GLOBAL AMPHIBIAN DECLINES: ARE EXPOSURES TO POLYBROMINATED DIPHENYL ETHERS A CONTRIBUTING FACTOR?

by

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GLOBAL AMPHIBIAN DECLINES: ARE EXPOSURES TO POLYBROMINATED DIPHENYL ETHERS A CONTRIBUTING FACTOR?

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ABSTRACT

Over the past 30 years amphibian populations have been declining globally. Exposure to environmental contaminants could be contributing to this global decline. Polybrominated diphenyl ether 47 (PBDE-47, a flame retardant) is a ubiquitous aquatic contaminant that leaches out of plastics, furniture, vehicles, and electronics into the air, soil, and water. Studies have shown that this organobromide compound may have thyroid-disrupting activity that alters the growth and development of exposed organisms. In this study, African clawed frog (*Xenopus laevis*) tadpoles were exposed to three environmentally-relevant concentrations of PBDEs via their diets for 21 days beginning at developmental stage 52. Tadpole mass, hind-limb length and developmental stage were evaluated on days 0, 7, 14 and 21, and expression of thyroid-related genes were measured at the conclusion of the exposure. Xenopus tadpoles exposed to the highest PBDE concentration were significantly smaller and less developed than controls. The expression of beta transcription element-binding protein (BTEB), thyroid-stimulating hormone beta (TSHß), deiodinase 1 (DI1), deiodinase 2 (DI2), thyroid receptor alpha (TRα), and thyroid receptor beta (TRß) were all significantly decreased in the medium and high dose groups compared to the control. These results indicate that PBDE-47 is a thyroid-disruptor that inhibits metamorphosis and growth through alterations in thyroid-related gene expression. Future studies aimed at uncovering the mechanism by which PBDE-47 alters thyroid-related gene expression are warranted to decipher their effects in amphibians.

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INTRODUCTION

Over the past 30 years, amphibian populations have been declining globally (Collins and Storfer, 2003). This global trend is worrisome to both herpetologists and environmentalists as amphibians are ecological indicators that reflect the health of the environment. Amphibians also serve as biological pest controllers in their respective ecosystems, making their presence an important aspect of the ecosystem. Several hypotheses have been put forward to explain declining amphibian populations including the presence of contaminants in the environment that effect organism survival and fitness by hindering growth and maturity, which can cause an increased risk of predation or decreased ability to reproduce.

Though there are a wide variety of environmental contaminants that could impact the health of amphibians, contaminants with the potential to act as endocrine disruptors are particularly concerning as exposures to such contaminants can alter endocrine-mediated processes such as growth, development and reproduction. Polybrominated diphenylethers (PBDEs) are a class of organobromide compounds with suspected endocrine disrupting activity. Over 200 congeners of PBDEs have been produced for use as flame-retardants in a wide variety of consumer goods including plastics, furniture, vehicles, and electronics. Recent evidence has shown that PBDEs are able to leach out of these materials and accumulate in the environment due to their resistance to biological degradation (Vane et al., 2009; Dinn et al., 2012). PBDEs have been detected in soil, dust, outdoor and indoor air, sewage sludge, and in the bodies of both aquatic and terrestrial organisms (De Wit et al., 2002; Gill et al., 2004). In the United States, fish have been found to have high concentrations of PBDEs, indicating that aquatic organisms

have high exposure to these compounds (Costa et al., 2008). Additional evidence has shown that PBDEs bioaccumulate in the adipose tissue of organisms due to their lipophilic nature and resistance to degradation (Hale et al., 2003; Athanasiadou et al. 2008; Kuo et al. 2010). PBDEs have been labeled as contaminants of emerging concern by the Environmental Protection Agency due to their ubiquity and persistence in the environment, as well as their suspected endocrine disrupting activity (EPA, 2013).

In recent years, a growing body of evidence has shown that exposures to PBDEs can have a wide variety of adverse biological effects, including functioning as an endocrine disruptor due to its activity as an androgen antagonist (Stoker et al., 2005) and estrogen antagonist (Hamers et al., 2006; Hamers et al., 2008) causing negative reproductive effects (Kuriyama et al., 2005). However, the most profound effects have been seen in the thyroid gland. Studies have shown that PBDEs can decrease the level of thyroid hormones, usually thyroxine (T4), in organisms across age groups (Richardson et al., 2006; Zhou et al., 2001; Talsness, et al., 2004). Many scientists speculate that this is due to the similar structure of PBDE and thyroxine (Fig. 1), which causes the PBDE to bind competitively to the thyroid hormone transport protein, transthyretin (McDonald, 2002; Meerts et al.). Without binding of this transport protein,

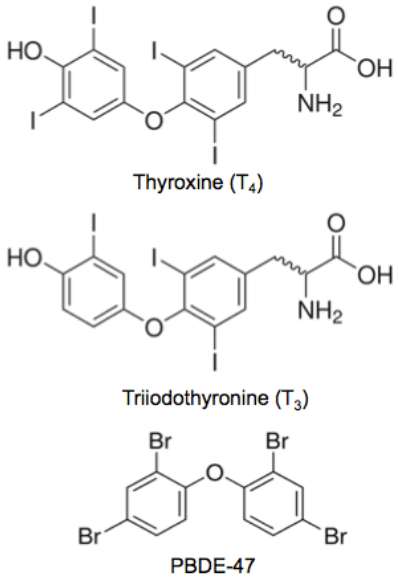

Figure 1: Structure of (a) T4, (b) T3, and (3) PBDE-47, adapted from Laura Macaulay. Retrieved 2015 from the Duke University Superfund Research Center website, http://sites.nicholas.duke.edu/superfund/early-life-exposures-to-flame-retardants-exploring-the-consequences/.

thyroid hormones are quickly degraded by phagocytosing leukocytes in the blood (Seymuor and Green, 1973). Thyroid hormones are important in the growth and development of vertebrates, and a change in their levels could result in hindered growth or maturation. This is especially true for amphibians, as thyroid hormones initiate and regulate the metamorphosis of tadpoles into frogs (Bartels et al., 2013). Much of the current literature demonstrating the effects of PBDEs on the thyroid system involves mice and fish, demonstrating that exposure to PBDEs decreases T4 levels in mouse pups (*Mus musculus*) pups (Talsness, et al. 2004) and alters thyroid hormone-related gene expression in fathead minnows (*Pimephales promelas*) (Lema et al., 2008). However, there is a paucity of information regarding the effects of PBDEs on thyroid function in amphibians.

The objectives of this study were to determine whether PBDE-47 exposure (1) alters the growth and metamorphosis of African clawed frog (*Xenopus laevis)* tadpoles and (2) induces alterations in the expression of thyroid-related genes.

MATERIALS AND METHODS

Test organisms. African clawed frogs (*Xenopus laevis*) were obtained from Nasco (Fort Aat developmental stages 47-49 were used. A total of 200 tadpoles were randomly assigned to four groups (n=50/group). Each group consisted of two replicates. They were housed at room temperature with a 16-hour light and 8-hour dark photoperiod. They were fed Sera Micron, with each group of 20 tadpoles receiving 600 mg/day on days 0-4, 400 mg/day days 5-10, 560 mg/day on days 11-14, and 480 mg/day on days 15-21. Feces and uneaten material were removed and 2/3 of the test solution was replaced each day. The tanks used to house the tadpoles were 30L in volume. The water reached a volume of

21L, and 7L of the water remained in the tank during the exchange of dirty and clean water. MS-222 was used for euthanization for post-mortem collection of tissues.

Experimental design and exposure regime. There were three experimental groups exposed via diet to: 0.05mg/L, 0.5mg/L, and 5mg/L of PBDE-47. The control group was fed regular SeraMicron. The tadpoles were randomly assigned to the four test groups and were tested in duplicates. The exposure duration lasted 21 days. On days 0, 7 and 14, 8 tadpoles from each group were removed and euthanized for mass, histology, and gene expression analysis.

PBDE food preparation.

PBDE food was prepared by dissolving 4mL stock solution of PBDE-47 into 4mL of hexane. 5.4mL of this PBDE-hexane solution was added to 5,400mg of SeraMicron for the high dose group. 800µL of the PBDE-hexane solution was added to 7.2 mL of hexane for the medium dose group and then 5.4mL of the total solution was dissolved in 5,400mg of SeraMicron. 800µL of the PBDE-hexane solution was then added to 14.4mL of hexane for the low dose group and then 5.4mL of the total solution was added to 5,400mg of SeraMicron. The solutions were allowed 24 hours for the hexane to evaporate.

Assessment of growth and development. Tadpole growth and development were assessed on days 7, 14 and 21 of the exposure period. On days 7 and 14, a subset of tadpoles from each replicate (n = 4) was randomly selected so that growth and developmental metrics could be assessed. Tadpoles were euthanized in a lethal dose of tricaine methanosulfate (MS-222), staged using a developmental chart (Daudin, 1967) and weighed. In addition, hind limb length was determined using digital calipers. At the

termination of exposure on day 21, the remaining tadpoles were euthanized for the determination of stage, mass and hind limb length. A subset of these tadpoles was randomly selected for the collection of tail and brain tissue for gene expression analysis and carcasses for PBDE analysis as described below.

PBDE Analysis.

Tadpole carcass samples were prepared by removing viscera. Carcass tissue was then homogenized using a bead beater with 200 ng isotopically labeled $^{13}C_{12}$ PBDE-47 in ethyl acetate as the internal standard. Samples were then filtered and 25% of the filtrate was reserved for extraction and evaporated under nitrogen gas. Following evaporation, samples were resuspended in approximately 1-2mL of acetonitrile and incubated at -20°C for 30 minutes. Samples were then filtered and solvent was evaporated under nitrogen gas. Finally, samples were resuspended in 100µL of ethyl acetate. Samples were analyzed on an Agilent Technologies 5973 mass spectrometer with a 6890N GC System (Santa Clara, CA) equipped with an Agilent 30 m X 0.25 mm X 0.025 um DB-5 capillary column. The GC was operated in splitless injection mode under constant flow of helium carrier gas at 1.1 mL/min with an inlet temperature of 265 C. The GC oven was programmed for an initial 1min. hold at 40 C, and then ramped at 15 C/min. to a final hold at 300 C for 10 min. The mass spectrometer was operate in the selected ion mode with the following m/z targets: $^{13}C_{12}$ PBDE-47: 498, 496, 338, 336 and PDBE-47: 486, 484, 326,324. Standard curves were prepared using PBDE 47 concentrations from 20 ppm to 16 ppb and $^{13}C_{12}$ PBDE-47 was at 500 ppb. Within each set of samples an additional solvent blank was prepared to ensure quality control.

Gene expression analysis. Gene expression analysis was used to determine whether there was a change in the relative gene expression of the thyroid receptor TRalpha (TRα), TRbeta (TRß), deiodinase 1 (DI1), deiodinase 2 (DI2), and deiodinase 3 (DI3), basic transcription element-binding protein (BTEB), and thyroid stimulating hormone beta (TSHß). Seven tadpoles from each group were randomly selected prior to euthanization for gene expression analysis at the conclusion of the study. The tail and brain of each tadpole was removed for analysis. Samples were flash frozen in liquid nitrogen immediately after collection and stored in -80º until used.

Total RNA was extracted from tails and brains using the Maxwell® simply RNA LEV kit (Promega, Madison, WI) coupled with the Maxwell® Research System. Briefly, tissues were homogenized manufacturer-supplied homogenization solution with a Misonix Microson XL-2000 Ultrasonic Cell Disruptor (QSonica, LLC., Newton, CT). Total RNA was purified per manufacturer instruction without exception.

The quantity and quality of the isolated total RNA was determined via the Nanodrop 1000 (Thermo Scientific). All samples had A260:A280 ratios > 1.8 confirming the purity of the extracted total RNA.First-strand cDNA was synthesized using the iScript cDNA Synthesis Kit (Bio-Rad Inc., Hercules, CA) per protocol. A thermal cycler carried out the synthesis reactions under 25ºC for 5 minutes, 42º for 30 minutes, and 85ºC for 5 minutes.

To quantify the expression of the selected target genes, qPCR reactions were performed in triplicate using a CFX Connect real-time PCR detection system managed by CFX Manager Software version 3.0 (Bio-Rad, Hercules, CA). Each reaction contained 0.4 µl of cDNA, 5 µl SYBR-Green (Bio-Rad) and 300 nM of forward and reverse primer

(totaling to 10 µl per reaction) and all reactions were carried out in white shell/clear 96-well PCR plates (Bio-Rad). Reactions were carried out via a PTC-100 thermal cycler (25°C for 5 minutes, 42°C for 30 minutes, and 85°C for 5 minutes) (Table 1). Results were quantified by the standard curve method using serial diluted cDNA samples and standards and were normalized by with relative L8 expression.

Table 1. Gene Sequence, Annealing Temperatures, and Efficiencies

Gene	Forward Sequence (5'→ 3')	Reverse Sequence (5' → 3')	Temp
L8	TCC GTG GTG TGG CTA TGA ATC C	GAC GAC CAG TAC GAC GAG CAG	61ºC
BTEB	CGT GGC AAA GTT TAT GGG	GGA TGG AAG TCG GTA TGG	55ºC
TSHß	AGA GTG CGC TTA CTG CCT TG	GGT AGG AAA AGA GCG GGT TC	55ºC
TRa	CG TTG GCA TGG CAA TGG	TGA GCT TCT GTT ACA ATG CGA	61ºC
TRß	TGT TTT GTG AGC TGC CAT GT	ACA CCC CCA AGT CAA AGA TG	60ºC
DI1	AAG AAC ACC GGC TGA ACA AT	CAA TGC TTT GCA ACA TCA GG	60ºC
DI2	GGC TGC GCT GTG TGT GGA A	AGG CTG GCA GCT GGC TTA	61ºC
DI3	GGC TGC GCT GTG TGT GGA A	AGG CTG GCA GCT GGC TTA	61ºC

Statistical analysis. To determine if any of the measured endpoints were significantly different between the groups, a one-way analysis of variance (ANOVA) was conducted using the statistical software JMP version 11.2.0 (SAS Institute).Dunnett's test was used to determine which groups were significantly different from the control. α was set at 0.05.

RESULTS

PBDE body burden.

Body burden PBDE-47 concentrations are reported in Table 2 and reflect the dose-dependent uptake of PBDE-47 into the tissues of exposed tadpoles.

Developmental Stage.

Significant differences in the developmental stage of tadpoles were noted on days 7, 14 and 21 of the exposure period (Fig. 2, ANOVA, $P < 0.0001$). Specifically, tadpoles in the high-dose PBDE group had significantly lower developmental stages than the corresponding control group on days 7, 14 and 21. In addition, tadpoles in the medium-dose PBDE group showed a significant decrease in stage on day 7 relative to controls.

Table 2. PBDE-47 concentrations (mean ± standard deviation) of tadpoles from the control, low-dose, medium-dose and high-dose groups at the end of the exposure period. n = 5.

	PBDE-47 Body Burden (μg/g)
Control	0.25 ± 0.13
Low	3.66 ± 4.90
Medium	26.30 ± 7.08
High	61.72 ± 21.70

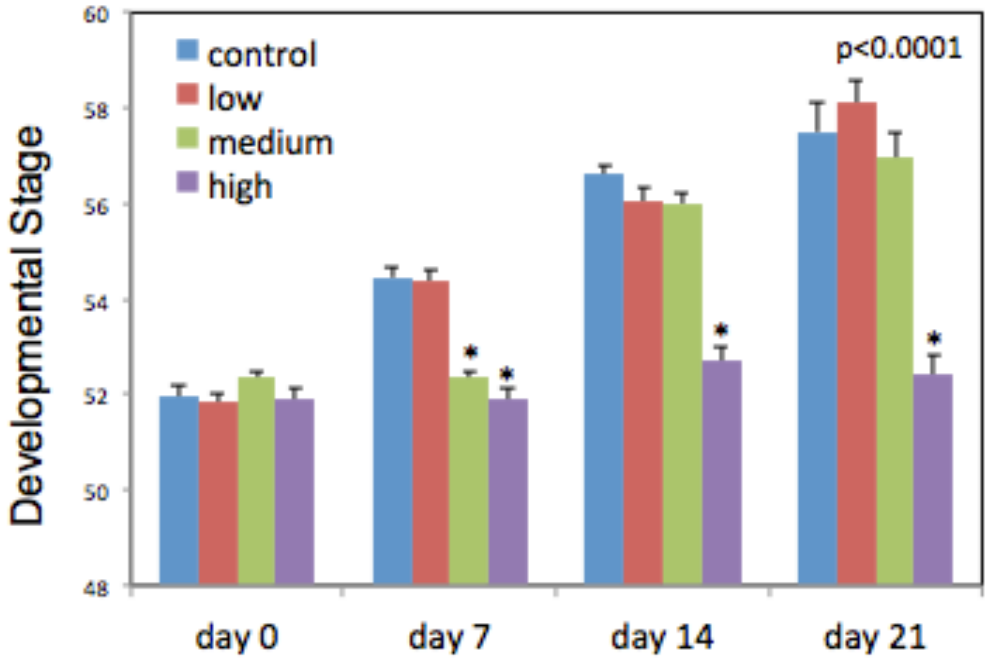

Figure 2. Mean developmental stage of tadpoles in the control (blue bar), low-dose PBDE (red bar), medium-dose PBDE (green bar) and high-dose PBDE (purple bar) groups on days 0, 7, 14, and 21 of the exposure period. Error bars indicate standard error. * indicate significant differences from the control group within the specific time period. n = four on day 0, eight on days 7 and 14, and 17-24 on day 21.

Mass and hind limb length.

There were significant differences in tadpole mass on days 7, 14 and 21 (Fig. 3, ANOVA, $P < 0.001$ for each time period). Specifically, tadpoles in the high-dose group had significantly lower masses than control tadpoles on days 7, 14, and 21. In contrast, the low-dose group showed a significant increase in mass when compared to the control on day 21. There were also significant differences in tadpole hind limb length on days 14 and 21 (Fig. 4, ANOVA, $P < 0.005$ on day 14, $P < 0.001$ on day 21) in the high-dose group relative to the control.

Gene expression.

At the conclusion of the exposure, no significant differences in the expression of any of the genes measured were noted in tail tissue (data not shown, $P > 0.05$ for each gene). However, analysis of brain tissue revealed significant differences in the expression of BTEB (Fig. 4A, $P < 0.0005$), TSHß (Fig. 4B, $P < 0.005$), DI3 (Fig. 4C, $P < 0.0007$), TRα (Fig. 4D, $P < 0.0025$) and TRß (Fig. 4D, $P < 0.0003$). For each of these genes, tadpoles in both the medium- and high-dose groups had significantly lower expression relative to the controls. Significant differences in DI2 expression in the brain were also noted (Fig. 4C, $P < 0.0002$) with tadpoles in each of the PBDE exposure groups experiencing significant decreases in expression compared to the controls. There were no significant differences in brain DI1 expression (Fig. 4C, $P = 0.16$).

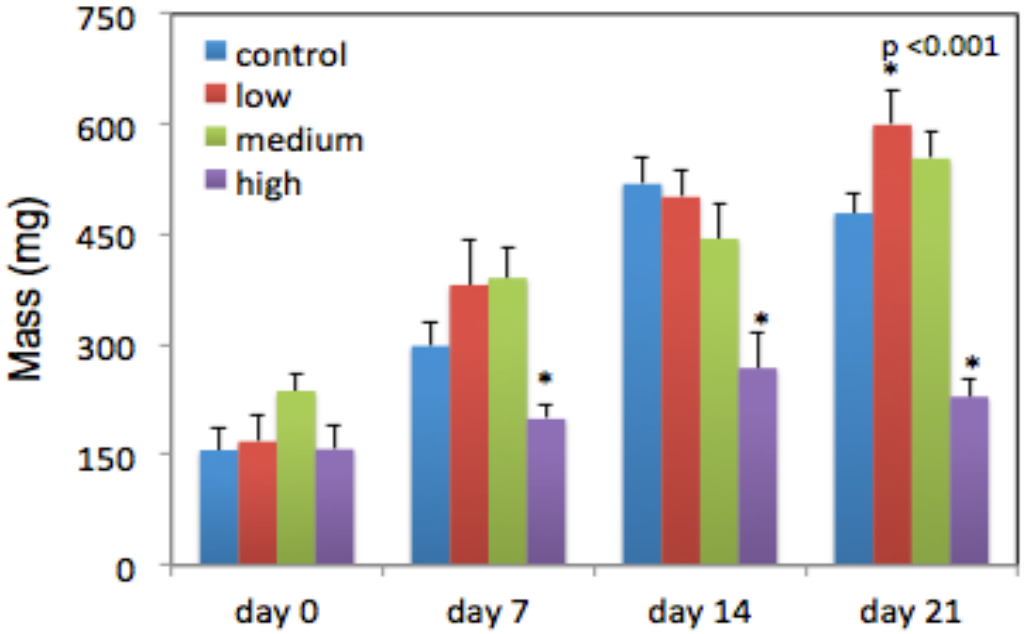

Figure 3. Mass of tadpoles exposed to 0.05mg of PBDE-47, 0.5mg of PBDE-47, and 5mg of PBDE-47 compared to the control on day 0, 7, 14, and 21. Error bars indicate standard error. p-value<0.001. n = 4 on day 0, 8 on days 7 and 14, and 17-24 on day 21.

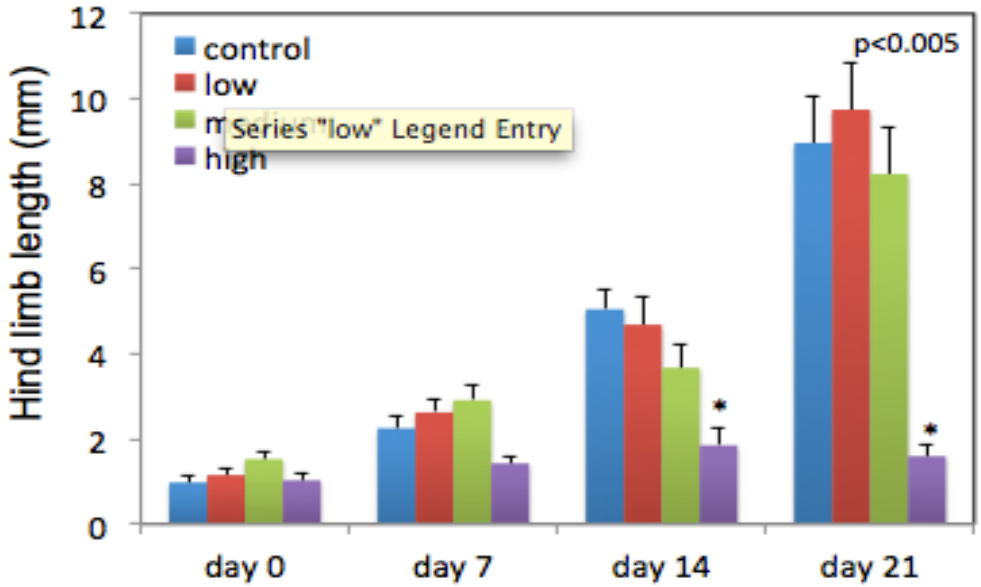

Figure 4. Hind limb length of tadpoles exposed to 0.05mg of PBDE-47, 0.5mg of PBDE-47, and 5mg of PBDE-47 compared to the control on day 0, 7, 14, and 21. Error bars indicate standard error. p-value<0.001. n = 4 on day 0, 8 on days 7 and 14, and 17-24 on day 21.

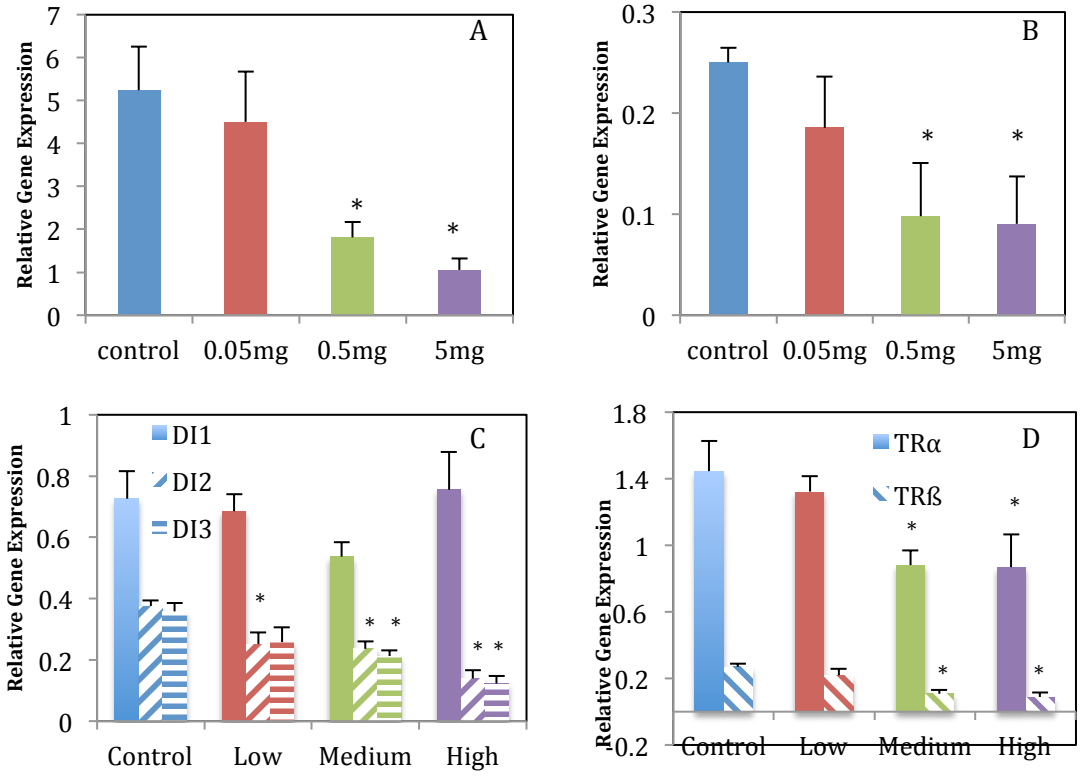

Figure 5. Relative gene expression of (a) BTEB, (b) TSHß), (c) Deiodinase 1, 2, and 3, and (d) thyroid receptors α and ß on day 21 of tadpoles exposed to 0.05mg of PBDE-47, 0.5mg of PBDE-47, and 5mg of PBDE-47 compared to unexposed controls. *n*=6. Error bars indicate standard error. * indicates statistical significance with p-value<0.05.

DISCUSSION

The goal of this study was to determine whether exposure to PBDE-47 alters the growth and metamorphosis of African clawed frog tadpoles and/or induces alterations in the expression of thyroid hormone related genes. The decrease in stage, mass, and the expression multiple thyroid related genes in the high dose group, and decreases in gene expression in the medium group, indicates that PBDE-47 exposures alter the thyroid signaling system and leads to adverse effects at the whole-organism level.

Developmental stage, mass, and hind limb length

The mean developmental stage of *Xenopus* tadpoles in the high dose group was significantly decreased on days 7, 14, and 21 relative to the controls suggesting that

metamorphosis was inhibited due to PBDE-47 exposure. Similarly, Balch et al. (2006) found a delay in metamorphosis after exposing *Xenopus laevis* tadpoles to PBDE-71 through intraperitoneal injections with body burdens over 100 times greater than the high-dose group of the present study. PBDE-71 is less able to move into adipose tissue, which may explain the differences in body burden; however, both have been shown to inhibit metamorphosis in *Xenopus* tadpoles. For amphibians, a decrease in the progression through metamorphosis, such as that observed in the current study, suggests an inhibition of T3 activity due to a decrease in circulating T3 levels or the inability for T3 to bind to its receptors (Miyata and Ose, 2012). Regardless of the mechanism, this data shows that PBDE-47 is capable of inhibiting metamorphosis in *Xenopus* tadpoles. Since PBDEs have been found in aquatic systems (Rayne et al., 2003), these compounds could be contributing to the global loss of amphibian populations due to hindered growth and the inability to reach reproductive age from PBDE exposure.

The hind limb growth is a hallmark event in metamorphosis. Here, there was a significant decrease in limb length among tadpoles in the high dose group on days 14 and 21 relative to the controls. This is consistent with the staging data, as a decrease in limb length is typically associated with inhibited metamorphosis.

The masses of tadpoles exposed to the high dose group exhibited a significant decrease on days 7, 14, and 21. This demonstrates the ability of high doses of PBDE-47 to inhibit growth as well as metamorphosis. The low dose group had a significant increase in mass at day 21 relative to the controls, suggesting that low levels of PBDE-47 may promote growth. During amphibian metamorphosis, thyroid hormones promote metamorphosis, rather than promoting overall growth (Krollos, 1961). Therefore, it

would be expected that tadpoles with hindered metamorphosis, such as those in the high dose group, would continue to grow. However, this was not observed in the present study suggesting that the reduction in mass in the high dose group may be attributed to the effect of PBDE-47 on something other than the thyroid-signaling system. This reduction in mass could be due to the alterations in growth hormone or growth factors, but more studies would be needed to assess this hypothesis. An alternative explanation could be that the highest dose of PBDE-47 caused a significant amount of stress in the organisms to the point that their bodies were struggling to perform homeostatic processes and did not have enough energy or resources to undergo metamorphosis or growth. This reduction in growth due to increased stress has been seen in fish (McCormick et al., 1998), rats (Marti et al., 1993), and amphibians (Crespi and Denver, 2005) with associated alterations in stress-related hormones, such as cortisol. Due to these possible factors, PBDE-47 reduced growth and mass of *Xenopus* tadpoles.

Deiodinase gene expression

Deiodinases are a group of enzymes that determine the amount of cytoplasmic T3 in a system and aim to modulate nuclear T3 concentrations and thyroid hormone saturation (Bianco and Kim, 2006). These enzymes cleave T4 as it enters the cell and either activates it into T3 or deactivates it into reverse T3 (rT3) or T2. (Fig. 6).

Deiodinase 1 (DI1) can either activate or inactivate T4 (Fig. 6) based on how much T3 is in the system (Moreno et al., 1994). DI1 activity is induced by T3, so if there were a decrease in T3 levels, DI1 activity would be reduced. There were no changes in gene expression in DI1 for all groups tested. However, this is mostly likely due to DI1 being primarily expressed in the liver and kidney. DI1 is not typically expressed in the

brain, so this may explain why alterations in its expression were not observed. Chen et al. (2010) also did not find alterations in whole-body DI1 expression after dietary exposure to PBDE-47 in larval zebrafish.

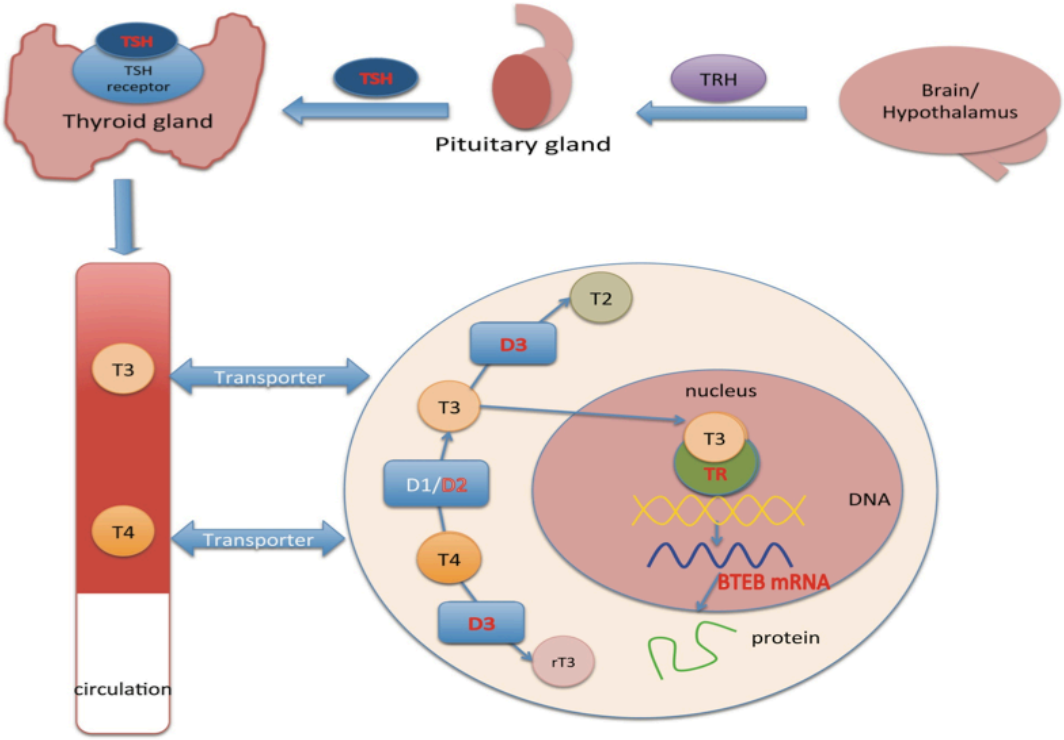

Figure 6. Graphic depiction of the thyroid hormone signaling system. Red letters indicate down-regulated mRNA transcripts. White letters indicate unaltered transcripts. Black letters indicate unmeasured transcripts. Thyroid regulating hormone (TRH), thyroid stimulating hormone (TSH), T2 (diiodothyronine), T3 (triiodothyronine), T4 (thyroxine), rT3 (reverse triodothyronine), D1 (deiodinase 1), D2 (deiodinase 2), D3 (diodinase 3), BTEB (basic transcription element-binding protein).

Deiodinase 2 (DI2) converts T4 into the more active thyroid hormone T3 (Fig. 6) and is expressed in various tissues including the brain (Guadano-Ferraz et el., 1997). DI2 expression is repressed by T3 and stimulated by TSH. There were decreases in DI2 expression in all of the exposed groups. This decrease in DI2 expression could be caused by the agonistic activity of PBDE-47. The exact mechanism by which PBDEs alter thyroid function is unknown; however, evidence from this study is consistent with the hypothesis that PBDE acts as a thyroid hormone agonist. Another source of the decrease

could be due to the reduction in TSHß expression; since TSH stimulates DI2 expression, a reduction in TSH could cause a reduction in DI2 expression. However, the reduction of TSHß transcripts is most likely due to the agonistic activity of PBDE-47 as well, indicating that PBDE-47 is altering multiple genes in a similar way that T3 does.

Deiodinase 3 (DI3) converts T3 and T4 into their inactive forms, T2 and rT3 respectively (Fig. 6). DI3 is also expressed in the brain, and its expression is induced by T3. DI3 expression showed significant decreases in the medium and high dose groups, which would be indicative of reduced T3 levels in the system. This down-regulation could be attributed to the decrease in TRα expression. TRα mediates up-regulation of DI3 in the presence of T3, but since TRα was down-regulated, TRα could not initiate the increased expression of DI3 as it normally would. This caused the decrease of DI3 expression seen in the medium and high dose groups.

BTEB and TSHß gene expression

The high and medium dose groups exhibited a significant reduction in BTEB gene expression. BTEB expression is directly regulated by the amount of T3 in the system (Denver et al., 1999). Therefore, a decrease in BTEB expression reflects a decrease in T3. This decrease in T3 could explain the reduction in metamorphosis seen in the high dose group. The medium dose group is being affected on the RNA level, but it has not caused a phenotypic change at that amount of PBDE exposure. Lema et al. (2008) also found a decrease in BTEB expression in the brain of male fathead minnows exposed to dietary PBDE-47, consistent with the present findings.

TSHß was significant decreased in the medium and high dose groups as well. T3 inhibits TSHß transcription, and the agonistic activity of PBDE-47 could have directly

caused this decrease in TSHß expression. A decrease in TSH levels in the system would cause a decrease in T4 production, reducing the overall amount of thyroid hormone in the body. This reduction could cause the inhibition of metamorphosis seen in the high dose group. The medium dose group saw decreases in TSHß as well, but the changes in gene expression were not yet great enough to cause an alteration in metamorphosis on the organismal level. A similar study done by Hallgren and Darnerud (2002) exposed rats to PBDE-47 and did not find any alterations in serum TSH; however, they did not investigate the mRNA expression of the TSH subunits. Chen et al. did not find alterations in TSHß after exposing zebrafish to PBDE-47; this may be due to the smaller doses of PBDE-47 administered to the fish compared to this study.

Thyroid receptor gene expression

Thyroid receptor expression is extremely variable and depends on the amount of substrate in the system. A study by Barca-Mayo et al. (2011) showed that normal physiological levels of T3 increased TRα and TRß mRNA transcripts, but an increase from the normal levels induced a decrease in mRNA transcripts for both receptors. This indicates that if there is a high amount of T3, the expression of thyroid receptors will decrease. A reduction in both TRα and TRß were found in the medium and high dose groups, which could have been caused by the high levels of PBDE-47. Since PBDE-47 has a similar structure to T3, the excessive amount of PBDE-47 could have induced this decrease in the expression of the receptors. A study performed by Lema et al. (2008) found decreased T4 levels in the serum and a decrease in TRß in the brain of male and female fathead minnows after dietary exposure to PBDE-47, which are consistent with the results found in the present study. However, Lema et al. (2008) also found an increase

in TRα in female brains, which is inconsistent with the present results possibly due to sex-specific gene expression, which was not possible to investigate in *Xenopus* tadpoles.

CONCLUSION

Overall, this data supports the hypothesis that PBDE-47 can inhibit metamorphosis and growth through alterations in thyroid related gene expression. Hallgren and Darnerud (2002) found a decrease in TH levels after PBDE-47 exposure and attributed this decrease to the binding of PBDE-47 to the TH transport protein transthyretin. If PBDE-47 is binding to transthyretin and preventing T3 from binding to it, then that would cause a decrease in T3 in the system due to the instability and degradation of T3. This decrease in T3 would then cause a decrease in BTEB expression, as seen in this study. However, after binding transthyretin, PBDE-47 may enter cells and act as a T3-agonist. This would explain the decreases seen in TSHß, DI2, TRα, and TRß, since high levels of T3 cause decreases in these genes. DI3 expression was decreased primarily due to the decrease in TRα expression, which DI3 uses as its T3 receptor. This data indicates that PBDE-47 may have thyroid-hormone agonistic activity upon certain genes, which prevents T3 from promoting metamorphosis in developing tadpoles, leading to a decrease in metamorphosis. With this inhibition of metamorphosis, PBDEs in aquatic systems may be antagonizing the global decline of amphibian populations due to the inability for these organisms to reach reproductive maturation. Further research should address the molecular function that PBDE-47 plays in the body to determine the true mechanism of its thyroid-disrupting abilities as well as potential effects on the amphibian reproductive system.

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