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Neurobehavioral effects of two metabolites of BDE-47 (6-OH-BDE-47 and 6-MeO-BDE-47) on zebrafish larvae

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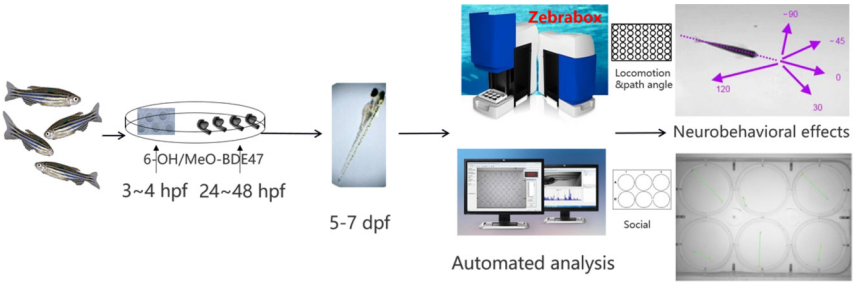
HIGHLIGHTS

- The neurobehavioral toxicity of 6-OH/MeO-BDE47 were evaluated with zebrafish larvae.
- 6-OH-BDE47 performed inhibiting effects on routine and average turns.
- Different from 6-OH-BDE-47, 6-MeO-BDE47 mainly promoted responsive turns.
- 6-MeO-BDE-47 performed more adverse effects on social activity.

GRAPHICAL ABSTRACT



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ABSTRACT

Two metabolites, OH-BDEs and MeO-BDEs, of polybrominated diphenyl ethers (PBDEs) were ubiquitously detected in animal tissues and environmental samples, drawing a widely public concern to their toxicity. The comparison of toxicity between PBDEs and their metabolites has been a focus in recent years, however, comparisons seldom involve neurobehavioral toxicity of PBDEs metabolites in published works. In this study, zebrafish larvae were exposed to 6-OH-BDE-47 and 6-MeO-BDE-47 and their neurobehavioral traits (including locomotion, path angle, and social activity) were recorded using the instrument Zebrabox; meanwhile, light illumination was used as stimuli in the test duration. The results showed larvae were more active in dark periods than light periods, and preferred turning right (+) to left (−). Effects of the two metabolites varied in different behavioral indicators. They induced different effects on path angle but did not reverse the left-right asymmetry. 6-OH-BDE-47 did not induce the effects on larval locomotion and social activity, but mainly decreased average and routine turn numbers; 6-MeO-BDE-47 promoted larvae responsive turns but inhibited social activity. This study offered new experimental means to the neurobehavioral toxicity of various PBDE metabolites. Further studies may focus on the toxic mechanisms of specific neurobehavioral traits.

1. Introduction

Polybrominated diphenyl ethers (PBDEs) have been widely detected in sediment, atmosphere, surface water, and marine environment though most of commercial PBDEs were banned by the Stockholm Convention (Wu et al., 2015). Their potential hazards attracted public attention after decades of studies (Wang et al., 2015), especially because of their well-known neurodevelopmental toxicity and endocrine disruption (Lv et al., 2015).

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PBDEs may be metabolized through oxidation or hydroxylation reaction during the accumulation process in the environment (Wang et al., 2012), producing two main kinds of metabolites, hydroxylated polybrominated diphenyl ethers (OH-BDEs) and methoxylated polybrominated diphenyl ethers (MeO-BDEs). Actually, these two metabolites could also be natural products, without the in *vivo* biotransformation processes (EL et al., 2005; Wan et al., 2009). Recent studies have begun to pay close attention to the toxicity of these two metabolites, especially OH-PBDEs. For example, 6-OH-BDE-47 could cause disruption of oxidative phosphorylation and interfere the steady state of T4 (Boxtel et al., 2008). However, neurobehavioral toxicity studies on MeO-PBDEs and further the comparison between OH-PBDEs and MeO-PBDEs were generally sparse. Thus the comparison of neurobehavioral toxicity among PBDEs, OH-PBDEs and MeO-PBDEs is of vital importance.

The behavior is mainly controlled by animal central nervous system, thus the changes in behavior indicated the dysfunctions of central nervous system (Balthazart et al., 2005). Zebrafish (*Danio rerio*) larvae are the most widely used model organism for neurobehavioral studies (Ulhaq et al., 2013). The zebrafish embryos usually hatch from the chorion at 2–3 days post fertilization (dpf) and larvae of 4 dpf have fundamental swimming ability. Besides, larvae can exhibit many other testable behaviors, such as turning, path angle, avoidance, social activities and so on (Kalueff and Cachat, 2011). Most of present neurobehavioral studies focused on swimming ability effects, but lack of those on the other diverse behavioral indicators related to brain functions. Taken PBDEs as an example, Usenko et al. (2011) evaluated the developmental and behavioral effects of six PBDEs congeners on embryonic zebrafish. Chou et al. (2010) found chronic exposure of 2,2′,4,4′-tetrabromodiphenyl ether (BDE-47) could alter the locomotion behavior in juvenile zebrafish. In addition, some other behaviors of zebrafish larvae exposed to PBDEs were also involved in several recent studies (Zhao et al., 2014; Xu et al., 2017a, b; Zhang et al., 2017). Studies of PBDEs metabolites on neurobehavioral toxicity are much fewer than PBDEs. Since PBDEs metabolites have shown neurotoxicity and endocrine disrupting effects like PBDEs, it is speculated they would also perform neurobehavioral effects on animals (Macaulay et al., 2015a).

In this study, we investigated three kinds of neurobehaviors, including fundamental locomotion, path angle and two-fish social activity, of zebrafish larvae affected by two metabolites of BDE-47, 6-OH-BDE-47 and 6-MeO-BDE-47. We aimed to demonstrate that early life exposure to PBDE metabolites can lead to neurodevelopmental effects, and proved some characteristic neurobehavioral changes of zebrafish larvae were induced by 6-OH/MeO-BDE-47 exposure.

2. Methods and materials

2.1. Experimental animals and BDE-47 treatments

Embryos were collected by mating healthy adult Tuebingen zebrafish, cultured under a 14:10 h light: dark cycle room at 28 ± 0.5 °C according to the standard methods (Westerfield, 2007). About seventy embryos (3–5 h post fertilization) were selected and exposed to 7 dpf in a glass culture dish (basal diameter was 6 cm) with 16 mL exposure solution. The treated embryos were placed in an illumination incubator with the temperature and light conditions identical to the culture room.

Our experiments had five groups, including four exposure groups and one control group. The nominal concentrations of 6-OH-BDE-47 and 6-MeO-BDE-47 purchased from Accustandard (New Haven, USA) were 5 μg/L and 50 μg/L, respectively. The concentration was selected based on previous studies (Macaulay et al., 2015a; Zhang et al., 2017). The 10% Hanks (pH = 7.2, 0.137 M NaCl, 5.4 M KCl, 0.25 mM Na_2HPO_4, 0.44 mM KH_2PO_4, 1.3 mM $CaCl_2$, 1.0 mM $MgSO_4$, and 4.2 mM $NaHCO_3$) and 0.1% DMSO (v/v) were used as control solution and were added in each group. The exposure solution was half renewed every day in the whole exposure duration.

2.2. Analysis of 6-OH-BDE-47 and 6-MeO-BDE-47

2.2.1. 6-OH-BDE-47 analysis

Exposure solution were collected, diluted and eluted out from the SPE cartridge with an elution phase of 3 × 2 mL of 2% formic acid in acetone (Xu et al., 2017a). The eluent was collected in a conical centrifuge tube and concentrated to about 500 μL by a stream of nitrogen. A 2 mL volume of hexanes was added to the tube. After vortexing and separation by centrifugation, the hexanes phase was transfer to another conical centrifuge tube. The process was repeated twice. The hexanes solution was then evaporated to dryness under a stream of nitrogen. The residue was then reconstituted in 200 μL acetonitrile.

6-OH-BDE-47 analysis was performed using a UPLC-MS/MS (Waters Xevo TQ MS, Milford, MA, USA). The mobile phase consisting of water (Solvent A) and acetonitrile (Solvent B). Elution was started at a composition of 40% of solvent A and 60% of solvent B for 1 min, then changed linearly to 15% of solvent A and 85% of solvent B from 1 to 4 min. The mobile phase composition was changed to 0% of solvent A and 100% of solvent B at 4 min and kept for 2 min, and at last changed linearly to 40% of solvent A and 60% of solvent B from 6 to 9 min (Sun et al., 2012). The flow rate was 0.4 mL min^{-1}. The column temperature was set at 50 °C and the volume injected onto the column was 5 μL. Mass spectrometric detection was completed using ESI source in the negative ion multiple-reaction monitoring (MRM) mode. The MRM transitions were 500.7 → 80.8 and 500.7 → 78.7.

2.2.2. 6-MeO-BDE-47 analysis

6-MeO-BDE-47 was identified by a gas chromatography system (Agilent 7890A) with ECD detector equipped with a DB-5 fused silica capillary column (30 m × 0.32 mm ID × 0.25 μm). Nitrogen was used as the carrier gas with a flow rate of 58 cm/s. The programmatic process of column temperature was set at 150 °C for 1min, increased to 200 °C by 20 °C/min and then increased to 280 °C by 5 °C/min and held for 5 min. The temperature of the injection port was 250 °C.

2.3. Behavior test protocol

Three behavior indicators, the locomotion, path angle, and two-fish social activity, were adopted to evaluate the effects induced by two BDE-47 metabolites, and only healthy larvae were used. Behavior tests were performed on a Zebrabox platform (ViewPoint, France), which could also give enhanced light stimulus to animals. The whole test lasted for 50 min, including a first 10 min of light adaption, followed by two repeated cycles with 10 min of dark period and 10 min of light period, namely the actual test duration was 40 min. Locomotion and path angle test were performed with larvae of 5 dpf, 6 dpf, and 7 dpf, while social activity test was performed using larvae of 5 dpf and 6 dpf.

2.3.1. Locomotion

In locomotion test, larvae were transferred into a 48-well microplate from the glass culture dish. Each well accommodated one larva with 1 mL exposure solution and each group had about 20 larvae. The system can record the swimming distance of larvae during the test time.