Bioaccumulation of decabromodiphenyl ether affects the antioxidant system in the clam *Mactra veneriformis* 

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**ABSTRACT**

Antioxidant enzymes play vital roles against oxidative stress induced by decabromodiphenyl ether (BDE-209), being widespread in marine environment. However, the effect of BDE-209 on antioxidant enzymes remains poorly understood in marine bivalves. In this study, the clams *Mactra veneriformis* were exposed to 0.1, 1, and 10 μg/L BDE-209 for 7 days and then maintained in clean seawater for 3 days as the depuration. The bioaccumulation of BDE-209 and the effects on superoxide dismutase, catalase, and glutathione peroxidase were investigated. BDE-209 accumulation was concentration-dependent and decreased by 36%–52% after recovery. Malondialdehyde contents increased in a time- and dose-dependent manner. mRNA expression and activity of antioxidant enzymes changed with different patterns and recovered after depuration. These results suggested that antioxidant systems were triggered to protect the clams from oxidative damage caused by BDE-209. Thus, this research is helpful in elucidating the effect of BDE-209 on antioxidant system in marine bivalves.

**1. Introduction**

Polybrominated diphenyl ethers (PBDEs) are aromatic compounds containing bromine atoms, and 209 PBDEs congeners vary by the number and position of bromine atoms in the benzene ring. PBDEs have been widely used as flame retardants in industrial and consumer products such as textiles, furniture, plastics, printed circuit boards, and building materials (de Wit et al., 2010). Commercial PBDEs are typically pentabromodiphenyl ether (PentaBDE), octabromodiphenyl ether (OctaBDE), and decabromodiphenyl ether (BDE-209). BDE-209 and PentaBDE have been listed as persistent organic pollutants by the Stockholm Convention (Stockholm Convention, 2018). Although bills have been introduced by the European Union, the United States, and Canada to prohibit the use of PentaBDE, OctaBDE, and BDE-209, the latter is still produced and used in multiple areas (Akortia et al., 2016).

PBDEs can be easily released into the environment during production and use, polluting ecosystems. PBDEs have been detected in a variety of environmental media and organisms, including seawater, sediment, air, shellfish, human, polar bear, and Antarctic penguin (Jin et al., 2008; Krieger et al., 2016; Mwangi et al., 2016; Roscales et al., 2018; Zota et al., 2018). BDE-209 is the dominant PBDEs congener found abundantly in the environment and biota. The ocean is the final destination of many pollutants, and PBDEs pollution has been recorded in marine environments worldwide, and is especially common in China. Concentrations of PBDEs in sediments and shellfish have been shown to be as high as 1800 ng/g dry weight and 720 ng/g lipids, respectively, in Laizhou Bay, around by multiple flame-retardant production plants (Jin et al., 2008). In the surface sediments and waters of Dongjiang River in the Pearl River Delta, where the largest global electronics production base is located, BDE-209 concentrations reached 7340 ng/g dry weight (Ma et al., 2005) and 65.2 ng/L (Guan et al., 2007), respectively. The toxicity and environmental behavior of BDE-209 have been studied less than those of low-brominated congeners. BDE-209 is known to exert toxic effects on the endocrine, nervous, cardiac, and reproductive systems in rats, zebrafish, and other terrestrial or freshwater vertebrates (He et al., 2011; Milovanovic et al., 2015; Zhu et al., 2016; Han et al., 2017), while less attention has been paid to the impacts of BDE-209 on marine organisms. The toxic effect of PBDEs is yet to be fully explored in the marine organisms.

Bivalves are a good indicator of marine environmental pollution, and offer a powerful tool in toxicological research. Shellfish biomonitoring programs are widespread. For example, the United States' Mussel...
Watch Program, which began in 1986, has analyzed more than 100 pollutants in bivalve tissues collected along coastal and Great Lake waters (Bricker et al., 2014). The Asian Mussel Watch Program, which was launched in 2003, was designed to monitor PBDEs and organochlorine pesticides in the coastal waters of Asian countries; BDE-209 has been measured in offshore mussels from China, South Korea, Japan, and Indonesia, suggesting that BDE-209 pollution is widespread in the marine environments of these countries (Ramú et al., 2007).

The good correlation has been found between effects of organic pollutants in mussels and effects of the same pollutants in humans, which indicates that these pollutants can affect the whole food chain (Van Beneden, 1994). Therefore, the study of the effects of PBDEs on shellfish may provide useful information of the effects of PBDEs on other organisms and on whole ecosystems (Barón et al., 2016). Barón et al. (2016) evaluated the genotoxicity and growth effects of BDE-209 on the mussel Mytilus galloprovincialis and found DNA damage and micronuclei formation. Mussels, however, are not highly distributed in the tidal flat areas that lack attachments, requiring a different indicator organism for toxicological research and environmental monitoring in these ecosystems, to provide scientific data for PBDEs ecological risk assessment. *Mactra veneriformis* is an infaunal suspension-feeding bivalve, widely distributed in the coastal areas of China, Japan, and South Korea, and is of economic importance in China. The clam can efficiently accumulate pollutants, and has evolved to respond to pollutants rapidly (Wang et al., 2005; Fang et al., 2010; Shi et al., 2018), making it a good candidate of experimental organism in toxicology research.

Oxidative stress is a known pathway of pollutant toxicity, and studies have shown that BDE-209 can stimulate the production of reactive oxygen species (ROS), leading to oxidative stress (Milovanovic et al., 2015; Zhang et al., 2016a). The antioxidant system can scavenge ROS and protect the body from oxidative damage using enzymes such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx). These enzymes in aquatic animals are sensitively responsive to PBDEs and considered as biomarkers of water pollution (Xie et al., 2014; Horion et al., 2015; Shenai-Tirodkar et al., 2017). However, to our best knowledge, few studies have focused on the effect of BDE-209 on antioxidant enzymes in marine bivalves.

The present study aims at elucidating the effect of BDE-209 on antioxidant systems in *M. veneriformis*. Clams were exposed to BDE-209 for 7 days and recovered in clean seawater for 3 days. First, the bioaccumulation of BDE-209 and the level of oxidative damage marker malondialdehyde (MDA) were measured to assess the oxidative stress caused by BDE-209. Then, the gene expression and enzyme activities of SOD, CAT, and GPx were determined in the digestive gland. Third, the correlations between antioxidant response, MDA level, and BDE-209 concentration were analyzed. Thus, this study benefits the toxic effect of BDE-209 on marine shellfish and facilitates ecological risk assessment of offshore PBDEs pollution.

2. Materials and methods

2.1. Clams and exposure

Adult *M. veneriformis* were collected from the Dongying coastal area (average shell length 3.77 ± 0.28 cm; shell height 3.15 ± 0.31 cm; shell width 2.46 ± 0.38 cm; weight 12.93 ± 0.52 g) and acclimated for 14 days in our laboratory. Healthy and regular-shaped *M. veneriformis* were placed into glass aquariums at random; each aquarium was loaded with 30 L seawater and 60 clams. One control group, one solvent control group, and three exposure groups were included, for a total of six replicates for each group. In order to identify the uptake of the contaminant by clams and possible losses of the contaminant, three blank groups without clams corresponding to three exposure groups were also set up. BDE-209 concentrations in blank groups were same as that in exposure groups.

DNA damage and micronuclei formation were induced in *M. galloprovincialis* exposed to 0.03 and 0.3 μg/L BDE-209 for 6 days (Barón et al., 2016). Exposure to 0.004 and 0.04 μM (3.884 and 38.84 μg/L) BDE-209 for 7 days increased hepatic GPx activity in *Carassius auratus* (Zhao et al., 2011). The bioaccumulation of BDE-209 increased with time in Japanese medaka *Oryzias latipes* under the exposure concentration of 0.001–1 μg/L (Luo et al., 2013). Parental exposure to environmentally relevant concentrations of PBDEs (0.16, 0.8, 4.0 μg/L) adversely affected the neurodevelopment of zebrafish offspring (Chen et al., 2012). Accordingly, 0.1–10 μg/L BDE-209 could be accumulated in aquatic animals and cause toxicological effect. Furthermore, the concentrations of 0.1 and 1 μg/L were in the same order as BDE-209 contents in coastal waters and sediments (Ma et al., 2005; Guan et al., 2007; Cristale et al., 2013). Although higher than those detected in waters (Guan et al., 2007), the concentration of 10 μg/L was often used for short-term exposures to produce distinctly identifiable effects. To identify the effects of BDE-209 on antioxidant system in *M. veneriformis* and compare these effects with that on other species, exposure concentrations of 0.1, 1, and 10 μg/L were used in this study.

A stock solution of 1g/L was prepared by dissolving BDE-209 (CAS No. 1163-19-5, purity > 98.5%, Accustandard Inc, New Haven, CT, USA) in dimethyl sulfoxide (DMSO, Sinopharm Chemical Reagent Co., Beijing, China), and added to the aquariums to obtain the required concentrations and 0.05% v/v DMSO. In the solvent control group, only DMSO was added at a final concentration of 0.05% v/v. In the control group, only natural seawater was added. The aquariums were continuously aerated throughout the experimental period, the water was changed every day at a fixed time, followed by the addition of the relevant volumes of BDE-209 and DMSO, and the clams were fed with the algae Nitzschia closterium at an approximate concentration of 1 × 10⁵ cells/ml. 2 h before the water change. The experimental conditions were as follows: Dissolved oxygen, 7.7–8.2 mg/L; pH, 8.1; salinity, 31; temperature, 16 ± 1 °C. The seawater was sampled immediately after (0 h) and before (24 h) water change, and BDE-209 concentrations were measured.

The experimental period lasted 10 days. Clams *M. veneriformis* in the exposure groups were exposed to BDE-209 for 7 days and recovered in clean seawater for 3 days, while the clams in the control group and the solvent control group were cultured in clean seawater and seawater containing DMSO (0.05% v/v) for 10 days, respectively. On days 0, 1, 3, 5, 7, and 10, the digestive glands of the clams were isolated. Half of the digestive gland from one clam was used to measure mRNA levels, and the other half was used to measure enzyme activities and MDA content. Digestive glands from 10 clams were pooled into one replicate, and 3 replicates were performed for each experimental group at each sampling point. Thus, 30 clams from each experimental group were sampled at each sampling point, and 180 clams from each experimental group were sampled at all the sampling points. On days 7 and 10, extra digestive glands were sampled to measure BDE-209 accumulation. Digestive glands from 20 clams were pooled into one replicate, and 3 replicates were performed for each experimental group at each sampling point. Thus, 60 clams from each experimental group were sampled at each sampling point, and 120 clams from each experimental group were sampled at all the sampling points. Taken together, 300 clams were sampled in each experimental group during the whole experiment. All the samples were rapidly frozen in liquid nitrogen and stored at −80 °C.

2.2. Measurement of BDE-209 concentrations in seawater and clams

BDE-209 contents in seawater and clam samples were determined by gas chromatography–mass spectrometry according to the method of Sha et al. (2015) with some modifications. Seawater samples were extracted twice with dichloromethane (HPLC grade, Sinopharm Chemical Reagent Co.), then evaporated at 40 °C by rotary evaporator (Büchi Rotavapor R-200, Flawil, Switzerland). After re-dissolving the samples using n-hexane (HPLC grade, Sinopharm Chemical Reagent Co.),
anhydrous sodium sulfate (HPLC grade, Sinopharm Chemical Reagent Co.) was added to remove water, and the supernatant was used for measurements.

The clam samples were dried with 10 g anhydrous sodium sulfate, then extracted twice ultrasonically with 25 mL n-hexane : acetone (1:1 v:v) mixture solution. After centrifugation at 6000 rpm for 10 min, the supernatant was evaporated to near dryness by rotary evaporator, and then re-dissolved in 5 mL n-hexane. Next, 3 mL of concentrated sulfuric acid (98%, HPLC grade, Sinopharm Chemical Reagent Co.) were added, and the samples were vortexed for 3 min and then centrifuged at room temperature and 6000 rpm for 20 min. Five milliliters of 20 g/L anhydrous sodium sulfate solution were then added to the supernatant, which was allowed to settle. The supernatant was subjected to a stream of nitrogen gas at 40 °C and re-dissolved in n-hexane. After removing the water with anhydrous sodium sulfate, the supernatant was used for BDE-209 measurements.

An Agilent 7890B gas chromatographer (with EI source) and 5977 mass spectrometer (Agilent Technologies, Santa Clara, CA, USA) was used to determine BDE-209 concentrations in a splitless mode. The injection temperature was 280 °C, the flow rate was 1.5 mL/min, and the injection volume was 2 μL. The temperature of the ion source and quadrupole were 230 °C and 150 °C, respectively. High-sensitivity tuning of selected ion monitoring mode was applied. The charge to mass ratio of the quantitative ion was 799.5 m/z, and the charge to mass ratios of the qualitative ions were 641.5 and 721.5 m/z.

### 2.3. Analysis of mRNA expression levels

Total RNA was extracted from M. veneriformis digestive glands using a TRIzol kit (Invitrogen, Carlsbad, CA, USA). After DNase digestion, cDNA was synthesized using oligo dT(18) primers (Promega, Madison, WI, USA) and M-MLV reverse transcriptase (Promega). cDNA was amplified using a SYBR Green qRT-PCR kit (Applied Biosystems, Foster City, CA, USA) and an ABI PRISM 7500 real-time PCR system (Applied Biosystems), and a dissolution curve was generated. PCR conditions were set as follows: Pre-denaturation at 95 °C for 5 min, 1 cycle; denaturation at 95 °C for 15 s; annealing at 60 °C for 30 s, 40 cycles. Due to its stable expression, β-actin was used as the internal reference. Table 1 lists the primers used in the experiment. The detailed procedure was published previously (Fang et al., 2010).

### 2.4. Determination of enzyme activity and MDA contents

Samples were incubated in an ice-water bath, and 0.1 M Tris – HCl buffer (pH 7.4, containing 1 mM EDTA, Sinopharm Chemical Reagent Co.) was added at a ratio of 1:9 (v:v), then homogenized for 1 min intermittently with a homogenizer (IKA T10, Staufen im Breisgau, Germany) at a rotation speed of 13,000 × g; after centrifugation at 4 °C and 13,000 × g, the homogenate was intermittently with a homogenizer (IKA T10, Staufen im Breisgau, Germany) at a rotation speed of 18,000 rpm. The homogenate was then re-dissolved in 5 mL n-hexane. Next, 3 mL of concentrated sulfuric acid (98%, HPLC grade, Sinopharm Chemical Reagent Co.) were added, and the samples were vortexed for 3 min and then centrifuged at room temperature and 6000 rpm for 20 min. Five milliliters of 20 g/L anhydrous sodium sulfate solution were then added to the supernatant, which was allowed to settle. The supernatant was subjected to a stream of nitrogen gas at 40 °C and re-dissolved in n-hexane. After removing the water with anhydrous sodium sulfate, the supernatant was used for BDE-209 measurements.

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### 3. Results

#### 3.1. BDE-209 concentrations in seawater and clams

The detection limits for BDE-209 in seawater and biological samples were 150 ng/L and 25 μg/kg, respectively. No BDE-209 was detected in the seawater and biological samples from the control group and solvent control group, or in seawater from 0.1 μg/L group. After 24 h, BDE-209 concentrations dropped to 11.09%, 6.99%, 63.78%, and 46.93% of the initial values in 1 and 10 μg/L exposure groups and 1 and 10 μg/L blank groups, respectively (Table 2). Within 24 h of exposure to 1 and 10 μg/L, the absorption of BDE-209 by clams accounted for 52.69% and 39.94% decrease of the water concentrations, respectively. BDE-209 accumulation in M. veneriformis was concentration-dependent, and peaked in 10 μg/L group on day 7. After 3 days of recovery in clean seawater, BDE-209 accumulation decreased significantly to 54.38%, 48.09%, and 64.12% of the accumulation on day 7 in 0.1, 1, and 10 μg/L groups, respectively (Table S1). In the clams exposed to 0.1 and 1 μg/L BDE-209, mRNA expression and activity of SOD increased in time-dependent manner and peaked on day 7. In clams exposed to 10 μg/L BDE-209, SOD showed a biphasic response and peaked on day 5. SOD mRNA expression exhibited dose-dependent increase. After 3 days of recovery in clean seawater, mRNA expression of SOD returned to the control level in all the exposure groups; SOD activity decreased and was still significantly higher than the control level in 1 and 10 μg/L groups. SOD mRNA expression was positively correlated with SOD activity (P < 0.05) at each sampling time during exposure period (Table S2); no significant correlation was found on day 10 (P > 0.05).

#### 3.2. SOD mRNA expression and activity

BDE-209 elevated mRNA expression and activity of SOD (Fig. 1). Both exposure dose and duration had significant effect on SOD (Table S1). In the clams exposed to 0.1 and 1 μg/L BDE-209, mRNA expression and activity of SOD increased in time-dependent manner and peaked on day 7. In clams exposed to 10 μg/L BDE-209, SOD showed a biphasic response and peaked on day 5. SOD mRNA expression exhibited dose-dependent increase. After 3 days of recovery in clean seawater, mRNA expression of SOD returned to the control level in all the exposure groups; SOD activity decreased and was still significantly higher than the control level in 1 and 10 μg/L groups. SOD mRNA expression was positively correlated with SOD activity (P < 0.05) at each sampling time during exposure period (Table S2); no significant correlation was found on day 10 (P > 0.05).

#### 3.3. CAT mRNA expression and activity

BDE-209 could induce the elevation of CAT mRNA expression and

### Table 1

| Primer name | Sequence (5′→3′) |
|-------------|------------------|
| SODR (reverse) | AGCATGACCAACAACTGCT (Fang et al., 2010) |
| CATR (reverse) | TTGTGGTGGAGATGACCTCT (Dong et al., 2019) |
| GPr (forward) | TATTTGCGCAACATGCCGAGT (Dong et al., 2019) |
| GPr (reverse) | GGAGGCTGGAATGATTGAGT (Dong et al., 2019) |
| β-actin (forward) | TGGCATGCTGGGTGAAGAGT (Fang et al., 2010) |
| β-actinR (reverse) | GATGGATTGGCTGTTAGAGT (Fang et al., 2010) |
activity (Fig. 2). Both dose and duration could significantly affect CAT response (Table S1). CAT mRNA expression and activity started to increase significantly on day 3 and on day 1, respectively, and peaked on day 7. CAT mRNA expression increased in time-dependent manner. CAT activity increased in time- and dose-dependent manner. After 3 days of recovery in clean seawater, CAT mRNA expression in all the exposure groups subsided to control levels, and enzyme activity decreased but was still significantly higher than the control level. The correlation between CAT gene expression and enzymatic activity changed with duration (Table S2).

3.4. GPx mRNA expression and activity

Both dose and duration could significantly affect mRNA expression and activity of GPx (Table S1). In the exposure groups, GPx expression increased significantly from day 3, peaked on day 5, and recovered to control levels after 3 days in clean water (Fig. 3). In clams exposed to 0.1 μg/L BDE-209, GPx activity increased significantly from day 3 and peaked on day 5. In clams exposed to 1 and 10 μg/L, GPx activity increased significantly on day 1 and peaked on days 5 and 3, respectively. There was no significant difference in GPx activity between exposure groups and the controls on day 7 or after recovery in clean water. The correlation between GPx mRNA expression and enzymatic activity changed with duration (Table S2).
3.5. MDA content following BDE-209 exposure

MDA contents in the exposure groups increased significantly from day 3, reached the climax on day 7, and returned to the control level on day 10 (Fig. 4). The increase exhibited time- and dose-dependent manner.

4. Discussion

4.1. BDE-209 concentrations in seawater and bioaccumulation in clams

BDE-209 concentrations in seawater of the exposure groups were 57.25%–69.01% of the nominal concentrations after spiking of the pollutants. A toxicological study by waterborne exposure in the rotifer Brachionus plicatilis reported that BDE-209 concentrations in seawater measured 41.5% of the set concentration (Zhang et al., 2016b). In a zebrafish study, BDE-209 concentrations in water were about 80% of the nominal exposure concentrations (Zhu et al., 2016), while a study in M. galloprovincialis found much lower BDE-209 concentrations in seawater, of only 0.035%–0.15% of the nominal concentrations (Barón et al., 2016). Because of the high lipophilicity of PBDEs, their solubility in water is low, requiring a cosolvent. In this study and the reported work in B. plicatilis and zebrafish, DMSO was used as cosolvent, while acetone was used in the study in M. galloprovincialis. The solubility of BDE-209 in water may vary by solvent, and different measurement methods may also account for the different ratios between the actual and nominal concentrations. BDE-209 concentrations in seawaters decreased with time. Same scenario was also found in other researches (Vidal-Liñá et al., 2015; Barón et al., 2016). Uptake by clams could not fully explain the decrease of BDE-209 concentrations. 36.22% and 53.07% of BDE-209 was lost in 1 and 10 μg/L groups within 24 h, which might be due to the absorption of BDE-209 by particulates and volatilization of BDE-209.

Bioaccumulation in M. veneriformis was positively correlated with the exposure concentration, which indicated that the accumulation of BDE-209 occurred in the test clam under set concentrations and the accumulation depended on exposure concentration. Pollutants were taken up into the body of filter feeding shellfish while filtering. Each mussel M. galloprovincialis could accumulate 1.9, 1.7, and 1.6 μg BDE-209 after exposure to 0.02, 0.03, and 0.3 μg/L BDE-209 for 6 days (Barón et al., 2016). The bioaccumulation in M. galloprovincialis was on the same order as that in M. veneriformis. Fishes take up and accumulate BDE-209 from water through gill and skin. When exposed to 0.1 and 1 μg/L BDE-209 for 15 days, the Japanese medaka O. latipes accumulated 53.6 and 36.2 ng/g wet weight BDE-209, respectively (Luo et al., 2013), which was less than the bioaccumulation observed in our study. Thus, M. veneriformis is able to accumulate BDE-209 efficiently, making it as a suitable model organism for studying the toxicological effects of BDE-209. After recovery in clean seawater for 3 days, BDE-209 contents in M. veneriformis decreased considerably. Depuration of contaminant by aquatic organisms has been reported in previous studies and the depuration period varied with species and contaminant (Vidal-Liñá et al., 2015; Aouini et al., 2018). The elimination of BDE-209 in M. veneriformis was probably due to the case that highly brominated PBDEs are more likely to be metabolized. Compared with low brominated congeners, highly brominated congeners had shorter half-life and faster elimination rates (Feng et al., 2012). The metabolism in fishes has shown that BDE-209 could be readily metabolized into debrrominated PBDEs, methoxylated PBDEs and hydroxylated BDEs (Luo et al., 2013). BDE-209 was also easily biodegraded into lower brominated PBDEs in invertebrate earthworms (Zhang et al., 2014). However, the metabolism of BDE-209 was not clear in bivalves and should be studied further.

4.2. Effects of BDE-209 on the antioxidant system

A dominant PBDE congener in the environment, BDE-209 has been shown to cause oxidative stress in human erythrocytes, rats, ...
antioxidant enzymes might increase via ROS mediated pathway (Fatima et al., 2014; Horion et al., 2015; Shenai-Tirodkar et al., 2017). In this study, gene expression of a CAT subtype and a GPx subtype was tested and the enzyme activity consisting of all isozymes was determined. It could also explain that CAT and GPx activities increased significantly on the first day after exposure, while the expression of CAT and GPx did not change significantly.

Molecular markers indicate the biological effects of pollutant stresses, which can be used as biomarkers of exposure, especially at low concentrations. Antioxidant enzymes are sensitive biomarkers of aquatic pollution, and have been widely studied in aquatic organisms (Xie et al., 2014; Horion et al., 2015; Shenai-Tirodkar et al., 2017). In the present research, SOD, CAT, and GPx showed quick and sensitive response to BDE-209 stress with time- or dose-dependent manner. Their transcripts and activities reduced sharply after recovery in clean seawater. Especially, mRNA expression of SOD, CAT, and GPx subsided to the control level after 3 days of removal from contaminated seawater. Moreover, the fluctuation range of mRNA expression was larger than that of enzymatic activity. These findings suggested that mRNA expression might be more responsive than enzymatic activity. Transcription level reflects the immediate state of cells and quantifies the toxicity of potentially harmful substances (Brulle et al., 2010). Enzymatic activity indicates pollution from the physiological condition (Jemec et al., 2010). Transcripts and activities belong to genic and physiological endpoints, and always mismatched as discussed above. Thus, transcriptional and protein levels complement each other to provide comprehensive overview on ecotoxicological effect induced by contaminations. mRNA expressions and enzymatic activities would be used jointly as potential biomarkers of aquatic pollution in view of health status of organisms.

5. Conclusion

We assessed BDE-209 bioaccumulation and the response of the antioxidant system in M. veneriformis. The clams could accumulate BDE-209 efficiently during exposure period and eliminate BDE-209 considerably after recovery in clean seawater. BDE-209 caused oxidative stress. The clams suffered more oxidative stress as MDA accumulated gradually over time. SOD, CAT, and GPx were activated and involved in resisting oxidative stress sequentially. In future, researches on the mechanism of antioxidant response to BDE-209 should be carried out.
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