloromethane and cleaned up with gel permeation chromatography on Biobeads SX-3 and fractionated on Florisil, silica and alumina. Approximately 25 grams of each tissue homogenate was spiked with 13C-labeled brominated diphenylether standards and analyzed in low light conditions to protect against photo-degradation. For PBDE congeners that were not detected, the concentration

was assumed to be zero. Forty-three congeners were analyzed with detection limits ranging from less than 1 pg/g – 34.6 pg/g depending on the congener. The highest detection limits were for the mono- and deca-BDEs. Percent recoveries of PBDEs ranged from 84-105%, with an average recovery rate of 96%. For most congeners, blanks were below detection limits. Small concentrations were detected for a few congeners, but were more than 2 orders of magnitude below samples (blanks were not subtracted from sample measurements).

PCBs were analyzed by AXYS Analytical Services utilizing a method in accordance with USEPA Method 1668, Revision A: Chlorinated Biphenyl congeners in water, soil, sediment and tissue by HRGC/HRMS. The analysis included a procedural blank, a known control sample and a duplicate analysis. Percent recoveries of PCBs ranged from 94-106%, with an average recovery rate of 100%. For most congeners, blank concentrations were below detection limits. Small concentrations were detected for a few congeners, but were more than 2 orders of magnitude below samples (blanks were not subtracted from sample measurements).

Approximately 10 grams of each sample homogenate was spiked with labeled quantification standards and soxhlet extracted with dichloromethane. After extraction, the homogenates were further refined using gel permeation and an SPB Octyl column, followed by fractionation using adsorption column chromatography on carbon for some congeners. Analytes are presented on a wet weight basis. For PCBs that were not detected, the concentration was assumed to be zero. All 209 congeners were analyzed, with detection limits ranging from less than 1 to 4.6 pg/g depending on the congener.

All data used in this study were conducted in accordance with the AXYS accredited quality assurance and quality control program and went through a rigorous quality assurance review by EPA Region 10. Data quality objectives included measures of precision, accuracy, representativeness, comparability and completeness. Differences in PBDEs and PCBs among WB, FNS, and FS were examined using one-way ANOVA followed by Dunnett's pairwise multiple comparison t-test ($p \leq 0.05$). Statistics and graphs were developed with SPSS v 11.5 and Microsoft Excel 2000.

RESULTS AND DISCUSSION

Levels of PBDEs (w/w) in three tissue types, as well as the percent reduction in contaminant burden, are presented in Table 1. Total PBDEs were found at the highest concentrations in WB samples (2.3 µg/kg), followed by FS (1.8 µg/kg) and FNS (1.5 µg/kg). Given the lipophilic nature of these chemicals, it is not surprising that PBDEs were found at higher levels in WB tissue compared with fillets. A statistically significant difference in mean concentrations of PBDEs (w/w) in WB vs. FNS was observed (Dunnett's t-test, $p = 0.044$). However, there was no statistically significant difference between tissue types when PBDEs were lipid normalized (one-way ANOVA, $p = 0.34$).

Table 1. Average wet weight PBDEs and PCBs and lipid content of different tissue types.

Tissue Type	Percent lipid	Mean PBDEs (ug/kg) +/- SE	Percent PBDE reduction	Mean PCBs (µg/kg) +/- SE	Percent PCB reduction
WB	9.4	2.3+/-0.28	-	15.3+/-0.96	-
FS	8.8	1.8+/-0.19	17.8	12.6+/-1.9	17.7
FNS	6.1	1.5+/-0.19*	34.8	10.2+/-1.7*	33.4

Percent reduction in contaminants for FS and FNS is relative to WB samples.
*statistically significant decrease in contaminant levels compared to WB samples ($P \leq 0.05$).

To determine the effectiveness of different preparation methods to reduce contaminant burden, total PBDE levels in FS and FNS were compared to WB tissue. A 17.8% reduction in PBDEs was calculated between WB and FS samples, and 17% reduction between FS and FNS samples. Careful filleting and the removal of skin results in a reduction of PBDEs by approximately one-third the original amount found in WB. It is important to note that half of this reduction was achieved by skin removal, which is often a preferred part of the fish among anglers. The lack of significant difference among lipid normalized samples suggests that the accumulation of PBDEs among tissue types is a function of the lipid content.

Figure 2 shows the five most dominant BDE congeners in various tissue samples with their corresponding standard error. BDE-47 was detected at the highest concentrations, representing over half of the PBDEs in the three tissue types. BDE-99 was the second highest congener at 15.3%, 15% and 15.2% among WB, FS and FNS, respectively. The next three dominant congeners, BDE-49, BDE-100 and BDE-154, comprised approximately 20% of the total PCBs for all tissue types when combined.

The prevalence of BDE-47, a major component of the penta formulation, is consistent with other studies in which BDE-47 accounted for more than 50% of the total PBDEs in tissue (She et al. 2002, Rayne et al. 2003). BDE-99 was the next most abundant congener detected in this study and is also a large component of the penta formulation. Bioavailability research on pike using labeled isotopes determined that BDE-47 had a 90% uptake rate through the G.I. tract, followed by 62% uptake for BDE-99 (Burreau 1999).

Many factors likely influence the uptake of PBDEs in fish, especially trophic status, diet and mobility. Unlike salmon, resident fish do not migrate and have smaller home ranges. As a result, PBDEs are usually higher in resident fish compared to salmon, especially in areas with effluent discharge and sediment contamination. In Washington State, levels of PBDEs ranged from 1.4 µg/kg in rainbow trout to 1250 µg/kg in mountain white fish (Johnson and Olson 2001). In Virginia, PBDEs ranged from less than 5 µg/kg to 47900 µg/kg (lipid basis) in carp, catfish and bass (Hale et al. 2001).

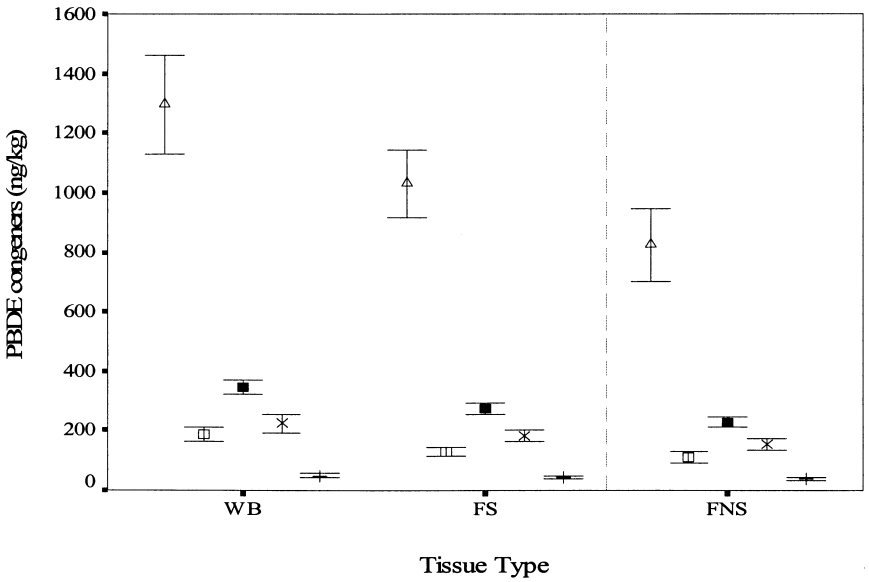

Figure 2. Congener profiles for the five most commonly detected brominated diphenyl ethers (ng/kg w/w) among different tissue types +/- one SE (Δ= BDE-47, □= BDE-49, ■=BDE-99, x = BDE-100 and +=BDE-154).

In salmon, PBDE levels may vary between different species, among wild versus farmed salmon and within-species from distinct geographic locations. In a recent study, chinook salmon from Oregon, British Columbia and Alaska had mean PBDE concentrations that were higher than chum, coho and sockeye salmon (Hites et al. 2004). Interestingly, wild-caught chinook from British Columbia had the highest levels of PBDEs in this study, including comparison with farm-raised salmon. Another study found that farm-raised salmon had higher levels of PBDEs compared with wild-caught salmon, which was attributed to contamination of commercial fish feed (Easton et al. 2002).

Total PCBs were approximately seven times higher compared to total PBDEs in all three tissue types (Table 1). WB samples had the highest concentration of PCBs at 15.3 μg/kg, followed by FS at 12.6 μg/kg and FNS at 10.2 μg/kg (w/w). A statistically significant difference in total PCBs (w/w) was observed between WB and FNS PCBs (Dunnett's t-test, $p = 0.037$).The percent reduction of contaminants between tissue types was similar to the PBDE results, with 17.7% PCB reduction between WB and FS, and 19.1% PCB reduction between FS and FNS. There was no statistically significant difference between tissue types for lipid normalized PCBs (one-way ANOVA, $p = 0.486$), similar to the findings for PBDEs. This suggests that PCB accumulation is positively correlated to lipid content, as would be expected given the lipophilic nature of these compounds.

Figure 3 depicts homologue profiles for PCB congeners in various tissue samples. The pentachlorinated homologue was the most prevalent group, which

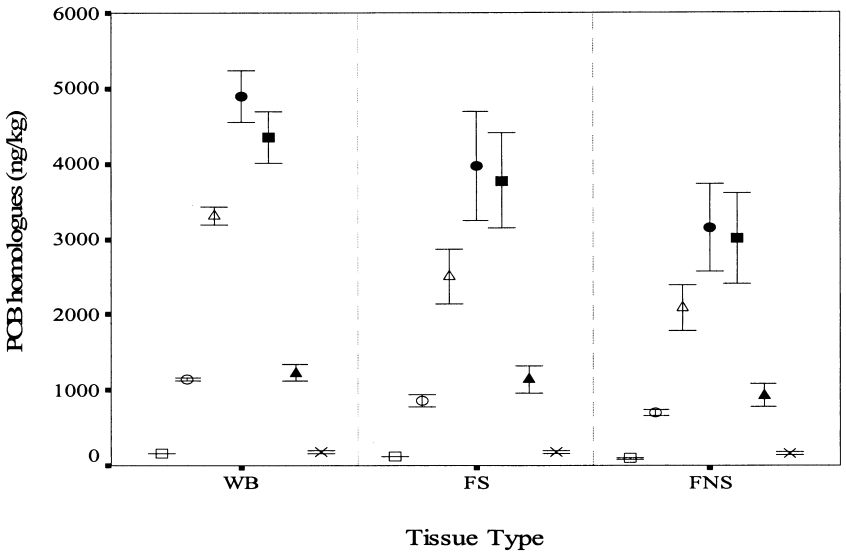

Figure 3. Homologue profiles for polychlorinated biphenyls (ng/kg w/w) among tissue types in salmon +/- one standard error (□= di-PCBs, ○ =tri-PCBs, Δ = tetra-PCBs, ●=penta-PCBs, ■=hexa-PCBs, =hepta-PCBs, x = octa-PCBs).

compromised 31.9%, 31.6% and 38.8%, among WB, FS and FNS, respectively. The hexachlorinated homologue was the next dominant group with 28.4%, 30% and 29.5% among WB, FS and FNS, respectively. The concentration of the pentachlorinated homologue did not decrease between FS and FNS samples, whereas the hexa and tetrachlorinated homologue concentrations declined. This suggests that the pentachlorinated homologues may have differential accumulation patterns between the skin and intramuscular fat compared with other PCB homologues.

The results of this study demonstrate that simple preparation methods will reduce exposure to PCBs and PBDEs for fish consumers, which accumulate in the fatty portions of fish. These measures include removing the organs, gut contents, side, belly and back fat, and skin, prior to ingestion. Since PCBs may cause developmental deficits as a result of prenatal exposure (Jacobsen et al. 1990, Stewart et al. 2000), education and outreach should continue to be directed towards women of childbearing age, especially those who want the benefits of a fish-rich diet, but are concerned about exposure to contaminants.

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