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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