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TBBPA chronic exposure produces sex-specific neurobehavioral and social interaction changes in adult zebrafish

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ABSTRACT

The toxicity of tetrabromobisphenol A (TBBPA) has been extensively studied because of its high production volume. TBBPA is toxic to aquatic fish based on acute high concentration exposure tests, and few studies have assessed the behavioral effects of low concentration chronic TBBPA exposures in aquatic organisms. The present study defined the developmental and neurobehavioral effects associated with exposure of zebrafish to 0, 5 and 50 nM TBBPA during 1–120 days post-fertilization (dpf) following by detoxification for four months before the behaviors assessment. These low concentration TBBPA exposures were not associated with malformations and did not alter sex ratio, but resulted in reduced zebrafish body weight and length. Adult behavioral assays indicated that TBBPA exposed males had significantly higher average swim speeds and spent significantly more time in high speed darting mode and less time in medium cruising mode compared to control males. In an adult photomotor response assay, TBBPA exposure was associated with hyperactivity in male fish. Female zebrafish responses in these assays followed a similar trend, but the magnitude of TBBPA effects was generally smaller than in males. Social interaction evaluated using a mirror attack test showed that 50 nM TBBPA exposed males had heightened aggression. Females exposed to 50 nM TBBPA spent more time in the vicinity of the mirror, but did not show increased aggression toward the mirror compared to unexposed control fish. Overall, the hyperactivity and social behavior deficits ascribed here to chronic TBBPA exposure was most profound in males. Our findings indicate that TBBPA can cause developmental and neurobehavioral deficits, and may pose significant health risk to humans.

1. Introduction

Tetrabromobisphenol A (TBBPA) is used primarily for printed circuit boards, paper processing, the manufacture of textiles, and accounts for 60% of global brominated flame retardant (BFRs) use (Batterman et al., 2010; D'Hulst et al., 2009; Morris et al., 2004). TBBPA and its derivatives are introduced to the environment by the processes of production, use, and disposal (Morris et al., 2004). A wide range of TBBPA concentrations are found in aquatic environments. For example, TBBPA ranged from 38.97 to 672.64 ng L^{-1} (0.07–1.24 nM) in water samples collected from Bo Sea sites (Zhang et al., 2011), and from 850 to 4870 ng L^{-1} (1.56–8.96 nM) in water samples collected from Chaohu Lake, Anhui (industrial active site) (Liu et al., 2016). In mammals TBBPA is rapidly excreted, thus TBBPA levels measured in human serum samples are generally very low, suggesting that the potential health risk for the general human population may be low (Colnot et al., 2014).

In rodents, developmental TBBPA exposures cause various developmental, reproductive and endocrine toxicities (Cope et al., 2015; Lilienthal et al., 2008; Nakajima et al., 2009; Viberg and Eriksson, 2011; Williams and DeSesso, 2010). Earlier studies suggested that perinatal TBBPA exposure altered motor activity, but inconsistencies across the studies cast some doubt on the behavioral effects (Cope et al., 2015; Williams and DeSesso, 2010). One study in Wistar rats showed that parental TBBPA exposure affected the auditory response with a predominant cochlear effect in female pups and more apparent neural effects in male pups (Lilienthal et al., 2008). However, in mice, exposure to TBBPA during neonatal development of the brain did not affect behavior, learning, or memory in adult mice (Cope et al., 2015; Viberg and Eriksson, 2011). Yet, acute treatment of mice with TBBPA (5 mg/kg body weight) 3 h before an open-field test was associated with increased horizontal movement, freezing behavior, and spontaneous alternation in a Y-maze test (Nakajima et al., 2009). The species

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differences may reflect metabolic differences and the exposure/detoxification times used.

The zebrafish (*Danio rerio*) model has been used extensively to elucidate the mechanisms underlying behavioral toxicities (Belanger et al., 2013; Nishimura et al., 2015). In particular, the zebrafish was employed for identifying sex-specific effects on social interactions (Dahlbom, Backstrom et al. 2012; Weber et al., 2015). Under the aegis of environmental chemical hazard (such as PFOS and BPA), adult zebrafish have been used to detect chemical bioactivity effects on swim behavior, cognition and social interaction (Chen et al., 2013; Saili et al., 2012; Weber et al., 2015). The toxicity of TBBPA is well studied in zebrafish, including studies focused on interacting molecular pathways (De Wit et al., 2008; Hu et al., 2009), developmental toxicity (McCormick et al., 2010; Wu et al., 2015), reproduction toxicity (Kuiper et al., 2007) and endocrine axis perturbation (Chan and Chan, 2012). We previously reported that exposure to TBBPA during early life stage was associated with behavior alterations in larval zebrafish (Bai et al., 2013; Noyes et al., 2015). However, most of the previous studies focused on high exposure concentrations at the micromolar level, and mainly focused on TBBPA neurotoxicity in zebrafish larvae. Zebrafish studies yet to evaluate whether adult neurobehavioral and social impacts result from chronic exposure TBBPA exposure at environmental relevant concentrations. Here we used the zebrafish model to assess the behavioral effects of chronic (from development through adulthood) low concentrations TBBPA.

2. Materials and methods

2.1. Fish husbandry and embryo collection

Adult zebrafish of wild-type strain (AB) line were raised and maintained under standard laboratory conditions of 28 °C with a 14:10 light/dark photoperiod in a recirculation system according to standard zebrafish breeding protocols. Water supplied to the system was filtered by reverse osmosis (pH 7.0–7.5), and Instant Ocean® salt was added to the water to raise the conductivity to 450–1000 μS/cm (system water). The adult fish were fed twice daily with zebrafish diet (Zeigler, Aquatic Habitats, Apopka, Florida) and live artemia (Jiahong Feed Co., Tianjin, China). The use of zebrafish in this research protocol was approved by the Institutional Animal Care and Use Committee at the Wenzhou Medical University.

Zebrafish embryos were obtained from spawning adults in tanks overnight with a sex ratio of 1:1. Embryos were collected within 0.50 h of spawning and rinsed in an embryo medium (EM: 0.137 M NaCl, 5.4 mM KCl, 0.25 mM Na2HPO4, 0.44 mM KH2PO4, 1.3 mM CaCl2, 1.0 mM MgSO4 and 4.2 mM NaHCO3) (Westerfield, 1995). Fertilized embryos with normal morphology were staged under a dissecting microscope SMZ 1500 (Nikon, Japan) according to standard methods (Kimmel et al., 1995).

2.2. TBBPA stock solutions and exposure protocols

TBBPA (3,3′,5,5′-tetrabromobisphenol A, CAS#79-94-7, purity > 97%, Sigma) stock solution (0.05 and 0.5 mM) was prepared by dissolving in 100% dimethyl sulfoxide (DMSO) and stored at −20 °C. A working solution was prepared by dilution of the stock solution immediately prior to experimental use. A serial dilution of 100%DMSO was made that was 10,000 times more concentrated to create a dilution with a final DMSO concentration of 0.01%. High quality embryos at 8 h post-fertilization (hpf) were exposed to three treatment groups: DMSO vehicle control (0.01% v/v), TBBPA at 5 and 50 nM. The zebrafish were exposed during 1–120 days post-fertilization (dpf) and then maintained in fish water for four months. For embryo stage exposure, the embryos with intact chorions were randomly distributed into a Petri dish (100 mm) and exposed (100 embryos with 40 mL solution per treatment) for 5 days without medium change, and all embryos hatched and survived without malformation in this stage. At 5 dpf larvae were transferred into 2 L tanks until 21 dpf when 60 juveniles in each group were separated into three replicate tanks with a total of 20 fish in a 9 L tank until the end of the experiment at 9 months. Larval-adult (5–250 dpf) exposure solutions were renewed (95% of volume) every 3 days. Each tank was checked for dead fish on a daily basis and water quality was monitored on a weekly basis. Feeding was initiated at day 5 and larvae between 5 and 21 dpf were fed three times daily with larval diet (Aquatic Habitats, Inc.; USA) and after 21 dpf were fed twice daily with freshly hatched live Artemia (Jiahong Feed Co., Tianjin, China).

2.3. Adult zebrafish growth evaluation

At 120 dpf exposure, survival and growth were evaluated, and all fish were visually sorted by sex. Before the behavior and social interaction evaluations at 9 months of age, a subset of female (n = 15; 5 fish per replicate) and male fish (n = 15; 5 fish per replicate) from each treatment group were anesthetized in 0.03% MS-222, and used for measurements of standard body length (from snout to the fork point of caudal fin), wet weight. Condition factor [K = weight (g) × 100 / length^3 (cm)] was calculated to evaluate the overall fitness.

2.4. Adult zebrafish movement behavior

Adult zebrafish locomotor activity was monitored with the ZebraCube Video-Tracking system (version 3.5, ViewPoint Life Science, France) equipped with a Sony one-third inch CCD monochrome camera (Model DR2-HIBW-CSBOX, 30 fps) and an infrared filter. All of the recording hardware was linked to a computer program and kept insulated from the lab environment in a sealed, opaque plastic cube (ZebraCube, ViewPoint Life Science). Data (number of movements, distances travelled and total duration of movements) were collected every 60 s and analyzed. Parameters of the videotrack system were: detection threshold, 35; movement threshold, inact/small, 2.0 cm/s, small/large, 8.0 cm/s. Briefly, 24 male/female adult fish in each treatment group from three replicate experiments were used for these assays. Adult behavior tests were carried out with males and females separated into two different test groups according to size and body type differences. Individual fish (n = 24) from each treatment group were placed in a 24-well-plate (made by ground glass to inhibit fish seeing each other and each well was 9 cm length × 7.5 cm width × 6 cm height). Each well contained 150 mL of fish water and one fish. The room temperature was controlled at 28 °C. Fish were given 2 h to acclimate prior to the test in the morning and the test was carried out from 12:00–16:00 pm. The free swimming movement was measured in 10 min of visible light with 5 min acclimation first. And the photomotor response (PMR) behavior was measured as swimming activity during 2 cycles of alternating 5 min dark-5 min light with 5 min acclimation first.

2.5. Aggression behavior test

Aggression was evaluated using mirror attack test method referenced from previous publications (Pham et al., 2012; Weber et al., 2015). The chamber was a 12 × 12 cm × 8 cm, square aquarium with four mirrored walls. Each chamber contained 150 mL of fish water and one fish. The room temperature was controlled at 28 °C and the test was carried out from 12:00–16:00 pm. Individual adult zebrafish (n = 24 per group) fasted during the morning, were placed into the separate 4-mirror chambers and monitored simultaneously by the ZebraCube system for 2 min. Attacks (lunges, biting) at the mirror image were counted and proximity to the mirror was defined as the frequency of crossing into an established mirror zone. The percent time spent in the mirror zone (≤1.0 cm away from the surrounding mirrors) and the average movement speed were quantified. The basic tracking setting was the same as in the movement test detailed in Section 2.3.