# VISUAL TOXICITY OF BDE-99 IN LARVAL ZEBRAFISH: AN ADVERSE OUTCOME MEDIATED BY THYROID HORMONE SIGNALING

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## Introduction

Vision is a primary route for individuals to acquire information from the external environmental. Impaired visual function often leads to the incapability of foraging and escaping from dangers in individuals, thus reducing the the individual survival rate and affecting the population continuity.[1] Owing to the special physiological position of the visual system, especially for the aquatic organism, the visual system is in direct contact with environmental pollutants and becomes a susceptible target of environmental toxicants.[2] Therefore, investigating the visual toxicity helps evaluate the ecological risk of environmental pollutants from a more comprehensive perspective.

Polybrominated diphenyl ethers (PBDEs), a conventional category of brominated flame retardant additives used in various household items and industrial materials, are known to disrupt the homeostasis of thyroid hormone and retinoic acid[3,4], which are two crucial factors involved in the development and appropriate functioning of eye. However, the visual toxicity of PBDEs and potential mechanism are rarely reported. Here, we use zebrafish larvae as the model organism and investigate the visual toxicity of 2, 2', 4, 4', 5-pentabromodiphenyl ether (BDE-99) and the underlying mechanism of visual injury in larval zebrafish.

## Materials and methods

**Chemicals and exposure design.** BDE-99 (99.1%, AccuStandard) was dissolved in dimethyl sulfoxide (DMSO) (≥99.5%, Sigma-Aldrich) and diluted to 5μg/L and 50 μg/L with sterile Hanks buffer solution. The whole exposure lasted from 3 hours post fertilization (hpf) to 120 hpf. 10% Hanks buffer solution with 0.01% DMSO was used as the negative control. Larvae at 120 hpf were used for subsequent experiments.

**Locomotion and vision-guided behavior.** Locomotor test was carried out in the Zebrabox platform (Viewpoint). Phototaxis was performed in a rectangular box (10 × 4 × 2 cm, length × width × height) with a board separating the apparatus into two compartments; one side is illuminated with a light, whereas the other side is kept dark. Optokinetic response (OKR) test was performed using the VisioBox platform (Viewpoint).

**Quantitative Real-Time Polymer Chain Reaction (qRT-PCR).** Total RNA was extracted from ~ 30 larvae and then reverse transcribed into cDNA. qRT-PCR was performed with SYBR Green Mix on a 7500 Real-time PCR System (Applied Biosystems).

**TUNEL staining.** After 120-exposure, larvae were fixed in 4% paraformaldehyde overnight and sectioned on a cryostat at a thickness of 10 μm. TUNEL staining was performed according to the instructor of DeadEnd Fluorometric TUNEL system (Promega).

**Molecular docking.** The combing energy between thyroid hormone receptor and BDE-47, BDE-99 and BDE-209 was calculated using AutoDock Vina.

**T3 and TR antagonist 1 treatments.** To investigate the role of thyroid hormone signaling in visual impairments, 3,3’,5-triiodo-L-thyroine (T3) exposure was used as the positive control. To further verify the role of thyroid hormone receptor, embryos were co-exposed to TR antagonist 1 and BDE-99.

## Results and discussion.

**BDE-99 induced visual function deficits in larval zebrafish.** Phototaxis is an innate behavior of zebrafish larvae when sensing the stimulation of light. After high concentration of BDE-99 exposure (50 μg/L), the phototactic behavior of larvae was remarkably reduced (Figure 1A). To determine whether the inhibition of phototaxis was a result of disturbed light sensing ability, the locomotor activity of larval zebrafish was investigated. Interestingly, the swimming ability of larvae was not apparently reduced (Figure 1B &1C), which indicates the light sensing ability of larvae was disturbed after BDE-99 exposure. The OKR results showed that the saccadic response of larvae was significantly suppressed after 5 μg/L of BDE-99 exposure (Figure 1d), which further confirmed the visual function of zebrafish larvae was impaired after BDE-99 treatment.

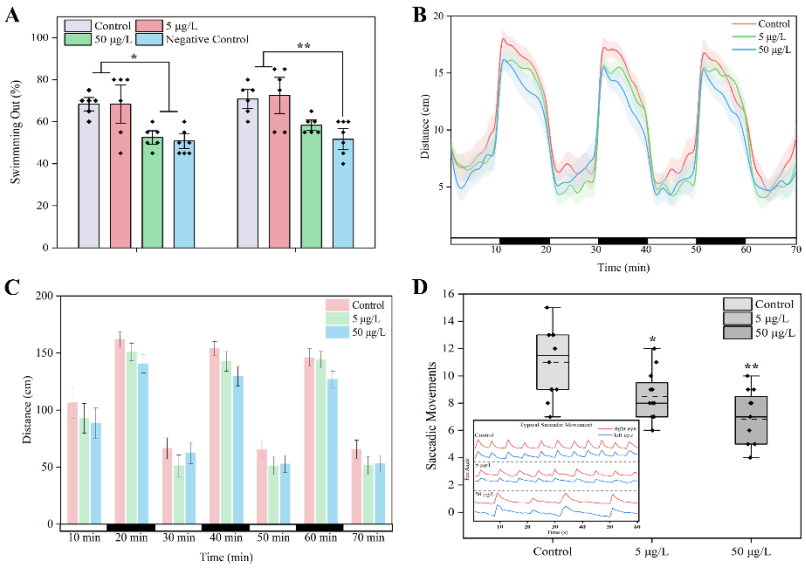


**Figure 1. BDE-99 induced visual function deficits in zebrafish larvae.** (A) Effects of BDE-99 on the phototactic behaviors of zebrafish larvae. (B) The swimming distance of larval zebrafish per minute. The shadow area stands for the standard error of mean (C) The total swimming distance of larval zebrafish in a light/dark period. (D) The optokinetic responses of larval zebrafish with the stimulation of black-white stripes.

**BDE-99 impaired the retina development of larval zebrafish.** The transcriptional levels of retinal nerve cells were altered after BDE-99 exposure. BDE-99 significantly downregulated the expression of *gnat2* and *vsx1*, which are the markers of cone photoreceptor and bipolar cell, respectively, whereas upregulated the expression of *gfap*, the marker of Müller glial cell (Figure 2A). Downregulation of *gnat2* and *vsx1* implied the dysfunction of retina nerve cells. TUNEL staining demonstrated that apoptosis occurred in the retina after high dose of BDE-99 exposure (Figure 2B), and upregulation of glial cells might be the self-protection process in response to the retinal injury.

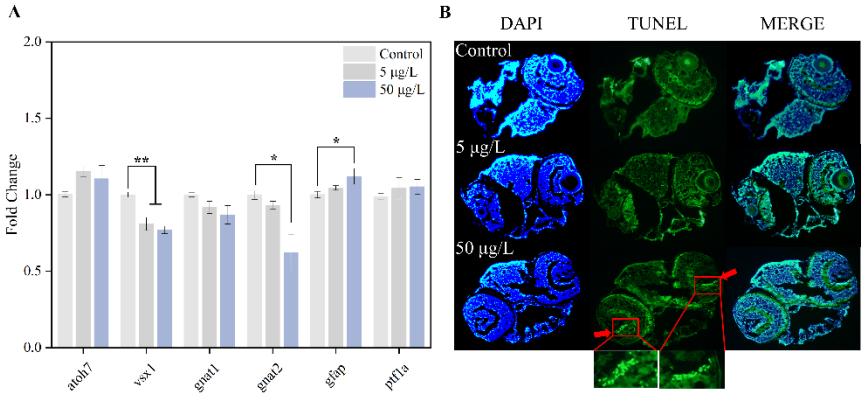


Figure 2. BDE-99 impaired the retina development of larval zebrafish. (A) Relative mRNA levels of retinal nerve cells after BDE-99 exposure. (B) TUNEL staining fluorescent images of the retina. TUNEL positive cells was labeled with green fluorescence.

**BDE-99 disturbed the color vision of larval zebrafish.** Opsins are G protein-coupled receptors, responsible for the primary visual information input, specifically expressed on the photoreceptors. After BDE-99 exposure, except for *opn1lw1* was upregulated, other opsins were all downregulated (Figure 3A). Given each opsin is sensitive to a specific spectral wavelength peak and range, the dysregulation of opsin expression might lead to the dysfunction of color vision. We modified the OKR test by replacing the black-white strips with red-white, green-white and blue-white strips, which represents the long, middle and short wavelength light stimulation, respectively. The modified OKR test showed that the larval zebrafish were less sensitive to middle and short wavelength light (Figure 3B), which was correspondence with the results of qPCR.

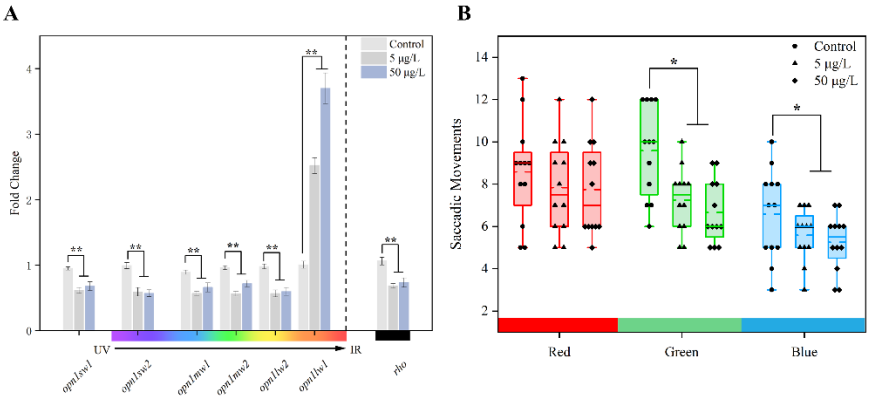


Figure 3. BDE-99 disturbed the color vision of larval zebrafish. (A) Relative mRNA expression of opsins of the larval zebrafish after BDE-99 exposure. (B) Optokinetic response of larval zebrafish to the stimulation of red-white, green-white and blue-white strips.

**Thyroid hormone signaling plays the key role in mediating the impaired color vision induced by BDE-99.** Thyroid hormone is an essential factor for the expression of long wavelength sensitive cones in the human retinal organoids.[5] Here, we exposed larvae to T3 to identify the role of thyroid hormone in the color vision of larvae. T3 treatments displayed similar results in both gene expression and OKR tests, indicating the BDE-99 might impact the color vision via simulating T3 (Figure 4A & 4B). Compared with our previous studies, we found an interesting phenomenon that BDE-47 showed a similar impact on the zebrafish larvae, whereas BDE-209 resulted in complete different outcomes in the color vision development. We constructed the molecular docking model of thyroid hormone receptor and BDE-47, BDE-99 and BDE-209, respectively. The molecular docking results showed that both BDE-47 and BDE-99 possessed high affinity to thyroid hormone receptor, while BDE-209 could hardly bind with thyroid hormone receptor, which further confirmed our hypothesis. Then, we conducted a rescue experiment by exposing larvae to the mixture of BDE-99 and TR antagonist 1, a molecule with high affinity to both thyroid hormone α and β. The results showed that TR antagonist 1 treatment completely rescued the color vision deficits caused by BDE-99. Therefore, we speculated that BDE-99 could simulate the T3 function by combining with thyroid hormone receptor and consequently regulate the expression of opsins, which finally disrupts the visual function of zebrafish larvae.

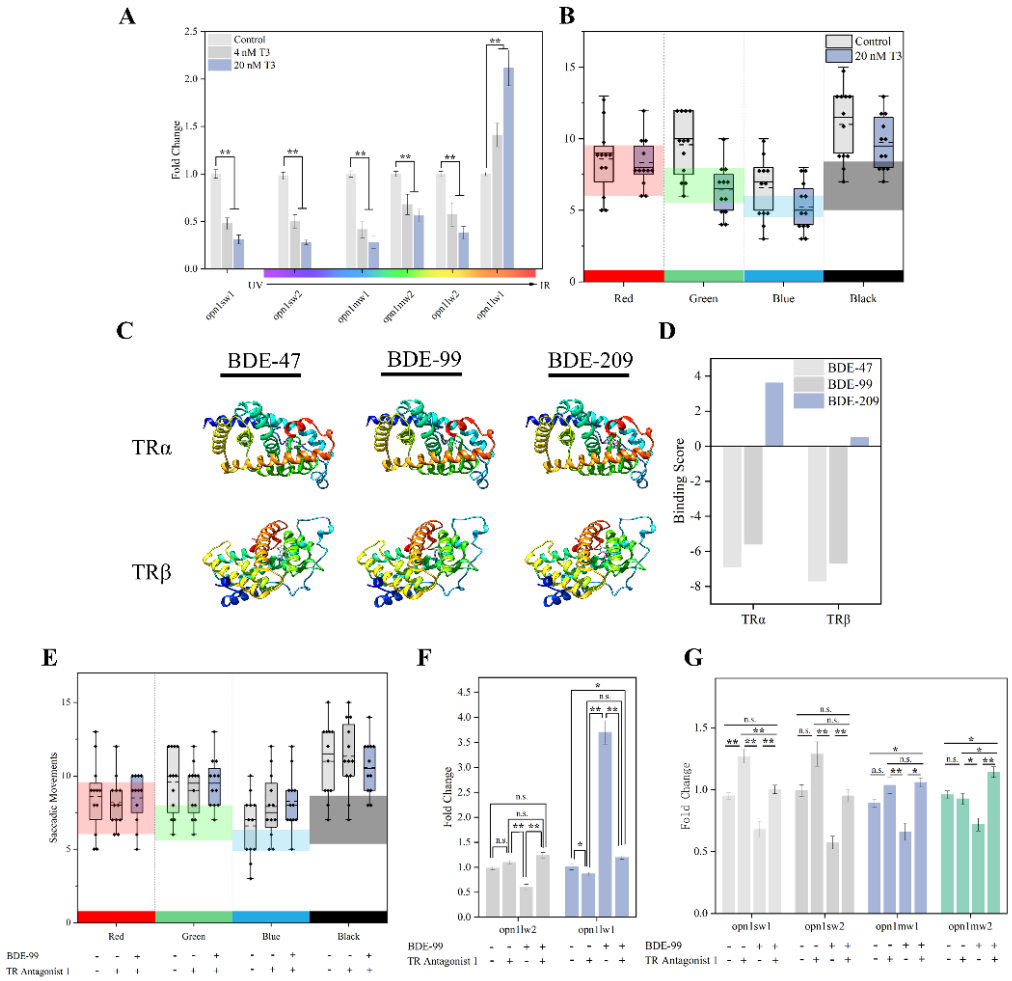


Figure 4. The role of thyroid hormone signaling in BDE-99 induced color vision deficits. (A) Relative mRNA levels of opsins after T3 treatment. (B) Optokinetic responses of zebrafish larvae after T3 Treatment. Shadow area stand for the results of BDE-99 exposure. (C) Molecular docking of PBDEs and thyroid hormone receptor. (D) Quantification of the binding ability of PBDEs to the thyroid hormone receptor. Minus binding score indicates a high affinity to the receptor. (E) Optokinetic responses after the co-exposure of BDE-99 and TR antagonist 1. Shadow area stand for the results of BDE-99 exposure. (F-G) Relative mRNA levels of opsins after the co-exposure of BDE-99 and TR antagonist 1.

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**References.**

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