Responses of growth inhibition and antioxidant gene expression in earthworms (*Eisenia fetida*) exposed to tetrabromobisphenol A, hexabromocyclododecane and decabromodiphenyl ether

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ABSTRACT

Tetrabromobisphenol A (TBBPA), hexabromocyclododecane (HBCD) and decabromodiphenyl ether (BDE 209), suspected ubiquitous contaminants, account for the largest volume of brominated flame retardants (BFRs) since penta-BDE and octa-BDE have been phased out globally. In this paper, the growth inhibition and gene transcript levels of antioxidant enzymes (superoxide dismutase (SOD), catalase (CAT)) and the stress-response gene involved in the prevention of oxidative stress (Hsp70) of earthworms (*Eisenia fetida*) exposed to TBBPA, HBCD and BDE 209 were measured to identify the toxicity effects of selected BFRs on earthworms. The growth of earthworms treated by TBBPA at 200 and 400 mg/kg dw were inhibited at rate of 13.7% and 22.0% respectively, while there was no significant growth inhibition by HBCD and BDE 209. A significant ($P < 0.01$) up-regulation of SOD expression level was observed in earthworms exposed to TBBPA at 50 mg/kg dw (1.77-fold) and to HBCD at 400 mg/kg dw (2.06-fold). The transcript level of Hsp70 gene was significantly up-regulated ($P < 0.01$) when earthworms exposed to TBBPA at concentration of 50–200 mg/kg (2.16–2.19-fold) and HBCD at 400 mg/kg (2.61-fold). No significant variation of CAT gene expression in all the BFRs treatments was observed, neither does all the target gene expression level exposed to BDE 209. Assessed by growth inhibition and the changes at mRNA levels of encoding genes in earthworms, TBBPA showed the greatest toxicity, followed by HBCD and BDE 209, consistent with trends in molecular properties. The results help to understand the molecular mechanism of antioxidant defense.

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1. Introduction

Brominated flame retardants (BFRs) are synthetic chemicals used globally to reduce the likelihood of ignition of materials and/or decrease the rate of combustion. The production and use of penta-BDE, octa-BDE, and some other BFRs have been banned regionally, e.g. in European Union, North-East Atlantic countries or even worldwide since they are listed as or suspected to be persistent organic pollutants (Kemmlin et al., 2009; Covaci et al., 2011; Ni et al., 2013). Currently, tetrabromobisphenol A (TBBPA), hexabromocyclododecane (HBCD) and decabromodiphenyl ether (BDE 209, the fully brominated congener of poly-brominated diphenyl ethers, PBDEs) account for the largest volume of BFRs. However, they have been identified to be ubiquitous contaminants where employed by the elevated concentration in biota tissues and abiotic matrices (e.g. in air, indoor dust, water, soil, sewage sludge, sediments etc.) and their thyroid hormone disrupting, endocrine disrupting toxicity, neurotoxicity, reproductive and development

toxicity (de Wit et al., 2006; Gauthier et al., 2007; Law et al., 2008, 2014; Covaci et al., 2009, 2011).

Soils are a major reservoir and sink for PBDEs, HBCD and other BFRs because of their high octanol–water partition coefficient, low water solubility and low vapor pressure (Covaci et al., 2006; Yang et al., 2010; Gevaio et al., 2011). Therefore, soils play an important role in the distribution and biogeochemical cycling of the BFRs, the effects of these BFRs on terrestrial invertebrates, with earthworms as the model species, which live in the soils in the whole or part of the life cycle could be the subject of intensified research.

Organisms remove reactive oxygen species (ROS) and then defend oxidative stress by antioxidative enzymes such as catalase (CAT), superoxide dismutase (SOD), and peroxidase (POD). Stress-responsive proteins such as heat shock protein 70 (Hsp70) also protect the cell from the toxin (Homa et al., 2005, 2007; Chen et al., 2011a). Evidence indicate that the induction of the antioxidant enzymatic system and Hsp70 may serve as potential biomarkers of environmental pollution in toxicology (Song et al., 2009; Webb and Gagnon, 2009; Xue et al., 2009; Zhang et al., 2009). Gene expression profiling is a useful supplement for protein level detection, as the mRNA level represents a snapshot of the

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cell activity, and has been performed to better identify biomarkers of toxicity to predict potential hazardous chemicals (Olsvik et al., 2005; Brulle et al., 2010). Previous research has detected that the gene expression of antioxidant enzymes and Hsp70 in earthworms is involved in the oxidative stress responses to heavy metals (Homa et al., 2007; Brulle et al., 2010; Xiong et al., 2014) and organic chemicals (Chen et al., 2011a,b). Reports indicated that BDE 209 and TBBPA induced the ROS and activities of selected antioxidant enzymes in earthworms (Xue et al., 2009; Xie et al., 2011; Zhang et al., 2014), however, little is known about the transcriptional regulation of antioxidant and stress-response gene at the mRNA level with respect to the response of earthworms to HBCD, BDE 209 and TBBPA, and warrant more thorough investigation.

In this paper, the growth inhibition and gene expression levels of SOD, CAT and Hsp70 of earthworms (*Eisenia fetida*) exposed to TBBPA, HBCD and BDE 209 were detected to identify the toxicity effects of selected BFRs on earthworms. The study may help to understand the molecular mechanism of antioxidant defense and provide early markers for the toxic damage of cellular proteins by assessing the changes in mRNA levels of encoding antioxidant enzyme genes and stress induced proteins involved in the prevention of oxidative stress.

2. Materials and methods

2.1. Earthworms and chemicals

Mature earthworms (*E. fetida*) of age 3 months with a well-developed clitellum were obtained from a farm in Beijing. They were removed from the soil 24 h before use and stored in Petri dishes on damp filter paper (in the dark at $20 \pm 1^\circ\text{C}$) to void their gut contents.

Decabromodiphenyl ether (BDE 209) (CAS No. 1163-19-5, 98% purity) was purchased from J&K Scientific Chem Service (China). Tetrabromobisphenol A (CAS No. 79-94-7, 98% purity), was produced by TCI (Shanghai) Chemicals; hexabromocyclododecane (CAS No. 3194-55-6, 95.0% purity), was produced by TCI Chemicals (Japan). N-hexane (analytical grade) was purchased from Sinopharm Chemical Reagent Co, Ltd. (SCRC) (Shanghai, China). All the other chemicals and reagents (analytical grade) were also purchased from Sinopharm Chemical Reagent Co, Ltd. (SCRC) (Shanghai, China). All aqueous solutions were prepared by using reagent water from a Milli-Q Gradient system (Millipore Company, Bedford, USA).

2.2. Acute toxicological tests

The earthworm cultivation method followed the guideline of the OECD method (OECD 1984). The artificial soils consisted of 70% quartz sand, 20% kaolin clay and 10% sphagnum peat and the moisture content was adjusted to about 35% by adding deionized water. The pH is adjusted to 6.0 ± 0.5 with calcium carbonate.

BDE 209 was spiked with n-hexane (20 ml) as a carrier and thoroughly mixed into the artificial soils at concentrations of 0, 1, 10, 100 and 200 mg/kg dry soil. TBBPA and HBCD were dissolved with 20 ml acetone and thoroughly mixed into the soils at concentrations of 0, 50, 100, 200, and 400 mg/kg dry soil, respectively. Four replicates were used for each dose; 750 g of wet artificial soils was added to each container. Controls and solvent controls were four containers with BFRs replaced by deionized water or acetone. Before introducing the

earthworms, every container was placed in an exhaust hood for 2–4 days to ensure that the n-hexane or acetone had completely evaporated. 10 earthworms (0.35–0.45 g each) were then placed into each container. The containers were kept in an incubation chamber ($20 \pm 1^\circ\text{C}$), $83 \pm 3\%$ relative humidity with continuous illumination at 400–800 lx throughout the test period.

During the period of exposure, earthworms were taken from the containers, cleaned with deionized water, weighed, and then put back to the containers at days 4, 7, 10, and 14. After exposure for 14 days, three earthworms were selected from each container randomly to be used to determine gene expression levels, respectively.

2.3. Growth inhibition of earthworms

The growth inhibition rate (GIR, %) and instantaneous growth rate (IGR, d^{-1}) were calculated as:

$$\text{GIR}_n = \frac{W_0 - W_{14}}{W_0} \times 100\%$$

$$\text{IGR}_n = \frac{\ln W_j - \ln W_i}{t_j - t_i}$$

where GIR_n is the growth inhibition rate for dose group *n* during the 14 d exposure period, IGR_n is the instantaneous growth rate for dose group *n* from day *i* to *j*, W_0 is the weight on day 0, W_{14} is the weight on day 14, W_i is the weight on day *i* and W_j is the weight after *j* days of exposure.

2.4. Gene transcript levels

Earthworms which were selected from each container randomly cleaned with deionized water, left in petri dishes with moist filter paper to clear out their gut contents for 24 h and then ground immediately in liquid nitrogen. All expression analyses were conducted on total RNA isolated using the TRIzol Reagent (Invitrogen, USA), according to the manufacturer's instructions. RNA purity and integrity were measured by agarose gel electrophoresis (2%) to ensure that absorbance ratios ($A_{260/280}$) were between 1.8 and 2.0. First-strand cDNA synthesis was performed using a superscriptTM reverse transcriptase (Invitrogen, USA), according to the manufacturer's instruction. Reverse transcription and quantitative PCR (RT-qPCR) of the selected genes (β -actin, SOD, CAT and Hsp70) were conducted using the Real-Time PCR kit (TaKaRa, Japan).

The Mastermix contained (final concentrations) the following: 2.0 μl cDNA template, 0.4 μl each of forward and reverse primer (concentration of 10 μM), 6.8 μl ddH₂O, 10 μl SYBR[®] Premix Ex TaqTM (2 $\times$) and 0.4 μl ROX Reference Dye(50 $\times$)*2 (TaKaRa, Japan). The following RT-qPCR reactions were performed with the 7300 Real-Time PCR system (ABI, USA): initial denaturation step (95 $^\circ\text{C}$, 10 min), amplification and quantification step repeated 40 times (95 $^\circ\text{C}$ for 15 s, annealing temperature 55 $^\circ\text{C}$ for 15 s, 72 $^\circ\text{C}$ for 45 s) and melting curve step (one cycle of 95 $^\circ\text{C}$ for 15 s, 55 $^\circ\text{C}$ for 30 s, and 95 $^\circ\text{C}$ for 15 s).

Sequence of primers used for RT-qPCR is shown in Table 1. Primers of β -actin, SOD, CAT and Hsp70 used for earthworms (*E. fetida*) were selected from references (Brulle et al., 2006; Chen et al., 2011b).

Table 1
Sequence of primers used for RT-qPCR.

Gene	Accession number	Forward primer	Reverse primer
β -actin	GU177854	5'-TCCATCGTCCACAGAAAG-3'	5'-AAATGTCCTCCGCAAGCT-3'
SOD	GU177856	5'-TGCTCACTTCAACCCATT-3'	5'-TTGGCAACACCACTTCA-3'
CAT	GU177857	5'-CATTGCGGATGGAACTA-3'	5'-CCAAGGACAACAACCTGCTC-3'
Hsp70	GU177858	5'-CCAAGGACAACAACCTGCTC-3'	5'-CGGCGTCTTCACCATTC-3'

The efficiencies (E) of amplification of target gene and reference gene were calculated from the given slope of the standard curve according to the equation $E = 10^{(-1/\text{slope})} - 1$. They were in the ranged from 0.98 to 1 (Pearson correlation coefficient $r^2 > 0.99$), and a strong linearity was observed.

The relative quantification of target genes with reference gene (R) was calculated according to the formula $R = (E_{\text{Tg}})^{\text{CPTg}} / (E_{\text{act}})^{\text{CAct}}$, target gene (Tg), β -actin gene(Act) (Pfaffl, 2001; Brulle et al., 2006).

2.5. Statistical analysis

The data for growth inhibition and gene expression levels were each subjected to a one-way analysis of variance (ANOVA) calculating the LSD to contrast the differences between treatment means, with assuming a normal distribution and the homogeneity of variances, which were tested respectively by the Shapiro–Wilk test and Levene's test. The paired sample t-test was applied to the instantaneous growth rates to test the differences of the growth rates in the different intervals. Logarithmic transformation was applied to the dependent variables in gene expression levels analysis. The Pearson correlation was used in correlation analysis. A probability level of 0.05 was used in all procedures (equivalent to 95% confidence) and data were presented as means $\pm$ standard deviation (SD). All statistical analyses were performed using SPSS 18.0[®] software and all charts were designed using Origin 8.6 software.

3. Results

3.1. Growth inhibition

The growth inhibition rates of earthworms exposed to TBBPA, HBCD and BDE-209 over 14 days are shown in Fig. 1. Growth inhibition rates of earthworms exposed to TBBPA were shown to be dose-dependent over 14-day ($P < 0.01$). The growth inhibition of earthworms treated with the higher doses (200 and 400 mg/kg dw) was significantly higher than those for treatments at the controls and lower concentrations (50, 100 mg/kg dw) ($P < 0.05$). However, no significant concentration-related growth inhibition in earthworms from exposure to HBCD and BDE 209 was observed ($P > 0.05$).

The instantaneous growth rates of the earthworms exposed to TBBPA during the 0–14th days are shown in Fig. 2a. The negative growth rates portended growth inhibition of earthworms. The growth rates during 0–4th day of all groups were significantly lower than those during 4th–7th and 7th–10th days ($P < 0.01$), and they showed negatively dose-dependent during each of the intervals, the minimum value

appeared at the concentration of 400 mg/kg dw ($P < 0.05$). The growth rates increased after the 4th day, becoming of positive values in the groups of controls and low doses (50 mg/kg dw).

The negative growth rates of earthworms exposed to HBCD appeared during the 0–4th day and 10th–14th day, the growth rates during 0–4th day of all groups were significantly lower than those during 4–7th and 7th–10th days ($P < 0.01$), however, no significant difference was obtained between the HBCD treatments and controls during each of the intervals ($P > 0.05$) (Fig. 2b).

The negative effect on growth rates of earthworms exposed to BDE 209 appeared during the whole 14 days, the growth rates during 0–4th day of all groups were significantly higher than those during all the other intervals ($P < 0.01$), no significant difference among different BDE 209 dose groups was shown during each of the exposure period ($P > 0.05$) (Fig. 2c).

3.2. Gene expression levels

Transcriptional levels of target genes (SOD, CAT and Hsp70) of earthworms exposed to TBBPA are shown in Fig. 3. No significant variations of CAT gene expression level between TBBPA treated groups and controls, deionized water (CK) and acetone control (CKs), were observed ($P > 0.05$). Transcriptional levels of SOD and Hsp70 genes showed significant variations between TBBPA dose groups and the controls throughout the 14-day acute tests. With the increase of the concentrations, up-regulation of SOD expression level was observed in earthworms, significant up-regulation of SOD gene expression level appeared at the concentration of 50 mg/kg dw (1.77-fold, $P < 0.01$). Earthworms exposed to TBBPA expressed a significant up-regulation of Hsp70 gene expression levels (50 mg/kg dw, 2.19-fold, $P < 0.01$; 100 mg/kg dw, 2.17-fold, $P < 0.01$; 200 mg/kg dw, 2.16-fold, $P < 0.01$).

Transcriptional levels of target genes (SOD, CAT and Hsp70) of earthworms exposed to HBCD are shown in Fig. 4. With increase of the concentrations, up-regulation of SOD and Hsp70 expression level was observed in earthworms. Significantly, higher level of SOD and Hsp70 gene expression level appeared at the concentration of 400 mg/kg dw (SOD, 2.06-fold, $P < 0.01$; Hsp70, 2.61-fold, $P < 0.01$). No significant variations of CAT gene expression level between HBCD treated groups and controls were observed ($P > 0.05$).

Transcriptional levels of target genes (SOD, CAT and Hsp70) of earthworms exposed to BDE-209 showed variations between control and BDE-209 treated groups, however no significant differences ($P > 0.05$) were detected for all the target genes (Fig. 5).

Fig. 1. Growth inhibition rates of earthworms exposed to TBBPA, HBCD and BDE-209 over 14 days (** $P < 0.01$ compared with the control groups).

Fig. 2. Growth rates of earthworms exposed to TBBPA (a), HBCD (b) and BDE-209 (c) over different exposure intervals.

Fig. 3. Changes in target genes in earthworm *Eisenia fetida* tissues with different TBBPA treatments after 14 day exposure. (**P < 0.01 compared with the controls group).

4. Discussion

Mortality and biomass change are applied frequently for assessing the toxicity of pollutants since they show the responses of organisms reliably and are easy to detect. In this study, the earthworms survived in all the BFR treatments, meaning that the nonlethal effects of the selected BFRs on earthworms were observed.

The effects of TBBPA and HBCD on growth of earthworms have been scarcely reported. PBDEs are the most extensively investigated BFRs and evidences showed that some monomers of PBDEs significantly inhibited the growth of the earthworm (*E. fetida*). The authors found that the growth inhibition rates of *E. fetida* treated by 400 mg/kg dw BDE 47 reached 36.4% after 14-day exposure (Xu et al., 2015). BDE 71 (1000 mg/kg dw) had significant effects on growth (GI = 25%) on *E. fetida* after 14-day exposure (Zhu et al., 2009). In this study, the growth of earthworms treated by TBBPA at 200 and 400 mg/kg dw was inhibited at rates of 13.7% and 22.0% respectively, while no significant growth inhibition by HBCD (at concentration of 50–400 mg/kg) and BDE 209 (at concentration of 1–200 mg/kg) was noticed (Fig. 1). The growth inhibition by exposure to the high concentration of TBBPA may be explained by the strategy of reducing food intake to avoid toxins and consuming more energy to combat deleterious effects of

Fig. 4. Changes in target genes in earthworm *Eisenia fetida* tissues with different HBCD treatments after 14 day exposure. (**P < 0.01 compared with the control group).

Fig. 5. Changes in target genes in earthworm *Eisenia fetida* tissues with different BDE 209 treatments after 14 day exposure.

contaminants, which was observed in crayfish (Rowe et al., 2001) and isopods (*Porcellio dilatatus*) (Ribeiro et al., 2001). The absence of growth inhibition in earthworm exposed to BDE 209 over the 14-day exposure in the present research is in agreement with previous report of BDE 209 (1000 mg/kg dw) on growth of the earthworms (*E. fetida*) after 28-day exposure (Xie et al., 2013).

The growth rates observed during the 0–14 day exposure period help to understand the dynamics of growth inhibition to earthworms. The growth rates for 0–4th day had a negative value, decreased with the increase of TBBPA dose and significantly lower than those during the 4th–7th, 7th–10th, and 10th–14th days (Fig. 2a), it indicated that the growth inhibition appeared during the first 4 days of exposure to TBBPA, and it increased with the increase of TBBPA dose. Lower growth rates of earthworms exposed to HBCD were appeared at 0–4th day, while that of BDE 209 appeared later at 4th–7th days, possible reflecting the different absorption rates of the two BFRs by earthworms. However, no significant difference between controls and HBCD or BDE 209 groups of earthworms were shown during each of the exposure periods, indicating that the earthworms' growth was inhibited by nutrient deficiency or the other environmental factors, but not by the exposure to HBCD or BDE 209.

Oxidative stress can generate DNA damage and produce oxidative damage to macromolecules, leading to apoptotic mechanisms and finally damaging different cellular organelles (Sabatini et al., 2009). Oxidative stress is generated by the excessive reactive oxygen species (ROS) in living organisms, caused by exposure to heavy metals and organic contaminants (Saint-Denis et al., 2001; Lin et al., 2010). Oxidative stress was produced in earthworms exposed to BDE 209, HBCD and TBBPA by the generation of excessive hydroxyl radical ROS (Xue et al., 2009; Jin et al., 2010; Marvin et al., 2011; Xie et al., 2011; Zhang et al., 2014).

To scavenge ROS and combat oxidative stress, organisms possess antioxidant defense mechanisms. Superoxide dismutase (SOD) converts superoxide radical to hydrogen peroxide, thereby protecting cells from the toxic effects of superoxide radical (Liu et al., 2011; Hackenberger et al., 2012). The up-regulation of SOD gene expression indicates an adaptive onset of the antioxidant defense system in zebrafish to methylmercury (Gonzalez et al., 2005), in earthworms exposed to BDE 47 (Xu et al., 2015) and tonalide (AHTN) (Chen et al., 2011b). In this study, the significant up-regulation (P < 0.01) of SOD expression level was observed in earthworms exposed to TBBPA at 50 mg/kg dw and to HBCD at 400 mg/kg dw, indicating that transcription of SOD might be stimulated by oxidative stress generated by superoxide anion radical and the enhanced SOD gene expression was required to protect earthworms from oxidative stress and to counteract

ROS toxicity. That there is no variation of SOD expression level exposed to BDE 209 and low dose HBCD shows the lack of the oxidative stress on earthworms.

Catalase (CAT) is one of the important antioxidant enzymes which can decompose hydrogen peroxide into oxygen and water. Previous reports have shown the up-regulation of CAT expression level in oysters exposed to cadmium (Jo et al., 2008), in earthworms exposed to AHTN (Chen et al., 2011a) to detoxify the oxidative stress, and its down-regulation in earthworms exposed to BDE 47 (Xu et al., 2015) when the generated superfluous ROS exceeds the capacity of scavenging ROS by CAT. In the present research, there is no variation of CAT gene expression in the earthworms in all the TBBPA, HBCD and BDE 209 treatments, indicating a balance of generation and scavenge of hydrogen peroxide radical in earthworms.

Heat shock proteins (Hsps) participate in multiple biological processes by promoting the proper folding of newly synthesized polypeptides into functional proteins and the repair or disposal of misfolded, damaged, proteins. In the present research, the expression of Hsp70 gene was significantly up-regulated when earthworms were exposed to TBBPA at concentrations of 50–200 mg/kg dw and HBCD at 400 mg/kg dw, but no variation when exposed to BDE 209. The results are contrary to the research in earthworms exposed to BDE 47 (Xu et al., 2015) and in aquatic animals exposed to 4-nonylphenol, 4-t-octylpheno (Rhee et al., 2009), carbon tetrachloride and fenitrothion (Lee et al., 2006), but are in agreement with researches on up-regulation of Hsp70 gene expression in copepod exposed to bisphenol A (Rhee et al., 2009), in earthworms induced by the exposure of heavy metals (Homa et al., 2007). These results all provide evidence that the Hsp70 gene expression is involved in oxidative stress responses. The results of this work also suggest the protective roles of Hsp70 in oxidative stress induced by TBBPA and HBCD in earthworms.

The comparative analysis of the different responses of growth, SOD gene expression, Hsp70 gene expression of earthworms to the BFRs may help to understand the toxicity order of TBBPA, HBCD and BDE 209. Only TBBPA significantly inhibited the growth of earthworms, with HBCD and BDE 209 showing no toxic effects on the growth; the SOD gene expression level was up-regulated by exposure to TBBPA at 50 mg/kg dw (1.77-fold, $P < 0.01$), and to HBCD at 400 mg/kg (2.06-fold, $P < 0.01$), but no up-regulation by exposure to BDE 209, it indicated that the toxicity of the BFRs to SOD gene expression was in decreasing order of TBBPA, HBCD and BDE 209; the gene expression levels of Hsp 70 in TBBPA treatments were up-regulated in 50, 100, and 200 mg/kg dw groups, the up regulation appeared at 400 mg/kg dw HBCD and no up-regulation by exposure to BDE 209 was observed, TBBPA showed the greatest toxicity to Hsp 70 gene expression, followed by HBCD and BDE209.

In general, the toxicity integrating the growth inhibition and antioxidant response gene expression of the three BFRs ranks in the decrease order of TBBPA, HBCD and BDE 209. The difference may be related to their chemical properties (Table 2). Compared to HBCD and BDE 209, TBBPA is the smallest molecule, has the highest water solubility and lowest distribution coefficient between organic matter and water, so that it is most readily cross membranes, and has the highest rate of toxic effects. On the contrary, BDE 209 has the largest molecules, lowest water solubility and is extremely hydrophobic, tightly bound in soils, showing why BDE 209 has little effects on the growth and genes expression levels of earthworms. HBCD shows medium toxicity to the genes with its chemical properties ranking between TBBPA and BDE 209.

5. Conclusions

The growth of earthworms treated by TBBPA at 200 and 400 mg/kg dw was significantly inhibited. Little variation of CAT gene and significant up-regulation of Hsp70 and SOD gene expression levels were observed in earthworms exposed to TBBPA (Hsp70: at dose 50–200 mg/kg; SOD: at dose 50 mg/kg dw).

Table 2
Selected chemical properties of the BFRs.

Chemicals	Skeletal formula	Molecular weight	Water solubility (mg/l)	log Kow
TBBPA		543.9	4.16	4.5
HBCD		641.7	0.002–0.048	5.6
BDE 209		959.2	0.02–0.03	10

Data sources: a) WHO: Environmental Health Criteria 172. Tetrabromobisphenol A and derivatives. Geneva, Switzerland: International Programme on Chemical Safety, World Health Organization; 1995;

b) Covaci et al. (2006). Hexabromocyclododecanes (HBCDs) in the Environment and Humans: A Review. Environmental Science & Technology 40, 3679–3688.

c) WHO: Environmental Health Criteria 162. Brominated diphenylethers. Geneva, Switzerland: International Programme on Chemical Safety, World Health Organization; 1994.

SOD and Hsp70 gene expression was both up-regulated by HBCD at the concentration of 400 mg/kg. No significant variation of growth and CAT gene transcript level by HBCD was observed.

The growth inhibition and all the target gene expression levels of the BDE 209 groups were not significantly different when compared with controls.

Assessed by growth inhibition and the changes in mRNA levels of encoding genes in earthworms, TBBPA showed the greatest toxicity, followed by HBCD and BDE 209. The results help to understand the molecular mechanism of antioxidant defense. The transcriptional responses of the antioxidant genes may provide early warning molecular biomarkers for identifying the BFR toxicity.

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