Bioaccumulation, Biotransformation, and Biochemical Effects of Brominated Diphenyl Ethers in Juvenile Lake Trout (Salvelinus namaycush)

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Juvenile lake trout (Salvelinus namaycush) were exposed to three dietary concentrations (0, ~2.5, and ~25 ng/g per BDE congener) of 13 BDE congeners (3–10 Br atoms) in the laboratory for 56 days, followed by 112 days of clean food, to examine bioaccumulation parameters and potential biochemical effects. The bioaccumulation of BDEs by the trout was highly influenced by biotransformation, via debromination, which resulted in bioaccumulation parameters that were much different than would be expected based on studies of chlorinated organic compounds (e.g., PCBs). Half-lives (t1/2's) for some BDE congeners (e.g., BDE-85 and -190) were much lower than expected based on their Kow, which was likely due to biotransformation, whereas t1/2's of other BDE congeners (e.g., BDE-66, -77, -153, and -154) were much longer than anticipated based on Kow. This was likely because the metabolites of BDEs formed via debromination had the same chemical structure of these BDE congeners, which supplemented measured concentrations. The detection of three BDE congeners (an unknown penta, BDE-140, and an unknown hexa) in the fish that were not present in the food or in the control fish provide further evidence for the debromination of BDEs. Half-lives of BDEs ranged from 38 ± 9 to 346 ± 173 days and biomagnification factors ranged from 1.6 (BDE-190) to 45.9 (BDE-100), but these bioaccumulation parameters need to be viewed with caution because they were highly influenced by debromination and relative abundance of individual BDEs that the fish were exposed to. CYP 1A enzyme activity, measured as EROD, and free tri-iodothyronine (T3) concentrations in the plasma of lake trout varied significantly throughout the experiment but were not related to BDE exposure. In contrast, plasma levels of thyroxine (T4) were lower in both groups of PBDE-exposed fish compared with control fish after 56 days of exposure, and after 168 days in the high dose, suggesting that PBDEs may influence thyroid homeostasis at levels that are higher than what is normally found in the environment.

Introduction

Heightened interest in the brominated diphenyl ether (BDE) flame retardants can be partly attributed to their exponential increase in the environment over the last 25 years. Although still referred to as a “new” or “emerging” contaminant, BDEs were first detected in fish from Sweden in 1981 (1). In that early study, BDE levels were reported to be ~3–4 times higher than those of PCBs and DDT in muscle and liver of pike (Esox lucius). After that time, and for almost 20 years, concern over their environmental occurrence waned. It was not until a recent report by Norén and Mėronytė in 2000 (2) showing exponentially increasing levels of BDEs in human breast milk from 1972 to 1997 that they re-emerged as a potential environmental threat. Today, BDEs are perhaps one of the more heavily studied classes of persistent organic pollutants (POPs).

The physical chemical properties of BDEs are similar in magnitude to those of other chlorinated POPs [e.g., poly-chlorinated biphenyls (PCBs)] of concern (3, 4). For example, the octanol–water partition coefficient (Kow) of BDEs, widely regarded to be a useful indicator of chemical bioaccumulation, puts them in the “obligatory” range (log Kow 5.5–7) for bioaccumulation (4, 5). Laboratory experiments have shown that BDEs bioaccumulate but it varies widely for the congener. A laboratory study on blue mussels (6) (Mytilus edulis) found that BDEs 47, 99, and 153 [numbering follows the IUPAC convention established for PCB congener (7)] has higher bioconcentration factors than PCB congeners with a similar number of chlorines. In zebrafish (Danio rerio) exposed to BDE contaminated food, the highest accumulation was observed for BDE 47 (8). Burreau et al. attributed the high biomagnification factor of BDE 47 in pike (Esox lucius) to its high assimilation efficiency (9). Kierkegaard et al. exposed rainbow trout (Oncorhynchus mykiss) to BDE 209 and found that less than 1% of the congener was taken up from food by the organism (10). Stapleton et al. exposed carp (Cyprinus carpio) to a number of BDE congeners and found that BDE 47 was taken up the most rapidly (11). Despite this work there has not been a definitive study that has examined a widerange of BDE congener. These data are not only critical for ecological risk assessments but are critical for modeling the environmental behavior of PBDEs.

There is concern about increasing concentrations of PBDEs found in aquatic biota because there is evidence they may exert toxic effects similar to those of PCBs, dioxins, and furans (12). For example, PBDEs are comparable to mono-ortho-substituted PCBs in terms of their potencies to interact with the cellular Ah receptor (12). PBDEs may also exert effects via thyroid hormone disruption by accelerating clearance rates of thyroid hormones, competing for cellular thyroid hormone binding sites, altering deiodinase enzyme activity, or in the case of hydroxylated PBDE metabolites, displacing thyroid hormones from the plasma transport protein, tran-sthyretin (12, 13).

In this study, we chose to examine the bioaccumulation parameters (depuration rate (Kd), half-life (t1/2), biomagnification factor (BMF), and assimilation efficiencies (αi) of...
comprehensive suite of BDE congeners in the laboratory to
develop quantitative structure-activity relationships (QSARs).
Our objectives were achieved by exposing juvenile lake trout
(Salvelinus namaycush) to environmentally relevant doses of
13 BDE congeners via the diet. A depuration period during
which all fish were fed unspiked food was also included to
examine elimination. Biochemical parameters, including CYP
IA enzyme activity and plasma concentrations of thyroid
hormones, at various periods of the accumulation and
depuration phases of the experiment were also examined.

Materials and Methods

Standards and Reagents. The following BDE and chlorinated
diphenyl ether (CDE) congeners were obtained from Well-
ington Laboratories (Guelph, ON, Canada) at purities greater
than 96%: 2,4,4'-tribromodiphenyl ether (BDE-28), 2,2',4,4'-
tetrabromodiphenyl ether (BDE-47), 2,3,4,4'-tetrabromo-
diphenyl ether (BDE-66), 3,3',4,4'-tetrabromodiphenyl ether
(BDE-77), 2,2',3,4,4'-pentabromodiphenyl ether (BDE-85),
2,2',4,4',5-pentabromodiphenyl ether (BDE-99), 2,2',4,4',6-
pentabromodiphenyl ether (BDE-100), 2,2',3,4,4',5-hexa-
 bromodiphenyl ether (BDE-138), 2,2',4,4',5,5-hexamethyl-
phenyl ether (BDE-153), 2,2',4,4',5,6-hexamethylphenyl ether
(BDE-154), 2,2',3,4,4',5,6-hexabromodiphenyl ether (BDE-
183), 2,3,3',4,4',5,6-heptabromodiphenyl ether (BDE-190),
2,2',3,3',4,4',5,5,6-decabromodiphenyl ether (BDE-209), 2,2',4-
trichlorodiphenyl ether (CDE-17), 2,2',4,4',5-pentachlo-
rodiphenyl ether (CDE-99), and 2,2',3,3',4,4',5-heptachlo-
 rodiphenyl ether (CDE-170). Technical-grade BDE-209 that
was used to fortify food was obtained from Great Lakes
Chemical Corporation (Indianapolis, IN) and a 36 congener
analytical standard was obtained from Cambridge Isotope
Laboratories (Andover, MA). Commercial fish food (Martin’s Feed
Mills, Elmina, ON, Canada) was placed in a pre-cleaned
Hobart blender. Corn oil, which was spiked with known
amounts of each BDE congener, was transferred to the
blender containing the food. After 20 min of gentle stirring,
an aqueous gelatin binder (40 g of gelatin in 1.5 L) was added.
Stirring continued until a firm consistency was observed (~20
min). The resulting spiked food was air-dried for 40 min, extruded
through a 4-mm-diameter nozzle, thoroughly
dried at 10 °C for 48 h, and crushed into pellets. Control food
was prepared in the same manner minus the test compounds.
Concentrations of each BDE congener were determined in
control and spiked food (see Table 1) using the same analytical
techniques used to determine concentrations in the lake trout
tissue described below.

Food Preparation. Commercial fish food (Martin’s Feed
Mills, Elmina, ON, Canada) was placed in a pre-cleaned
Hobart blender. Corn oil, which was spiked with known
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Experiment. Juvenile lake trout (initial mean weights, 55 ± 5 g)
were exposed to the spiked food for 56 d, followed by
112 d of depuration. The daily feeding rate was equal to 1.5%
of the mean weight of the lake trout, adjusted after each
sampling period based on the mean weight of the lake trout,
adjusted after each
112 d of depuration. The daily feeding rate was equal to 1.5%
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made with the injector temperature set isothermally at 280°C. To minimize degradation of BDE-209, a faster temperature program was employed to reduce the residence time on the column. The initial oven temperature was set at 90°C with no hold time, ramped at 25°C/min to 300°C, and held for 10 min. The ECD detector was held isothermally at 300°C. All samples were quantified using the BDE standard from CIL.

**GC-MS Analysis.** All analyses were performed on Agilent 5973 GC-MSD fitted with a 30 m × 0.25 mm i.d. (0.25 µm film thickness) DB-5 capillary column (J&W Scientific, CA). UHP helium was used as the carrier gas. Splitless injections of 2 µL were made by a 7683 Agilent autosampler with the injector set isothermally at 260°C. The initial oven temperature was set at 90°C with no hold time, ramped at 20°C/min to 250°C with no hold time, and ramped at 5°C/min to 300°C and held for 22 min. Confirmation of penta- and hexa-BDE unknowns was done in the electron ionization mode under selected ion monitoring (SIM) conditions using two ions in the [M−28]⁺ cluster: pentabDEs (m/z 404/406) and hexa-BDEs (m/z 484/486). Quantitation was achieved by comparing the integrated response of each unknown to the response of the nearest eluting BDE from the CIL's external standard.

**Data Analysis.** Concentrations of BDEs in muscle tissue of control fish were subtracted from those in fish exposed to treated food. Concentrations in fish from the low- and high-dose treatment were also corrected for growth, lipid percent, and recovery corrected using CDE (17, 99, 170), all of which were greater than 72%.

Growth rates were determined by fitting all fish and liver data to a linear growth model

\[ W_t = W_0[1 + b \times t \text{ (day)}] \]

where \( W_0 \) is the liver or body weight at time 0 (days), \( W_t \) is the weight at \( t \) = 0 days, and \( b \) is the growth rate. BDE concentrations in the fish were lipid normalized and corrected for growth dilution by multiplying the concentrations by a factor \((1 + b \times t)\). Assimilation efficiencies \( (\alpha) \) were calculated by using the equation

\[ \alpha = \frac{\text{control-corrected concentration in fish} \times \text{(mass of fish)}}{\text{control concentration in food} \times \text{(mass of food eaten)}} \]
Depuration rates ($k_d$) were calculated by fitting the depuration period data to a first-order decay curve,

\[ \text{In concentration} = a + b \times \text{time (day)} \]

where $a$ is a constant and $b$ is the depuration rate. Depuration half-life ($t_{1/2}$) was calculated by the formula

\[ t_{1/2} = \frac{\ln(2)}{k_d} \]

The equilibrium biomagnification factors (BMFs) were predicted from the equation (17)

\[ \text{BMF} = \frac{\alpha \times F}{k_d} \]

where $F$, the feeding rate, was 1.5% of the body weight of the fish and corrected for the lipid percentage of the food.

Results and Discussion

Bioaccumulation. All BDEs were measurable in fish in the low and high dose on the first collection day (after 7 days of exposure). BDE concentrations in the trout did not achieve steady state within the 56-day uptake phase for the high-dose treatment but appeared to be approaching steady state for the low-dose treatment (Figure 1). The shape of the uptake curves were mostly linear and very similar for most BDE congeners within treatment, and in most cases the uptake rate constants ($g$ d$^{-1}$) were similar for both treatments.

For most BDEs, depuration from fish was a first-order process, although many of the BDEs showed an initial rapid depuration for the first 14 days of depuration followed by a slower depuration rate over the remainder of the experiment (Figure 1). For other congeners (e.g., BDE-77, -99, and -100) there were increases in the tissue concentrations at the later stages of the depuration phase. In those cases, concentrations on those sampling days were omitted in the calculation of depuration rates (Table 1). Half-lives of BDEs, which are based on depuration rates, were highly variable among BDEs and between treatments (Table 1), and no clear trend with log $K_{ow}$ or Br number was found. Halflives ranged from 38 days (BDE-190) to 210 days (BDE-77) for the low-dose treatment. Half-lives were generally longer in the high-dose treatment and ranged from 26 days (BDE-209) to 346 days (BDEs-47, -77, and -183). Rankings of the BDEs based on $t_{1/2}$'s were different for the low- and high-dose treatments.

The assimilation efficiencies (AEs) were calculated based upon the concentrations of BDEs at days 7, 14, and 28 of the uptake phase (see below). AEs of the BDEs were similar between treatment groups and ranged from 0.3% for BDE-209 to 52.9% for BDE-28 (Table 1). There were no clear trends in AEs based on either brominonumber or log $K_{ow}$. The BMFs in this study were all >1 except for BDE-209 and ranged from 1.6 (BDE-190) to 45.9 (BDE-100) (Table 1).

Biological Effects. Whole body growth rates did not vary between BDE exposed and control lake trout, suggesting that BDEs did not have any negative effect on the growth of the fish (Table 2). Liver somatic index (LSI = liver weight/whole fish weight × 100) also did not vary between treatments but lipid percentages were slightly lower after day 168 compared to those after day 56 (Table 2). No mortalities occurred in any of the treatment groups.

Hepatic phase I enzyme activity, measured as ethoxyresorufin-O-deethylase (EROD) is shown in Figure 2 for each of the dose groups on days 0, 14, and 56 of the uptake phase and days 14 and 56 of the depuration phase. While there was some variability of EROD activity between sample periods within each of the dose groups, no significant differences were detected between dose groups at any of the time periods examined.

Concentrations of free tri-iodothyronine (T3) and thyroxine (T4) in plasma for days 0, 7, 14, 28, and 56 of the uptake phase and day 112 of the depuration phase are shown in Figure 3b. Tri-iodothyronine (T3) concentrations in the plasma of lake trout from all three groups varied throughout the experiment, but no consistent differences related to PBDE exposure levels were evident. In contrast, thyroxine levels (T4) were lower in both groups of PBDE exposed fish after 56 days of uptake. Significantly lower T4 was maintained in the high dose group even after 112 days of depuration when concentrations of many of the congeners were far lower than their maximal concentrations during the experiment.

Bioaccumulation, Biotransformation, and Bioformation. The bioaccumulation of BDE congeners is not a simple process in fish and is much more difficult to assess compared to chlorinated organic contaminants such as PCBs. Significant biotransformation, likely debromination, of some BDEs increases their elimination rates and confounds efforts to measure assimilation efficiency, depuration rate, and BMF. High biotransformation has been found to confound efforts to measure these parameters for chlorinated alkanes (18). Biotransformation of brominated organic compounds, including BDEs, has been noted in fish as well as other biota (11, 19, 20). Another significant effect of biotransformation is that of bioformation of some BDE congeners via debromination of higher brominated BDEs. This appeared to supplement the concentration of these less brominated BDEs, producing slower depuration rates and longer expected half-lives. Formation of lower brominated BDEs from highly brominated BDEs in fish has been observed previously (21).

The evidence for biotransformation of BDEs comes from contrasting their half-lives to those of chemicals with a similar $K_{ow}$. The half-lives of BDE-47, -66, -77, and -183 were all greater than 200 days, at least that are considered recalcitrant. The half-lives of BDE-85 (209) following 14 days, low and high, respectively) and -190 (28 days, low dose) were 1/2 those estimated for PCB 156 (101 days) in similar-sized rainbow trout (23). The log $K_{ow}$ of PCB 156 has been reported to be 7.2 (24), very similar to BDE-85 and -190 (log $K_{ow}$ of 7.4 and 8.5, respectively (4)), and this PCB is considered recalcitrant in fish based on the chlorine pattern. The most reasonable explanation for the lower $t_{1/2}$'s of these BDEs would be biotransformation.

Evidence for the debromination of higher brominated BDEs to lower brominated BDEs by lake trout was also found by comparing BDE half-lives to chemicals with a similar $K_{ow}$ that are considered recalcitrant. The half-lives of BDE-47, -66, -77, -99, and -153 were all greater than 200 days, at least for one of the exposure treatments. These values are much greater than PCBs of similar $K_{ow}$'s in rainbow trout (Onchorhynchus mykiss) of similar size (23). Bioformation of these congeners via debromination of higher brominated congeners during depuration would supplement measured concentrations and produce artificially long half-lives and consequently greater BMFs. Unfortunately, there are no analytical methods that could distinguish between the BDE that was bioaccumulated from BDE formed via debromination. Greater bioaccumulation of p,p' DDE and heptachlor epoxide in aquatic food webs has been attributed to bioformation by biota (25).

Further evidence that debromination may have supplemented concentrations is observed in concentrations of BDE-100 from the high-dose group. Concentrations of this congener increased over the final 98 days of the depuration...
period when the trout were exposed to negligible quantities of this chemical. Further, if debromination of some BDE congeners was supplementing other BDEs used in this experiment, then shorter half-lives would be expected for the source BDEs. This was the case, steeper depuration curves and shorter $t_{1/2}$ values of BDE-85, -183, -190, and -209 suggest that these could be the source of BDE congeners for debrominated metabolites.

The strongest evidence that debromination was resulting in the formation of other BDEs was the elution patterns of BDEs in the exposed lake trout. An examination of these profiles in fish after 56 days of exposure to the high BDE treatment revealed a number of BDE congeners that were absent in the fish food, in the control lake trout, or in technical...
The % lipid is an average (± standard deviation) of the fish (n = 5) sampled on that day. *Liver somatic index (± standard deviation) calculated as (liver weight ÷ whole fish weight) × 100.

BDEs formed via debromination could also be derived by their selective accumulation from the technical mixture if present as minor impurities. To check for this possibility, the following approach was taken: at day 56 of the high-dose exposure, the maximum concentration of BDE-209 present in the fish tissue was ~200 ng/g. The end result of a 10-g fish sample used for extraction, and a final sample volume (for GC-injection) of 100 μL, is a solution containing 20 ng/μL of BDE-209. A concentrated solution of technical BDE-209, 25 ng/μL, was therefore prepared and 2 μL injected onto the GC-MSD and the characteristic penta- and hexa-BDE ions monitored under SIM conditions. At this concentration, none of the three BDE congeners were detected, solidifying our hypothesis that these three congeners were formed by in vivo debromination.

Assessing the trophic transfer of BDEs in aquatic food webs is even more difficult, and variable BDE BMFs between studies would appear to be unavoidable. Biotransformation of chlorinated organic contaminants has been shown to vary between fish species, both in rate and chemicals that are susceptible to biotransformation. It appears likely that debromination of brominated organic contaminants by animals has been observed in other experiments (11, 19–21). Penta- and hexa-BDE congeners were also observed in common carp exposed only to BDE-209 (21). Lower bromobenzene metabolites were found in rats fed a diet contaminated with hexabromobenzene (19) and debromination of highly brominated biphenyls were also observed in Atlantic salmon (20).

Interestingly, the source of the BDE-140 (2,2′,3,4,4′,6′-BDE-140) in the fish could only be from debromination of BDE-209, as the substitution pattern of this congener cannot be derived from the higher brominated BDE congeners BDE-183 and -190. This assumes that the lake trout could not shift bromine atoms between carbons, for which there is no evidence for or against this. The structure of the two other BDEs which were observed in the lake trout extracts could not be positively identified. The addition of BDE-209 to this experiment clearly influenced bioaccumulation parameters determined for all other BDEs because this congener can be made into any other BDE via debromination. Debromination likely varies depending on the position of the bromine atom on the carbon ring; however, there are little data to provide guidance.

When this study began, only technical BDE-209 was commercially available. One might argue that the proposed model is shown in parentheses. b The % lipid is an average (± standard deviation) of the fish (n = 5) sampled on that day. *Liver somatic index (± standard deviation) calculated as (liver weight ÷ whole fish weight) × 100.
is weaker than the Cl–C bond. There is also evidence that fish age and water temperature can also influence biotransformation of chlorinated contaminants (A. Buckman, unpublished data). It is likely that this is also true for BDEs. Depuration rates ($k_d$) of BDE-47 (0.32 d$^{-1}$), BDE-154 (0.28 d$^{-1}$), and BDE-100 (0.23 d$^{-1}$) derived from a dietary study on juvenile common carp of similar size to the fish in this study (11) were 3–4 times higher than those of this study. A difference in biotransformation capacities between lake trout and carp is likely to cause such a discrepancy.

These bioformation-enhanced BMFs provide a new layer of complexity to BDE environmental behavior and present new challenges to models developed to assess food web dynamics of contaminants. Clearly, since BDEs can be debrominated to lower brominated BDEs, the exposure profile in the environment could have a significant influence on bioaccumulation and estimated BMFs. For example, BMFs of BDEs are likely to be much higher in fish that are exposed to these BDEs as well as BDE-209 than fish that are not exposed to BDE-209.

It is difficult to speculate on the mechanism or enzymes involved in debromination of BDEs. The structural similarity of BDEs to thyroxine (T4) presents a possible mechanism for debromination of BDEs; BDEs could be debrominated to less brominated congeners in a manner similar to the deiodination of T4 to T3. Variation in T3 levels across all treatments and reduced T4 levels in PBDE-treated trout in this study provides some evidence that deiodinase enzymes may play a role in PBDE debromination.

Other biotransformation pathways for PBDEs may also be important. For example, CYP 1A and 2B enzymes are associated with hydroxylation of aromatic contaminants, such as PCBs. Hydroxylated PBDEs have been found in the blood of Lake Ontario lake trout (A. Buckman, unpublished data) and Baltic salmon (Salmo salar) (26), suggesting CYP involvement in PBDE biotransformation. Biotransformation and bioformation make bioaccumulation parameters of BDEs generated in this experiment specific to this exposure regime and fish species. As discussed above, variable biotransformation among species and dif-

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**FIGURE 4.** GC-MS chromatograms showing three of the bioformed BDEs (bioformed products are asterisks). Top trace is the penta-BDEs (m/z 404) and the bottom is hexa-BDEs (m/z 484).
The assimilation efficiencies of the BDEs in this study are lower than those reported for PCBs in rainbow trout (50–60%) (18, A. Buckman, unpublished data). Burreau et al. found higher PBDE AEs, ranging from 90% to 35% for tetra- to hexa-BDEs, which may in part be due to the means by which BDEs were exposed to pike (Esox lucius) or the biotransformation capacity of this species. In the Burreau et al. (9) study, fish were exposed by spiking BDE congeners into lipid from trout muscle tissue that was then injected into the fish. Gobas et al. (27) and Burreau et al. (9) noted that the medium in which the chemical is administered can have an effect on AEs and perhaps the high lipid results in greater AEs. However, Drouillard and Norstrom found that lipid did not influence the dietary assimilation of PCBs in birds (28). Assimilation efficiencies should be independent of biotransformation and bioconversion (28) but the methods to determine assimilation efficiencies used in this work are sensitive to biotransformation and generally reduce the estimated AE (18). Estimating AEs in fish is difficult in part because feces are hard to collect. Studies with birds by litter mates collection and AEs for OCs have been found to be around 90% for compounds within a log Kow range of 5–7.5 (28). Nichols et al. reported an AE for 2, 2′, 5, 5′-tetrachlorobiphenyl in rainbow trout of >90% (29). It may well be that assimilation efficiencies of hydrophobic OCs in fish are >50%.

The BMFs calculated for the BDEs were all >1 except for BDE-209, implying that PBDEs will biomagnify in fish. However, BMFs are inversely proportional to measured depuration rates and the BMFs reported here may be artificially high due to the confounding effects of bioconversion on estimated t1/2. BMFs are also related to the assimilation efficiencies, and if the assimilation efficiencies in this study are confounded (see above), BMFs could be underestimated due to biotransformation or overestimated due to bioconversion kinetics. BMFs generated from this work need to be viewed and used with caution. However, even the shortest BDE half-lives are in a range that would suggest that biomagnification could occur in fish.

There are a few studies on BMFs of BDE congeners in the natural environment, which suggest that BDE biomagnify. Burreau et al. found similar biomagnification potentials (B-values) for tetra- and penta-BDEs and considerably lower B-values for hexa-BDEs in fish species from the Baltic Sea (30). Boon et al. examined the biomagnification of six BDE congeners (three to six Br atoms) in a North Sea food web and found highest BMFs for the fish to marine mammal feeding relationship (31).

Relationships between Bioaccumulation Parameters and Kow and Br Number. Not surprisingly, efforts to relate bioaccumulation parameters with physical-chemical properties were generally not successful. Relationships between log Kow and t1/2, assimilation efficiency, uptake rates, and BMFs have been reported for many recalcitrant OCs (18). No relationships between bioaccumulation parameters and Kow or bromine numbers were found in this experiment. This is most likely due to the impact of biotransformation and bioformation of the BDE congeners. However, Burreau et al. (9) found AEs of BDEs had negative relationships with degree of bromination but not with Kow.

Biological Parameters and Biochemical Effects. Results from this study suggest that toxicity of PBDEs mediated by induction of CYP 1A enzymes via the Ah receptor is not likely in fish at environmentally relevant doses. Despite using PBDE exposure concentrations above those observed in the environment, we observed no increase in EROD activity in the lake trout exposed to BDEs compared with those fish that were not exposed. A similar conclusion about CYP 1A mediated toxicity by PBDEs was also reached by Dameruddin et al. (32) after considering the available literature. Others have suggested that some PBDE congeners are inducers of phase I enzyme activity while others are inhibitory (33). Chen et al. (34) examined the induction of EROD by 12 different PBDE congeners using 6 different cell types, including 2 from fish, and found that PBDEs were 2–5 orders of magnitude less potent than TCDD for inducing this enzyme activity. Perhaps more importantly, they determined that the most prominent congeners, 47 and 99, did not induce EROD in any of the cell lines tested. Tjamhoud et al. (35) reported significant inhibition of EROD activity in rainbow trout (Oncorhynchus mykiss) after 6 and 22 days of exposure to feeding containing BDE-47, but at concentrations that are well above observed environmental concentrations of PBDEs (5.3–21 mg/kg). EROD was not induced in three spined stickleback (Gasterosteus aculeatus) fed a freeze-dried diet of chironomids containing the technical blend of tetra- and pentabrominated diphenyl ethers, Bromkal 70-SDE, for 3 months (36). Boon et al. (37) also reported no induction of EROD in Atlantic salmon (Salmo salar) orally dosed with penta- or octa-PBDEs. Impurities in the technical mixtures of PBDEs are speculated to contribute to some reports of Ah activation reported in the literature (12, 34).

Minor alterations in thyroid homeostasis were observed in the lake trout exposed to BDEs in this experiment. There was significant variation in free triiodothyronine (T3) concentrations in the plasma of lake trout from all 3 groups throughout the experiment, but no consistent differences related to PBDE exposure levels. In contrast, thyroxine levels (T4) were lower in both groups of PBDE exposed fish after 56 days and in the high-dose group after 168 days. This is similar to what has been observed in mammals exposed to PBDEs. For example, several studies using rats and mice have shown that exposure to PBDEs can reduce circulating plasma concentrations of T4 (38–40). One study exposed adult female rats to PBDEs and documented lower circulating T4 in both the adults and their pups (41). At least one study has shown that pure PBDE congeners were equally or more effective at eliciting thyroid responses compared with the technical mixtures (32).

Proposed mechanisms for the reduction of thyroxine seen in these experiments include increased clearance via induced phase II enzymes, interference with binding to plasma transport proteins, and effects to the thyroid gland itself, reducing production of the hormone (38). Competitive inhibition of thyroxine binding to the plasma transport...
proteins transthyretin appears to require hydroxylation of the parent PBDE compounds, specifically at the para or meta positions (13). Transthyretin has not been found in rainbow trout at significant concentrations but other thyroid transport proteins may be influenced by OH—BDEs. Hydroxylated PBDE metabolites have been detected in salmon from the Baltic Sea (26) and lake trout from Lake Ontario (A. Buckman, unpublished data) and are likely generated by phase I enzyme mediated metabolism of the parent compounds.

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