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Prenatal transfer of decabromodiphenyl ether (BDE-209) results in disruption of the thyroid system and developmental toxicity in zebrafish offspring

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ABSTRACT

Decabromodiphenyl ether (BDE-209) was one of most widely-used polybrominated diphenyl ether (PBDE) flame retardants and is frequently detected in both abiotic and biotic samples from environment. However, knowledge of its transgenerational risks is limited. Here, 4-month-old zebrafish were exposed to various concentrations of BDE-209 (0, 3, 30 or 300 μg/L) for 28 days and spawned in clean water without BDE-209. Concentrations of triiodothyronine (T3) and thyroxine (T4) as well as expressions of genes involved in the hypothalamic–pituitary–thyroid (HPT) axis were measured in offspring after exposure of adult zebrafish to BDE-209. BDE-209 was accumulated in adult fish and F1 eggs, which suggests transfer of this compound from adult fish to their offspring. Exposure of BDE-209 to parents resulted in developmental abnormalities in offspring and a significant decrease in T4 concentrations in F1 larvae 120 h post-fertilization (hpf). Furthermore, expressions of several genes involved in the HPT axis were also altered. Expressions of thyroid hormone receptor α (*tr-α*), thyrotropin releasing hormone (*trh*), thyroid stimulating hormone β (*tsh-β*) and deiodinase 1 (*dio 1*) were significantly down-regulated in F1 individuals, while expressions of thyroid stimulating hormone receptor (*tshr*) and transthyretin (*ttr*) were significantly up-regulated. These results suggest that exposure of parent zebrafish to BDE-209 can cause developmental toxicity in offspring and disruption of the thyroid endocrine system of offspring.

1. Introduction

2,2′,3,3′,4,4′,5,5′,6,6′-Decabromodiphenyl ether (BDE-209) is the predominant component of commercial mixtures of polybrominated diphenyl ethers (PBDEs) that are used as additive flame retardants in a number of products (e.g. textiles, electronics, furniture and plastics) (de Wit, 2002; Hardy, 2002; Zou et al., 2007). BDE-209 has been phased out of use by most manufacturers and its use was recently banned in the manufacture of products sold within the European Union and North America (Munoz-Arnanz et al., 2011). However, BDE-209 and related metabolites are still detected in the environment, and especially in developing countries such as China (Li et al., 2016). For example, 7340 ng/g of BDE-209 has been detected in river sediments while 65 ng/L BDE-209 has been detected in the waters of the Pearl River (Guan et al., 2007). Furthermore, BDE-209 concentrations in fish within rivers near the southern Chinese town of Guiyu have been reported as high as 28 μg/g wet weight (Luo et al., 2007). Thus, considering the bioaccumulation of BDE-209 in food chains, further studies are needed to evaluate the potential for environmental health risks.

Some PBDE congeners have molecular structures that resemble thyroid hormones (THs) and BDE-209 has been demonstrated to disorder the dynamic nature of the thyroid endocrine system in fishes and mammals (Yu et al., 2015). For example, histological abnormalities of the thyroid gland and alterations of concentrations of triiodothyronine (T3) and thyroid-stimulating hormone (TSH) in the blood plasma were observed in Sprague-Dawley rats that were exposed to BDE-209 (Lee

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et al., 2010). Waterborne exposure to BDE-209 that was accumulated into, and biotransformed by larvae of zebrafish, resulted in disruption of their thyroid endocrine systems and the presence of developmental abnormalities during early life stages (Chen et al., 2012b). Similarly, concentrations of total T3 and T4 in the blood plasma of fathead minnows (*Pimephales promelis*) were significantly lower after exposure to BDE-209 in their diet (Noyes et al., 2013). Recently, BDE-209 has been detected in human milk, semen, cord blood and even fetuses (Liu et al., 2012; Sudaryanto et al., 2008; Xu et al., 2013; Zhao et al., 2013), which suggests that BDE-209 has the potential to be transferred to offspring. However, it has remained unclear whether this vertical transfer of BDE-209 results in thyroid endocrine dysfunction and further induces developmental toxicity in progeny.

Previous studies have confirmed that some PBDEs could be transferred to the eggs of fish after exposure of adult females (Nyholm et al., 2008), which resulted in adverse effects during embryogenesis (Ostrach et al., 2008). Exposure to environmentally relevant concentrations of DE-71 for 150 days resulted in PBDE accumulation in adult zebrafish and their eggs, and the induction of developmental neurotoxicity in F1 individuals (Chen et al., 2012a). Long-term exposure to relatively small doses of DE-71 significantly altered production of T3 and T4, caused developmental toxicity of offspring as well as transcription of genes involved in HPT axis in F0 and F1 generations (Yu et al., 2011). Here, zebrafish were used as a model to assess potential *trans*-generational toxicity and disruption of the thyroid system in F1 individuals after exposure of adult fish to BDE-209. It was hypothesized that exposure of adults to BDE-209 would result in accumulation in F0-generation fish, and the subsequent transfer of the compound and its adverse effects to the thyroid system and development of F1 larvae.

2. Materials and methods

2.1. Reagents or chemicals

2,2′,3,3′,4,4′,5,5′,6,6′-Decabromodiphenyl ether (BDE–209 > 98% purity) was purchased from Wellington Laboratories (ON, Canada). Dimethyl sulfoxide (DMSO) and 3-aminobenzoic acid ethyl ester methanesulfonate salt (MS-222) were obtained from Sigma (St. Louis, MO, USA). SYBR Green kits, TRIzol reagent, and reverse transcription reagents were obtained from Takara (Dalian, Liaoning, China). Triiodothyronine (T3) and thyroxine (T4) enzyme immunoassay (EIA) kits were obtained from Wuhan EIAab Science Co. Ltd. (Wuhan, China). All other reagents (e.g., ethyl alcohol, isopropanol, chloroform, acetone and isooctane) that were used in this study were analytical grade.

2.2. Zebrafish maintenance and BDE-209 exposure

Stock solutions of BDE-209 were prepared in dimethyl sulfoxide (DMSO). Four-month-old mature zebrafish (wild type) were cultured at 28 ± 0.5 °C on a 12:12 light/dark cycle in carbon-filtered water, using previously described methods (Dang et al., 2015). Fish were exposed to one of four concentrations of BDE-209 (0, 3, 30, or 300 μg/L; corresponding to 0, 3.12, 31.2 and 312 nM). Prior to exposure, 80 females and 80 males were randomly divided into 16 tanks (15 L tanks containing 10 L of carbon-filtered water) and maintained for 2 weeks. Each tank contained either 10 male or 10 female fish, and there were 4 replicate tanks (2 male tanks and 2 female tanks) for each concentration group. Adult zebrafish were fed thrice daily with brine shrimp (*Artemia nauplii*) and 50% of the water in each tank was replaced daily with freshly carbon-filtered water including the appropriate concentrations of BDE-209. The final concentration of DMSO was 0.01% (v/v) in the control and treatment groups which corresponds to a level that has been confirmed to not affect zebrafish reproductive system (Han et al., 2013). During exposure, adult zebrafish mortality was recorded. After 28 days of exposure, male and female fish were paired and placed in clean water (without PBDEs), and embryos were collected. A subset of the collected embryos were used to quantify BDE-209 in the F1 generation, and the remainder from each group were then transferred into culture dishes for evaluation of offspring developmental toxicity at 72 or 120 h post-fertilization (hpf). During the cultivation period, dead larvae were removed from the culture dishes and the embryonic culture solution was renewed daily. Endpoints of developmental toxicity at 120 hpf included survival rate, body length and malformation rate. Heart rate and hatching rate were recorded at 72 hpf. Four replicate dishes were included for each concentration, and each dish consisted of 150 eggs. For the calculations of survival, hatching and malformation rates, all the 150 eggs were used, and for measurement of heart rates and body length, twenty larvae from each dish, a total of 80 larvae, were used. All studies were conducted in accordance with the guidelines for animal experimentation from the Institutional Animal Care and Use Committee (IACUC) of Nanjing University.

2.3. Quantification of BDE-209

Concentrations of BDE-209 were quantified in adult zebrafish (each group consisted of 3 females and 3 males) and their embryos (each treatment group consisted of 3 replicates, and each replicate included 100 eggs). Quantification of BDE-209 concentrations in egg and adult fish was conducted as described previously (Zhu et al., 2014) with slight modifications. The overall protocol consisted of sample extraction, cleaning, analysis and quality assurance and quality control (QA/QC). First, eggs and adult zebrafish from each group were weighed and then thoroughly homogenized in a 1:1 mixture of acetone: isooctane and ultrasonic extraction for 120 mins, followed by drying under a nitrogen atmosphere at room temperature. 4 ng of ^{13}C BDE-209 (as an internal standard) was added to each treated sample. Second, extracts were dissolved in 1 mL isooctane, filtered with a 0.2 μm nylon mesh filter, and dried under a nitrogen atmosphere in an auto sampler vial. ^{13}C12-PCB-208 (4 ng) was added as the internal standard before sample analysis. Finally, quantification of BDE-209 was conducted via gas chromatography-mass spectrometry using a 6890A/5975C Gas Chromatograph–Mass Spectrometer (GC/MS) (Agilent Technologies, Santa Clara, CA, USA). A DB-5HT fused silica capillary column (15 m × 0.25 mm i.d., 0.1 μm film thickness; J & W Scientific) was used as the analytical column. Recoveries of ^{13}C BDE-209 were in the range of 80–120%. The limit of detection (LOD) was calculated as three-times the standard deviation (SD) from six runs that were conducted for ongoing precision and recovery. The LOD was defined as a signal: noise (S/N) ratio of 3 and, on average, was 0.5 ng for BDE-209. Samples with BDE-209 concentrations less than the LOD were considered to have no detectable BDE-209.

2.4. Measurement of thyroid hormones in offspring

Thyroid hormone measurements were conducted using Uscnlife EIA kits as previously described (Yu et al., 2010). Briefly, 120-hpf larval samples were homogenized in 0.4 mL ELISA buffer. Each sample was completely disrupted by intermittent ultrasonic oscillation for 5 min on ice, and was then continuously vortexed for 10 min. Samples were centrifuged at 5000 × *g* for 10 min at 4 °C and supernatants were collected and stored at −80 °C for measurement of T3 and T4. Detection limit of T3 and T4 were 123.5 pg/mL and 1.46 ng/mL, respectively. In this study, two hundred larvae were pooled to produce one replicate for the measurement of thyroid hormones, and three replicates were included for each concentration. In zebrafish, deiodinases play a key role in converting T4 to T3 and thus the ratio of T3 to T4 indirectly reflects deiodinase activity.

2.5. Quantitative real-time polymerase chain reaction (qRT-PCR)

qRT-PCR was performed following the guidelines of minimum information for publication of quantitative real-time PCR experiment

(MIQE) guidelines (Bustin et al., 2009). In this study, expressions of genes along the HPT axis of zebrafish, including corticotropin releasing hormone (*crh*), thyrotropin releasing hormone (*trh*), thyriod-stimulating hormone β (*tsh-β*), thyrotropin-releasing hormone receptor (*trhr*), thyriod-stimulating hormone receptor (*tshr*), sodium iodide symporter (*nis*), transthyretin (*ttr*), thyroglobulin (*tg*), thyroid hormone receptor-α (*tr-α*), thyroid hormone receptor-β (*tr-β*), deiodinase 1 (*dio 1*) and deiodinase 2 (*dio 2*), were examined. Twenty larvae per sample were homogenized for isolation of total RNA using the TRIzol reagent according to manufacturer's instructions. RNA concentrations were measured using a NanoDrop 2000 (Thermo Fisher Scientific, USA) and RNA purity was verified by determining the A260/A280 ratio and confirmed by using agarose-formaldehyde gel electrophoresis with ethidium bromide staining. RNA integrities (RIN) were ≥8.0 and purities of RNA were between 1.8 and 2.0 (260 nm/280 nm ratio), indicating high purity. For each sample, 500 ng of total RNA was reverse transcribed to first-strand cDNA using the PrimeScript™ RT reagent kit (Takara, Dalian, Liaoning, China). qRT-PCR was performed with SYBR Green Premix ExTaq^{II} kits (Takara, Dalian, Liaoning, China) following manufacturer instructions. Primer sequences of genes along the HPT axis of zebrafish were obtained by using the NCBI primer designing tool (http://www.ncbi.nlm.nih.gov/tools/primer) and melting curves were performed to verify primer specificity in each assay. A list of primers is provided in Table 1 and the ribosomal protein L8 (*rpl8*) gene was used as an internal reference (Chen et al., 2012b). Thermal cycling was done at 95 °C for 5 min, followed by 40 cycles of 95 °C for 15 s and 60 °C for 1 min. Each group included 6 biological replicates and the relative gene expression compared to the control group was calculated using the $2^{-D\Delta CT}$ method (Livak and Schmittgen, 2001).

2.6. Statistical analyses

Statistical analyses were performed with the SPSS 18.0 (SPSS Inc., Chicago, IL, USA) for Windows. Normality and homogeneity of data were examined using the Kolmogorov–Smirnow and Levene's tests, respectively. Differences between the control and treatment groups were evaluated using a one-way analysis of variance (ANOVA) by Tukey's multiple range test (If necessary, data were log-transformed to meet parametric assumptions, and the differences were then evaluated by ANOVA) (Ankley et al., 2009). *P* values less than 0.05 were considered statistically significant. All data are expressed as the mean ± standard error (SEM).

Table 1
Sequences of primers for the genes tested.

Gene name	Sequence of the primer (5′-3′)	Accession number
rpl8	Forward: TTGTTGGTGTTGTTGCTGGT Reverse: GGATGCTCAACAGGGTTCAT	NM_200713
crh	Forward: TACGCACAGATTCTCCTCGC Reverse: GGGGAGAAGTCGGGTTTCTG	NM_001007379
trh	Forward: CCCCAACCTCAGAACCAGTC Reverse: TGCTTCCAGAACAGACCGAC	NM_001012365
tshβ	Forward: AATGAAGGTTGCCGTGCCTA Reverse: AGGATCTGCATGTGAAGGGC	AY135147.1
trhr	Forward: ACTGTCCTCTACGGCCTCAT Reverse: TTCCTCCTTTGTGTCCGACG	NM_001114688
tshr	Forward: ACTGTCCTCTACGGCCTCAT Reverse: TTCCTCCTTTGTGTCCGACG	NM_001145763
nis	Forward: GCCACAGATTTCTGACACGC Reverse: AAGACTGGAACAGCCCGATG	NM_001089391
ttr	Forward: CTCCTGGTGTGTATCGGGTG Reverse: AGGATGTCAGTCATGTGCCTT	BC081488
tg	Forward: ACACGCGTGCAAAAACTCTC Reverse: CAAAGCTGAGCCTCCTGGAA	NM_001329865.1
trα	Forward: TGAAGGTGGAGTGTCCAACAG Reverse: AATGGATGTGGTTTGGAGGTGC	NM_131396
trβ	Forward: CACATGCTGTGTTGCAGCTT Reverse: TCATAAGAGCCAGAGCCCCT	NM_131340
dio1	Forward: CTGGACCGACAGAAGACGAG Reverse: TGCGACATTGCTGAAGTCCT	BC076008
dio2	Forward: CTCGGACACTTGGCTTGACT Reverse: TTGGATCAGGACGGAGAGGT	NM_212789

3. Results

3.1. Adult fish survival

No mortality was observed in F0-generation fish exposed to 0, 3, 30 or 300 μg/L BDE-209 after 28 days of exposure.

3.2. Developmental toxicity in offspring

Exposure of adults to BDE-209 caused significant effects on development of offspring (Fig. 1). Larval survival (71.13 ± 3.36%, $P = 0.023$) and hatching rates (64.53 ± 3.01%, $P = 0.047$) were significantly lower from adults that were exposed to 300 μg/L BDE-209 compared to the control group (Fig. 1A and B). Rates of malformations of offspring (8.17 ± 2.78%, $P = 0.040$) (malformation including yolk sac or pericardial edema and spinal curvature) were greater in larvae from adults exposed to the greatest concentration group (Fig. 1D). Heart rates of offspring were not different between any of the treatment groups and that of the control (Fig. 1C). Compared with control group, exposure of adults to 30 or 300 μg/L BDE-209 significantly inhibited body length development in larvae (Fig. 1E).

3.3. Bioaccumulation and transfer of BDE-209

Exposure to BDE-209 resulted in a dose-dependent bioaccumulation in adult fish (Table 2). No BDE-209 was detected in the female and male in the control group. BDE-209 concentrations were 0.09, 0.31 and 1.02 μg/g (wet weight) in female fish exposed to 3, 30 or 300 μg BDE-209/L, respectively. In male fish, BDE-209 concentrations were 0.17, 0.39 and 0.81 μg/g (wet weight) when exposed to 3, 30 or 300 μg/L, respectively. BDE-209 concentrations in eggs were 0.15, 0.65 and 2.13 μg/g when adult fish were exposed to 3, 30, or 300 μg/L BDE-209, respectively.

3.4. Concentrations of thyroid hormones in offspring

Exposure of adults to BDE-209 resulted in significantly different concentrations of thyroid hormones in F1-generation larvae (120 hpf) (Fig. 2). Compared to the control group (385.65 ng/g), whole-body concentrations of T4 were significantly lower in F1-generation larvae derived from adults exposed to 300 μg/L BDE-209. Concentrations of T3 were not significantly different in larvae derived from adults exposed to any concentration of BDE-209 compared with the control group. T3 to T4 concentration ratios were significantly greater ($P = 0.037$) in larvae derived from adults exposed to the greatest concentration of BDE-209 compared to the control group.

3.5. Transcriptional responses in offspring

Only expression of *tr-α* (0.87-fold, $P = 0.015$) in F1 larvae (120 hpf) was significantly lower than that of controls when adults were exposed to 3 μg/L BDE-209. Relative to that of controls, exposure of adults to 30 μg BDE-209/L significantly up-regulated expressions of *tshr* (1.98-fold, $P = 0.003$) and *ttr* (1.57-fold, $P = 0.001$) and down-regulated expression of *tr-α* (0.84-fold, $P = 0.004$) in larvae. Expressions of *trh* (0.86-fold, $P = 0.046$), *tsh-β* (0.70-fold, $P = 0.042$), *tr-α* (0.75-fold, $P = 0.001$) and *dio1* (0.62-fold, $P = 0.018$) were significantly lower in offspring derived from adults exposed to 300 μg BDE-209/L, while the expressions of gene *tshr* (1.92-fold, $P = 0.003$) and *ttr* (1.36-fold, $P = 0.045$) were significantly increased after parental exposure to 300 μg BDE-209/L compared to the control group (Fig. 3).

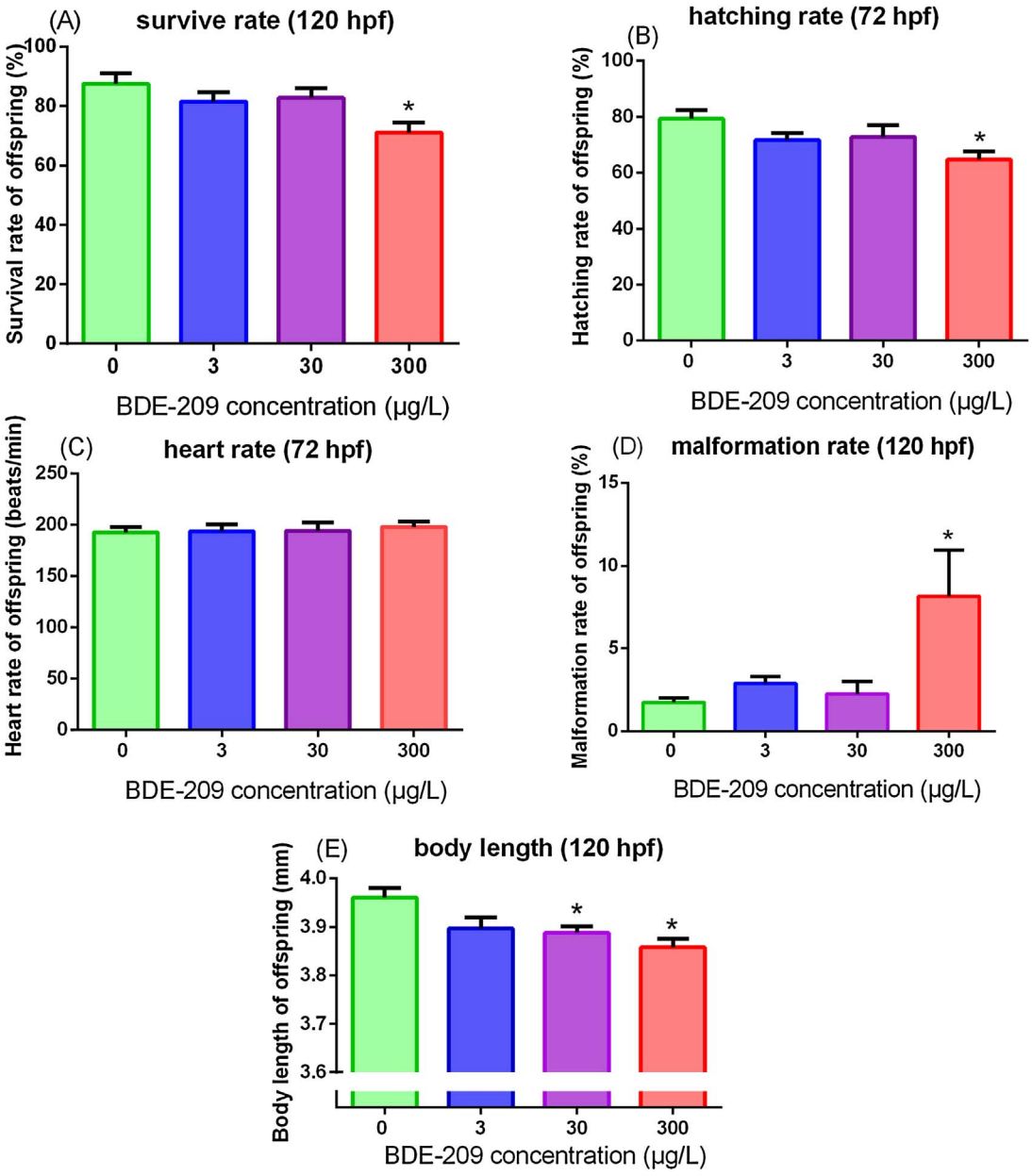

Fig. 1. Developmental endpoints of offspring larvae derived from adult zebrafish exposed to BDE-209 (0, 3, 30, 300 μg/L) for 28 days. (A) survival rate; (B) hatching rate (C) heart rate; (D) malformation rate; (E) body length; Values represent mean ± SEM. Significant differences from controls are indicated by $^{*}P < 0.05$.

Table 2
Bioaccumulation of BDE-209 was measured in F0 adult zebrafish after exposure to BDE-209 for 28 days and their F1 eggs derived from adult zebrafish exposed to BDE-209.

Nominal concentration of BDE-209	Measured concentration of BDE-209		
	Egg (μg/g)	Adult Female fish (μg/g wet weight)	Adult Male fish (μg/g wet weight)
0 μg/L	ND	ND	ND
3 μg/L	0.15 ± 0.07	0.09 ± 0.04	0.17 ± 0.03
30 μg/L	0.65 ± 0.20	0.31 ± 0.07	0.39 ± 0.12
300 μg/L	2.13 ± 0.68	1.02 ± 0.19	0.81 ± 0.11

The values represent mean ± standard error (SEM) of 3 replicate.
ND: not detectable.

4. Discussion

Our results demonstrated that BDE-209 can be transferred to offspring after exposure to adult zebrafish. Several studies have reported that some PBDEs, including DE-71, BDE-28, and BDE-183 could be transferred to offspring via waterborne exposure of adults (Nyholm et al., 2008; Ostrach et al., 2008; Yu et al., 2011). Results are consistent with these findings, where BDE-209 was measured in eggs after exposure of adults to BDE-209 for 28 days. Moreover, concentrations of BDE-209 in offspring were greater than that of their exposed parents, which could be potentially due to differences in the lipid composition of eggs and whole bodies (Tocher, 2003). Collectively, our results indicate that BDE-209 can accumulate in offspring after waterborne exposure of adult fish.

Exposure of adults to BDE-209 resulted in adverse effects in the development of offspring including reduced survival and hatching rates, decreased body length, and elevated malformation rates. Developmental endpoints in offspring, including survival rate, hatching rate, malformation rate and body length are essential parameters to evaluate the transgenerational effects of chemicals (Wang et al., 2015; Yu et al., 2017, 2011). Although the design of this study was not to

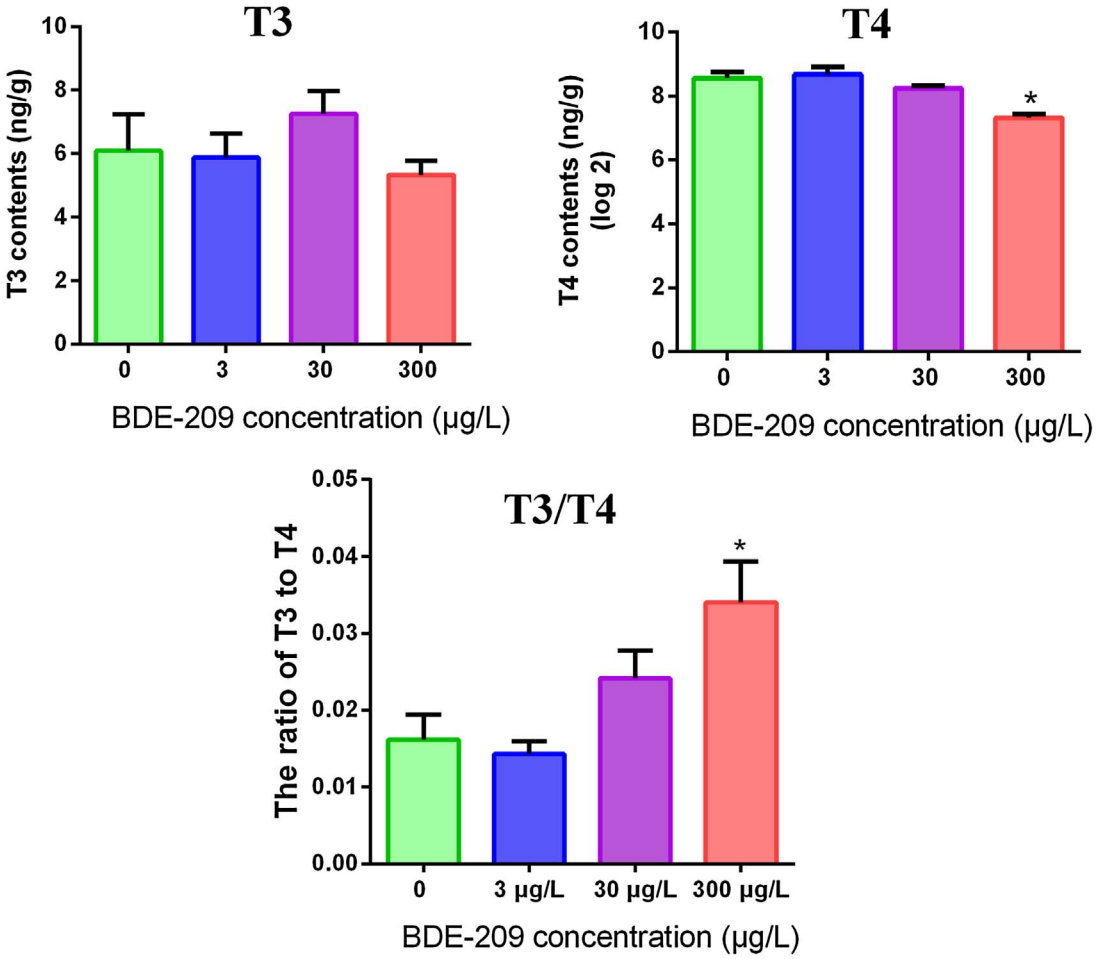

Fig. 2. Whole-body T3 and T4 levels in 120 hpf larvae derived from adult zebrafish exposed to BDE-209 (0, 3, 30, 300 μg/L) for 28 days. Values represent mean ± SEM of three replicate samples (T4 data are expressed as log 2–transformed units). Significant differences from controls are indicated by $^{*}P < 0.05$. Each concentration group contains 3 biological replicates.

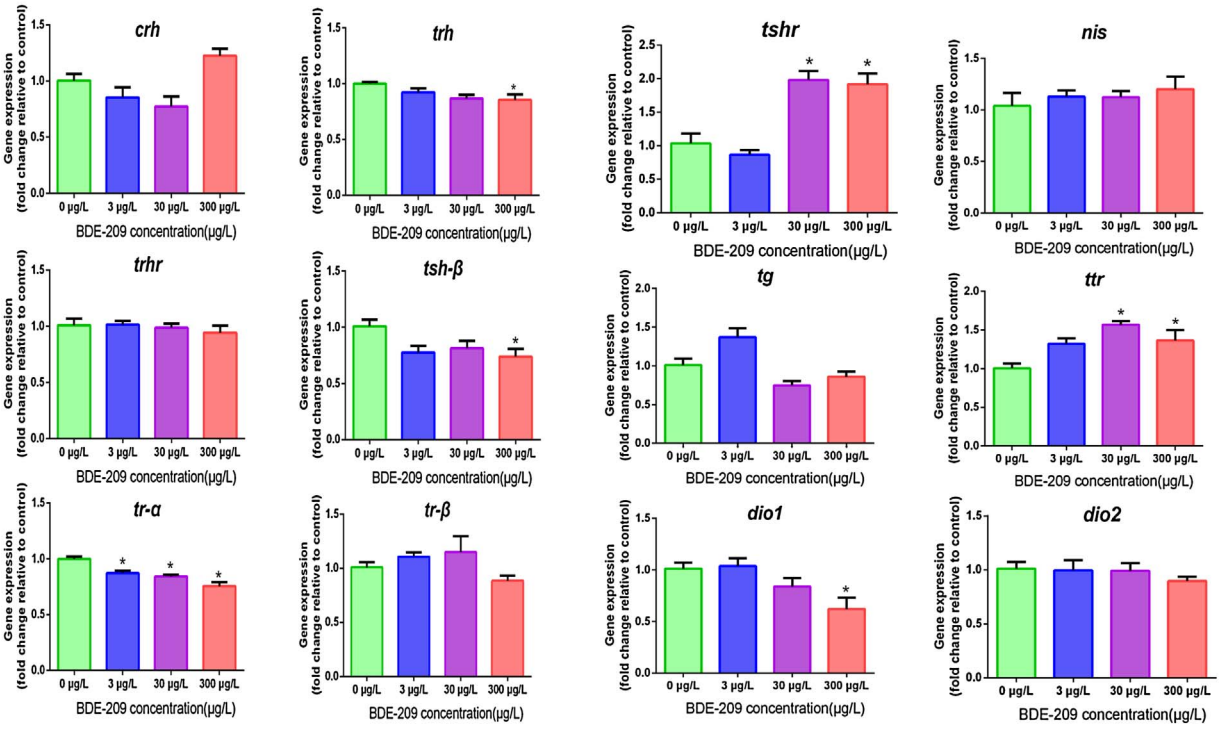

Fig. 3. Expression of genes involved in the HPT axis in offspring larvae (120 hpf) derived from adult zebrafish exposed to BDE-209 (0, 3, 30, 300 μg/L) for 28 days. Values represent mean ± SEM of six replicate samples. Significant differences from controls are indicated by $^{*}P < 0.05$. Each concentration group contains 6 biological replicates.

develop a detailed understanding of the mechanisms of toxicity, our results suggest that exposure of adults to BDE-209 results in significant developmental toxicity in offspring.

The significantly lower whole-body concentrations of T4 in F1 larvae of parents exposed to BDE-209 might be responsible for the observed developmental abnormalities in offspring. In zebrafish, T4 plays a crucial role in growth and development of larvae during the transition to larval phase (Liu and Chan, 2002). A previous study showed that parental exposure to tris(1,3-dichloro-2-propyl)phosphate (TDCIPP) for 3 months resulted in a significant decreased in the-whole-body T4 content of offspring and subsequent developmental toxicity, including decreased survival rates and elevated malformation rates (Wang et al., 2015). Our results are consistent with above study, where parental exposure to BDE-209 resulted in the reduction of whole-body T4 content and decreased body length of offspring. Although whole-body concentrations of T3 in larvae were not altered by the exposure of their parents to BDE-209, the increased T3/T4 ratios observed here indirectly suggests dysfunction of the thyroid endocrine system (Carr and Patino, 2011). Similarly, elevated T3/T4 ratios are associated with developmental abnormalities (decreased survival rate and body mass) in zebrafish larvae after direct waterborne exposure to BDE-209 (Chen et al., 2012b).

Expression of *tr-α* in offspring was significantly down-regulated in a dose-dependent manner after exposure of parents to BDE-209. Thyroid hormones (THs) in fish bind to corresponding thyroid receptors (TRs) in order to regulate transcription of target genes in the corresponding organs (Marchand et al., 2001). Therefore, the reduction of *tr-α* transcription in offspring might disorder response cascades of THs by affecting THs to bind TR-α. Thus, the developmental toxicity of offspring observed in the present study might be attributed to the transfer of BDE-209, which then causes disruption of T4 production and subsequent decreased transcription of *tr-α* in offspring. However, in a recent study, it was reported that significant increases in T3 content and up-regulation of *tr-α* were observed in zebrafish embryos/larvae that were exposed to BDE-209 (Chen et al., 2012b). The difference in expression of *tr-α* between the present study and the recent study might be due to different responses in T3 content, which were caused by different exposure concentrations and modes.

Down-regulation of *trh* and *tsh-β*, which are involved in the HPT axis, might be associated with reduced T4 content in offspring. In this study, parental exposure to BDE-209 resulted in significantly decreased whole-body T4 content, which was accompanied by down-regulation of *trh* and *tsh-β* in offspring. In vertebrates, Thyrotropin releasing hormone (TRH) stimulates thyroid stimulating hormone (TSH) secretion from the anterior pituitary and the TSH then activates TH synthesis and release from the thyroid gland. Thus, the down-regulation of *trh* and *tsh-β* might be responsible for the observed decrease in T4 content.

Up-regulation of *tshr* and *ttr* was considered as a negative feedback of transcriptional level for the reduction of T4 levels. The dynamic nature of THs in fishes is closely related to the regulation of HPT axis, which is responsible for maintaining homeostasis of the thyroid endocrine system through various feedback mechanisms coordinating biosynthesis, secretion, transport and the metabolism of THs (Chiamolera and Wondisford, 2009). In teleost fish, TSH synthesis and release is regulated by TSHR in thyrocytes. In addition, TTR plays an important function in TH transport via blood circulation and also regulates the supply of circulating THs to corresponding target organs (Power et al., 2001). Significant up-regulation of *ttr* in zebrafish larvae has been shown to accompany decreased T4 content after exposure to tetrabromobisphenol A (TBBPA) (Chan and Chan, 2012). Moreover, exposure to the model chemical metyrapone (MET) resulted in significant up-regulation of *tshr* expression and decreased T4 content in zebrafish larvae (Liu et al., 2013). Thus, it can be hypothesized that the increased transcription of *tshr* and *ttr* is a feedback mechanism that might result from decreased whole-body T4 content.

In summary, BDE-209 can be bioconcentrated in adult fish and subsequently transferred to offspring. The results here demonstrate that exposure of parent zebrafish to BDE-209 can result in dysfunction of the thyroid endocrine system and adverse developmental effects in offspring. Our findings emphasize the importance of considering transgenerational effects of chemicals, and particularly BDE-209, in environmental risk assessments. The mechanisms underlying the transgenerational effects of BDE-209 remain unknown, and thus further studies are needed to uncover the mystery.

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