Effects of 2, 2', 4, 4'-tetrabromodiphenyl ether on the path angle and social activity of zebrafish larvae

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Introduction

Polybrominated diphenyl ethers (PBDEs), a class of environmental endocrine disruptor chemicals commonly used as flame retardants, are ubiquitous in the environment and bioaccumulate in humans and wildlife. Commercial octa-BDE and penta-BDE were the first brominated persistent organic pollutants (POPs) listed in the Stockholm Convention. Neurobehavioral tests of zebrafish have been widely adopted in the development of psychopathic drugs [1, 2] and research on environmental neurotoxic pollutants [3-5]. Studies on neurobehavior toxicity of zebrafish larvae mainly focused on the fundamental swimming ability exposed to pollutants, however, studies on other types of zebrafish larvae behaviors, including path angle and social activity, have rarely been reported. 2, 2', 4, 4'-tetrabromodiphenyl ether (BDE-47), as a typical PBDE congener and a major constituent of animal tissues and environmental samples, was selected as a representative PBDE to demonstrate the speculation that PBDEs may induce neurobehavioral effects of zebrafish larvae. Our results showed that BDE47 could affect the two behavior.

Material and methods

Fifty embryos that reached the blastula stage (3-5 hpf) and developed naturally were exposed in 6-well microplates (Corning, NewYork, US) containing 5 ml of BDE-47 (Accustandard, Connecticut, US) at nominal concentrations of 5μg/L and 500 μg/L. Two treated groups and a control group received 10% Hanks and 0.1% DMSO. To maintain the BDE-47 concentration, half of the exposure solution was renewed every day.

A Zebrabox platform (ViewPoint, lyon, France) was adopted to record the larval neurobehavioral tests with the VideoTrack software version 3.5. The path angle refers to the angle of the path of motion relative to the swimming direction, based on the angle measured on the path of the larvae. On the day of testing (5 and 6 dpf), the 6-well microplate, with one larva in each well, was placed in the Zebrabox, which was a light-tight chamber with infrared illumination from the bottom. Visible light and infrared light were used during the light and dark periods, respectively. Larvae were adapted to the light condition in the box for 10 min before testing to minimize any unintended disturbance. Then, there were three circles where the light was alternated between ON to OFF in ten-minute intervals. The total testing time was 70 min. To characterize the rotation angles, 6 classes from -180° to +180° were divided and defined as in **Table 1**.

The two-fish social assay was performed at 5, 6, and 7 dpf. Two larvae were placed together in each well of a 6-well plate. The distances between the two animals at each sampling time were recorded, and a valid social "contact" was defined as a distance less than 1 cm. The total testing duration and lighting settings were the same as those in the path angle test.

Table 1 Definition of different path angles

Definition	Range of path angle
straight motion/no turn	-10° ~0° (-), 0° ~+10° (+)
average turn	-10° ~-90° (-), +10° ~+90° (+)
responsive turn	-180° ~-90° (-), +90°~+180° (+)

Results and discussion

The actual concentration of BDE-47 was detected using GC-ECD method, which were 1.63 ± 0.12 μg/L and 240.7 ± 23.5 μg/L, in the nominal 5 μg/L and 500 μg/L exposure groups respectively.

The low-dose BDE-47 group showed a significant decrease in straight motion compared with the control group at 5 pdf, especially during the dark period (**Fig. 1**). Under natural conditions, zebrafish larvae are active during the day and quiescent at night. Zimmerman et al. reviewed the evidence for the presence of sleep states of zebrafish [6]. There was a transiently hyperactive period before a quiescent state during continuing darkness, which was considered the state of light-to-dark adaptation. During our intermittent dark test, the larvae became more active from light to dark. The times of straight motion and the total times of turns in the exposure groups became fewer compared with the control group, indicating that exposure to BDE-47 disturbed the state of dark adaption of zebrafish larvae.

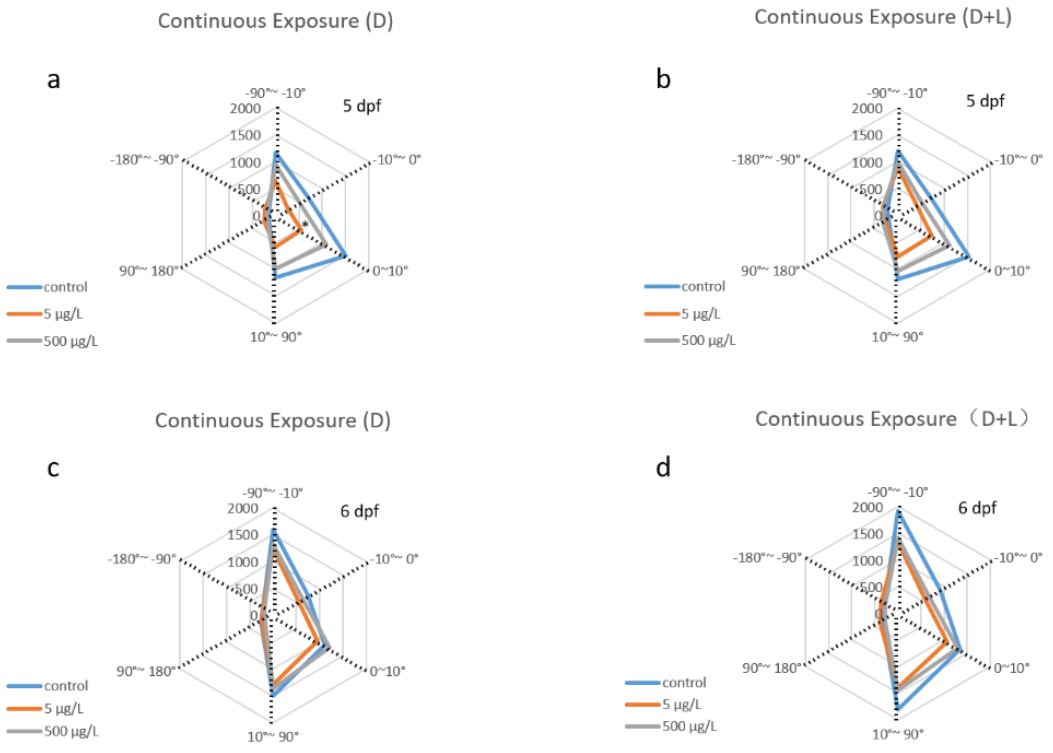

Fig. 1 Effects of BDE-47 on the path angles of larval zebrafish exposed to BDE-47, **a and c:** dark period; **b and d:** dark and light period. The numbers across the lines are the turning numbers of each path angle class. Data are presented as the mean (n = 6) ± SEM (*p < 0.05 compared with control).

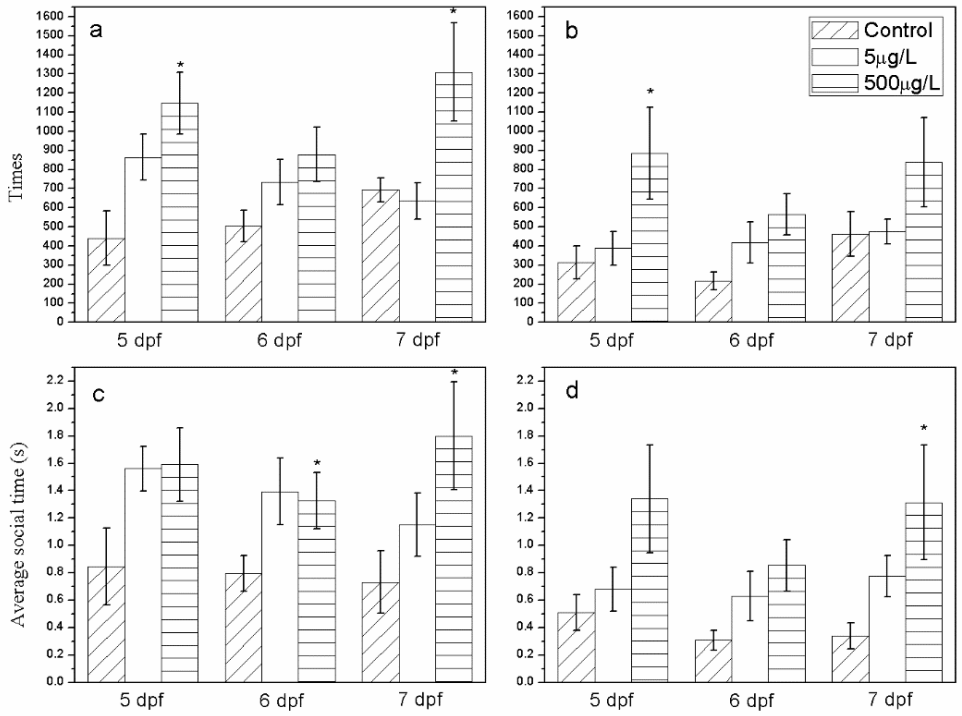

Fig. 2 Effects of BDE-47 on the social activity of larval zebrafish exposed to BDE-47 (**a and c**: dark period; **b and d**: light period). Data are presented as the mean (n = 6) ± SEM (*p < 0.05 compared with control)

The two-fish assay was designed to study the disruption of social activity by BDE-47 in a relatively limited space. The recorded parameters, such as times and duration of the valid contacts of two individuals, could be the basis of more complex interactions in the system with more fish. From 5 to 7 dpf, the number of social contacts in the three groups all had an increasing trend both in the dark and light periods (**Fig. 2a-b**), and the high concentration group showed a significant difference compared with the control group at 5 dpf and 7 dpf in the dark period (**Fig. 2a**). The average social time per contact showed a similar trend (**Fig. 2c-d**). The results indicated that the high concentration of BDE-47 treatment influenced the social behavior. One study demonstrated that the social behavior of zebrafish larvae was altered after exposure to retinoic acid (RA) [7], and our previous study showed that BDE-47 could disturb RA signaling in zebrafish [8]. Therefore, the mechanisms by which BDE-47 altered the social behavior of zebrafish larvae probably correlated with its ability to disturb RA signaling.

Acknowledgements

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