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Disease susceptibility of salmon exposed to polybrominated diphenyl ethers (PBDEs)

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ABSTRACT

The health effects of the flame retardant polybrominated diphenyl ethers (PBDEs) in fish are not well understood. To determine the potential effects of this ubiquitous contaminant class on fish health, juvenile subyearling Chinook salmon (*Oncorhynchus tshawytscha*) were fed a diet that reflected the PBDE congeners found in the stomach contents of subyearling Chinook salmon collected from the highly urbanized and industrialized lower Willamette River in the Columbia River Basin of North America. The diet, consisting of five PBDE congeners (BDE-47, BDE-99, BDE-100, BDE-153 and BDE-154), was fed to the salmon at 2% of their body weight in food per day for 40 days. Two concentrations of the diet (1× and 10× PBDE) were fed to the salmon. The 1× PBDE diet reflected the concentration of PBDEs (190 ng PBDEs/g food) found in the stomach contents of juvenile subyearling Chinook salmon; the 10× diet was prepared at 10 times that concentration. The fish were then exposed to the marine bacterial pathogen *Listonella anguillarum* to assess susceptibility to infectious disease. Juvenile Chinook salmon fed the 1× PBDE diet were more susceptible to *L. anguillarum* than salmon fed the control diet. This suggests that juvenile salmonids in the lower Willamette River exposed to PBDEs may be at greater risk for disease than nonexposed juvenile salmonids. In contrast, salmon that consumed the 10× PBDE diet were not more susceptible to the pathogen than salmon fed the control diet. The mechanisms for the dichotomous results observed in disease susceptibility between salmon fed the 1× and 10× PBDE diets are currently not known but have also been observed in other species exposed to PBDEs with respect to immune function.

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1. Introduction

Activities that degrade the habitat and result in ecosystems that are contaminated with persistent organic pollutants (POPs) can influence the health of endangered salmonid populations (Loge et al., 2005; Spromberg and Meador, 2005). Earlier studies have determined that juvenile subyearling Chinook salmon (*Oncorhynchus tshawytscha*) are exposed to varying levels of the following legacy and nonlegacy organic pollutants in various industrialized waterways (McCain et al., 1990; Johnson et al., 2007a, 2007b; Sloan et al., 2010): polychlorinated biphenyls (PCBs), polycyclic aromatic hydrocarbons (PAHs), organochlorine pesticides (OCP) such as DDTs (dichloro-diphenyl-trichloroethane), and polybrominated diphenyl ethers (PBDEs). Juvenile subyearling Chinook salmon in industrialized waterways exposed to relatively high concentrations of ΣPCBs and ΣPAHs have been found to be immunosuppressed and more susceptible to infectious disease, as well as having reduced growth and survival compared to fish not exposed to organic pollutants (Arkoosh et al., 1991, 1998; Varanasi et al., 1993). Therefore, contaminant exposure has the potential to alter the ability of salmon to respond to infectious agents as well as specifically altering immune function.

Recently, a monitoring study determined that juvenile subyearling Chinook salmon from the heavily urbanized and industrialized lower Willamette River in the Columbia River Basin in North America are exposed to the emerging contaminant PBDE (Sloan et al., 2010). PBDEs are a class of flame retardants that are added to a number of household and commercial items including televisions, computers, electronic equipment, and furniture (de Wit, 2002). These compounds have been measured in air, water, fish, birds, marine mammals, and humans (Hites, 2004), with levels in the environment that have been increasing over time (Koizumi et al.,

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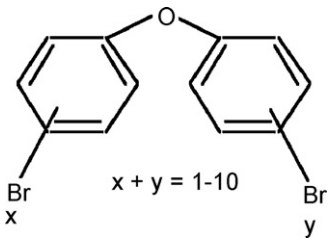

Fig. 1. General structure of PBDEs.

2005). Fig. 1 shows the general structure of PBDEs, which are characterized by two brominated phenyl rings that are connected by an ether bridge. In theory, all of the hydrogen groups can be exchanged with bromine, creating the possibility for 209 structurally different PBDEs (Hamers et al., 2008). PBDEs are structurally similar to PCBs and they follow the same nomenclature (Ballschmitter and Zell, 1980). Typically, three different mixtures of PBDEs have been commercially produced: penta-BDE, octa-BDE, and deca-BDE. Each mixture varies by the degree of bromination (Hamers et al., 2008). The movement away from using PBDEs in electronic equipment has begun recently due to concerns about their potential health effects (de Wit, 2002). Since 2004, octa-BDE is no longer produced in the European Union, United States and the Pacific Rim. Also as of 2007, there is no current production of penta-BDE in Europe, Japan, Canada, Australia and the United States. The European Union banned the use of all PBDEs in electronic equipment in 2006. In the United States, Washington and Maine have banned the use of penta-, octa-, and deca-PBDEs. Other states have also recently introduced legislation to ban PBDEs.

To date, little research has been conducted on fish to determine the biological effects of PBDE exposure including the contaminant's effect on disease susceptibility and immune function. However, since PBDEs are structurally similar to PCBs, the potential exists for this emerging contaminant to influence these biological functions. PBDEs have been shown to detrimentally affect immune cells in lake trout (*Salvelinus namaycush*) by decreasing thymocyte viability and increasing apoptosis and necrosis of thymocytes (Birchmeier et al., 2005). Some work on this topic has been performed with aquatic mammals. For example, thymic atrophy has been found to be associated with elevated PBDE levels in harbor porpoises (*Phocoena phocoena*) from contaminated sites in the North and Baltic Seas near Germany (Beineke et al., 2005). The porpoises from these contaminated sites also had greater bacterial infections than porpoises sampled from less polluted sites.

PBDE concentrations detected in juvenile subyearling Chinook salmon sampled from the Columbia River Basin in the United States (Sloan et al., 2010) were comparable to or greater than average concentrations of PBDEs reported in benthic invertebrate species from other sites in the United States, Europe, and Asia (Boom et al., 2002; Bayen et al., 2003; Voorspoels et al., 2003; Cai-Hong et al., 2007; Svendsen et al., 2007). In the present study, juvenile subyearling Chinook salmon were fed a diet containing an environmentally relevant concentration of PBDEs (38 ng/g wet or 190 ng/g dry weight) intended to mimic levels found in the stomach contents of subyearling Chinook salmon from the lower Willamette River of the Columbia River Basin as well as a diet containing PBDEs at 10 times that concentration. Higher concentrations of contaminants that are not environmentally relevant are frequently used in toxicology studies in order to ensure an induction of clear phenotypic effects (Lema et al., 2007), such as an alteration in disease resistance. Fish fed either the contaminated or the control diets were then challenged with the marine pathogen *Listonella anguillarum* to determine if exposure to PBDEs could alter the host's susceptibility to an infectious disease.

2. Materials and methods

2.1. Raising Chinook salmon

Eyed subyearling Chinook salmon (*O. tshawytscha*) eggs were transported from the Elk River Fish Hatchery, Port Orford, OR, to the Fish Disease Laboratory (FDL) at the Newport Research Station, Newport, OR. The eggs were housed in a vertical incubator chamber with a flow of 1 L/min of charcoal-filtered fresh water maintained at 100% oxygen saturation and 10 °C. The incubation chamber was checked twice daily for morbid eggs or larvae. After hatching, the Chinook salmon fry were transferred to and housed in three 1.8 m × 0.61 m × 0.76 m rectangular fiberglass flow-through aquaculture tanks. When the fry reached a weight of approximately 1.5 g, they were transferred to and housed in two 1.8 m × 0.91 m flow-through circular aquaculture tanks. The juvenile salmon smolts were fed with a commercially prepared Salmon Grower #4 Low Fat pellet (Rangen Inc., Buhl, ID) 6 times per week. The Salmon Grower #4 Low Fat pellet was 45.0% crude protein (min), 7.0% crude fat (min), 5.0% crude fiber (max), 12.0% ash (max), and 1.0% phosphorus (min).

2.2. Lethal concentration (LC) response curve for L. anguillarum

A 7-day lethal concentration response (LC) curve was developed with *L. anguillarum* (strain 1575) to determine the virulence of the pathogen on juvenile subyearling Chinook salmon as described by Arkoosh et al. (2005). In brief, a stock solution of *L. anguillarum* was grown as a suspension culture in Tryptic Soy Broth (TSB), supplemented with 1.5% sodium chloride, at 25 °C. Eight serial dilutions were generated with the stock solution (2.4×10^9 cfu/mL) and seawater in 11.36 L containers used for the disease challenge. An additional control container was generated using sterile media in place of the stock bacterial solution. To achieve a stocking density of 50 g/L, 20 juvenile Chinook smolts, approximately 15 g each, were placed in each container for 1 h under static conditions and aeration. After the 1-h exposure, the fish were transferred into 0.91 m diameter fiberglass aquaculture tanks, with a flow rate of 1 L/min of aerated, sand-filtered, and UV-irradiated seawater. Morbid fish were collected twice daily over a 7-day period.

2.3. Preparation of PBDE-contaminated diet

We produced a diet representing the PBDE concentration and congener composition found in subyearling Chinook salmon stomach contents from the lower Willamette River near Portland, OR. Stomach contents contained a mean ΣPBDE concentration of 38 ng/g wet weight (Sloan et al., 2010). For preparation of the experimental diet, wet weight BDE values for individual congeners were converted to dry weight values (Meador et al., 2002). In brief, stomach contents of subyearling Chinook salmon from the lower Willamette River were found to contain the following five congeners: BDE-47 (75 ng/g dry weight), BDE-99 (89 ng/g dry weight), BDE-100 (18 ng/g dry weight), BDE-153 (2.8 ng/g dry weight) and BDE-154 (1.3 ng/g dry weight) for a final PBDE concentration of 190 ng/g dry weight (Sloan et al., 2010). Preparation of the contaminated diet involved a serial two-step process wherein specific PBDE compounds were diluted in methylene chloride and isooctane by Accustandard Inc. (New Haven, CT) to create a concentrated stock solution of PBDEs, and then the stock solution was further diluted in methylene chloride and applied to the food pellets. Specific details of each step are provided below.

The stock solution of PBDEs was prepared using selected congeners diluted in a specified volume of methylene chloride and isooctane (4.5:5.5, v/v). The BDE congeners were weighed and dissolved by Accustandard Inc. (New Haven, CT) in 10 mL of sol-