Alteration of thyroid hormone concentrations in juvenile Chinook salmon (*Oncorhynchus tshawytscha*) exposed to polybrominated diphenyl ethers, BDE-47 and BDE-99

Mary R. Arkoosh[a,*], Ahna L. Van Gaest[b], Stacy A. Strickland[b], Greg P. Hutchinson`[b], Alex B. Krupkin[b], and Joseph P. Dietrich[a]

[a] Environmental & Fisheries Science Division,
Northwest Fisheries Science Center
National Marine Fisheries Service,
National Oceanic and Atmospheric Administration,
2032 South East OSU Drive,
Newport, OR USA 97365

[b] Frank Orth & Associates,
Under Contract to Northwest Fisheries Science Center
National Marine Fisheries Service,
National Oceanic and Atmospheric Administration,
2032 South East OSU Drive,
Newport, OR USA 97365

*corresponding author, mary.arkoosh@noaa.gov, (541) 867-0327

1

Abstract

Polybrominated diphenyl ethers (PBDEs) have been used as flame-retardants in consumer products and are currently detected in salmon globally. The two most predominant PBDE congeners found in salmon are BDE-47 (2,2',4,4'-tetrabromodiphenyl ether) and BDE-99 (2,2',4,4',5-pentabromodiphenyl ether). In the present study, groups of juvenile Pacific Chinook salmon were fed five environmentally relevant concentrations of either BDE-47 (0.3-552 ng total PBDEs/g food), BDE-99 (0.3-580 ng total PBDEs/g food), or nearly equal mixtures of both congeners (0.7-690 ng total PBDEs/g food) for 39-40 days. The concentrations of circulating total thyroid hormones, thyroxine (T_4) and 3,5,3'-triidothyronine (T_3), were measured using a hormone-specific time-resolved fluoroimmunoassay to determine if PBDE exposure disrupts the hypothalamic-pituitary-thyroid endocrine axis. The concentrations of both circulating T_4 and T_3 were altered in juvenile salmon by dietary uptake of BDE-99. Exposure to BDE-47 did not alter either T_3 or T_4 circulating hormone concentrations. However, exposure to a mixture of BDE-47 and BDE-99 reduced T_3 in fish with lower concentrations of total whole body PBDEs than with either congener alone at equivalent PBDE whole body concentrations. Accordingly, the disruption of PBDEs on circulating thyroid hormone concentrations has the potential to impact a number of critical functions in juvenile salmon including growth, parr-smolt transformation, and immunological processes.

Keywords

PBDE; Chinook salmon; thyroid hormones; T3, T4; endocrine disruption

1. Introduction

Polybrominated diphenyl ethers (PBDEs) are flame-retardants historically added to a number of products for consumer protection to impede combustion. However, PBDEs disassociate from these products and have been detected in a number of aquatic environments within several mammalian (Hites, 2004; Krahn et al., 2007; Nelson et al., 2015; Lavandier et al., 2016) and fish species (Hites et al., 2004; Sloan et al., 2010; Arkoosh et al., 2011; Good et al., 2014; Cappelletti et al., 2015; Nugegoda and Kibria, in press). Fish exposed to PBDEs during early life stages have altered growth, behavior, neurological and endocrine development, immune function and an increased susceptibility to disease; while adult fish exposed to PBDEs can have impaired reproduction (Lema et al., 2007; Arkoosh et al., 2010; Chen et al., 2012; Noyes and Stapleton, 2014; Arkoosh et al., 2015; Yu et al., 2015).

Studies have demonstrated that PBDEs have the potential to act as endocrine disrupting compounds capable of altering the concentration of thyroid hormones in fish by a number of mechanisms (brief summary in Table 1 and reviewed in Johnson et al., 2014; Noyes and Stapleton, 2014; Yu et al., 2015; Nugegoda and Kibria, in press). The production of thyroid stimulating hormone (TSH) by the pituitary in teleost fish is controlled by negative feedback of thyroid hormones 3,5,3'-triiodothyronine (T_3) and thyroxine (T_4) (Eales and Brown, 1993; Blanton and Specker, 2007; Noyes and Stapleton, 2014). In brief, the central hypothalamic-pituitary-thyroid (HPT) endocrine axis of teleost fish is responsible for regulating the production of T_4. The follicles of the thyroid produce T_4 after stimulation by TSH from the pituitary. T_4 is secreted into the plasma by the follicles. The secreted T_4 is converted to the biologically active T_3 in the peripheral tissues by deiodination of the outer ring of T_4.

Theoretically, a total of 209 PBDE congeners exist and have the potential to accumulate in the environment, but only a select few are routinely reported (Birnbaum and Staskal, 2004; Law et al., 2014). The limited number of PBDEs found in the environment is due to their instability and the congeners used in the three major commercial mixtures (i.e. PentaBDE, OctaBDE, and DecaBDE) added to consumer products (Birnbaum and Staskal, 2004). Despite the efforts to phase out the production of these commercial mixtures (EPA, 2010; Shaw et al., 2010; EPA, 2014), the potential for exposure will continue to exist due to the continued use or recycling of products containing the flame retardant (Ghosh et al., 2013; Noyes et al., 2013) and to existing contamination of sediment and biota (Desforges et al., 2014).

PBDEs have been detected in salmon located throughout the world, from freshwater Chinook salmon (*Oncorhynchus tshawytscha*) in the Great Lakes, to farmed Atlantic salmon (*Salmo salar*) in the Baltic Sea, to wild Chinook salmon off Chile, and Chinook salmon from the west coasts of Canada and the United States (Manchester-Neesvig et al., 2001; Hites et al., 2004; Montory et al., 2010; Sloan et al., 2010; Arkoosh et al., 2011; Ikonomou et al., 2011). The greatest whole body concentrations of total PBDEs were detected in juvenile Chinook salmon from the Puget Sound, Washington state, as high as 13,000 ng/g lipid (Sloan et al., 2010). The lower brominated congeners BDE-47 (2,2'4,4-tetraBDE) and BDE-99 (2,2'4,4',5-pentaBDE) were the most predominant congeners found in the whole bodies and stomach contents of the salmon (Sloan et al., 2010). The objective of this study was to determine if the two most predominant PBDE congeners and a mixture of these congeners disrupt the HPT axis in juvenile salmon as demonstrated by a change in the concentration of thyroid hormones. Prior studies, Table 1, have examined the effects that individual PBDE congeners produce in freshwater 'teleost models' such as the fathead

minnow, *Pimephales promelas*, (Noyes et al., 2013; Noyes and Stapleton, 2014) and zebrafish, *Danio rerio,* (Yu et al., 2010; Yu et al., 2011). This study examines the activity of the HPT endocrine axis in an anadromous species, Chinook salmon, exposed to dietary PBDEs. Investigating the effects of exposure in fish to individual PBDE congeners found in the environment, as well as relevant mixtures of congeners is the first step in determining risk of exposed species in the wild.

2. Materials and methods

2.1. Salmon and exposure to PBDEs

Juvenile Chinook salmon, originating from Garrison Springs Hatchery, Washington, USA, were exposed to PBDEs at NOAA's National Marine Fisheries Service's Newport Research Station in Newport, Oregon. The Chinook salmon and had transitioned into seawater between 4.5 to 5.5 months post-hatch according to an ocean-type life history (Healey, 1991). Stock concentrations of BDE-47 (550 µg/ml) and BDE-99 (550 µg/ml; AccuStandard; New Haven, CT) were prepared in methylene chloride. Five concentrations of BDE-47 and five concentrations of BDE-99 were produced from the appropriate stock. Each of the five individual BDE-47, individual BDE-99, and mixed PBDE dietary treatments were prepared as described by Dietrich et al. (2015). In brief, aliquots of the various concentratons of PBDE in methylene chloride were added to batches of low-fat (no oil spray) dry food pellets (Rangen Inc.; Buhl, ID) in a 1:1 (kg:liter) ratio for the production of BDE-47, BDE-99 and mixed PBDE dietary treatments 1-5. Additional control diets (Treatment 0) were prepared in the same manner by mixing 1 kg of food to 1 liter of methylene chloride without PBDE congeners. Batches of the food pellets were mixed in stainless steel bowls, dried, and then stored in glass jars. The glass jars were previously fired at 450 °C for 17 to 19 hrs to remove contaminants. The jars were covered with aluminum foil, secured with a plastic lid, and stored at 4°C until use. Five gram samples were removed from each diet preparation for chemical analysis (Sloan et al., 2004) of 11 PBDE congeners: BDEs 28, 47, 49, 66, 85, 99, 100, 153, 154, 155, and 183. The BDE-47, BDE-99, and total PBDE concentrations determined in the congener dietary treatments are presented in Table 2, with detailed description of the chemical analysis presented in Dietrich et al. (2015). The total PBDE whole body concentration was determined by summing the concentrations of the 11 BDE congeners.

The mean weights (±SD) of Chinook salmon prior to BDE-47, BDE-99, and mixed diet exposure were 9.1 (1.6) g, 8.6 (0.5) g, and 5.4 (1.3) g, respectively. Fish were fed the treatments between 6 to 7.5 months post-hatch. Fish were fed daily rations of either the individual (BDE-47 or BDE-99) congener dietary treatments for 40 days or the mixed PBDE congener dietary treatments for 39 days. The feed duration and concentrations resulted in the Chinook salmon having whole body concentrations of BDE-47 or BDE-99 that spanned global ranges measured in salmon from wild and aquaculture settings (Manchester-Neesvig et al., 2001; Hites et al., 2004; Johnson et al., 2010; Montory et al., 2010; Sloan et al., 2010; Arkoosh et al., 2011; Ikonomou et al., 2011). During the individual PBDE congener exposures, two replicate tanks of 175 fish were fed each concentration. During the mixed PBDE congener exposure, three replicate tanks of 285 fish were fed each concentration. Food rations were weighed into separate

glass jars, specific to an individual tank, each day and stored at room temperature for up to 8 hrs. Daily rations were calculated based on numbers of fish per tank and an estimated fish mass. Rations were adjusted daily to reflect estimated fish growth and mortalities found in tanks in order to consistently feed 2% of fish body mass. The mean weights (±SD) of Chinook salmon after dietary treatment with BDE-47, BDE-99, and mixed diet exposure were 16.9 (3.4) g, 16.4 (4.1) g, and 10.6 (1.4) g, respectively.

2.2. Chemistry analysis

Chemistry analysis was completed as per Dietrich et al. 2015 (2015), on salmon whole bodies collected one day after completing each dietary treatment exposure. The sample and analysis plan included: five individual fish per feed tank (10 fish per concentration) for the individual PBDE congener dietary treatments; and two composites of five fish per feed tank (six composite samples, or 30 total fish, per concentration) for the mixed PBDE congener dietary treatments. All chemistry samples were analyzed for the same 11 PBDE congeners as the food. The BDE-47, BDE-99, and total PBDE whole body concentrations are presented in Table 2.

2.3. T_3 and T_4 measurement

After the dietary PBDE exposures, subsets of juvenile Chinook salmon were euthanized and blood was immediately collected to determine levels of total circulating thyroid hormones, triiodothyronine (T_3) and thyroxine (T_4). Total T_3 and T_4 levels were each determined in the plasma from 10 samples per treatment. All necropsies occurred within 4 days of the last feeding. Blood samples were collected from the caudal vein after removal of the caudal peduncle using heparinized Natelson tubes. The blood samples were spun in a microcentrifuge for 10 minutes at 8000 x g. Plasma was separated from red blood cells by pipet and stored at -80°C. For the individual congener PBDE exposures, each T_3 and T_4 sample represented an individual fish. For the mixed-congener PBDE exposures, each sample represented a composite of the plasma collected from 5 fish, and T_3 and T_4 levels were determined from the same composite.

The concentrations of total T_4 and T_3 in the plasma were determined using hormone-specific time-resolved fluoroimmunoassays. Manufacturer protocols were followed to complete the dissociation-enhanced lanthanide fluoroimmunoassays (DELFIA; Perkin-Elmer). Briefly, plasma samples were assayed in 96-well microtiter plates. Each plate was run with either a T_3 or T_4 standard curve to quantify hormone levels. The concentrations of the T_3 standards ranged from 0.05 - 6.5 ng/ml. The concentration of the T_4 standard ranged from 1.8 – 233 ng/ml. Aliquots from a pool of salmon plasma were also added to each plate at high (25 µl), medium (10 µl), and low (1 µl) levels to ensure consistency of measurements between plates. Two replicate wells were run for each sample and standard concentrations.

2.4. Data analysis

Differences in mean thyroid hormone levels were tested among the treatments of the BDE-47, BDE-99, and mixed BDE diets separately, using one-way ANOVA (SYSTAT 13; Systat Software, Inc.). Post-hoc analysis, Tukey's Honestly Significant Difference, was used for comparisons of T3 or T4 levels among all six dietary treatments. A significance level (α) was set at 0.05 for all comparisons.

3. Results

Juvenile Chinook salmon did not have significantly altered total T_4 concentrations ($p \geq 0.184$) among the six BDE-47 dietary treatments (Figure 1a). However, juvenile Chinook salmon fed the BDE-99 dietary treatments did have significantly altered total T_4 concentrations (Figure 1b; $p \leq 0.010$). Specifically, juvenile Chinook salmon from BDE-99 Treatment 3 (24.1 ng total PBDEs/g ww) had significantly lower total T_4 than fish in Treatment 1 (2.1 ng total PBDEs/g ww, $p \leq 0.010$; Figure 1b) and in Treatment 2 (6.8 ng total PBDEs/g ww, $p \leq 0.029$). Finally, juvenile Chinook salmon fed the mixed BDE congener diets did not have significantly altered total T_4 concentration in the plasma (Figure 1c; $p \geq 0.742$).

Similar to T_4, juvenile Chinook salmon from the BDE-47 dietary treatments did not have significantly altered total T_3 concentrations (Figure 2a; $p \geq 0.722$). However, juvenile Chinook salmon fed the BDE-99 and mixed dietary treatments did have significantly altered total T_3 concentration ($p \leq 0.029$ and $p \leq 0.017$, respectively). Specifically, juvenile Chinook salmon from BDE-99 Treatment 5 (219 ng total PBDEs/g ww) had significantly lower total T_3 than fish in Treatment 3 (24.1 ng total PBDEs/g ww ($p \leq 0.009$; Figure 2b). Finally, juvenile Chinook salmon from the mixed BDE congener Treatment 3 (36.8 ng total PBDEs/g ww) had significantly less total T_3 than fish in Treatment 0 ($p \leq 0.010$; Figure 2c). No other significant differences in total T_3 concentrations were observed between the different BDE-99 or mixed-congener dietary treatments.

4. Discussion

In the present study, juvenile Chinook salmon transitioned into seawater and exposed to environmentally relevant concentrations of either BDE-99 or a mix of BDE-47 and BDE-99 congeners had plasma thyroid hormone concentrations that were significantly reduced. By contrast, salmon exposed to only environmentally relevant concentrations of BDE-47 did not have altered thyroid hormone concentrations. Earlier studies have found that freshwater fish exposed to PBDEs have altered thyroid hormones concentrations (Table 1). For example, fathead minnows exposed to BDE-47 have decreased levels of T_4 (Lema et al., 2008). Similarly, fathead minnows exposed to BDE-209 also had decreased levels of T_4, as well as T_3 (Noyes et al., 2013). T_4 levels were also reduced in juvenile crucian carp (*Carassius auratus,* (Song et al., 2012)) and juvenile lake trout (*Salvelinus namaycush,* (2004)) after exposure to mixtures of PBDE congeners. However, adult zebrafish exposed to DE-71 were found to have an increased concentration of T_4 and no changes in the concentration of T_3 (Yu et al., 2011) while larval zebrafish exposed to DE-71 were found to have a decreased concentration of T_4 (Yu et al., 2010). The ultimate effect of PBDE exposure on thyroid hormones appears to be dependent on a number of variables, including: the species of the fish and its life-stage; the PBDE congener; route of exposure (i.e. through the diet, aqueous exposure); exposure concentration, and the duration of exposure.

A number of internal mechanisms can play a role in altering thyroid hormone homeostasis in fish due to PBDE exposure (Dishaw et al., 2014; Noyes and Stapleton, 2014). One potential mechanism responsible for the reduction in circulating plasma thyroid hormone concentrations may be the ability of PBDEs to attach to thyroid hormone binding protein (Meerts et al., 2000; Morgado et al., 2007). The majority of thyroid hormones circulating in the plasma are attached to thyroid hormone binding proteins. Transthyretin (TTR) is the major thyroid hormone binding protein in fish (Yamauchi et al., 1999) capable of binding both T_3 and T_4 (Morgado et al., 2006). PBDEs have been found to inhibit the *in vitro* binding of T_3 to fish TTRs (Morgado et al., 2007). Although yet to be studied in fish, both BDE-47 and BDE-99 can inhibit the ability of T_4 to bind to male rat TTR, potentially resulting in increased metabolism of T_4 in circulation (Meerts et al., 2000). Once the hormone is displaced from TTR, increased metabolism of the hormone may occur, resulting in reduced amounts of circulating hormone (Meerts et al., 2000; Noyes and Stapleton, 2014), as observed in the current study with T_3 and T_4. Congeners BDE-47, BDE-49, and BDE-99 can inhibit T3 from binding to sea bream TTR *in vitro* (Morgado et al., 2007). The 50% inhibitory concentrations (IC_{50}) of BDE-47 and BDE-99 were nearly equivalent, 5.2 and 6.7 nM, respectively. By contrast, the IC_{50} of BDE-49 was about 10-fold lower, at 0.5 nM (Morgado et al., 2007). The ability of Chinook salmon to debrominate BDE-99 to BDE-49 (Browne et al., 2009; Roberts et al., 2011; Dietrich et al., 2015), a congener with an ability to bind strongly to TTR, may partially explain why we observed significantly altered thyroid hormone concentrations in fish exposed to BDE-99 alone and in a mixture, but not in fish exposed to BDE-47 alone.

The reduction in plasma T_3 observed in this study may also be due to the ability of PBDEs to act as a substrate and competitively inhibit T_4 from binding to deiodinase (DI) enzymes. DI enzymes catalyze the transformation of thyroid hormones by removing iodine from the inner and outer rings (Roberts et al., 2011). The conversion of T_4 to the more biologically active T_3 occurs by cleaving an iodine atom in the meta position and appears to be controlled in peripheral tissues (Eales and Brown, 1993). The livers of juvenile fathead minnows fed a diet containing BDE-209 for 28 days were found to have a 74% reduction in the rates of deiodination. Even after a 14-day depuration period, DI activity in the minnows was still reduced by 48% (Noyes et al., 2011). The metabolites of BDE-209 were also found to have distinctly different abilities to bind to DI enzymes in the minnows (Noyes et al., 2011). Our findings of reduced T_3 concentrations support the hypothesis that BDE-99 may inhibit the transformation of T_4 to T_3 by competing with T_4 for DI catalysis. Conversely, our findings of no decrease in T_3 after BDE-47 exposure suggests that BDE-47's ability to act as a substrate and bind to DI enzymes may be less than that of BDE-99 or its metabolites. However, little information is available on the varying capacity of DI enzymes in fish to bind to different BDE congeners and requires further study (Noyes et al., 2011).

Thyroid hormone concentrations may be responding to PBDE exposures non-monotonically. A non-monotonic response due to endocrine disrupting chemicals occurs when the response generated to the contaminant is not linear (Vandenberg et al., 2012; Lagarde et al., 2015). Although not significant, trends were observed in the thyroid hormone levels of salmon exposed to PBDEs that were suggestive of a non-monotonic thyroid hormone response in juvenile salmon. These trends included: 1) an initial increase of T_4 in fish exposed to BDE-99 with a mean body burden of 2.1 ng total PBDEs/g ww followed by a subsequent decrease in T_4 in fish with greater mean PBDE body burdens (6.8-219 ng total PBDEs/g ww); 2) a maximum of total T_3 occurred in fish exposed to BDE-99

with a mean body burden of 24.1 ng total PBDEs/g ww then decreased in fish with the greatest PBDE body burdens (219 ng total PBDEs/g ww); and 3) a minimum of total T_3 occurred in fish exposed to the mixture diet with a mean body burden of 36.8 ng total PBDE/g ww with an increase in T_3 in fish with lower or greater mean PBDE body burdens. Classical risk assessment techniques used with monotonic dose responses to determine no observed adverse effect level and the lowest observed adverse effect level should not be applied to non-monotonic dose responses (Vandenberg et al., 2012; Arkoosh et al., 2015; Lagarde et al., 2015). Seven potential mechanisms have been described (Vandenberg et al., 2012) that can result in non-monotonic dose response curves due to endocrine disrupting chemicals, including: cytotoxicity, cell and tissue specific receptors and cofactors, receptor selectivity, receptor down-regulation and desensitization, receptor competition, endocrine negative feedback loops, and other downstream mechanisms. However, few studies exist that have examined these mechanisms experimentally (Lagarde et al., 2015).

The central HPT endocrine axis (thyroid follicles) and peripheral tissues, via the activity of enzymes (DI) and membrane transporters (*mct8* and *oatp 1c1*), have the ability to partially compensate for alterations in the production of thyroid hormones due to both internal and external influences (Noyes et al., 2013; Noyes and Stapleton, 2014). This ability to partially compensate for thyroid hormone concentrations can complicate extrapolating functional consequences or effects of PBDE exposures that result in altered circulating T_3 and T_4 hormone concentrations on critical teleost processes. Despite the actions of these compensatory mechanisms, this current study demonstrates that changes in thyroid hormone levels occur that may have serious impacts on juvenile fish health and survival.

Thyroid hormones play key roles in a number of critical processes in juvenile fish including growth, the transitional period from fish fry to larva (Reddy and Lam, 1992; Power et al., 2001), the parr-smolt transformation (Dickhoff et al., 1997), as well as immune system development and function (Lam et al., 2005). Consequently, the observed reduction in thyroid hormone concentrations found in juvenile fish after exposure to a mixture of PBDEs or BDE-99 may impact these processes. This current study also demonstrates that lower whole body concentrations of a mixture of PBDEs have the ability to reduce T_3 in fish more so than fish exposed to individual congeners at equivalent PBDE whole body concentrations. Therefore, when considering risk assessments of PBDE exposure on juvenile salmon, not only is the total concentration of the contaminant important, but also the specific PBDE congeners.

Acknowledgements

Funds for this work were provided by NOAA and US EPA, Region 10, Puget Sound Science and Technical Studies Assistance Program; EPA-R10-PS-1004, Federal Grant No. 13-923270-01. We thank Dr. Andrew Dittman from NOAA's Northwest Fisheries Science Center for determining the concentrations of total T3 and T4 in the plasma samples.

241 **References**

242

243 Arkoosh, M.R., Boylen, D., Dietrich, J.P., Anulacion, B.F., Ylitalo, G.M., Bravo, C.F., Johnson, L.L., Loge, F.J.,
244 Collier, T.K., 2010. Disease susceptibility of salmon exposed to polybrominated diphenyl ethers (PBDEs). Aquat.
245 Toxicol. 98, 51-59.

246 Arkoosh, M.R., Strickland, S.A., Van Gaest, A.L., Ylitalo, G.M., Johnson, L., Yanagida, G.K., Collier, T.K.,
247 Dietrich, J.P., 2011. Trends in organic pollutant in juvenile Chinook salmon with different outmigration histories
248 through the Lower Snake and Middle Columbia Rivers. Sci Total Environ 409, 5086-5100.

249 Arkoosh, M.R., Van Gaest, A.L., Strickland, S.A., Hutchinson, G.P., Krupkin, A.B., Dietrich, J.P., 2015. Dietary
250 exposure to individual polybrominated diphenyl ether congeners BDE-47 and BDE-99 alters innate immunity and
251 disease susceptibility in juvenile Chinook salmon. Environ. Sci. Technol. 49, 6974-6981.

252 Birnbaum, L.S., Staskal, D.F., 2004. Brominated flame retardants: Cause for concern? Environ. Health Perspect.
253 112, 9-17.

254 Blanton, M.L., Specker, J.L., 2007. The hypothalamic-pituitary-thyroid (HPT) axis in fish and its role in fish
255 development and reproduction. Crit. Rev. Toxicol. 37, 97-115.

256 Browne, E.P., Stapleton, H.M., Kelly, S.M., Tilton, S.C., Gallagher, E.P., 2009. *In vitro* hepatic metabolism of 2,2
257 ',4,4 ',5-pentabromodiphenyl ether (BDE 99) in Chinook Salmon (*Onchorhynchus tshawytscha*). Aquat. Toxicol. 92,
258 281-287.

259 Cappelletti, N., Speranza, E., Tatone, L., Astoviza, M., Migoya, M.C., Colombo, J.C., 2015. Bioaccumulation of
260 dioxin-like PCBs and PBDEs by detritus-feeding fish in the Rio de la Plata estuary, Argentina. Environ. Sci. Pollut.
261 Res. 22, 7093-7100.

262 Chen, Q., Yu, L.Q., Yang, L.H., Zhou, B.S., 2012. Bioconcentration and metabolism of decabromodiphenyl ether
263 (BDE-209) result in thyroid endocrine disruption in zebrafish larvae. Aquat. Toxicol. 110, 141-148.

264 Desforges, J.P.W., Dangerfield, N., Shaw, P.D., Ross, P.S., 2014. Heightened biological uptake of polybrominated
265 diphenyl ethers relative to polychlorinated biphenyls near-source revealed by sediment and plankton profiles along a
266 coastal transect in British Columbia. Environ. Sci. Technol. 48, 6981-6988.

267 Dickhoff, W.W., Beckman, B.R., Larsen, D.A., Duan, C., Moriyama, S., 1997. The role of growth in endocrine
268 regulation of salmon smoltification. Fish Physiol. Biochem. 17, 231-236.

269 Dietrich, J.P., Strickland, S.A., Hutchinson, G.P., Van Gaest, A.L., Krupkin, A.B., Ylitalo, G.M., Arkoosh, M.R.,
270 2015. Assimilation efficiency of PBDE congeners in Chinook salmon. Environ Sci Technol 49, 3878-3886.

271 Dishaw, L.V., Macaulay, L.J., Roberts, S.C., Stapleton, C.M., 2014. Exposures, mechanisms, and impacts of
272 endocrine-active flame retardants. Curr. Opin. Pharm. 19, 125-133.

273 Eales, J.G., Brown, S.B., 1993. Measurement and regulation of thyroidal status in teleost fish. Rev. Fish Biol. Fish.
274 3, 299-347.

275 EPA, 2010. DecaBDE phase-out initiative.
276 http://www.epa.gov/oppt/existingchemicals/pubs/actionplans/deccadbe.html.

277 EPA, 2014. An alternatives assessment for the flame retardant decabromodiphenyl ether (DecaBDE).
278 http://www.epa.gov/dfe/pubs/projects/decaBDE/deca-report-chapter1.pdf.

279 Feng, C.L., Xu, Y.P., Zhao, G.F., Zha, J.M., Wu, F.C., Wang, Z.J., 2012. Relationship between BDE 209

280 metabolites and thyroid hormone levels in rainbow trout (*Oncorhynchus mykiss*). Aquat. Toxicol. 122, 28-35.

281 Ghosh, R., Lokman, P.M., Lamare, M.D., Metcalf, V.J., Burritt, D.J., Davison, W., Hageman, K.J., 2013. Changes
282 in physiological responses of an Antarctic fish, the emerald rock cod (*Trematomus bernacchii*), following exposure
283 to polybrominated diphenyl ethers (PBDEs). Aquat. Toxicol. 128, 91-100.

284 Good, T.P., Pearson, S.F., Hodum, P., Boyd, D., Anulacion, B., Ylitalo, G., 2014. Persistent organic pollutants in
285 forage fish prey of *Rhinoceros auklets* breeding in Puget Sound and the northern California xurrent. Mar. Pollut.
286 Bull. 86, 3670378.

287 Healey, M.C., 1991. Life history of Chinook salmon. in: Groot, C., Margolis, L. (Eds.). Pacific salmon life histories.
288 UBC Press, Vancouver, pp. 313-393.

289 Hites, R.A., 2004. Polybrominated diphenyl ethers in the environment and in people: A meta-analysis of
290 concentrations. Environ. Sci. Technol. 38, 945-956.

291 Hites, R.A., Foran, J.A., Schwager, S.J., Knuth, B.A., Hamilton, M.C., Carpenter, D.O., 2004. Global assessment of
292 polybrominated diphenyl ethers in farmed and wild salmon. Environ Sci Technol 38, 4945-4949.

293 Ikonomou, M.G., Teas, H.J., Gerlach, R., Higgs, D., Addison, R.F., 2011. Residues of PBDEs in northeastern
294 pacificmarine fish: Evidence for spatial and temporal trends. Environ. Toxicol. Chem. 30, 1261-1271.

295 Johnson, L.L., Anulacion, B.F., Arkoosh, M.R., Burrows, D.G., Da Silvia, D.A.M., Dietrich, J.P., Myers, M.S.,
296 Spromberg, J., Ylitalo, G.M., 2014. Effects of legacy persistent organic pollutants (POPS) in fish-current and future
297 challenges. in: Tierney, K.B., Farrell, A.P., Brauner, C.J. (Eds.). Organic chemical toxicology of fishes. Acadmic
298 Press, New York, pp. 53-140.

299 Johnson, L.L., Willis, M.L., Olson, O.P., Pearce, R.W., Sloan, C.A., Ylitalo, G.M., 2010. Contaminant
300 concentrations in juvenile fall Chinook salmon from Columbia River hatcheries. N. Am. J. Aquacult. 72, 73-92.

301 Krahn, M.M., Hanson, M.B., Baird, R.W., Burrows, D.G., Emmons, C.K., Ford, J.K.B., Jones, L.L., Noren, D.P.,
302 Ross, P.S., Schorr, G.S., Collier, T.K., Boyer, R.H., 2007. Persistent organic pollutants and stable isotopes in biopsy
303 samples (2004/2006) from Southern Resident killer whales. Mar. Pollut. Bull. 54, 1903-1911.

304 Kuiper, R.V., Vethaak, A.D., Canton, R.F., Anselmo, H., Dubbeldam, M., van den Brandhof, E.J., Leonards, P.E.G.,
305 Wester, P.W., van den Berg, M., 2008. Toxicity of analytically cleaned pentabromodiphenyl ether after prolonged
306 exposure in estuarine European flounder (*Platichthys flesus*), and partial life-cycle exposure in fresh water zebrafish
307 (*Danio rerio*). Chemosphere 73, 195-202.

308 Lagarde, F., Beausoleil, C., Belcher, S.M., Belzunces, L.P., Emond, C., Guerbet, M., Rousselle, C., 2015. Non-
309 monotonic dose-response relationships and endocrine disruptors: a qualitative method of assessment. Environ.
310 Health 14.

311 Lam, S.H., Sin, Y.M., Gong, Z., Lam, T.J., 2005. Effects of thyroid hormone on the development of immune system
312 in zebrafish. Gen. Comp. Endocrinol. 142, 325-335.

313 Lavandier, R., Areas, J., Quinete, N., de Moura, J.F., Taniguchi, S., Montone, R., Siciliano, S., Moreira, I., 2016.
314 PCB and PBDE levels in a highly threatened dolphin species from the Southeastern Brazilian coast. Environ. Pollut.
315 208, 442-449.

316 Law, R.J., Covaci, A., Harrad, S., Herzke, D., Abdallah, M.A.E., Femie, K., Toms, L.M.L., Takigami, H., 2014.
317 Levels and trends of PBDEs and HBCDs in the global environment: Status at the end of 2012. Environ. Int. 65, 147-
318 158.

319 Lema, S.C., Dickey, J.T., Schultz, I.R., Swanson, P., 2008. Dietary exposure to 2,2 ',4,4 '-tetrabromodiphenyl ether

(PBDE-47) alters thyroid status and thyroid hormone-regulated gene transcription in the pituitary and brain. Environ. Health Perspect. 116, 1694-1699.

Lema, S.C., Schultz, I.R., Scholz, N., Incardona, J.P., Swanson, P., 2007. Neural defects and cardiac arrhythmia in fish larvae following embryonic exposure to 2,2',4,4'-tetrabromodiphenyl ether (PBDE 47). Aquat. Toxicol. 82, 296-307.

Manchester-Neesvig, J.B., Valters, K., Sonzogni, W.C., 2001. Comparison of polybrominated diphenyl ethers (PBDEs) and polychlorinated biphenyls (PCBs) in Lake Michigan salmonids. Environ. Sci. Technol. 35, 1072-1077.

Meerts, I.A.T.M., van Zanden, J.J., Luijks, E.A.C., van Leeuwen-Bol, I., Marsh, G., Jakobsson, E., Bergman, A., Brouwer, A., 2000. Potent competitive interactions of some brominated flame retardants and related compounds with human transthyretin *in vitro*. Toxicol. Sci. 56, 95-104.

Montory, M., Habit, E., Fernandez, P., Grimalt, J.O., Barra, R., 2010. PCBs and PBDEs in wild Chinook salmon (*Oncorhynchus tshawytscha*) in the Northern Patagonia, Chile. Chemosphere 78, 1193-1199.

Morgado, I., Hamers, T., Van der Ven, L., Power, D.M., 2007. Disruption of thyroid hormone binding to sea bream recombinant transthyretin by ioxinyl and polybrominated diphenyl ethers. Chemosphere 69, 155-163.

Morgado, I.M., Sauer-Eriksson, E., Power, D.M., 2006. Thyroid hormone binding by recombinant sea bream transthyretin: The role of the N-terminal region. J Exp Zool Part A 305a, 158-158.

Nelson, C., Drouillard, K., Cheng, K., Elliott, J., Ismail, N., 2015. Accumulation of PBDEs in an urban river otter population and an unusual finding of BDE-209. Chemosphere 118, 322-328.

Noyes, P.D., Hinton, D.E., Stapleton, H.M., 2011. Accumulation and debromination of decabromodiphenyl ether (BDE-209) in juvenile fathead minnows (*Pimephales promelas*) induces thyroid disruption and liver alterations. Toxicol. Sci. 122, 265-274.

Noyes, P.D., Lema, S.C., Macaulay, L.J., Douglas, N.K., Stapleton, H.M., 2013. Low level exposure to the flame retardant BDE-209 reduces thyroid hormone levels and disrupts thyroid signaling in fathead minnows. Environ. Sci. Technol. 47, 10012-10021.

Noyes, P.D., Stapleton, C.M., 2014. PBDE flame retardants. Toxicokinetics and thyroid hormone endocrine disruption in fish. Endocrine Disruptors 2, e29430-29431-29425.

Nugegoda, D., Kibria, G., in press. Effects of environmental chemicals on fish thyroid function: Implications for fisheries and aquaculture in Australia. Gen. Comp. Endocrinol.

Power, D.M., Llewellyn, L., Faustino, M., Nowell, M.A., Bjornsson, B.T., Einarsdottir, I.E., Canario, A.V.M., Sweeney, G.E., 2001. Thyroid hormones in growth and development of fish. Comparative Biochemistry and Physiology C-Toxicology & Pharmacology 130, 447-459.

Reddy, P.K., Lam, T.J., 1992. Effect of thyroid-hormones on morphogenesis and growth of larvae and fry of telescopic-eye black goldfish, *Carassius auratus*. Aquaculture 107, 383-394.

Roberts, S.C., Noyes, P.D., Gallagher, E.P., Stapleton, H.M., 2011. Species-specific differences and structure-activity relationships in the debromination of PBDE congeners in three fish species. Environ Sci Technol 45, 1999-2005.

Shaw, S.D., Blum, A., Weber, R., Kannan, K., Rich, D., Lucas, D., Koshland, C.P., Dobraca, D., Hanson, C., Birnbaum, L.S., 2010. Do the fire safety benefits justify the risks. Rev. Environ. Health 25, 261.

Sloan, C.A., Anulacion, B.F., Bolton, J.L., Boyd, D., Olson, O.P., Sol, S.L., Ylitalo, G.M., Johnson, L.L., 2010.

359 Polybrominated diphenyl ethers in outmigrant juvenile Chinook salmon from the Lower Columbia River and estuary
360 and Puget Sound, Washington. Arch Environ Contam Toxicol 58, 403-414.

361 Sloan, C.A., Brown, D.W., Pearce, R.W., Boyer, R.H., Bolton, J.L., Burrows, D.G., Herman, D.P., Krahn, M.M.,
362 2004. Extraction, cleanup, and gas chromatography/mass spectrometry analysis of sediments and tissues for organic
363 contaminants. U.S. Dept. of Commerce, NOAA Tech. Memo., NMFS-NWFSC-59, 47 p.

364 Song, Y., Wu, N.X., Tao, H., Tan, Y.F., Gao, M., Han, J.L., Shen, H.T., Liu, K.C., Lou, J.L., 2012. Thyroid
365 endocrine dysregulation and erythrocyte DNA damage associated with PBDE exposure in juvenile crucian carp
366 collected from an e-waste dismantling site in Zhejiang Province, China. Environ. Toxicol. Chem. 31, 2047-2051.

367 Tomy, G.T., Palace, V.P., Halldorson, T., Braekevelt, E., Danell, R., Wautier, K., Evans, B., Brinkworth, L., Fisk,
368 A.T., 2004. Bioaccumulation, biotransformation, and biochemical effects of brominated diphenyl ethers in juvenile
369 lake trout (*Salvelinus namaycush*). Environ. Sci. Technol. 38, 1496-1504.

370 Vandenberg, L.N., Colborn, T., Hayes, T.B., Heindel, J.J., Jacobs, D.R., Lee, D.H., Shioda, T., Soto, A.M., vom
371 Saal, F.S., Welshons, W.V., Zoeller, R.T., Myers, J.P., 2012. Hormones and endocrine-disrupting chemicals: Low-
372 dose effects and nonmonotonic dose responses. Endocr. Rev. 33, 378-455.

373 Yamauchi, K., Nakajima, J., Hayashi, H., Hara, A., Yamauchi, K., 1999. Purification and characterization of
374 thyroid-hormone-binding protein from masu salmon serum - A homolog of higher-vertebrate transthyretin. Eur. J.
375 Biochem. 265, 944-949.

376 Yu, L.Q., Deng, J., Shi, X.J., Liu, C.S., Yu, K., Zhou, B.S., 2010. Exposure to DE-71 alters thyroid hormone levels
377 and gene transcription in the hypothalamic-pituitary-thyroid axis of zebrafish larvae. Aquat. Toxicol. 97, 226-233.

378 Yu, L.Q., Han, Z.H., Liu, C.S., 2015. A review on the effects of PBDEs on thyroid and reproduction systems in fish.
379 Gen. Comp. Endocrinol. 219, 64-73.

380 Yu, L.Q., Lam, J.C.W., Guo, Y.Y., Wu, R.S.S., Lam, P.K.S., Zhou, B.S., 2011. Parental transfer of polybrominated
381 diphenyl ethers (PBDEs) and thyroid endocrine disruption in zebrafish. Environ. Sci. Technol. 45, 10652-10659.
382

Table 1. Summary of recent studies assessing the response of thyroid hormones (T_3 and T_4) [a] in fish after exposure to PBDEs relative to control fish.

Genus species/ Common name (age at exposure)	Reference	PBDE congener or mixture	Exposure concentrations	PBDE body burdens	T_3 response [b,c,d]	T_4 response
Carassius auratus/ Carp (juvenile)	(Song et al., 2012)	e-waste recycling	Carp sampled from a contaminated river with e-waste	7.7-703.31 ng ΣPBDE /g wet weight (BDE-28, 47, 153, 154, 100) in the muscle of carp	**Not available**	**↓T_4**
Danio rerio/ Zebrafish (embryos to adult)	(Yu et al., 2011)	DE-71	1, 3, 10 µg/l aqueous exposure (BDE-47, 99, 199, 153, 154)	7,706 – 55,029 ng ΣPBDE /g wet weigth	**(-)TT_3**	**↑TT_4**
Danio rerio/ Zebrafish (embryos)	(Yu et al., 2010)	DE-71	1, 3, 10 µg/l aqueous exposure	Not available	**Not available**	**↓T_4**
Danio rerio/ Zebrafish (larval)	(Chen et al., 2012)	BDE-209	0.08, 0.36, 1.92 mg/l aqueous exposure	2,351-38,627 ng BDE-209 /g wet weight	**↑T_3**	**↓T_4**
Danio rerio/ Zebrafish (adult)	(Kuiper et al., 2008)	DE-71	5, 16, 50, 160, 500 µg/l aqueous exposure	8.8-460 µg ΣPBDE /g wet weight	**↑T_3**	**↑T_4**
Oncorhynchus mykiss/ Rainbow trout (juvenile)	(Feng et al., 2012)	BDE-209	50-10,00 ng/g wet weight i.p. injection	38.51-80.29 ng ΣPBDE /g	**(-)TT_3, ↓FT_3**	**↑TT_4 ↓↑FT_4**
Pimephales promelas/ Fathead minnows (adult)	(Lema et al., 2008)	BDE-47	2.4, 12.3 µg/pair/day dietary exposure	Not available	**(-)TT_3**	**↓TT_4**
Pimephales promelas/ Fathead minnows (adult)	(Noyes et al., 2013)	BDE-209	3, 300 ng/g body weight/day dietary exposure	Not available	**↓TT_3**	**↓TT_4**
Platichthys flesus/ European flounder (adult)	(Kuiper et al., 2008)	DE-71	Treatment of spiked sediment ($7x10^{-3}$ – 700 µg/g TOC) and food ($14x10^{-3}$ -14,000 µg/g)	0.13-71 ΣPBDE µg/g wet weight	**(-)T_3**	**↓T_4**
Salvelinus namaycush/ Lake trout (juvenile)	(Tomy et al., 2004)	Mixture of 13 congeners (BDE-28, 47, 66, 77, 85, 99, 100, 138, 153, 154, 183, 190, 209)	2.5-25ng/g per BDE congener dietary exposure	Body burdens of 13 individual BDE congeners were determined over a 63 day period	**(-)FT_3**	**↓FT_4**

[a]The concentrations of total (TT_3, TT_4), free (FT_3, FT_4), or undesignated (T_3, T_4) thyroid hormones were determined.
[b]The downward pointing arrow (↓ signifies a significant reduction in thyroid hormone concentration after exposure to at least one of the concentrations or doses of PBDEs.
[c]The upward pointing arrow (↑ signifies an increase in thyroid hormone concentration after exposure to at least one of the concentrations or doses of PBDEs.
[d]The dash (-) signifies no change in thyroid hormone concentration after exposure to PBDEs.

Table 2. Mean concentration of targeted (BDE-47 and BDE-99) and total PBDEs in the diets and whole bodies (modified from Dietrich et al. 2015 Dietrich et al. (2015)).

		PBDEs in diets (ng/g food)				PBDEs in salmon (ng/g wet weight)		
Dietary treatment		BDE-47	BDE-99	Total PBDEs[a]	Lipids in salmon, %	BDE-47	BDE-99	Total PBDEs[a]
BDE-47								
	0	0.3	<LOQ[b]	0.3	1.65	0.27	<LOQ	0.3
	1	5.0	<LOQ	5.0	1.83	2.09	<LOQ	2.1
	2	23	<LOQ	23	2.05	6.32	0.03	6.4
	3	84	<LOQ	84	1.91	18.4	<LOQ	18.4
	4	280	<LOQ	280	2.11	69.5	<LOQ	69.6
	5	550	0.2	552	1.58	149	0.02	150
BDE-99								
	0	0.3	<LOQ	0.3	1.87	0.26	0.05	0.3
	1	0.4	6.4	6.8	1.53	0.29	1.80	2.1
	2	0.5	29	30	1.62	0.28	6.57	6.8
	3	1.0	100	100	1.81	0.55	23.4	24.1
	4	3.0	290	290	1.65	1.04	74.4	75.9
	5	5.7	570	580	1.73	1.83	216	219
Mixed congener (BDE-47/BDE-99)								
	0	0.4	0.1	0.7	2.43	<LOQ	<LOQ	
	1	4.1	4.2	8.5	2.49	1.52	1.29	2.8
	2	16	17	34	2.05	5.60	5.27	10.9
	3	50	51	102	1.92	18.2	18.6	36.8
	4	160	170	330	2.71	46.7	51.7	98.6
	5	330	360	690	1.92	86	96.8	184

391 [a] Total PBDEs equals the sum of detected BDE congeners (BDEs 28, 47, 49, 66, 85, 99, 100, 153, 154,
392 155, and 183) with levels greater than the limit of quantification (LOQ), as described (Dietrich et al.,
393 2015).
394 [b] Less than the limit of quantification (LOQ).
395
396
397

Figure captions

Figure l. Concentration of plasma total T_4 in juvenile Chinook salmon exposed to (a) BDE-47, (b) BDE-99, or (c) Mixed PBDEs diets. Total T_4 levels was determined in the plasma from 10 samples per treatment. For the individual congener PBDE exposures, each T_4 sample represented an individual fish. For the mixed-congener PBDE exposures, each sample represented a composite of the plasma collected from 5 fish, and T_3 and T_4 levels were determined from the same composite. The numbers in the brackets ([]) represent the corresponding PBDE dietary treatment the fish were fed. Post-hoc analysis, Tukey's Honestly Significant Difference, was used for comparisons of T_3 levels among all six dietary treatments. A significance level (α) was set at 0.05 for all comparisons. Treatments that do not share a letter are statistically different.

Figure 2. Concentration of plasma total T_3 in juvenile Chinook salmon exposed to (a) BDE-47, (b) BDE-99, or (c) Mixed PBDEs diets. Total T_3 levels was determined in the plasma from 10 samples per treatment. For the individual congener PBDE exposures, each T_3 sample represented an individual fish. For the mixed-congener PBDE exposures, each sample represented a composite of the plasma collected from 5 fish, and T_3 and T_4 levels were determined from the same composite. The numbers in the brackets ([]) represent the corresponding PBDE dietary treatment the fish were fed. Post-hoc analysis, Tukey's Honestly Significant Difference, was used for comparisons of T_3 levels among all six dietary treatments. A significance level (α) was set at 0.05 for all comparisons. Treatments that do not share a letter are statistically different.

419

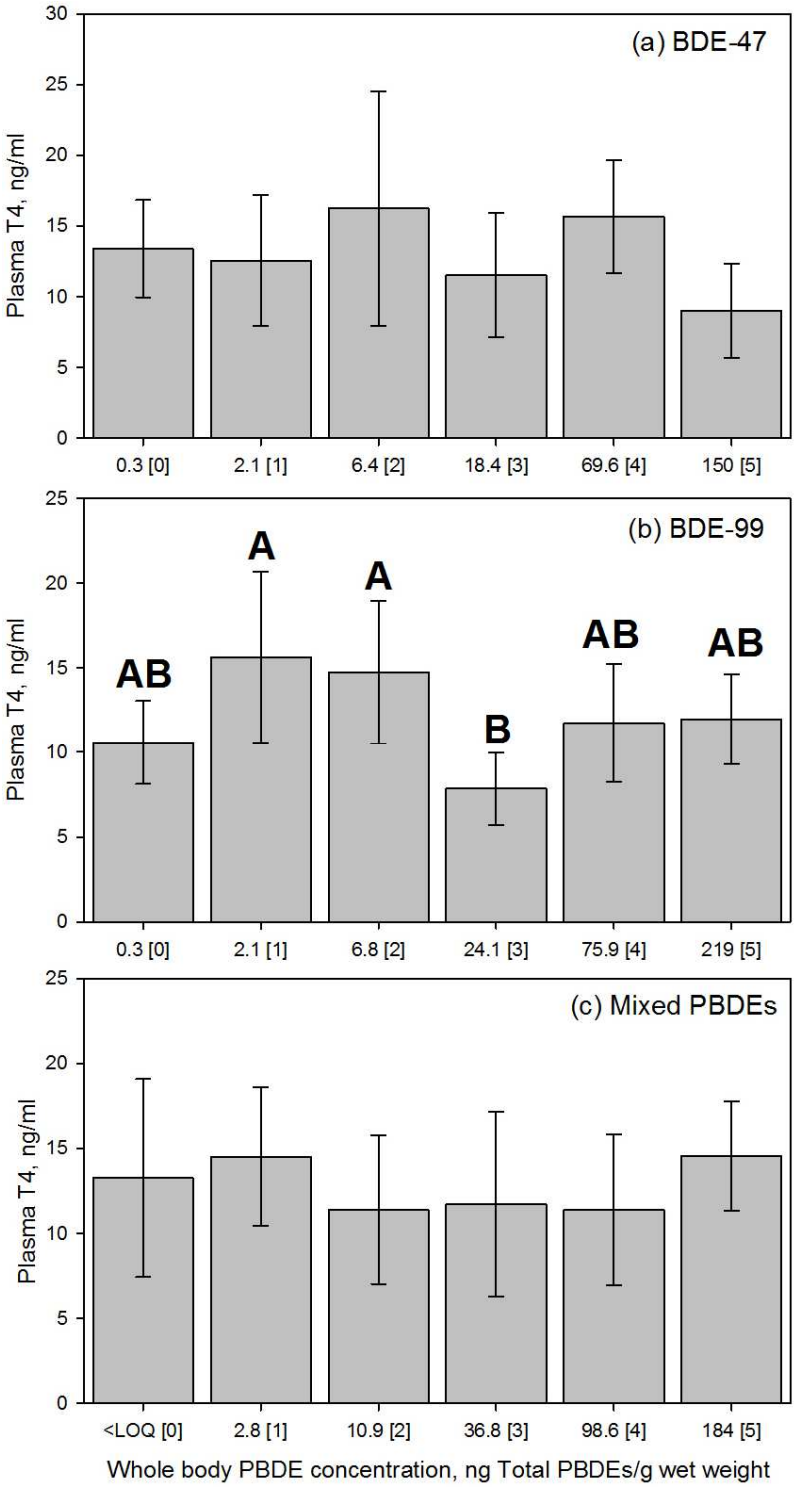

Figure 1. Plasma T4 (a) BDE-47, (b) BDE-99, and (c) Mixed PBDEs.

420

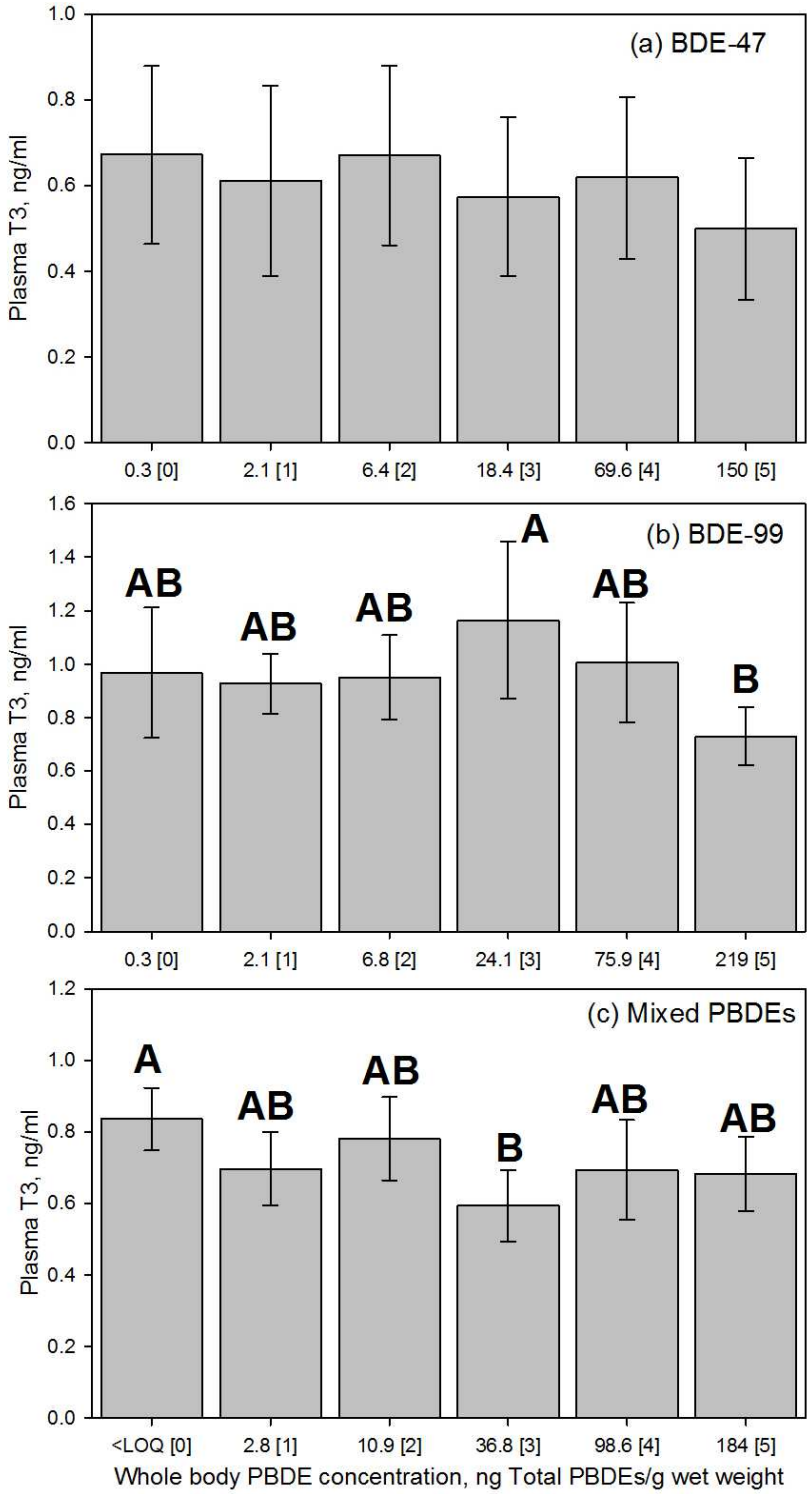

Figure 2. Plasma T3 (a) BDE-47, (b) BDE-99, and (c) Mixed PBDEs.

421