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Persisting Effects of a PBDE Metabolite, 6-OH-BDE-47, on Larval and Juvenile Zebrafish Swimming Behavior

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Abstract

Polybrominated diphenyl ethers (PBDEs) are persistent organic pollutants that are widely detected in the environment, biota, and humans. In mammals, PBDEs can be oxidatively metabolized to form hydroxylated polybrominated diphenyl ethers (OH-BDEs). While studies have examined behavioral deficits or alterations induced by exposure to PBDEs in both rodents and fish, no study to date has explored behavioral effects from exposure to OH-BDEs, which have been shown to have greater endocrine disrupting potential compared to PBDEs. In the present study, zebrafish (*Danio rerio*) were exposed during embryonic and larval development (0-6 days post fertilization, dpf) to a PBDE metabolite, 6-hydroxy, 2,2',4,4' tetrabromodiphenyl ether (10-50 nM) and then examined for short and long-term behavioral effects. Exposed zebrafish tested as larvae (6 dpf) showed an altered swimming response to light-dark transitions, exhibiting hypoactivity in light periods compared to control fish. When fish exposed from 0-6 dpf were tested as juveniles (45 dpf), they showed an increased fear response and hyperactivity in response to tests of novel environment exploration and habituation learning. These results demonstrate that early life exposure to a PBDE metabolite can have immediate or later life (more than a month after exposure) effects on activity levels, habituation, and fear/anxiety.

Keywords

PBDE; neurotoxicity; zebrafish; OH-BDE; metabolite; behavior

1 Introduction

Flame retardants are added to many consumer products, including electronics, plastics, baby products, and polyurethane foam used in upholstered furniture, to meet flammability standards (Alaee et al., 2003; Shaw et al., 2010). Some of the most widely used flame retardants in consumer products were the polybrominated diphenyl ethers (PBDEs) (Allen et

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al., 2008; Stapleton et al., 2005). Studies now demonstrate that PBDEs can escape from products over time and are ubiquitously detected in home environments, where young children can be exposed at levels higher than adults (Lorber, 2008). For example, PBDE serum levels in a toddler cohort in the US were significantly associated with PBDE levels measured in house dust and with residue levels measured on their hands, suggesting a role for hand to mouth contact (Stapleton et al., 2012). While PBDEs have been phased out from use in newly manufactured products sold in the United States starting in 2005 (PentaBDE in 2005, DecaBDE in 2013), products containing PBDEs are still widely present in homes and landfills, and contamination is expected to continue for decades (de Wit, 2002)

The metabolic products of PBDEs are also important to consider because some PBDE metabolites have been shown to be more toxic than the parent compounds in some bioassays (Dingemans et al., 2011, 2008; Kim et al., 2011). In mammals, PBDEs are oxidatively metabolized by cytochrome p450 enzymes to form OH-BDEs (Erratico et al., 2013; Feo et al., 2013). Interestingly, there are also some natural sources of brominated phenolic compounds (Figure 1), including methoxylated-BDEs (Me-O-BDE) and OH-BDEs that originate from marine algae and sponges (Kelly et al., 2008; Wan et al., 2009). Of note, naturally derived OH-BDEs tend to have the hydroxyl group in the ortho position relative to the diphenyl ether bond (Wan et al., 2009). There is also some mixed evidence to suggest that fish can interconvert Me-O-BDEs to OH-BDEs (Liu et al., 2012; Wan et al., 2010; Wen et al., 2015). PBDEs, Me-O-BDEs, and OH-BDEs have been measured in wildlife and humans, suggesting widespread exposure to these compounds (Table 1) (Chen et al., 2013; Fujii et al., 2012; Wiseman et al., 2011). The present study examined 6-OH-BDE-47, which has both natural and anthropogenic sources (Qiu et al., 2009, 2007; Wan et al., 2009). These multiple sources of 6-OH-BDE-47 indicate that there could be increased environmental exposures (Wan et al., 2009).

Studies in fish indicate that early life exposures to PBDEs/OH-BDEs can cause developmental toxicity (Lema et al., 2008; Usenko et al., 2012, 2011; van Boxtel et al., 2008). Studies examining PBDE exposure in rodents have also found neurodevelopmental and neurobehavioral effects (Costa and Giordano, 2007; Eriksson et al., 2001; Ta et al., 2011). For example, male mice exposed neonatally to a single dose of BDE-47 (10.5 mg/kg bw) showed decreased spontaneous activity (locomotion, rearing, total activity) 2 and 4 months after exposure (Eriksson et al., 2001). Epidemiological studies in humans have also observed significant positive associations in maternal PBDE exposure during pregnancy with deficits in motor behavior and cognitive function in children (Herbstman et al. 2010; Herbstman and Mall 2014). In the US, PBDE concentrations in cord blood have also been associated with neurodevelopmental effects (lower scores on tests of mental and physical development at ages 12-48 months and 72 months) in children (Herbstman et al., 2010). Therefore, studying the effects of these compounds on the lifetime development of neurobehavioral function is important.

Recent research has shown that metabolism of PBDEs to OH-BDEs increases their neurotoxic potential (Dingemans et al., 2011, 2008). In a series of studies, OH-BDEs (specifically 6-OH-BDE-47) were shown to disturb calcium homeostasis and neurotransmitter release in PC-12 cells. These effects were more severe than the effects

caused by the parent compound, BDE-47 (Dingemans et al., 2010a, 2010b, 2008). Furthermore, 6-OH-BDE-47 exposure during zebrafish larval development has shown thyroid hormone disruption (~1.3 μ M) and disrupted energy metabolism (oxidative phosphorylation) (~1 μ M) (Legradi et al., 2014; Liu et al., 2015; Usenko et al., 2012; van Boxtel et al., 2008). Adult neurogenesis is also a target of 6-OH-BDE-47 exposure, including effects on neuronal/oligodendrocyte differentiation in primary adult neural stem progenitor cells of adult mice (Li et al., 2013). To our knowledge, no studies have specifically examined effects of OH-BDEs on behavior.

In the present study, we performed acute exposures with 6-OH-BDE-47 to determine if a low-level (10-50 nM) early life exposure resulted in deficits in behavior immediately and later in life. Zebrafish are an appropriate model for neurotoxicology, including behavioral assays, due to their rapid and conserved development with other higher vertebrates (Bailey et al., 2013; Hill et al., 2005; Linney et al., 2004). During early development zebrafish can engage in relatively complex behaviors (e.g. swimming, exploration and prey capture) which make them an excellent candidate for high-throughput functional analyses (MacPhail et al., 2009; Padilla et al., 2011). Adult zebrafish engage in a wide range of behavior that includes the capacity for exploration and avoidance, associative and operant conditioning, social affiliation and more complex cognitive function (Bailey et al., 2013; Gerlai et al., 2000; Levin et al., 2007). Here we emphasize several domains of behavior in zebrafish using two well-documented tasks, novel tank-exploration and startle habituation (Bailey et al., 2013; Levin, 2011). Adult zebrafish also show similar chemically-induced behavioral changes to those replicated in rodent studies, validating their utility as a complex vertebrate model that could potentially serve as a useful intermediate model between *in vitro* cell-based studies to traditional rodent assays (Bencan et al., 2009; Eddins et al., 2010).

The primary objective of this study was to determine the persisting functional impact of early life exposure (4-hpf to 144-hpf) to low doses of 6-OH-BDE-47 (10-50 nM), a known neurotoxicant, during two distinct ages, the larval and juvenile stages in zebrafish. BDE-47 has been evaluated in zebrafish studies previously (Chen et al., 2012; Chou et al., 2010; Huang et al., 2015; Truong et al., 2014; Usenko et al., 2011), but 6-OH-BDE-47 has not been previously evaluated and was selected for further study because environmental exposures are likely.

2 Materials & Methods

2.1. Fish Husbandry

Adult wild-type (Tropical 5D) zebrafish (founder fish were from a population in the laboratory of Dr. David Volz at the University of South Carolina) were housed in a recirculating AHAB system (Aquatic Habitats, Apopka, FL, USA) according to standard protocols at 28°C on a 14:10 light/dark photoperiod. Adults were fed twice daily with hatched artemia and Ziegler's Adult Zebrafish Complete Diet (Aquatic Ecosystems, Apopka, FL, USA). Embryos were collected within one hour of fertilization after spawning of adult zebrafish in breeder tanks. Embryos were maintained in embryo media (5 mM NaCl, 0.17 mM KCl, 0.33 mM CaCl₂, 0.33 mM MgSO₄) within incubators under the same conditions as adults. Beginning on 6dpf, larvae were fed 3-4 times daily with Ziegler Larval

Diet (<50 microns), switching to larger particle size diets as the fish grew according to the manufacturer's recommendation (<100 microns, 100-150 microns, 150-250 microns, and 250-450 microns). Beginning at 7dpf, all larvae also received hatched artemia once a day. All fish care and experimental procedures were approved by the Duke University Institutional Animal Care & Use Committee.

2.2. Chemicals

6-hydroxy-2,2',4,4' tetrabromodiphenyl ether (6-OH-BDE-47) was purchased neat from Accustandard (New Haven, CT, USA) and was > 99.5% purity. Chlorpyrifos (CPF) was purchased from Chem Service >98.8% purity (West Chester, PA, USA). Chlorpyrifos (CPF) was selected as a positive behavioral control due to its established neurotoxicity in fish models (Aldridge et al., 2005; Eddins et al., 2010; Yen et al., 2011). Stock solutions were prepared by dissolving an appropriate amount of neat standard in DMSO and were assessed for purity prior to dosing using LC-MS-MS using published methods (Butt et al., 2011). Exposure solutions were prepared via serial dilution in glass amber vials from stock solutions with embryo media water with <0.1% DMSO solvent vehicle.

2.3 Larval Zebrafish Exposures and Assessment

2.3.1 Larval Exposures—Viable zebrafish embryos were staged according to standard zebrafish embryogenesis development (Kimmel et al., 1995). Embryos (with intact chorions) were placed in a 96-well plate (1 embryo/well insert) (Laboratory Supply Distributors, Millville, NJ, USA). The 0.5 mL glass inserts were baked prior to use at 450°C for 4 hours to reduce possible contamination. Embryos were dosed between 4-5 hours post fertilization (hpf; mid to late blastula period, sphere to 30% epiboly stage) by adding 1 µL of a concentrated stock solution to 0.25 mL embryo media using a multichannel pipettor to reach the desired concentration (10 nM or 50 nM 6-OH-BDE-47, 1 µM CPF, 0.004% DMSO, or embryo medium). Stock solutions were prepared in a 96-well dosing plate as described in Dishaw et al. (2014) to facilitate rapid, repeatable dosing. Testing concentrations were selected from range finding studies, and from previous studies that showed no morphological effects at these concentrations (Dishaw et al., 2014; Usenko et al., 2012). Each plate contained multiple doses of the chemical of interest, as well as a positive behavioral control (CPF), a vehicle control (DMSO) and a control receiving only embryo media (n=18-20 fish/dose/plate, 5 doses/plate, 2 plates). Embryos received a static exposure due to difficulties in removing media in the glass wells without damaging embryos. Embryos were evaluated on a daily basis for survival, abnormalities, and hatching success. Embryos were exposed from 4-hpf to 144-hpf, congruent with previous zebrafish behavioral studies (Dishaw et al., 2014). On morning of testing, plates were transferred from incubators to a light tight drawer, until testing between 13:00 and 16:00 h, the diurnal period with stable activity. Following testing, embryos were evaluated for malformations such as head, jaw, craniofacial, fin, spinal abnormalities, swim bladder inflation, and edema of the pericardial or abdominal regions. Embryos exhibiting malformations were excluded from analysis (~2-4 larvae were excluded from analyses per treatment due to morphological effects).

2.3.2 Larval Swimming Activity Behavior Assay—To study the effects of developmental exposures on behavior, larval swimming activity (total swimming distance, mm/min) during alternating periods of light and dark conditions was measured using a Daniovision observation chamber (Noldus Information and Technology, Wageningen, The Netherlands). The Daniovision chamber uses a 96-well plate holder with bottom illumination (LED display) and video recording (top-mounted firewire camera with infrared capability) and real-time tracking was controlled using EthoVision software (Noldus Image Analysis program Wageningen, The Netherlands). Ethovision tracking software was set to use a dynamic subtraction detection method to identify pixels darker than a pre-captured background reference image, using a pre-set minimum and maximum object size for detection

Zebrafish larvae are more active during the dark phases, presumably due to reduced predator threat, and less active during light phases (MacPhail et al., 2009). An experimental protocol was modified from existing literature (Padilla et al., 2011), and consisted of a 10 minute habituation period (dark), followed by alternating light (5400 lux) and dark (<1 lux) periods (10 minutes, 2 repetitions). Swimming activity during the baseline habituation period and the two alternating periods of light and dark was monitored for signs of hyper or hypoactivity. Data represent combined results from 2 different cohorts (n= 34-40 fish/treatment).

2.4 Larval zebrafish exposure and Juvenile assessment

2.4.1 Juvenile Zebrafish Exposed as Larvae—To characterize longitudinal effects of OH-BDE exposure, larval zebrafish received a static exposure to either 10 nM or 50 nM 6-OH-BDE-47, 0.05% DMSO, or 1 µM Chlorpyrifos, from 4-hpf to 6-dpf (same exposure period as larvae). Thirty viable embryos were placed in 15 mL of dosed embryo media within glass Petri dishes (Pyrex, 100mm by 20 mm) in an incubator. The experiment was repeated in duplicate (60 embryos/dose). At 6 dpf, embryos were removed from exposure solution using stainless steel tea strainers, rinsed in triplicate, and placed in 3-L aquaria on a circulating AHAB system containing fish water. Fish were raised in aquaria until 45-50 dpf, when they underwent behavioral testing (n=34-46 fish/treatment). The 45-dpf time point was the earliest age when fish had achieved sufficient size to be reliably tracked by the Noldus Image Analysis program EthoVision (Wageningen, The Netherlands) and was selected as the desired age. On the day of testing, fish were run on behavioral assays between the hours of 11:00 and 16:00, with the evening feeding withheld until the end of testing. The novel tank diving test was performed first, and 24-hours later, the startle-habituation test was performed. After testing, fish were euthanized using MS-222 (300-mg/L). Mortality across treatments ranged from 15%-30%, in accordance with some natural loss of larvae during development.

2.4.2 Juvenile Behavior Assay, Novel Tank Diving Test—The novel tank diving task has been described previously in detail (Levin et al., 2007; (Bailey et al., 2013; Bencan et al., 2009). This task measures the species-specific swimming pattern of initial bottom dwelling following introduction to a new environment with later exploration of higher levels of the water column. The task is designed to measure predator avoidance behavior (bottom

dwelling), similar to measures of thigmotaxis in rodent models (Levin, 2011; Levin et al., 2007). Here, the initial dive is characterized as predator avoidance activity, and the later fuller exploration of the tank is thought to be driven by food seeking. Deviation from this dive/explore pattern is considered a maladaptive response. Two measures of behavior during this test are reported: location from tank floor (cm) and swimming distance (cm), a measure of motoric function in this exploration task. Zebrafish are placed in a 1.5-liter plastic tank filled with approximately 1,350-mL of home tank water from the AHAB system. Tanks are positioned so that a Sony HD HandyCam can be used to record the images for tracking using Noldus Image Analysis program EthoVision (Wageningen, The Netherlands).

Zebrafish were individually (n=34-46 fish/treatment) tested in 1.5-liter plastic tanks filled with 1350-mL of tank water, the surface of the water was approximately 10 cm from the tank floor. The trapezoid shaped tanks were 22.9 cm along the bottom and 27.9 cm along the top. The diagonal side of the tank was 15.9 cm and the opposite vertical side was 15.2 cm, the same design as used previously (Levin et al., 2007). Behavior was tracked in real-time using EthoVision tracking software, which calculated distance from the tank floor and total distance traveled. Each trial was five-minutes in duration and commenced immediately after a fish was placed in the tank. The video signal was transmitted through a Sony camcorder that was positioned approximately 90 cm away.

2.4.3. Juvenile Behavior Assay, Startle Habituation Tap Test—One day after completion of the dive test, animals were tested for sensorimotor function and habituation learning using a startle test as previously described (Bailey et al., 2013; Eddins et al., 2010; Sledge et al., 2011). The startle test apparatus consisted of a flat white 20.4 cm by 38.1 cm surface with white 12.7 cm by 15.2 cm frontal and rear blocking barriers attached. On the flat surface there were arranged eight 5.1 cm by 7.6 cm clear cylindrical arenas made of Plexiglas in a 2 by 4 array. The apparatus was positioned between two white opaque barriers, which faced each other and projected a bare white screen. Each arena was attached to solenoids which delivered a mechanical tap to the bottom of the swimming chamber at specified intervals throughout a trial. A Sony camcorder was located approximately 71 cm above the apparatus. The solenoids were used to administer taps to the bottom of the cylindrical arena under control of the computer. Eight zebrafish were taken from the holding tanks and each one was placed into a cylindrical arena on the tap apparatus. Following a 10 minute acclimation period, the solenoids tapped the testing arena at 1 minute intervals for 10 consecutive trials. EthoVision software was used to calculate total distance traveled 5 seconds before and 5 seconds after delivery of the tap stimulus.

2.5. Statistical Analysis

Superanova (SAS, Cary, NC, USA) software was used for statistical analysis. Graphpad Prism v6 (San Diego, CA, USA) was used for creation of graphs. For all behavioral tests, no significant effects were observed between cohorts of animals, so data were combined. All data were log transformed prior to analysis. The Type 1 error rate (α) was set at 0.05 for all omnibus tests and Dunnett's post hoc comparisons.

For the larval swimming activity assay, mean distance traveled for each minute was calculated. For the novel tank diving test, the mean distance from tank floor and mean distance traveled (cm) was calculated for each minute of the task. For the tap test, the mean distance traveled for the 5 sec before and after each stimulus delivery was calculated. Repeated measures analysis of variance (RMANOVA) was used to assess statistical significance with a Dunnett's post hoc test to determine differences between control and each of the exposure groups.

3. Results

3.1. Developmental Toxicity

The doses selected for the behavioral exposures were below known overt toxicity thresholds (spine/fin abnormalities, edema, craniofacial malformations) in a previous study (LC_{50} value $\sim 1.9 \mu\text{M}$, malformations $\sim 1.3 \mu\text{M}$) (Usenko et al., 2012). However, to be cautious, any embryos that exhibited malformations were not included in the behavioral analysis so as to not conflate neurobehavioral abnormalities with morphological effects. There were no statistical differences between survival and malformation rates among treatment groups (data not shown).

3.2. Larval Locomotor Behavior

In the Daniovision locomotor activity assay, the main effect of light condition was significant ($F(1, 151) = 146.40$, $p < 0.0001$) with higher activity during the dark phases, as is typically observed for zebrafish in this test. The dark periods were associated with an increase in larval swimming activity, which gradually decreased over each dark period (Figure 2). This described pattern of behavior is characteristic of zebrafish larvae. There was a significant main effect of exposure ($F(3, 151) = 6.87$, $p < 0.0005$). There was also a significant exposure x light condition interaction ($F(3, 151) = 8.47$, $p < 0.0005$). Tests of the simple main effects of exposure condition in each lighting condition showed that during the light condition all three exposure conditions caused significant ($p < 0.01$) reductions in activity. Dunnett's post hoc tests showed that the $1 \mu\text{M}$ CPF (positive control) and the 50 nM 6-OH-BDE-47 exposure conditions caused significant ($p < 0.01$) decreases in locomotor activity during the light phases. In contrast, during the dark condition only the CPF exposure caused significant hypoactivity ($p < 0.01$). A comparable effect was seen during the acclimation dark period prior to the onset of the behavioral trial.

3.3. Juvenile Novel Dive Test

RMANOVA revealed a main effect of treatment ($F(3, 168) = 4.92$, $p < 0.005$) on distance to the floor with 10 nM or 50 nM 6-OH-BDE-47 exposed animals remaining closer to the floor than control animals (Figure 3A). Dunnett's post hoc tests showed that both the 10 nM ($p < 0.05$) and the 50 nM ($p < 0.01$) exposures caused the fish to dwell significantly closer to the bottom of the tank, which is indicative of an increased fear or anxiety response (Figure 3A) ($F(3, 168) = 4.92$, $p < 0.005$). Exposure to CPF did not cause any significant change on this measure. There was a significant interaction of exposure with minute of test ($F(12, 672) = 2.41$, $p < 0.005$). There was also a significant main effect of minute in the trial

($F(4,672)=6.58$, $p<0.0005$) with a significant ($F(1,672)=24.10$, $p<0.0005$) linear increase in distance swum across the five minutes of the test (Figure 3B).

3.4. Juvenile Startle Habituation Tap Test

There was a significant main effect of exposure on startle response to the tap stimulus ($F(3,171)=5.78$, $p<0.001$). Dunnett's post hoc tests showed that the juvenile fish that were developmentally exposed to 1 μ M CPF ($p<0.05$) or 50 nM 6-OH-BDE-47 ($p<0.01$) showed significantly increased swimming activity following the solenoid taps relative to control animals (Figure 4B). However, the hyperactivity was not just seen following the tap. There was also hyperactivity seen prior to the taps (Figure 4A) with a significant main effect of exposure ($F(3,171)=7.58$, $p<0.0005$). Dunnett's tests showed that 10 nM ($p<0.05$) and 50 nM 6-OH-BDE-47 ($p<0.01$) both showed hyperactivity relative to controls during the five seconds prior to the tap, as did CPF ($p<0.05$).

4. Discussion

As behavioral toxicologists have long been aware, chemical exposures early in life can shift developmental trajectories, negatively affecting later-life behavior (Weiss, 1983). As such, it is essential to characterize long-term consequences of early exposures, in addition to short-term or acute impairments. Development of the vertebrate nervous system requires coordination of cellular migration, differentiation, and synaptogenesis, which can be perturbed by chemical exposures and lead to neurologic problems (Bondy and Campbell, 2005; Weiss, 1983). The analysis in the present study has shown that developmental exposure to 6-OH-BDE-47 resulted in decreases in larval swimming activity (Figure 2), as well as long-term alterations in novel tank exploration behavior (Figure 3) and sensorimotor response/habituation (Figure 4).

Developmental exposures to a range of PBDE congeners have been shown to cause alterations in zebrafish spontaneous movement at 24 hpf, with the largest effects observed for BDE-47 (~54 μ M) (Usenko et al., 2011). Aqueous BDE-47 exposure (~54 μ M) also elicited decreases in swimming rate at 7 dpf (Usenko et al., 2011). Similar results were found by Chen et al. 2012, with BDE-47 exposure (0-20 μ M) altering spontaneous movement, touch response, and free swimming speed in zebrafish embryos/larvae. These behavioral changes were hypothesized to be from inhibited axonal growth of motor neurons (Chen et al., 2012). The doses of 6-OH-BDE-47 used in the present study are an order of magnitude lower than doses used in these PBDE studies, suggesting that OH-BDEs may have enhanced potency in behavioral effects relative to the parent chemicals. However, it is important to note that we did not evaluate BDE-47 in the present study, and can make no direct comparisons.

Exposure to chlorinated organophosphate flame retardants and chlorpyrifos (1-10 μ M) also has been shown to produce comparable behavioral effects (decreases in activity during dark phases) when exposed during early development (0-5 dpf) (Dishaw et al., 2014). In addition, larval exposures to 300 nM chlorpyrifos (0-5 dpf) showed decreased larval motility in a 2 minute period at 6 dpf (Yen et al., 2011).

In adult fish studies, fish exposed to BDE-47 (100 ng/g ww (from 21-56 dph) via the diet were evaluated for predator avoidance responses and locomotion (maximum velocity, total distance moved, percent time active). These BDE-47 treated fish showed a reduction in the total distance moved and percent time active, but not the maximum swimming speed following exposure to high doses of BDE-47 via the diet (Chou et al., 2010). Killifish larvae (*Fundulus heteroclitus*) receiving an aqueous exposure to PentaBDE commercial mixture DE-71 showed a decrease in their activity level and an elimination of a fear response at lower doses (0.001 and 0.01 µg/L), as well as lower predation rates at high exposures (10 and 100 µg/L). In the same study, the killifish showed reduced learning ability in later life (3 months after cessation exposure), with signs of hyperactivity (Timme-Laragy et al., 2006). Previous work from our laboratory has also shown that developmental exposure to 0.29 µM chlorpyrifos (0-5 dpf) resulted in greater startle response at 20 weeks of age (Eddins et al., 2010). Taken together, these results along with previous studies suggest that early life exposures to flame retardants, in addition to chlorpyrifos, can have effects on behavior during larval, juvenile, and adult life-stages.

While our studies were not designed to fully address the mechanism responsible for the observed behavioral changes from 6-OH-BDE-47 exposure, mechanisms of PBDE neurotoxicity have been reviewed previously, and will be briefly addressed here (Costa et al., 2014; Dingemans et al., 2011; Legler, 2008). One proposed mechanism of PBDE toxicity is through disrupted thyroid hormone signaling (Costa et al., 2014). Thyroid hormones are very important in regulating development and growth of the central nervous system, so thyroid disruption during larval development could have lasting effects on neurodevelopment (Howdeshell, 2002). Many *in-vitro* studies have also found that OH-BDEs can displace thyroid hormones from transporter proteins in serum (Meerts et al., 2000), as well as inhibit thyroid hormone regulating enzymes intracellularly (deiodinases, sulfotransferases) (Butt and Stapleton, 2013; Butt et al., 2011) and agonize/antagonize the thyroid receptor (Kojima et al., 2009; Ren et al., 2013; Takeuchi et al., 2011). 6-OH-BDE-47 specifically has been shown to affect thyroid receptor expression (Dong et al., 2014; Liu et al., 2015; Zheng et al., 2012) as well as other nuclear receptor pathways in early zebrafish (AhR, ER, and MR) (Liu et al., 2015). Additionally, 6-OH-BDE-47 exposure has been shown to affect expression of other thyroidal genes (deiodinases, thyroid releasing hormone, sodium iodide symporter, transthyretin, & others) in zebrafish embryos (Dong et al., 2013; Usenko et al., 2012; Zheng et al., 2012). Behavioral studies in hypothyroid rodents (induced by propylthiouracil) show decreases in learning and habituation in mazes, changes in anxiety-like behavior, and increases in locomotor activity (Negishi et al., 2005). Therefore, it is possible that endocrine disruption could contribute to the behavioral phenotypes observed in the present study.

In addition to effects on the thyroid system, OH-BDEs have been shown to affect nervous system signaling by interfering with neurotransmitter uptake and release (Dingemans et al., 2011; Mariussen and Fonnum, 2003). In PC-12 cells, 6-OH-BDE-47 exposure interfered with cellular calcium release, causing increased release of catecholamines (Dingemans et al., 2010a, 2010b, 2008). Alterations in neurotransmitter release could be causing some of the behavioral effects observed in the present study. Another proposed mechanism of PBDE-

induced neurotoxicity is oxidative stress and apoptosis. PBDEs can accumulate in neurons, and preferentially accumulate in the mitochondria (Huang et al., 2010). 6-OH-BDE-47 (~1 μ M) specifically was shown to uncouple the electron transport chain, leading to disrupted energy production in zebrafish PAC2 fibroblasts (van Boxtel et al., 2008). 6-OH-BDE-47 (~1.3 μ M) has also been shown to cause oxidative stress in the tails of zebrafish larvae using TUNEL staining (Usenko et al., 2012). More recently, *in vitro* studies with mixtures of OH-BDEs found in the environment were found to disrupt oxidative phosphorylation (through protonophoric uncoupling or inhibition of the electron transport chain) and to have synergistic effects in zebrafish PAC2 cells (Legradi et al., 2014). Additionally 6-OH-BDE-47 has been shown to impact multiple aspects of adult neurogenesis (including cytotoxicity, proliferation, neuronal/oligodendrocyte differentiation) in primary adult neural stem progenitor cells of adult mice (Li et al., 2013). While these behavioral assays cannot address specifically which biochemical mechanism underlying behavior is disrupted (neurodevelopment, neurotransmitter release, altered energy metabolism, etc.), these areas merit further study emphasizing the need to further study *in-vivo* impacts of the OH-BDEs.

6-OH-BDE-47 and BDE-47 have been widely detected in human serum, including pregnant women and cord blood (summarized in Table 1). While the levels of OH-BDEs detected in human serum are lower than the water concentrations used in this study, exposures during critical developmental windows present unique susceptibilities. Additionally, PBDEs are detected at higher concentrations in toddlers relative to adults (Stapleton et al., 2012) and this has raised concerns for their potential developmental toxicity and neurotoxicity (Costa and Giordano, 2007; Dingemans et al., 2011). Importantly, there is a high correlation between maternal total PBDEs and total OH-BDEs, and in some cases OH-BDE levels are higher in cord serum than in maternal serum (Chen et al., 2013), suggesting higher fetal exposures in-utero. In the present study, we were unable to measure the concentration of 6-OH-BDE-47 in the zebrafish tissues, thus we are limited in our understanding of the relationship between internal dose and behavioral effects, which is needed for risk assessment purposes.

In summary, this study has shown that short developmental exposures (0-6dpf) to 6-OH-BDE-47 alter the behavior of zebrafish larvae and juveniles. These behavioral changes may be due to OH-BDE disruption of normal brain development during a critical developmental window (Howdeshell, 2002). Given the widespread exposure to 6-OH-BDE-47 (both natural and anthropogenic sources), and the body of evidence supporting its role as a neurotoxicant (Dingemans et al., 2011, 2008; Li et al., 2013), it is important to understand the functional effects of developmental exposures. Future studies will seek to examine the precise mechanisms responsible for the observed behavioral effects.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

HPC	Halogenated Phenolic Compound
DMSO	Dimethyl Sulfoxide
FR	Flame Retardant
PBDE	Polybrominated Diphenyl Ether
OH-BDE	Hydroxylated Polybrominated Diphenyl Ether
CPF	Chlorpyrifos
TH	Thyroid Hormone
hpf	Hours Post Fertilization
dpf	Days Post Fertilization
DI	deiodinase enzyme
T4	Thyroxine
T3	Triiodothyronine
TR	Thyroid receptor

Bibliography

- Alaee M, Arias P, Sjödin A, Bergman A. An overview of commercially used brominated flame retardants, their applications, their use patterns in different countries/regions and possible modes of release. *Environ. Int.* 2003; 29:683–9. [PubMed: 12850087]
- Aldridge JE, Levin ED, Seidler FJ, Slotkin TA. Developmental exposure of rats to chlorpyrifos leads to behavioral alterations in adulthood, involving serotonergic mechanisms and resembling animal models of depression. *Environ. Health Perspect.* 2005; 113:527–31. [PubMed: 15866758]
- Allen JG, McClean MD, Stapleton HM, Webster TF. Linking PBDEs in house dust to consumer products using X-ray fluorescence. *Environ. Sci. Technol.* 2008; 42:4222–8. [PubMed: 18589991]
- Athanasiadou M, Cuadra SN, Marsh G, Bergman Å, Jakobsson K. Polybrominated Diphenyl Ethers (PBDEs) and Bioaccumulative Hydroxylated PBDE Metabolites in Young Humans from Managua, Nicaragua. *Environ. Health Perspect.* 2007; 116:400–408. [PubMed: 18335110]
- Bailey J, Oliveri A, Levin ED. Zebrafish model systems for developmental neurobehavioral toxicology. *Birth Defects Res. C. Embryo Today.* 2013; 99:14–23. [PubMed: 23723169]
- Bencan Z, Sledge D, Levin ED. Buspirone, chlordiazepoxide and diazepam effects in a zebrafish model of anxiety. *Pharmacol. Biochem. Behav.* 2009; 94:75–80. [PubMed: 19643124]
- Bondy SC, Campbell A. Developmental neurotoxicology. *J. Neurosci. Res.* 2005; 81:605–12. [PubMed: 16035107]
- Butt C, Stapleton HM. Inhibition of thyroid hormone sulfotransferase activity by brominated flame retardants and halogenated phenolics. *Chem. Res. Toxicol.* 2013; 26(11):1692–1702. [PubMed: 24089703]
- Butt CM, Wang D, Stapleton HM. Halogenated phenolic contaminants inhibit the in vitro activity of the thyroid-regulating deiodinases in human liver. *Toxicol. Sci.* 2011; 124(2):339–47. [PubMed: 21565810]
- Chen A, Park J-S, Linderholm L, Rhee A, Petreas M, DeFranco E. a, Dietrich KN, Ho S-M. Hydroxylated polybrominated diphenyl ethers in paired maternal and cord sera. *Environ. Sci. Technol.* 2013; 47:3902–8. [PubMed: 23506475]

- Chen XX, Huang C, Wang X, Chen J, Bai C, Chen Y, Dong Q, Yang D. BDE-47 disrupts axonal growth and motor behavior in developing zebrafish. *Aquat. Toxicol.* 2012; 120-121:35–44. [PubMed: 22609740]
- Chou C-T, Hsiao Y-C, Ko F-C, Cheng J-O, Cheng Y-M, Chen T-H. Chronic exposure of 2,2',4,4'-tetrabromodiphenyl ether (PBDE-47) alters locomotion behavior in juvenile zebrafish (*Danio rerio*). *Aquat. Toxicol.* 2010; 98:388–95. [PubMed: 20416957]
- Costa LG, de Laat R, Tagliaferri S, Pellacani C. A mechanistic view of polybrominated diphenyl ether (PBDE) developmental neurotoxicity. *Toxicol. Lett.* 2014; 230(2):282–94. [PubMed: 24270005]
- Costa LG, Giordano G. Developmental neurotoxicity of polybrominated diphenyl ether (PBDE) flame retardants. *Neurotoxicology.* 2007; 28:1047–67. [PubMed: 17904639]
- De Wit CA. An overview of brominated flame retardants in the environment. *Chemosphere.* 2002; 46:583–624. [PubMed: 11999784]
- Dingemans MML, de Groot A, van Kleef RGDM, Bergman A, van den Berg M, Vijverberg HPM, Westerink RHS. Hydroxylation increases the neurotoxic potential of BDE-47 to affect exocytosis and calcium homeostasis in PC12 cells. *Environ. Health Perspect.* 2008; 116:637–43. [PubMed: 18470311]
- Dingemans MML, Heusinkveld HJ, Bergman A, van den Berg M, Westerink RHS. Bromination pattern of hydroxylated metabolites of BDE-47 affects their potency to release calcium from intracellular stores in PC12 cells. *Environ. Health Perspect.* 2010a; 118:519–25. [PubMed: 20368133]
- Dingemans MML, van den Berg M, Bergman A, Westerink RHS. Calcium-related processes involved in the inhibition of depolarization-evoked calcium increase by hydroxylated PBDEs in PC12 cells. *Toxicol. Sci.* 2010b; 114:302–9. [PubMed: 20044592]
- Dingemans MML, van den Berg M, Westerink RHS. Neurotoxicity of brominated flame retardants: (in)direct effects of parent and hydroxylated polybrominated diphenyl ethers on the (developing) nervous system. *Environ. Health Perspect.* 2011; 119:900–7. [PubMed: 21245014]
- Dishaw LV, Hunter DL, Padnos B, Padilla S, Stapleton HM. Developmental Exposure to Organophosphate Flame Retardants Elicits Overt Toxicity and Alters Behavior in Early Life Stage Zebrafish (*Danio rerio*). *Toxicol. Sci.* 2014; 142(2):445–54. [PubMed: 25239634]
- Dong W, Macaulay LJ, Kwok KW, Hinton DE, Ferguson PL, Stapleton HM. The PBDE metabolite 6-OH-BDE 47 affects melanin pigmentation and *THRβ* mRNA expression in the eye of zebrafish embryos. *Endocr. Disruptors.* 2014; 2:e969072.
- Dong W, Macaulay LJ, Kwok KWH, Hinton DE, Stapleton HM. Using whole mount in situ hybridization to examine thyroid hormone deiodinase expression in embryonic and larval zebrafish: a tool for examining OH-BDE toxicity to early life stages. *Aquat. Toxicol.* 2013; 132-133:190–9. [PubMed: 23531416]
- Eddins D, Cerutti D, Williams P, Linney E, Levin ED. Zebrafish provide a sensitive model of persisting neurobehavioral effects of developmental chlorpyrifos exposure: comparison with nicotine and pilocarpine effects and relationship to dopamine deficits. *Neurotoxicol. Teratol.* 2010; 32:99–108. [PubMed: 19268529]
- Eriksson P, Jakobsson E, Fredriksson A. Brominated flame retardants: a novel class of developmental neurotoxicants in our environment? *Environ. Health Perspect.* 2001; 109:903–8.
- Erratico CA, Szeitz A, Bandiera SM. Biotransformation of 2,2',4,4'-tetrabromodiphenyl ether (BDE-47) by human liver microsomes: identification of cytochrome P450 2B6 as the major enzyme involved. *Chem. Res. Toxicol.* 2013; 26:721–31. [PubMed: 23537005]
- Feo ML, Gross MS, McGarrigle BP, Eljarrat E, Barceló D, Aga DS, Olson JR. Biotransformation of BDE-47 to Potentially Toxic Metabolites Is Predominantly Mediated by Human CYP2B6. *Environ. Health Perspect.* 2013; 121:440–446. [PubMed: 23249762]
- Fujii Y, Ito Y, Harada KH, Hitomi T, Koizumi A, Haraguchi K. Regional variation and possible sources of brominated contaminants in breast milk from Japan. *Environ. Pollut.* 2012; 162:269–74. [PubMed: 22243874]
- Gerlai R, Lahav M, Guo S, Rosenthal A. Drinks like a fish: zebra fish (*Danio rerio*) as a behavior genetic model to study alcohol effects. *Pharmacol. Biochem. Behav.* 2000; 67:773–82. [PubMed: 11166068]

- Herbstman JJB, Mall JJK. Developmental Exposure to Polybrominated Diphenyl Ethers and Neurodevelopment. *Curr. Environ. Heal. Reports*. 2014; 1:101–112.
- Herbstman JJB, Sjödin A, Kurzon M, Lederman S. a, Jones RS, Rauh V, Needham LL, Tang D, Niedzwiecki M, Wang RY, Perera F. Prenatal exposure to PBDEs and neurodevelopment. *Environ. Health Perspect.* 2010; 118:712–9. [PubMed: 20056561]
- Hill AJ, Teraoka H, Heideman W, Peterson RE. Zebrafish as a model vertebrate for investigating chemical toxicity. *Toxicol. Sci.* 2005; 86:6–19. [PubMed: 15703261]
- Howdeshell KL. A model of the development of the brain as a construct of the thyroid system. *Environ. Health Perspect.* 2002; 110(Suppl):337–48. [PubMed: 12060827]
- Huang SC, Giordano G, Costa LG. Comparative cytotoxicity and intracellular accumulation of five polybrominated diphenyl ether congeners in mouse cerebellar granule neurons. *Toxicol. Sci.* 2010; 114:124–32. [PubMed: 19969594]
- Huang Y, Zhu G, Peng L, Ni W, Wang X, Zhang J, Wu K. Effect of 2,2',4,4'-tetrabromodiphenyl ether (BDE-47) on sexual behaviors and reproductive function in male zebrafish (*Danio rerio*). *Ecotoxicol. Environ. Saf.* 2015; 111:102–108. [PubMed: 25450921]
- Kelly BC, Blair JD, Gobas FAPC, Ikononou MG. Hydroxylated and methoxylated polybrominated diphenyl ethers in a Canadian Arctic marine food web. *Environ. Sci. Technol.* 2008; 42:7069–77. [PubMed: 18939528]
- Kim KH, Bose DD, Ghogha A, Riehl J, Zhang R, Barnhart CD, Lein PJ, Pessah IN. Para- and ortho-substitutions are key determinants of polybrominated diphenyl ether activity toward ryanodine receptors and neurotoxicity. *Environ. Health Perspect.* 2011; 119:519–26. [PubMed: 21106467]
- Kimmel CB, Ballard WW, Kimmel SR, Ullmann B, Schilling TF. Stages of embryonic development of the zebrafish. *Dev. Dyn.* 1995; 203:253–310. [PubMed: 8589427]
- Kojima H, Takeuchi S, Uramaru N, Sugihara K, Yoshida T, Kitamura S. Nuclear hormone receptor activity of polybrominated diphenyl ethers and their hydroxylated and methoxylated metabolites in transactivation assays using Chinese hamster ovary cells. *Environ. Health Perspect.* 2009; 117:1210–8. [PubMed: 19672399]
- Legler J. New insights into the endocrine disrupting effects of brominated flame retardants. *Chemosphere.* 2008; 73:216–22. [PubMed: 18667224]
- Legradi J, Dahlberg A-K, Cenijn P, Marsh G, Asplund L, Bergman A, Legler J. Disruption of Oxidative Phosphorylation (OXPHOS) by Hydroxylated Polybrominated Diphenyl Ethers (OH-PBDEs) Present in the Marine Environment. *Environ. Sci. Technol.* 2014; 48:14703–11. [PubMed: 25422162]
- Lema SC, Dickey JT, Schultz IR, Swanson P. 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.* 2008; 116:1694–9. [PubMed: 19079722]
- Levin ED. Zebrafish assessment of cognitive improvement and anxiolysis: filling the gap between in vitro and rodent models for drug development. *Rev. Neurosci.* 2011; 22:75–84. [PubMed: 21615262]
- Levin ED, Bencan Z, Cerutti DT. Anxiolytic effects of nicotine in zebrafish. *Physiol. Behav.* 2007; 90:54–8. [PubMed: 17049956]
- Li T, Wang W, Pan Y-W, Xu L, Xia Z. A hydroxylated metabolite of flame-retardant PBDE-47 decreases the survival, proliferation, and neuronal differentiation of primary cultured adult neural stem cells and interferes with signaling of ERK5 MAP kinase and neurotrophin 3. *Toxicol. Sci.* 2013; 134:111–24. [PubMed: 23564643]
- Linney E, Upchurch L, Donerly S. Zebrafish as a neurotoxicological model. *Neurotoxicol. Teratol.* 2004; 26:709–18. [PubMed: 15451034]
- Liu F, Wiseman S, Wan Y, Doering JA, Hecker M, Lam MHW, Giesy JP. Multi-species comparison of the mechanism of biotransformation of MeO-BDEs to OH-BDEs in fish. *Aquat. Toxicol.* 2012; 114-115:182–8. [PubMed: 22446830]
- Liu H, Tang S, Zheng X, Zhu Y, Ma Z, Liu C, Hecker M, Saunders DMV, Giesy JP, Zhang X, Yu H. Bioaccumulation, biotransformation and toxicity of BDE-47, 6-OH-BDE-47 and 6-MeO-BDE-47 in early life-stages of zebrafish (*Danio rerio*). *Environ. Sci. Technol.* 2015

- Lorber M. Exposure of Americans to polybrominated diphenyl ethers. *J. Expo. Sci. Environ. Epidemiol.* 2008; 18:2–19. [PubMed: 17426733]
- MacPhail RC, Brooks J, Hunter DL, Padnos B, Irons TD, Padilla S. Locomotion in larval zebrafish: Influence of time of day, lighting and ethanol. *Neurotoxicology.* 2009; 30:52–8. [PubMed: 18952124]
- Mariussen E, Fonnum F. The effect of brominated flame retardants on neurotransmitter uptake into rat brain synaptosomes and vesicles. *Neurochem. Int.* 2003; 43:533–542. [PubMed: 12742101]
- Meerts IA, van Zanden JJ, Luijckx EA, van Leeuwen-Bol I, Marsh G, Jakobsson E, Bergman A, Brouwer A. Potent competitive interactions of some brominated flame retardants and related compounds with human transthyretin in vitro. *Toxicol. Sci.* 2000; 56:95–104. [PubMed: 10869457]
- Negishi T, Kawasaki K, Sekiguchi S, Ishii Y, Kyuwa S, Kuroda Y, Yoshikawa Y. Attention-deficit and hyperactive neurobehavioural characteristics induced by perinatal hypothyroidism in rats. *Behav. Brain Res.* 2005; 159:323–31. [PubMed: 15817195]
- Padilla S, Hunter DLL, Padnos B, Frady S, MacPhail RCC. Assessing locomotor activity in larval zebrafish: Influence of extrinsic and intrinsic variables. *Neurotoxicol. Teratol.* 2011; 33:624–30. [PubMed: 21871562]
- Qiu X, Bigsby RM, Hites RA. Hydroxylated metabolites of polybrominated diphenyl ethers in human blood samples from the United States. *Environ. Health Perspect.* 2009; 117:93–8. [PubMed: 19165393]
- Qiu X, Mercado-Feliciano M, Bigsby RM, Hites R. a. Measurement of polybrominated diphenyl ethers and metabolites in mouse plasma after exposure to a commercial pentabromodiphenyl ether mixture. *Environ. Health Perspect.* 2007; 115:1052–8. [PubMed: 17637922]
- Ren X-M, Guo L-H, Gao Y, Zhang B-T, Wan B. Hydroxylated polybrominated diphenyl ethers exhibit different activities on thyroid hormone receptors depending on their degree of bromination. *Toxicol. Appl. Pharmacol.* 2013; 268:256–263. [PubMed: 23402801]
- Roze E, Meijer L, Bakker A, Van Braeckel K.N.J. a, Sauer PJJ, Bos AF. Prenatal exposure to organohalogenes, including brominated flame retardants, influences motor, cognitive, and behavioral performance at school age. *Environ. Health Perspect.* 2009; 117:1953–8. [PubMed: 20049217]
- Shaw SD, Blum A, Weber R, Kannan K, Rich D, Lucas D, Koshland CP, Dobraca D, Hanson S, Birnbaum LS. Halogenated flame retardants: do the fire safety benefits justify the risks? *Rev. Environ. Health.* 2010; 25:261–305. [PubMed: 21268442]
- Sledge D, Yen J, Morton T, Dishaw L, Petro A, Donerly S, Linney E, Levin ED. Critical duration of exposure for developmental chlorpyrifos-induced neurobehavioral toxicity. *Neurotoxicol. Teratol.* 2011; 33:742–51. [PubMed: 21745564]
- Sniegowski LT, Moody JR. Determination of serum and blood densities. *Anal. Chem.* 1979; 51:1577–1578. [PubMed: 484867]
- Stapleton HM, Dodder NG, Offenberger JH, Schantz MM, Wise SA. Polybrominated Diphenyl Ethers in House Dust and Clothes Dryer Lint. *Environ. Sci. Technol.* 2005; 39:925–31. [PubMed: 15773463]
- Stapleton HM, Eagle S, Anthopolos R, Wolkin A, Miranda ML. Associations between polybrominated diphenyl ether (PBDE) flame retardants, phenolic metabolites, and thyroid hormones during pregnancy. *Environ. Health Perspect.* 2011; 119:1454–9. [PubMed: 21715241]
- Stapleton HM, Eagle S, Sjödin A, Webster TF. Serum PBDEs in a North Carolina Toddler Cohort: Associations with Handwipes, House Dust, and Socioeconomic Variables. *Environ. Health Perspect.* 2012; 120:1049–1054. [PubMed: 22763040]
- Ta TA, Koenig CM, Golub MS, Pessah IN, Qi L, Aronov PA, Berman RF. Bioaccumulation and behavioral effects of 2,2',4,4'-tetrabromodiphenyl ether (BDE-47) in perinatally exposed mice. *Neurotoxicol. Teratol.* 2011; 33:393–404. [PubMed: 21334437]
- Takeuchi S, Shiraishi F, Kitamura S, Kuroki H, Jin K, Kojima H. Characterization of steroid hormone receptor activities in 100 hydroxylated polychlorinated biphenyls, including congeners identified in humans. *Toxicology.* 2011; 289:112–21. [PubMed: 21843587]

- Timme-Laragy AR, Levin ED, Di Giulio RT. Developmental and behavioral effects of embryonic exposure to the polybrominated diphenylether mixture DE-71 in the killifish (*Fundulus heteroclitus*). *Chemosphere*. 2006; 62:1097–104. [PubMed: 16045967]
- Truong L, Mandrell D, Mandrell R, Simonich M, Tanguay RL. A rapid throughput approach identifies cognitive deficits in adult zebrafish from developmental exposure to polybrominated flame retardants. *Neurotoxicology*. 2014
- Usenko CY, Hopkins DC, Trumble SJ, Bruce ED. Hydroxylated PBDEs induce developmental arrest in zebrafish. *Toxicol. Appl. Pharmacol.* 2012; 262:43–51. [PubMed: 22546086]
- Usenko CY, Robinson EM, Usenko S, Brooks BW, Bruce ED. PBDE developmental effects on embryonic zebrafish. *Environ. Toxicol. Chem.* 2011; 30:1865–72. [PubMed: 21560146]
- Van Bostel AL, Kamstra JH, Cenijs PH, Pieterse B, Wagner JM, Antink M, Krab K, van der Burg B, Marsh G, Brouwer A, Legler J. Microarray analysis reveals a mechanism of phenolic polybrominated diphenylether toxicity in zebrafish. *Environ. Sci. Technol.* 2008; 42:1773–9. [PubMed: 18441834]
- Wan Y, Liu F, Wiseman S, Zhang X, Chang H, Hecker M, Jones PD, Lam MHW, Giesy JP. Interconversion of hydroxylated and methoxylated polybrominated diphenyl ethers in Japanese medaka. *Environ. Sci. Technol.* 2010; 44:8729–35. [PubMed: 20973477]
- Wan Y, Wiseman S, Chang H, Zhang X, Jones PD, Hecker M, Kannan K, Tanabe S, Hu J, Lam MHW, Giesy JP. Origin of hydroxylated brominated diphenyl ethers: natural compounds or man-made flame retardants? *Environ. Sci. Technol.* 2009; 43:7536–42.
- Wang H-S, Chen Z-J, Ho K-L, Ge L-C, Du J, Lam MH-W, Giesy JP, Wong M-H, Wong CK-C. Hydroxylated and methoxylated polybrominated diphenyl ethers in blood plasma of humans in Hong Kong. *Environ. Int.* 2012; 47:66–72. [PubMed: 22771521]
- Weiss B. Behavioral toxicology and environmental health science. Opportunity and challenge for psychology. *Am. Psychol.* 1983; 38:1174–87. [PubMed: 6196997]
- Wen Q, Liu H-L, Zhu Y-T, Zheng X-M, Su G-Y, Zhang X-W, Yu H-X, Giesy JP, Lam MHW. Maternal transfer, distribution, and metabolism of BDE-47 and its related hydroxylated, methoxylated analogs in zebrafish (*Danio rerio*). *Chemosphere*. 2015; 120:31–36. [PubMed: 24973617]
- Wiseman SB, Wan Y, Chang H, Zhang X, Hecker M, Jones PD, Giesy JP. Polybrominated diphenyl ethers and their hydroxylated/methoxylated analogs: environmental sources, metabolic relationships, and relative toxicities. *Mar. Pollut. Bull.* 2011; 63:179–88. [PubMed: 21439595]
- Yen J, Donerly S, Levin ED, Linney EA. Differential acetylcholinesterase inhibition of chlorpyrifos, diazinon and parathion in larval zebrafish. *Neurotoxicol. Teratol.* 2011; 33:735–41. [PubMed: 22036888]
- Zheng X, Zhu Y, Liu C, Liu H, Giesy JP, Hecker M, Lam MHW, Yu H. Accumulation and Biotransformation of BDE-47 by Zebrafish Larvae and Teratogenicity and Expression of Genes along the Hypothalamus-Pituitary-Thyroid Axis. *Environ. Sci. Technol.* 2012; 46:12943–51. [PubMed: 23110413]
- Zota AR, Park J-S, Wang Y, Petreas M, Zoeller RT, Woodruff TJ. Polybrominated diphenyl ethers, hydroxylated polybrominated diphenyl ethers, and measures of thyroid function in second trimester pregnant women in California. *Environ. Sci. Technol.* 2011; 45:7896–905. [PubMed: 21830753]

Highlights:

- 6-OH-BDE-47 is a natural marine product and metabolite of the flame retardant BDE-47, and is commonly detected in human tissues
- zebrafish exposed from 0-6dpf to 6-OH-BDE-47 showed altered larval swimming behavior, and altered adult behavior at 45 dpf
- Early life exposure to 6-OH-BDE-47 has lasting effects on zebrafish behavior later in life

Figure 1.

Potential sources of 6-OH-BDE-47 from oxidative metabolism of anthropogenic PBDEs, and from inter-conversion with marine natural products (Source: Erratico et al., 2013; Feo et al., 2013; Wan et al., 2010, 2009).

Figure 2.

Larval swimming distance under alternating conditions of ambient illumination in 6 dpf zebrafish. Panel A is the distance swum ($\pm$ SEM; $n=34-40$ larvae/treatment) by minute in alternating light/dark periods (10 min acclimation, followed by 10 minute light/dark transitions for a 50 minute trial). Panel B is the total distance swum ($\pm$ SEM) in the dark and light periods over the whole trial time. Note the main effect of 6-OH-BDE-47 exposure during the light phases, causing hypoactivity, and the reduction in swimming activity during the dark phases for chlorpyrifos. Asterisks indicate significant differences from controls ($p < 0.05$).

Figure 3.

Summary of data from the Dive Test. Average distance ($\pm$ SEM; $n=34-46$ fish/treatment) from the tank floor is plotted as a function of session minute for each treatment group (panel A). Bars with different letters indicate statistical differences from controls. Note that the 10 nM and 50 nM 6-OH-BDE-47 treated animals spent significantly more time near the bottom of the tanks, indicating an increased fear/anxiety response. In contrast, there was no statistical difference between treatment groups when looking at average total distance swum ($\pm$ SEM) over the duration of the trial (Panel B), therefore the capacity to swim is independent of exploration behavior exploration.

Figure 4.

Distance traveled during the 5 s preceding (panel A) and the 5 s following (panel B) delivery of the tap stimulus is plotted for each treatment group, error bars represent SEM (n=34-46 fish/treatment). Bars with different letters indicate statistical differences from controls. Note each bar represents the average distance traveled from a bin of two stimulus presentations. With repeated tap stimulus, all fish show typical attenuation in responding following stimulus delivery, i.e. habituation to the stimulus presentation. Treated fish showed an overall hyperactivity during the trial, manifesting in both the “pre” and “post” samples (Panel A & B).

Table 1

Sample serum concentrations of BDE-47 (nM) and 6-OH-BDE-47 (pM) in serum samples from the United States, Nicaragua and Hong Kong. When values were reported on a lipid mass basis, the molar concentration was estimated by assuming 0.6% serum lipids and a density of 1.024 g/mL (Sniegowski and Moody, 1979).

Survey of Concentrations of PBDE/OH-BDE Levels in Humans							
<i>Congener</i>	<i>Location</i>	<i>Sample Matrix</i>	<i>[Mean, nM]</i>	<i>[Median, nM]</i>	<i>50/95th percentile</i>	<i>Range, [nM]</i>	<i>Reference</i>
PBDE-47 [nM]	United States	Cord blood	0.89	0.16	nr	nr	(Qiu et al. 2009)
	United States	Maternal	0.22	0.19	nr	0-3.9	(Qiu et al. 2009)
	United States	Maternal	0.20 [*]	nr	nr	0.03-3.8	(Stapleton et al. 2011a)
	United States	Maternal	0.54 [*]	nr	0.53-1.55	.01-.23	(Zota et al. 2011)
	United States	general population	0.03	0.02	nr	.03-1.1	(Wang et al. 2012)
	United States	Maternal	0.15 [*]	0.14	nr	.09-1.2	(Chen et al. 2013)
	United States	Cord blood	0.21 [*]	0.23	nr	.01-2.4	(Chen et al. 2013)
	Nicaragua	occupational exposure	nr	nr	nr	.07-3.9 nM	(Athanasiadou et al. 2007)
6-OH-BDE-47 [pM]	United States	cord blood	121	12.26	nr	nr	(Qiu et al., 2009))
	United States	Maternal	3.70	2.45	nr	nr	(Qiu et al., 2009)
	United States	Maternal	2.1 [*]	2.45	nr	0.12-132	(Stapleton et al., 2011)
	United States	Maternal	0.31 [*]	nr	.28-1.8	nr	(Zota et al., 2011)
	Nicaragua	occupational exposure	nr	nr	nr	8.6-79.9	(Athanasiadou et al., 2007)
	United States	maternal	9.2 [*]	0.06	nr	4.9-164	(Chen et al., 2013)
	United States	cord blood	13.1 [*]	11.21	nr	4.9-336	(Chen et al., 2013)
	Hong Kong	general population	0.74	0.41	nr	0-3.6	(Wang et al., 2012)

* indicates geometric mean; nr: not reported.