Introduction

Recently, hydroxylated PBDEs (OH-BDEs) have been identified in human samples, including breast milk, cord blood and serum, and environmental samples (Houde et al., 2009; Lacorte and Ikonomou, 2009; Qiu et al., 2009; Yu et al., 2010). Widespread use resulted in the leaching of PBDEs from consumer products into the environment where they bioaccumulate in aquatic organisms (Ackerman et al., 2008; Houde et al., 2009; Wu et al., 2008). OH-BDEs are naturally produced in some algal species, at concentrations 10–200 times lower than parent PBDEs (Hakk et al., 2010; Malmberg et al., 2005; Malmvarg et al., 2008). As higher concentrations in the environment are detected, there is increasing concern regarding the toxicity of these compounds.

Current studies have found that OH-BDEs are more toxic than PBDEs (Dingemans et al., 2008; Meerts et al., 2001; Van Boxtel et al., 2009). OH-BDEs increased basal calcium levels and ablated the ability for calcium depolarization in cells, potentially leading to neu- rodisruption (Dingemans et al., 2008; Kim et al., 2011). Similarly, alteration of protein transport and carbohydrate metabolism genes was identified in zebrafish liver cells following exposure to 6-OH-

BDE 47 (Van Boxtel et al., 2009). PBDEs have the potential to disrupt thyroid hormone homeostasis and disrupt neurogenesis; however, whether hydroxylation alters these or other biological processes further, remains unknown. Depending on the congener, hydroxylation of the PBDE may increase its structural similarity to thyroid hormones. It is unknown if hydroxylated PBDEs disrupt thyroid hormone homeo-stasis or other biological processes. BDE 47 has been a high priority congener due to its persistence and abundance in the environment. BDE 47 is often one of the most abundant congeners in environmental samples (Gewurtz et al., 2011; Law et al., 2006; Meeker et al., 2009). In our previous study, we evaluated the toxicity of BDE 47 along with five additional congeners with varying physical-chemical structures (Usenko et al., 2011). BDE 47 was found to increase rates of spontaneous movement, induce malformations (curved body axis), and increase mortality during zebrafish development (Usenko et al., 2011). Due to the environmental importance and toxicity of BDE 47, there is increasing interest in its hydroxylated metabolites. Hydroxylated PBDEs have been identified as metabolites of BDE 47 in multiple species, including rats, mice, and humans (Erratico et al., 2011; Hakk et al., 2010; Lupton et al., 2009; Stapleton et al., 2009).

In this study, three OH-PBDEs are evaluated and compared: 3-hydroxy-2,2′,4,4′-tetrabromodiphenyl ether (3-OH-BDE 47), 5-hydroxy-2,2′,4,4′-tetrabromodiphenyl ether (5-OH-BDE 47), and 3-hydroxy-2,2′,4,4′-tetrabromodiphenyl ether (6-OH-BDE 47). Two of these congeners, 5-OH-BDE 47 and 6-OH-BDE 47, have been identified as...
the most abundant hydroxylated PBDE congeners in human serum (Zota et al., 2011). The use of these three congeners allows for comparisons of the influence of structure (i.e. hydroxyl group location) on the biological response. Zebrafish were used in the present study to examine the developmental effects of exposure to OH-PBDEs and to explore their mechanisms of action.

**Methods**

**Fish husbandry.** Adult wild-type (Tropical 5D) zebrafish were obtained from stocks at Oregon State University, Corvallis, OR, USA. Adult fish were housed at 28 °C on a 16:8 light/dark photoperiod in a recirculating system according to standard zebrafish protocols and as previously described (Westerfield, 1995). Zebrafish were spawned in tanks and embryos collected within 2 hours of fertilization. All rearing and experimental procedures were conducted in accordance with IACUC regulations and approved protocols.

**PBDE exposure experiments.** OH-PBDE standards were purchased from AccuStandard (New Haven, CT) and were ≥99.0% purity (Fig. 1). Stock solutions were prepared by dissolving neat standards in dimethylsulfoxide (>90% DMSO) (Sigma, St. Louis, MO, USA). Exposure solutions were prepared via serial dilution from stock solutions with culture water with a 0.5% DMSO solvent vehicle for all concentrations. Solvent controls were used for all studies, and were no observed adverse effects at concentrations up to 0.5% DMSO.

To prevent uptake inhibition of OH-PBDEs, embryos were dechorionated using 0.5 mg/ml protease E (Sigma, St. Louis, MO, USA) at 6 hours post fertilization (hpf), prior to exposure (Mizell and Romig, 1997). Protease should not increase the uptake of OH-BDEs, however further studies are necessary to confirm this hypothesis. To determine the concentration-response of each PBDE congener, zebrafish embryos were exposed to six concentrations of each congener from 6 hpf until 168 hpf, for comparison to our previous study on PBDEs (Usenko et al., 2011). Exposure solutions were prepared based on nominal calculations for 20 mg/L, then serial diluted to make 10, 5, 2.5, 0.635, and 0.156 ppm. This concentration range is similar to other studies assessing toxicity and biological interactions of OH-PBDEs (Dingemans et al., 2008). Exposure solutions were added to wells of a 48-well plate, and five embryos were added to each well in 0.5 ml of exposure water. For all experiments, 12 replicates of each treatment were used. Exposures were housed in a light-controlled incubator on a 14:10 light/dark cycle at 28 °C. Embryos were evaluated daily for mortality, malformations, and developmental progression as previously described (Usenko et al., 2007; Usenko et al., 2011). Stage of development was determined according to standard zebrafish embryogenesis staging (Kimmel et al., 1995).

**Cell death assay using acridine orange.** In preliminary studies, we determined the optimal exposure window for the cell death assay begins at 24 hpf. Embryos were exposed to 1.25, 2.5, 5, 10, or 20 mg/L of 3-OH-BDE 47, 5-OH-BDE 47, or 6-OH-BDE 47 at 24 hpf for 4 hours. Controls (0.5% DMSO exposure) were used in all studies. Embryos were subsequently rinsed with water and incubated in 5 µg/L acridine orange (Sigma, St. Louis, MO, USA) for 1 hour in the dark. Embryos were rinsed and placed in 0.03% tricaine (Sigma, St. Louis, MO, USA) for 1 minute, mounted in methyl cellulose, and viewed under a 498 nm filter. A Carl Zeiss Axio Observer microscope was used for all fluorescence viewing, and AxioVision software was used for image analysis. For each treatment, 12 embryos were imaged.

**Caspase-3 immunohistochemistry.** In order to determine if the observed cell death was primarily due to necrosis or apoptosis, immunohistochemistry for caspase-3 was conducted. Caspase-3 is a member of the caspase family of proteins, which play a key role in the apoptotic process. Therefore, the embryos were stained for the active form of caspase-3 in order to suggest apoptosis may be occurring. Exposure protocols were similar to those used in the acridine orange assay. After four hours of exposure, embryos were fixed in 4% paraformaldehyde overnight at 4 °C and rinsed 3 times in phosphate buffer solution (PBS). Embryos were permeabilized in 1% trypsin for 10 min on ice and rinsed thoroughly with PBS with 10% tween (PBST). Embryos were then blocked with 10% normal goat serum (Sigma, St. Louis, MO, USA), then incubated in caspase-3 antibody originated in mouse (Sigma, St. Louis, MO, USA) for 4 hours at room temperature. The embryos were rinsed 4 times for 20 min in PBST and incubated in goat-anti-mouse Alex Fluor 488 secondary antibody (Sigma, St. Louis, MO, USA) at a concentration of 1:1000 overnight at 4 °C. Lastly, the embryos were rinsed in PBST 4 times for 20 min, mounted, and imaged under the 488 nm filter using a Carl Zeiss Axio Observer microscope.

![Image](44)


Fig. 1. Chemical structures of the three hydroxylated BDE 47 congeners tested in the present study: (A) 3-OH-BDE 47, (B) 5-OH-BDE 47, (C) 6-OH-BDE 47.
Axio Observer microscope. AxioVision software was used for image analysis.

**Oxidative stress assay.** Embryos were dechorionated at 24 hpf and exposed to 0, 1.25, 2.5, 5, 10, or 20 mg/L of 3-OH-BDE 47, 5-OH-BDE 47, or 6-OH-BDE 47 for 4 hours. A control of 0.5% DMSO was used to detect background or autofluorescence. This exposure window was used to compare with the acidine orange assay. Embryos were rinsed then incubated in 10 mM dichlorofluoresceine (DCFDA) (Sigma, St. Louis, MO, USA ma) for 30 minutes. DCFDA is a dye that fluoresces when cleaved by reactive oxygen species (ROS) and is commonly used in PBDE exposure experiments (Giordano et al., 2008; He et al., 2008). To restrict movement during imaging, embryos were placed in 0.03% tricaine for 1 minute. The embryos were rinsed with fish water, then mounted in methylcellulose and imaged under 488 nm filter using a Carl Zeiss Axio Observer microscope and AxioVision software was used for image analysis. For each treatment, 12 embryos were imaged. Fluorescence indicates ROS is interacting with DCFDA in that particular region.

**RNA isolation.** Embryos were exposed to 0.625 ppm 6-OH-BDE 47 or 0.5% DMSO for the control from 24 to 28 hpf. This concentration was selected because it did not delay development immediately following exposure, but was still expected to elicit a response in our preliminary studies. This exposure window was selected to correspond with the cell death and oxidative stress assays. At the end of the exposure period, embryos were euthanized with tricaine and pooled into groups of 30 with 5 replicates per treatment. RNA was extracted using Trizol reagent (Sigma, St. Louis, MO, USA) according to manufacturer's guidelines. RNA was quantified using a Nano Drop 1000 Spectrophotometer (Thermo Scientific, Wilmington, DE) and checked for purity based on the 260/280 and 260/230 ratios.

**Gene expression analysis.** Real time polymerase chain reaction (RT-PCR) was used to assess changes in gene expression between control and treated embryos. Primers were designed using Invitrogen’s Primer Design and were purchased from MWG Operon (Huntsville, Alabama). A list of the primer sequences used in this study is given in Table 1. A one-step PCR protocol was used according to manufacturer’s instructions using Trizol reagent (Sigma, St. Louis, MO, USA) according to manufacturer's guidelines. RNA was quantified using a Nano Drop 1000 Spectrophotometer (Thermo Scientific, Wilmington, DE) and checked for purity based on the 260/280 and 260/230 ratios.

**Table 1**

<table>
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<th>Gene</th>
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<th>Reverse Primer</th>
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Fig. 2. Daily assessments of mortality were cumulated for each of the congeners tested (A) BDE 47, (B) 3-OH-BDE 47, (C) 5-OH-BDE 47, and (D) 6-OH-BDE 47. Embryos were exposed at 6 hpf to a concentration gradient of each congener and analyzed daily until 168 hpf. Significance noted by (*) indicating p<0.05 and is compared to control exposures.
for the Rotor-Gene SYBR Green RT-PCR kit on a Rotor-Gene Q (Qiagen, Valencia, CA). For each reaction, 50 ng of RNA was reverse transcribed at 55 °C for 10 min followed by a denaturing step at 95 °C for 5 min. Two-step cycling of alternating 95 °C for 5 s and 60 °C for 10 s was performed 40 times. Melt curve analysis was conducted for all samples after the PCR was completed. Each sample was normalized to glyceraldehyde-3-phosphate dehydrogenase (GAPDH) expression for that particular sample, and the fold change was calculated against the expression of control samples.

Statistical analysis. The average percent mortality or malformation was taken for each well and significance was determined using Fisher’s Exact Test compared to controls (N = 12). Developmental progression rates were determined by taking the average of the five embryos per well, and significance was determined using a one-way analysis of variance (ANOVA). Significance of gene expression data was determined using a Student’s T-Test, p < 0.05 as compared to gene expression in control embryos. All statistical analyses were performed using SigmaPlot (Systat Software, Inc., San Jose, CA).

Results

There was concentration-dependent induction of mortality for all three congeners (Fig. 2). Again, the embryos were most sensitive to 6-OH-BDE-47, followed by 5-OH-BDE-47, which was more toxic than 3-OH-BDE-47. The lethal concentration for which 50% of the animals died (LC50) was calculated using SigmaPlot and plotting the %mortality for each concentration. A best fit line was used to determine the approximate LC50 ($r^2 = 0.96–0.99$). 3-OH-BDE 47 had an LC50 of 3.8 ppm, 5-OH-BDE 47 was 3.05 ppm, and 6-OH-BDE 47 was 0.96 ppm. Mortality was statistically significant at 48 hpf for all OH-BDE 47 congeners, and the rates continued to increase at 72 hpf.

These results differed from BDE 47, where mortality was not observed until 144 hpf (Fig. 2A).

Malformations were also recorded daily. BDE 47 induced a curved body malformation, which was observed starting at 120 hpf (Fig. 3A). The hydroxylated BDE 47 congeners, however, induced fin malformations and pericardial edema, which were observed beginning at 48 and 120 hpf, respectively. 3-OH-BDE 47 induced fin malformations at 5, 10, and 20 ppm exposure concentrations (Fig. 3B). Fin malformations were only observed at in embryos exposed to 5 ppm 5-OH-BDE 47, and was only in the surviving few embryos at this concentration (Fig. 3C). 6-OH-BDE 47 induced fin malformations at 0.625 ppm, due to the 100% mortality at higher concentrations (Fig. 3D). Malformations were not recorded for higher concentrations due to mortality prior to onset of the malformation.

Pericardial edema was also observed in several of the exposures, but not all. Pericardial edema was not observed in BDE 47-exposed embryos at any concentration (Fig. 4A). All pericardial edema was observed starting at 96 hpf. In 3-OH-BDE 47 exposed embryos, pericardial edema was significant at concentrations of 0.625 ppm and greater (Fig. 4B). 3-OH-BDE 47 induced pericardial edema in a concentration-dependent manner. There was a slight increase in incidence of pericardial edema in 5-OH-BDE 47-exposed embryos at 0.625 and 2.5 ppm, but these are very low rates (although statistically significant) of pericardial edema (Fig. 4C). Finally, 6-OH-BDE 47 also induced pericardial edema in embryos exposed to 0.625 ppm (Fig. 4D). Again, no pericardial edema was observed at higher concentrations due to mortality.

At 24 hpf, all three congeners induced developmental arrest in a concentration-dependent manner (Fig. 5). Developmental arrest is a state at which the embryo remains intact, but does not continue to develop. At 24 hpf, 20 ppm 3-OH-BDE 47-exposed embryos had entered developmental arrest at the 18 hpf stage and never progressed...
Development was not delayed in any other 3-OH-BDE 47 exposure treatment at 24 hpf. At 48 hpf, the 20 ppm-exposed embryos were still developmentally at the 18 hpf stage. Furthermore, embryos exposed to 10 ppm 3-OH-BDE 47 had entered developmental arrest at the 30 hpf stage. No other concentration of 3-OH-BDE 47 induced a change in developmental progression over the course of the study.

5-OH-BDE 47 induced developmental arrest at lower concentrations than 3-OH-BDE 47. At 24 hpf, embryos exposed to 5 ppm and higher were developmentally delayed at stages that are statistically significant (17 hpf and lower). Embryos continued to develop slightly between 24 and 48 hpf, however, embryos exposed to 5 ppm had only progressed from the 17 hpf stage to the 25 hpf stage at 48 hpf (Fig. 5).

6-OH-BDE 47 had the greatest delay in developmental progression, with embryos exposed to 1.25 ppm and greater concentrations delayed at 24 hpf (Fig. 5A). At 24 hpf, embryos exposed to 20 ppm had 100% mortality by 24 hpf. Embryos exposed to 10 ppm did not continue to develop from the time of exposure until the assessment 18 hours later. Furthermore, at 48 hpf, the embryos were dead. Embryos exposed to 2.5 and 5 ppm 6-OH-BDE were severely delayed at 24 hpf (14 and 9 hpf stage, respectively) (Fig. 5A); however they did continue to develop, albeit at a delayed rate (17 and 15 hpf stage, respectfully at 48 hpf) (Fig. 5B).

**Cell death**

In order to look at effects at the cellular level, two assays were used: acridine orange (total cell death), and caspase-3 (apoptosis). Due to the fragile state of the embryos through the gastrula stage, exposures were conducted from 24 to 28 hpf. No background cell death was detected in controls (data not shown). Acridine orange staining revealed cell death throughout the body to the tip of the caudal fin (Fig. 4). Cell death may also be occurring at lower concentrations, however our system does not allow for quantification of measurements, therefore only concentrations of 2.5 ppm were used. Generally, fluorescence was observed from the caudal fin along the body with increasing fluorescence noted with increasing concentration of OH-PBDE congeners. Both 5-OH-BDE 47 and 6-OH-BDE 47 induced cell death at concentrations of 2.5 ppm, increasing from the fin to body region (Fig. 6). Alternatively, 3-OH-BDE 47 only induced cell death at 10 ppm (Fig. 6). Lower concentrations were not shown due to the lack of an image (see 2.5 ppm 3-OH-BDE 47 for an example in Fig. 6).

In addition, immunohistochemistry was performed with a monoclonal caspase-3 antibody, as previously described (Eimon et al., 2006). In the present study, there was not an observed increase in fluorescence between exposed and control embryos at any concentration (data not shown).

**Oxidative stress**

DCFDA was used to identify cells in vivo that are generating reactive oxygen species. DCFDA is first deacetylated by endogenous esterases to dichlorofluorescein (DCFH), which can react with several ROS to form the fluorophore DCF. There is a band of fluorescence along the body axis of exposed fish in proximity to the cells undergoing cell death (as measured by acridine orange). There was no observed fluorescence in any of the controls. A burst of reactive oxygen species was located in the center of the zebrafish body in the 10 ppm exposures for 5-OH-BDE 47 and 6-OH-BDE 47 (Fig. 7).
correlated to the top region of where the cell death was occurring in the acridine orange assay at 10 ppm (Fig. 6). There was no observed fluorescence in any of the controls.

Alterations in gene expression

Two genes commonly associated with oxidative stress, GST and GCLc, were not upregulated (Fig. 8). In fact, GST was significantly downregulated following treatment ($p = 0.003$). Cytochrome c oxidase (COX6a), a gene involved in oxidative phosphorylation, was not significantly upregulated compared to controls (Fig. 8). Heat shock protein 70 (HSP70), a gene involved in stress response was upregulated 55 fold compared to controls ($p = 0.007$) (Fig. 8).

All genes involved in thyroid signaling and function were upregulated when compared to controls. Dio2 was upregulated 18-fold while Dio1 was upregulated 7 fold ($p = 0.05$). Slc5r, a gene encoding the sodium-iodide symporter, was upregulated 8.5-fold ($p = 0.034$). The cholinergic receptor neuronal nicotinic alpha (ChRNA2) gene was upregulated nearly 27 fold compared to controls ($p < 0.001$). Cholinergic and thyroid-related genes were significantly upregulated following exposure to 6-OH-BDE-47.

To further investigate the potential for apoptosis following exposures, caspase-3 gene expression was examined. There was no change in caspase-3 gene expression between controls and embryos treated with 0.625 ppm 6-OH-BDE-47. Previously, cell death was observed at 5 ppm in the acridine orange study, but 0.625 ppm 6-OH-BDE-47 did not significantly increase expression of an apoptosis-related gene (Fig. 8).

Discussion

In the present study, OH-BDE-47 was more toxic to zebrafish when compared to our previous study of BDE-47 and elicited different types of responses than BDE-47 (Usenko et al., 2011). In our previous study, BDE-47 was one of the most potent PBDE congeners tested (out of seven) (Usenko et al., 2011); however, in this study all three hydroxylated PBDE-47 congeners demonstrated significantly more toxicity than the parent congener. When exposed, all three OH-BDE-47 congeners delayed development, induced mortality, and caused cell death and oxidative stress. These endpoints were not previously observed following zebrafish exposure to non-hydroxylated PBDEs, although oxidative stress and cell death have been reported in cell culture (He et al., 2008; Usenko et al., 2011). Additionally, mortality was not observed in BDE-47 exposures until 120 hpf, yet all three OH-BDE-47 exposures induced mortality within 72 hpf.

The location of the hydroxylation of BDE-47 greatly altered exposure outcome, with the greatest effects observed in the ortho-position (6-OH-BDE-47), followed by para- (5-OH BDE-47), and the least was meta (3-OH-BDE-47). Studies have found that the ortho-substituted congeners are primarily produced in nature by some algal species (Malmvam et al., 2008; Marsh et al., 2004), therefore the more potent congener might be released into the environment at a greater rate. In one study, congeners with para- substitutions, were found to be more abundant in human serum (Athanasiadou et al., 2008); although in another study, all hydroxylated metabolites had a hydroxyl group in the ortho-position (Yu et al., 2010). In rat liver microsomes treated with BDE-47, 4′-OH-BDE-49 and 3-OH-BDE-47 were the most abundant metabolites, also supporting para-substitutions, and CYP2A2 and CYP3A1 were the most active CYP enzymes tested (Erratico et al., 2011). Furthermore, the sum of OH-PBDEs in mink, hydroxylated metabolites comprised 38–32% of the excreted fraction of PBDEs following exposure to DE-71 (penta-mixture), demonstrating hydroxylation as a pathway for elimination of PBDEs (Zhang et al., 2008). Currently, more information regarding the metabolic and environmental transformation of PBDEs to OH-PBDEs is needed, and is an area of increasing interest and research.
uncertain. While delayed development is a generic response to chemical exposure in zebrafish, arrest occurring early in development is not a common result. Previous studies observed developmental arrest as the result of oxidative stress, disruption of cyclooxygenase activity (FitzGerald et al., 2002), or disruption of cell adhesion (i.e. e-cadherin) (Kane et al., 2005). In an anoxia-based study, embryos that had entered developmental arrest could be revived when subsequently placed in optimal conditions (Padilla and Roth, 2001); however, that was not the case with the hydroxylated PBDEs. When exposed to OH-BDE's developmental arrest could not be reversed even when rinsed and placed in optimal conditions (data not shown). This is possibly due to the bioaccumulative nature of these compounds or to an irreversible interaction with their biological targets. Similar effects were observed when zebrafish embryos were exposed to the drug, bromo-methoxy zidovudine (Uckun et al., 2005), which possess some structural similarities to hydroxylated PBDEs. Chemical-induced developmental arrest is an interesting endpoint for further study to determine the mechanism(s) of action leading to this adverse developmental outcome.

Oxidative stress is a common mode of action for many chemicals and diseases. To our knowledge, this is the first time DCFDA has been used in vivo. In this study, at concentrations from 2.5 ppm to 10 ppm, there was evidence of oxidative stress corresponding to regions of the body undergoing cell death as identified in the acridine orange assay. At the lower concentrations (i.e. 0.625 ppm), there was no evidence of oxidative stress, either through the DCFDA assay or through alterations in antioxidant response gene expression. These results are in agreement with a study involving 2-OH-

**Fig. 6.** Cell death was detected using acridine orange after exposure from 24 to 28 hpf. Representative images are shown from each exposure treatment with increasing exposures from left to right (2.5, 5, 10 ppm). (A-C) 3-OH-BDE 47, (D-F) 5-OH-BDE 47, (G-I) 6-OH-BDE 47. Fluorescence (white) indicates cells undergoing cell death. No fluorescence was detected in controls, therefore images not shown.

**Fig. 7.** Oxidative stress was detected using dichlorofluorescein diacetate (DCFDA) to indicate the presence of reactive oxygen species. Representative images are shown across the concentration range (2.5, 5.0, and 10 ppm) from left to right, with (A–C) 3-OH-BDE 47, (D–F) 5-OH-BDE 47, (G–I) 6-OH-BDE 47. No fluorescence was detected in controls, therefore images not shown.
BDE 47 and 2-OH-BDE 85, neither of which altered expression of oxidative stress related genes (Song et al., 2009).

Additionally, 6-OH-BDE 47 was found to uncouple of oxidative phosphorylation and inhibit the electron transport in zebrafish cells (Van Boxtel et al., 2009). This disruption of ATP synthesis could be partially responsible for the developmental arrest in zebrafish embryos observed in the present study. HSP90 is involved in the protection of proteins until they can be repaired, while HSP70 is involved in the removal of proteins that are damaged beyond repair (Proctor and Lorimer, 2011). Essentially, they act in opposing manners which is supported in the data. These results demonstrate that while standard oxidative stress genes were not upregulated at 0.625 ppm, the organisms were undergoing stress within a few hours of exposure.

All of the genes associated with thyroid function assessed in this study were upregulated. Thyroid hormones have similar chemical structures to some hydroxylated PBDEs: both are diphenyl ethers; however PBDEs have bromines rather than iodine atoms and thyroid hormones have an additional functional group. While further investigation is needed, this study demonstrates that 6-OH-BDE 47 disrupts normal thyroid hormone gene transcription during development. Thyroid hormones play an essential role during development, including the development of the cholinergic system.

A recent study found a positive correlation between human serum concentrations of OH-BDE and thyroid stimulating hormone (Zota et al., 2011). There was, however, no correlation between OH-BDE and thyroxine concentrations (Zota et al., 2011). Furthermore, structural analysis has found that 5-OH-BDE 47 and 6-OH-BDE 47 readily bind to transthyretin and thyroxine-binding globulin (Guo et al., 2010).

While uptake was not determined in this study, in our previous studies we determined that the water–octanol partitioning coefficient (log $K_{ow}$) was strongly correlated with toxicity of PBDEs (Usenko et al., 2011). If hydroxylated PBDEs follow similar uptake based on the calculated log $K_{ow}$ (log $P$; 6.13), it is likely that hydroxylated PBDEs are highly bioavailable (Wu et al., 2008). The potentially high internal dose could also explain the potency of the compounds, however it is not expected that one OH-BDE 47 congener would be preferentially bioavailable over another based on the physical-chemical properties.

Although concentrations used in this study are higher than what would be expected in the environment, the potential for maternal-fetal transfer increases concerns of exposure, even at low concentrations. Stapleton et al. reported that 6-OH-BDE 47 was detected in >67% of blood samples from pregnant women in the US (Stapleton et al., 2011). Human concentrations of hydroxylated PBDEs vary greatly, with some reports of $\sum$ OH/PBDE ranging from 2.9% to 10% (Athanasiadou et al., 2008; Lacorte and Ikonomou, 2009). Human exposure, however, is occurring and increasing concentrations are being reported. Reports of concentrations in other environmental media (i.e. water and sediment) are currently limited. Water concentrations were reported to be 0.63–1.0 pg/L of 5-OH-BDE 47 in China (Chang et al., 2012).

Primarily, organisms are exposed to hydroxylated PBDEs through metabolism of parent PBDEs. The increased toxicity of these congeners compared to the parent PBDEs raises concern about the risk of exposure, particularly during development. There is a great need to gather more information regarding blood and breast milk concentrations, routes and sources of exposure. While exposure is already occurring worldwide, it may be possible to mitigate or reduce the release or production of these chemicals, and there is little environmental concentration information currently available.

Currently, there is little information regarding the differential biological interaction of PBDEs compared to hydroxylated PBDEs. This study found that the location of the hydroxyl group greatly influences the toxicity of the compound. Our previous study found lower brominated congeners to elicit effects at the lowest concentrations; however Song et al. found that 2-OH-BDE 85 induced toxicity at lower concentrations than BDE 47 making toxicity of these hydroxylated congeners a significant concern (Song et al., 2009; Usenko et al., 2011). Further investigations are necessary to understand the full structure–activity relationships between numbers and locations of bromines as well as hydroxylation patterns that affect the toxicity of these compounds.

Conflict of interest statement

The authors declare that there are no conflicts of interest.

Acknowledgments

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