Chemosphere 193 (2018) 711–719

Contents lists available at ScienceDirect

Chemosphere

journal homepage: www.elsevier.com/locate/chemosphere

# Toxic effects and mechanism of 2,2′,4,4′-tetrabromodiphenyl ether (BDE-47) on *Lemna minor*

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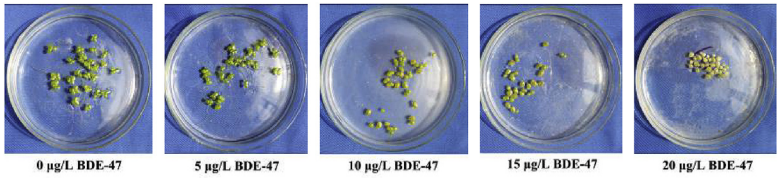
## HIGHLIGHTS

- BDE-47 at 5–20 μg/L had significant toxicity to *L. minor*.
- BDE-47 caused a direct injury to plasma membrane and thylakoid membrane of *L. minor*.
- BDE-47 had no effect on the enzyme activity of NR, POD, MDH in the cytoplasm.
- BDE-47 induced remarkable oxidative damage in frond cells of *L. minor*.

## GRAPHICAL ABSTRACT




## ARTICLE INFO

*Article history:*
Received 14 August 2017
Received in revised form
24 October 2017
Accepted 26 October 2017
Available online 17 November 2017

Handling Editor: A. Gies

*Keywords:*
2,2′,4,4′-tetrabromodiphenyl ether (BDE-47)
*Lemna minor*
Toxic effects
Toxic mechanism
Chronic toxicity
Direct toxicity

## ABSTRACT

To investigate the toxic effect and mechanism of 2,2′,4,4′-tetrabromodiphenyl ether (BDE-47) in aquatic plants, *in vivo* and *in vitro* exposure to BDE-47 were conducted. After 14-d exposure to 5–20 μg/L BDE-47, the growth of *Lemna minor* plants was significantly suppressed, and the chlorophyll and soluble protein contents in fronds markedly decreased. Accordingly, the photosynthetic efficiency (Fv/Fm, PI) decreased. When the thylakoid membranes isolated from healthy fronds was exposed to 5–20 mg/L BDE-47 directly *in vitro* for 1 h, the photosynthetic efficiency also decreased significantly. In both the *in vitro* (5–20 μg/L) and *in vivo* (5–20 mg/L) experiments, BDE-47 led to an increased plasma membrane permeability. Hence, we concluded that BDE-47 had a direct toxicity to photosynthetic membranes and plasma membranes. However, direct effects on the activities of peroxidase (POD), malate dehydrogenase (MDH) and nitroreductase (NR) were not observed by adding 5–20 mg/L BDE-47 into crude enzyme extracts. The malondialdehyde (MDA) and superoxide anion radical ($O_2^{\cdot}$) contents in the BDE-47 treated fronds were higher than those in the control fronds, suggesting that *L. minor* can not effectively relieve reactive oxygen species (ROS). The data above indicates that BDE-47 is toxic to *L. minor* through acting directly on biomembranes, which induces the production of ROS and thus causes remarkable oxidative damage to cells.



*Abbreviations:* PBDEs, Polybrominated diphenyl ethers; BDE-47, 2,2′,4,4′-tetrabromodiphenyl ether; SOD, superoxide dismutase; POD, peroxidase; CAT, catalase; NR, nitroreductase; GST, glutathione S-transferase; MDA, malondialdehyde; Fv/Fm, maximal efficiency of PSII photochemistry; PI, performance index; Chl, chlorophyll; ROS, reactive oxygen species; $O_2^{\cdot}$, Superoxide anion radical; MDH, malate dehydrogenase.

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https://doi.org/10.1016/j.chemosphere.2017.10.148
0045-6535/

## 1. Introduction

As a brominated flame retardant, polybrominated diphenyl ethers (PBDEs) have been widely used for a wide variety of industrial and consumer products, such as BDE-15, BDE-28, BDE-47, BDE-99, BDE-209. As such, PBDEs have become a new type of persistent organic pollutants, and their pollution levels in the environment have been increasing constantly in recent years (Shang et al., 2016). Many studies have found that PBDEs have ubiquitous toxicity to organisms (Maranghi et al., 2013; Macaulay et al., 2015), including both higher plants and lower plants (Kallqvist et al., 2006; Wang et al., 2014). The higher brominated PBDEs have more bromine atoms on the benzene ring, which affects the efficiency of PBDEs entering into cells, and their toxicity is relatively low. However, the low brominated PBDEs are easier to enter the cells and have higher toxicity to organisms (Bragigand et al., 2006). The metabolites of PBDEs produced by debromination, hydroxylation and redox, including lower brominated congeners, hydroxylated PBDEs (OH-PBDEs) and methoxylated PBDEs (MeO-PBDEs), are more stable *in vivo* and also exert adverse influences on organisms (Haglund et al., 1997; Qiu et al., 2007; Wan et al., 2010; Huang et al., 2013a). Knowledge on effective detoxification mechanism of PBDEs in organisms is scarce.

At present, a few studies have been carried out on the toxicity mechanism of PBDEs to plants. These studies have shown that PBDEs can cause the overproduction of reactive oxygen species (ROS) in plants (Wang et al., 2014), which poses an oxidative stress to plants, causes membrane damage, inactivates functional enzymes and decreases photosynthetic activities, leading to growth inhibition, senescence or even the death of the plants (Bhattacharjee, 2005; Kallqvist et al., 2006; Zhang et al., 2013b; Wang et al., 2014). PBDE-induced mechanisms of ROS in plants are still unclear. A number of studies in animal provide evidence that PBDEs induce $Ca^{2+}$ release from calcium stores (apoplast, mitochondria and endoplasmic reticulum), which disturbs the $Ca^{2+}$ balance in the cytoplasm (Kodavanti and Ward, 2005; Dingemans et al., 2010). High concentration of $Ca^{2+}$ in the cytoplasm stimulates mitochondrial ROS production (Camello-Almaraz et al., 2006). In turn, ROS causes membrane destabilization and affects membrane potential of mitochondrial, which further promotes the release of $Ca^{2+}$ and induces apoptosis (Shao et al., 2008a; Camello-Almaraz et al., 2006). Based on the PBDEs toxicity mechanism in animals, we speculate that PBDEs may also affect the biomembrane system of plants and induce oxidative injury.

*Lemna minor* is a model aquatic plant widely used for aquatic toxicological studies. The PBDEs in the aqueous phase are predominantly low brominated diphenyl ethers such as BDE-47, BDE-99, etc., which show a higher absorption rate by organisms than other forms of PBDEs (Huang et al., 2011). Therefore, the toxicity mechanism of PBDEs to plants was studied with BDE-47 as the stress factor. We systematically analyzed the direct and indirect toxicity of BDE-47 to the plasma membrane, photosynthetic system, antioxidant enzyme system, and important metabolic enzyme systems of *L. minor* in this paper.

## 2. Materials and methods

### 2.1. Plant culture and treatments

The duckweeds (*Lemna minor* L.) used in this research were collected from an artificial river in Qufu City, P.R. China. The duckweed plants were cultured under controlled conditions (temperature: 24 ± 2 °C, light/dark cycle: 14 h/10 h, and an irradiance: 100 μmol $m^{-2}$ $s^{-1}$ provided by cool white fluorescent lamps) with full strength Hoagland's solution for rapid multiplication as stock materials for this experiment (Zezulka et al., 2013). Then uniform duckweed plants were placed into glass dishes (10 plants per dish) and cultivated in Hoagland's solution (30 mL per dish) without (control) or with 5, 10, 15, and 20 μg/L BDE-47, respectively. The diameter of the dishes was 9.2 cm and the depth was 2.0 cm. BDE-47 standard (purity 98.6%, GC/MS certified) was obtained from AccuStandard (German) and used to prepare a stock solution (1 mg/L) in *N,N*-Dimethylformamide (DMF). A concentration series of BDE-47 (5, 10, 15, and 20 μg/L) was obtained by adding 1 mg/mL of BDE-47 stock solution to Hoagland's solution. The no-observed-effect concentration (NOEC) of DMF to duckweed is 2% (v/v). So the concentration of DMF in each treatment solution was all adjusted to 1%. The treatment solution was renewed every 2 d to ensure a stable BDE-47 concentration and reduce the growth of green algae in the culture medium. Ten replicates were prepared for each BDE-47 concentration. All duckweed plants were harvested for determination of physiological and biochemical indexes after 14-d exposure to BDE-47.

### 2.2. Growth inhibition

The growth inhibition of duckweeds by BDE-47 was defined as the decrease of root length and frond fresh weight. All plants in each dish were removed from the culture medium and the root length of each plant was measured. The average root length of all plants in each dish was used as one replicate. Then, the roots were cut off and the frond surface moisture was dried gently with absorbent paper. The total fresh weight of all fronds in each dish was weighted as one replicate. Ten replicates were included in the measurement of root length and frond fresh weight.

### 2.3. Photosynthesis analysis

The photosynthetic activity of the intact fronds and isolated thylakoid membrane was measured with a portable fluorometer (Plant Efficiency Analyser, Handy PEA; Hansatech Instrument Ltd., UK). The duckweed plants were dark-adapted for 1 h prior to the measurements. The photosynthetic activity of the thylakoid membrane was determined using 2 mL thylakoid membrane extract in a specifical tube of Handy PEA. The maximal photochemistry efficiency of PSII (Fv/Fm) and performance index (PI) were calculated according to the OJIP curve by Handy PEA (Kalaji et al., 2016).

### 2.4. Chlorophyll and soluble protein assays

The chlorophyll content of fronds was extracted with 80% acetone and determined according to the method of Porra (2002). Total soluble protein content in fronds was quantified according to the method of Bradford (1976), using bovine albumin for calibration.

### 2.5. Plasma membrane permeability testing

The toxicity of BDE-47 to plasma membranes is expressed as plasma membrane permeability (%), which was measured with whole duckweed plants. For *in vivo* toxicity experiment, the plants were harvested after 14-d exposure to BDE-47 (0, 5, 10, 15, and 20 μg/L, respectively) and rinsed with distilled water before the determination of the plasma membrane permeability. For direct toxicity experiments (*in vitro*), the healthy plants cultured in Hoagland's solution were moved into the culture medium containing 0, 5, 10, 15 and 20 mg/L BDE-47, respectively, and then vacuumized for 10 min to allow BDE-47 to penetrate into the plant tissue. These BDE-47-treated plants were rinsed with distilled