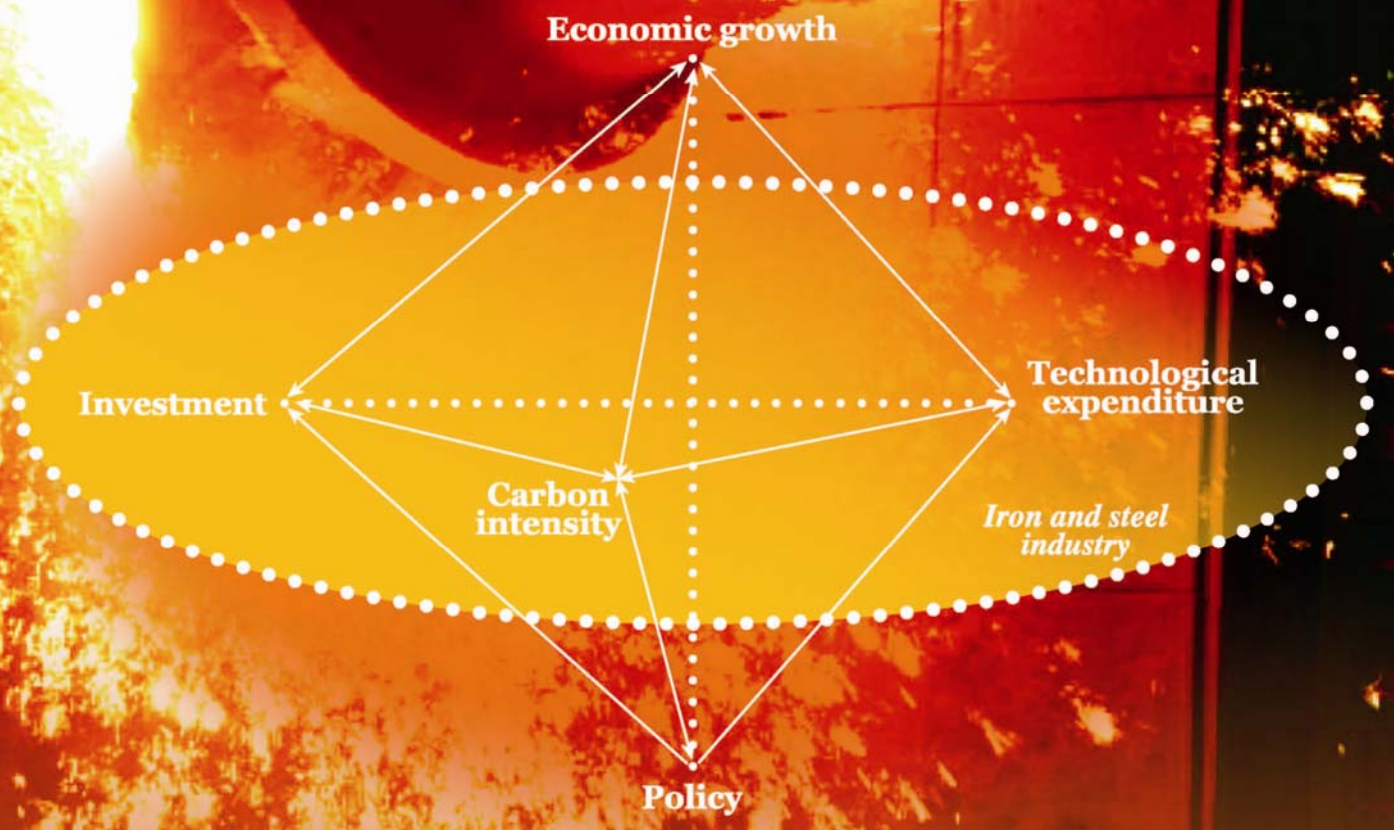
ISSN 1001-0742
CN 11-2629/X

JES

JOURNAL OF ENVIRONMENTAL SCIENCES

February 1, 2015 Volume 28
www.jesc.ac.cn



ELSEVIER
Science Press

Sponsored by
Research Center for Eco-Environmental Sciences
Chinese Academy of Sciences

Journal of Environmental Sciences Volume 28 2015

www.jesc.ac.cn

CONTENTS

Available online at www.sciencedirect.com

ScienceDirect

www.journals.elsevier.com/journal-of-environmental-sciences

JOURNAL OF ENVIRONMENTAL SCIENCES
www.jesc.ac.cn

Effects of two polybrominated diphenyl ethers (BDE-47, BDE-209) on the swimming behavior, population growth and reproduction of the rotifer *Brachionus plicatilis*

Jingjing Sha[1], *You Wang*[1], *Jianxia Lv*[2], *Hong Wang*[1], *Hongmei Chen*[1], *Leilei Qi*[1], *Xuexi Tang*[1,*]

1. *Department of Marine Ecology, College of Marine Life Sciences, Ocean University of China, Qingdao 266003, China. E-mail: shajingjingouc@163.com*
2. *Thermo Fisher Scientific (Beijing) Co., Ltd., Beijing 100080, China*

ARTICLE INFO

Article history:
Received 11 March 2014
Revised 8 July 2014
Accepted 31 July 2014
Available online 17 November 2014

Keywords:
BDE-47
BDE-209
Brachionus plicatilis
Swimming inhibition
Population growth
Reproductive toxicity

ABSTRACT

Polybrominated diphenyl ethers (PBDEs) are new kinds of persistent organic pollutants (POPs) and their potential threats to the equilibrium and sustainability of marine ecosystems have raised worldwide concerns. Here, two kinds of PBDEs, tetra-BDE (BDE-47) and deca-BDE (BDE-209) were applied, and their toxic effects on the swimming behavior, population growth and reproduction of *Brachionus plicatilis* were investigated. The results showed that: (1) The actual concentrations of BDE-47 and -209 in the seawater phase measured by GC–MS (Gas Chromatography–Mass Spectrometer) were much lower than their nominal concentrations. (2) In accordance with the 24-hr acute tests, BDE-209 did not show any obvious swimming inhibition to rotifers, but a good correlation did exist between the swimming inhibition rate and BDE-47 concentration suggesting that BDE-47 is more toxic than BDE-209. (3) Both BDE-47 and -209 had a significant influence on the population growth and reproduction parameters of *B. plicatilis* including the population growth rate, the ratio of ovigerous females/non-ovigerous females (OF/NOF), the ratio of mictic females/amictic females (MF/AF), resting egg production and the mictic rate, which indicate that these parameters in *B. plicatilis* population were suitable for monitoring and assessing PBDEs. Our results suggest that BDE-47 and -209 are not acute lethal toxicants and may pose a low risk to marine rotifers at environmental concentrations for short-term exposure. They also accumulate differently into rotifers. Further research data are needed to understand the mechanisms responsible for the effects caused by PBDEs and to assess their risks accurately.

Introduction

As a new kind of persistent organic pollutants (POPs), polybrominated diphenyl ethers (PBDEs) have 209 congeners that are distinguished by the number and location of bromine atoms in the benzene ring. The PBDEs have been widely used as additive brominated flame retardants (BFRs) in petroleum, textiles, building products, plastics, electronic circuits and other materials because of their stable chemical properties (Rahman et al., 2001). They have a high boiling point, low vapor pressure and low solubility in water (Darnerud et al., 2001; de Wit, 2002). Their structure, character, toxicity and distribution in the environment show many similarities with PCBs (polychlorinated biphenyls) and DDT (dichloro diphenyl trichloroethane) (Sellström et al., 1993). These compounds, which are quite resistant to physical, chemical, and biologic degradation, can easily bio-accumulate in

* *Corresponding author*. E-mail addresses: tangxx@ouc.edu.cn, tangmentg@163.com (X. Tang).

http://dx.doi.org/10.1016/j.jes.2014.07.020
1001-0742/

fatty tissues and biomagnify through food chains and eventually become concentrated in high trophic level organisms and humans threatening health (Talsness, 2008; Noyes et al., 2010). The PBDE levels in marine sediments and biota generally increase over time. The lower (tetra- and penta-) PBDEs accumulate and predominate in biota, while the higher congeners (deca-) are prevalent in marine environments and sediments (Martin et al., 2004). Therefore, governments and researchers have recently paid more attention to PBDE pollution especially PBDEs in the marine environment. Deca-BDE, which consists almost entirely of BDE-209, is the only PBDE mixture still used routinely, but is scheduled for phase out in the United States by the end of 2013 (USEPA, 2010; Roberts et al., 2011). Due to their bioaccumulation and biotransformation ability, lower brominated PBDEs are often detected in many organisms and environments (air, water, soil, sediment, *etc.*) (Letcher et al., 2010).

One of the most abundant PBDE congeners detected in animal and human tissues is 2,2′,4,4′-tetrabromodiphenyl ether (BDE-47). It can bioaccumulate rapidly in the lipid reservoirs of organisms (Lema et al., 2007; Viganò et al., 2011), and its biological absorption rate is higher than other PBDEs (Stapleton et al., 2009). The mass production of 2,2′,3,3′,4,4′,5,5′,6,6′-decabromodiphenyl ether (BDE-209) led to its higher levels in the environment *versus* other PBDEs. Research on the ecotoxicological effects of PBDEs has been mainly on the lower brominated diphenyl ethers including BDE-28, BDE-47 and BDE-99 which are more toxic and bioactive. It is demonstrated that deca-BDE appears to have very low toxicity, including acute, sub-chronic, chronic, reproductive/developmental toxicity on mammals, such as rats or mice (Hardy, 2002; Hardy et al., 2002, 2009; Xie et al., 2013). Nevertheless, studies have shown that BDE-209 has liver toxicity, reproductive toxicity, endocrine toxicity and potentially carcinogenic effects (Du et al., 2008). It also has toxic effects on the growth and inter-specific competition of marine microalgae (Zhang et al., 2013). However, there are only a limited number of published studies investigating the toxicity of BDE-47 and BDE-209 on invertebrates. Thus, we here assess the hazard risk posed by BDE-47 and BDE-209 to aquatic organisms because of their wide distribution in the environment.

Routine toxicity evaluation is increasingly in need of quick and convenient chronic toxicity test methods. Rotifers have become an important new tool for toxicity testing and environmental monitoring. They are ideal test animals for aquatic toxicology because of their rapid reproduction, short generation time, high sensitivity to toxic substances, cosmopolitan distribution and the commercial availability of resting eggs (Snell and Janssen, 1996). While the toxic effects of various pollutants on rotifers and other aquatic animals have been studied, there are still limited reports on the toxicity of PBDEs to rotifers.

The rotifer *Brachionus plicatilis*, one of the main components of freshwater and coastal marine plankton, plays an increasing role in assessing the impacts of environmental contaminants in aquatic ecosystems. In the rotifer life cycle, two types of females exist which differ in their reproductive mode. Asexual females produce eggs mitotically that develop into females. Given appropriate environmental conditions, asexual females produce sexual females. Sexual females subsequently produce eggs mitotically that develop into haploid males, or resting eggs if fertilized by males (Birky and Gilbert, 1971; Snell, 1987). Resting eggs are generally resistant to negative environmental conditions and can maintain their viability for long periods of time. When in a favorable environment, the resting eggs begin to hatch a new generation of amictic females and entering a period of parthenogenesis (Snell, 1986). Several studies have been previously designed to assess the effects of pesticides, heavy metals and environmental endocrine-disrupting chemicals on the reproduction of rotifers (Ferrando et al., 1993; Janssen et al., 1994; Snell and Carmona, 1995; Preston and Snell, 2001; Radix et al., 2002; Marcial et al., 2005). Results showed that sexual reproduction and resting egg production are among the most sensitive endpoints.

Despite the prevalence of PBDEs in aquatic systems, very limited information has been gathered about the toxic effects of PBDEs on rotifers. The objective of this study is to derive toxicological information related to the effects of the two PBDEs (BDE-47 and BDE-209) on rotifer *B. plicatilis*. We investigated not only acute toxicity of the two PBDEs, but also their effects on the swimming behavior, population growth and reproduction of *B. plicatilis*. Our study provided the first known data on the toxicity of these two PBDEs to *B. plicatilis* and establishes the framework for future laboratory experiments.

1. Materials and methods

1.1. Materials

1.1.1. Culture of the rotifers

The rotifer *B. plicatilis* used in this experiment was obtained by hatching resting eggs, which were collected from the sediment of the prawn breeding ponds of Ru Shan, Weihai, China. Stock rotifer cultures were kept under static-renewed conditions with a 12 hr: 12 hr light: dark cycle at (25 ± 1)°C, 80 μmol photons/(m^2·sec) in a GXZ-280 C intelligent illumination incubator (Jiangnan Instrument, Ningbo, Zhejiang, China). Seawater used for experiment, collected from Huiquan Bay, Qingdao, China, was filtered through a 0.45 μm pore size cellulose nitrate membrane filter and autoclaved at 121.3°C for 20 min. Salinity was around 31‰ and pH was 8.6. The rotifers were daily fed on *Chlorella* sp. at a concentration of 1.0×10^6 cells/mL. Algae were grown in a semi-continuous culture in sterile seawater enriched with f/2 nutrient medium. Before the experiments commenced, rotifers were cultured in the illumination incubator for at least two weeks.

The rotifers were acclimated to the experimental conditions for about 48 hr by inoculating them in tissue culture plates containing 24 wells at density of 10 ind./mL. The active and strong female individuals with amictic eggs were chosen and then placed in other tissue culture plates under the same conditions to observe the number of eggs hatched. Neonates that hatched within 2 hr were used for follow-up experiments.

1.1.2. Chemicals and experimental solutions

Standard solutions of BDE-47 (50 mg/L, solvent: isooctane), BDE-209 (100 mg/L, solvent: toluene) and BDE-47 (100% purity, Gas Chromatography–Mass Spectrometer (GC–MS), white solid powder) were purchased from AccuStandard Inc. (New Haven, Connecticut, USA). BDE-209 (99.5% purity, GC–MS, white solid powder) was provided by Dr. Ehrenstorfer Laboratories (Augsburg, Germany) and purchased from Quandao Company (Shanghai, China). The dichloromethane (99.0%), the nonane (99.0%) and the concentrated sulphuric acid (H_2SO_4, 96%) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Since the solubility of PBDEs in seawater is very low, we used dimethyl sulfoxide (DMSO, GC grade, ≥99.0%, liquid, Sigma-Aldrich, St. Louis, MO, USA) as a co-solvent. A stock solution was prepared by dissolving BDE-47 and BDE-209 in DMSO that was diluted to the desired concentration using seawater filtered through a 0.45 μm pore size membrane and sterilized at 121.3°C for 20 min.

BDE-47 stock solution preparation: BDE-47 stock solution of 2000 mg/L was prepared by injecting 2.5 mL DMSO into BDE-47 small bottle (5 mg BDE-47 inside), then diluted 10

times to 200 mg/L using filtered seawater. Standard solutions containing 0.8, 2.0, 6.0, 8.0, 10, 14, and 18 μg/mL of BDE-47 were prepared by diluting stock solution with filtered seawater.

BDE-209 stock solution preparation: BDE-209 stock solution of 2.0×10^4 mg/L was prepared by dissolving 100 mg BDE-209 in 5 mL DMSO and then diluted 10 times to 2000 mg/L using filtered seawater. Standard solutions containing 6.0, 10, 20, 30, 60, 90, and 120 μg/mL of BDE-209 were prepared by diluted BDE-209 stock solution in filtered seawater.

1.2. Chemical analyses

1.2.1. Chemical identification and sample preparation

The predicted concentrations of PBDEs in the seawater were very low because of their high lipophilicity of PBDEs. Therefore, it is difficult to prepare test solutions with correct concentrations (Tallarida and Murray, 1987; Breitholtz and Wollenberger, 2003). The chemical analysis of PBDEs was complemented by GC–MS. A quadratic calibration equation ($y = -5500.43 + 915761.00x + 190336.00x^2$, $R^2 = 0.99$) for BDE-47 (Fig. 1a) and a quadratic calibration equation ($y = 430.08 - 8725.85x + 16746.30x^2$, $R^2 = 1.00$) for BDE-209 (Fig. 1b) were obtained by external standard calibration and used for quantification. The calibration curves of BDE-47 and BDE-209 were determined by GC–MS using PBDE standard solutions. The two six-point calibration curves were used to verify the BDE-47 and BDE-209 concentration of the working experimental solutions, respectively.

For solution samples, to extract the PBDE congeners, 5 mL of *n*-hexane (HPLC grade, Sinopharm Chemical Reagent Co., Beijing, China) was added, and the BDE-47 and BDE-209 samples were vortexed for 30 sec, respectively. The *n*-hexane phase in the upper layer of each sample was dried over sodium sulfate and independent experiments were performed by triplicate (Akutsu et al., 2001). The extracts were concentrated in a vacuum evaporator (Büchi Rotavapor R-200, Flawil, Switzerland). The concentrated extracts were transferred to screw top vials for GC–MS analysis. The rotifers still alive at the end of the 7-day test were also taken to chemical analysis. Biological samples were vortexed with dichloromethane (3 × 2 mL) for 10 min to extract the analytes. The extracts were then cleaned with sulphuric acid, concentrated nearly to dryness by nitrogen evaporation and finally dissolved to nonane before GC–MS analysis (Nakari and Huhtala, 2008).

1.2.2. GC–MS analysis of actual PBDE concentration in seawater

All chemical analyses were performed on ISQ Single Quadrupole Gas Chromatograph and Mass Spectrometer (GC–MS) (Thermo Scientific Inc., Waltham, MA, USA). All samples were automatically injected with an AS 1310 Auto-sampler (Thermo Scientific Inc., Waltham, MA, USA). A splitless injection mode was used with temperature and pressure programs. The GC was equipped with a 15 m × 0.25 mm i.d. × 0.1 μm film thickness TG-5MS column (coating 5% diphenyl and 95% dimethyl polysiloxane, Thermo Scientific Inc., Waltham, MA, USA). A concentrated extract (1 μL) was injected onto the column. Temperature programming was 160 °C for 2 min then ramping at 35 °C/min to 320 °C followed by isothermal elution at 320 °C for 10 min. The SSL injector temperature was 300 °C and the MS transfer line temperature was isothermal at 300 °C. Helium was used as a carrier gas, at 1.5 mL/min, and the electron impact (EI mode) mass spectra were obtained at 70 eV. By this analysis procedure, the recoveries of the two PBDEs were in the range of 86.4%–98.2%.

1.3. Experimental design

1.3.1. Acute toxicity test

To choose appropriate toxicant concentrations for the population growth and reproduction experiments, range-finding tests consisting of five concentrations were conducted for 24 hr. The concentrations used here were 20, 40, 80, 100 and 120 mg/L for BDE-209, and 2, 6, 10, 14, 18 and 22 mg/L for BDE-47. Three replicates of each treatment concentration, including blank controls of filtered seawater and co-solvent controls were performed. The volume ratio of DMSO in the co-solvent control was the same as that of the highest concentration group. All treatments including controls contained ≤1.1% (V/V) DMSO in the BDE-47 tests and contained ≤0.6% (V/V) DMSO in the BDE-209 tests, which were within the safety range of DMSO.

Different concentrations of toxicants (1 mL) were injected into each well of 24-well tissue culture plates. Then 10 neonates (<2 hr) were placed in each well and starved during the experiment (ASTM, Reapproved, 2004). Both mortality and swimming behavior were monitored after 24 hr of exposure to toxic solutions. Mortality analysis was performed under a stereomicroscope. Rotifers that were completely motionless were counted as dead organisms. The term "motionless" means individuals that do not change their own barycenter position and do not move any appendages in 5 sec (Garaventa

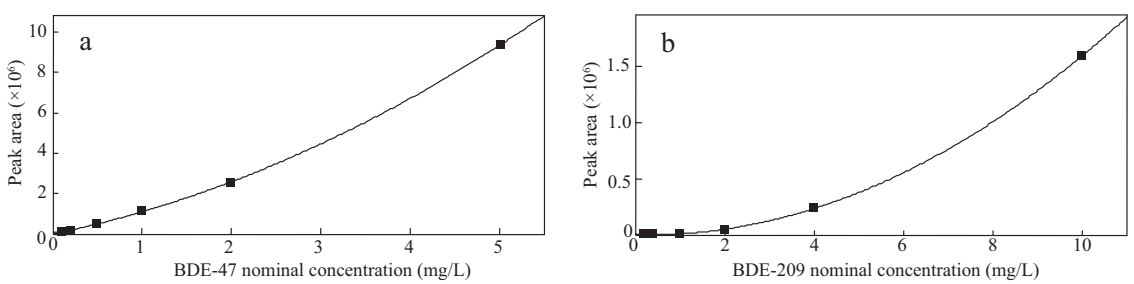


Fig. 1 – Calibration curves for BDE-47 and BDE-209 analysis. (a) A calibration curve for BDE-47 between nominal concentration of 0.1 and 5 mg/L and (b) a calibration curve for BDE-209 between nominal concentration of 0.2 and 10 mg/L.

et al., 2010). The experimental set up for swimming speed alteration test (SSA test) has been described by Faimali et al. (2006). The test is performed using a video camera-based system with image analysis software, designed to track and analyze linear swimming speed of aquatic invertebrates. The swimming behavior of the 24 hr old *B. plicatilis* specimens within view of the lens was examined. In the present study, rotifers whose swimming speed was less than 0.27 mm/sec were counted as swimming-inhibited organisms (Larsen et al., 2008).

1.3.2. Three-day population growth and seven-day resting egg production tests

Exposure concentrations were 0.8, 2, 6, 14 and 18 mg/L for BDE-47-DMSO solution, and 6, 10, 20, 30, 60, 90 and 120 mg/L for BDE-209-DMSO solution. Three parallel groups were also set for each concentration as well as a separate blank control and a co-solvent control. The volume ratio of DMSO in the co-solvent control was the same as that of the highest concentration group (0.9% (V/V) in the BDE-47 test and 0.6% (V/V) in the BDE-209 test). The test rotifers were fed daily with *Chlorella* sp. at a concentration of 1.0×10^6 cells/mL during the experimental period.

Neonates (<2 hr) were placed in 24-well tissue culture plates for toxicity testing. Following inoculation, every 24 hr all through the test we counted the number of neonates born and surviving adults, and removed dead adults. After 72 hr, amictic female rotifers, mictic females and females without eggs in each well were counted respectively and then returned to the 24-well plates. These were cultured for another 4 days to count the resting eggs. The types of female rotifers were identified according to the size, number and embryonic development condition of the eggs carried by mature rotifers similar to the work of Xi and Huang (2000) and Radix et al. (2002). From these counts, the ratio of ovigerous females to non-ovigerous females (OF/NOF) and the ratio of mictic females to amictic females (MF/AF) were calculated according to Radix et al. (2002). The population growth rate (r, day^{-1}) for each group was calculated according to the following equation:

$$r = (\ln N_t - \ln N_0)/t$$

where, N_t (females/mL) denotes the density of females at time t (day), N_0 is 10 females/mL, and t is 3 days. At the end of each test, the live adults were washed with $NaCl/NaH_2PO_4$ buffer for three times and frozen for subsequent chemical analyses.

1.4. Data statistical analysis

SPSS Statistics 17.0 and Excel were used to analyze the data. The 24 hr-LC_{50} and its 95% confidence limits were calculated by PROBIT analysis (Tallarida and Murray, 1987). The EC_{50} in the present study is the concentration that inhibited the swimming activity of 50% rotifer individuals. Curves were fitted by non-linear regression using OriginPro.8.0 (OriginLab Co., Northampton, MA, USA). Iteration was used to find the best fit and used a sigmoidal model. The 95% confidence intervals were provided by the regression algorithm. The difference between the test and control results was analyzed statistically with one-way analysis of variance (one-way ANOVA) and multiple comparisons (LSD test) with significance set at $p < 0.05$. No observed effect concentration (NOEC) and the lowest observed effect concentration (LOEC) were determined using Dunnett test ($p < 0.05$). Mean values and standard errors were calculated from the different replicates from each treatment ($n = 3$), and figures of population density were generated using Sigmaplot 10.0.

2. Results

2.1. Concentration of PBDEs in seawater

The measured concentrations of BDE-47 and BDE-209 in the seawater phase were much lower than the nominal values (Table 1). The BDE-47 ranged from 23.4% to 60.2% nominal concentrations, and BDE-209 ranged from 2.6% to 27.0% nominal values. At the nominal concentrations of 0.2 and 0.6 mg/L in the low-dose groups, the measured concentrations of BDE-47 were 39.5% and 50.3% nominal concentrations, while those of BDE-209 were 27.0% and 13.8% nominal concentrations, respectively. Moreover, the determined concentrations of BDE-47 were 50.3% and 39.2% nominal concentrations at the nominal concentrations of 6.0 and 10.0 mg/L, respectively. For BDE-209, the measured concentrations were 9.3% and 14.1% of the nominal concentrations.

2.2. Acute toxic effect of PBDEs on *B. plicatilis*

According to the 24-hr acute toxicity tests, there is no acute lethal effect for BDE-209. Even at the highest test concentration, 120 mg/L, none of the *B. plicatilis* died. No more than half of the individuals died at the highest test concentration of BDE-47, 22 mg/L. The DMSO had no observable effect on the rotifers in the co-solvent control. Rotifers in the BDE-209 test group showed no obvious swimming inhibition, while the swimming capability of some rotifers in the BDE-47 test group was inhibited. A clear dose–response was observed with increasing BDE-47 concentrations. The 24 hr-$LOEC_{SI}$ (the lowest observed effect concentration for swimming inhibition, 24 hr-$LOEC_{SI}$ = 2.0 mg/L) was derived from SSA test. The 24 hr-EC_{50} value resulted in an estimate of 9.7 mg/L (95% confidence interval: 8.3–11.3 mg/L) for larval *B. plicatilis*. A good correlation between swimming inhibition rate (inhibited individuals/total individuals) and BDE-47 concentration (R^2 = 0.99) was noted (Fig. 2).

2.3. Effects of PBDEs on population growth and reproduction

The growth curves of *B. plicatilis* in the culture with different concentrations of BDE-47 (Fig. 3a) and BDE-209 (Fig. 3b) are shown in Fig. 3. In the first 3 days, both of the growth with BDE-47 and BDE-209 was slow for *B. plicatilis*. Subsequently, rotifers in 0.8 mg/L group grew significantly faster than other groups of BDE-47 ($p < 0.05$) and entered the exponential phase. Rotifers in blank and DMSO controls also entered the exponential phase on day 3, while rotifers in the low toxicity groups (6, 10, 20 mg/L) of BDE-209 maintained a certain growth and entered the exponential phase on day 4. Furthermore, both of the growth of *B. plicatilis* with BDE-47 and

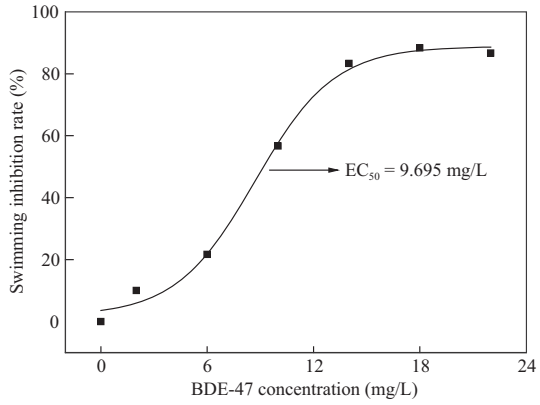


Fig. 2 – Boltzmann correlation between swimming inhibition rate and BDE-47 concentration.

BDE-209 were obviously suppressed relative to their respective control groups ($p < 0.01$). For *B. plicatilis*, the significant difference of population density was observed ($p < 0.01$) between 6.0 mg/L and control groups of BDE-47, however, the population density of the same concentration 6.0 mg/L group of BDE-209 was not significantly different from the control at the $p < 0.05$ level. The results showed that the impact of BDE-47 on the rotifer population density is greater than that of BDE-209.

The population growth and reproduction of *B. plicatilis* in relation to different concentrations of BDE-47 and BDE-209 are presented in Fig. 4. BDE-47 influenced the population parameters of *B. plicatilis* significantly according to one-way ANOVA analysis ($p < 0.05$). There was a highly significant correlation between the concentration of BDE-47 and the OF/NOF ratio as well as the population growth rate ($p < 0.001$) (Fig. 4a). Compared to the control and DMSO control, BDE-47 remarkably decreased the population growth rate ($p < 0.05$). Compared to the other test concentrations except 18 mg/L, BDE-47 at 14 mg/L increased significantly the resting egg production ($p < 0.05$). Moreover, BDE-47 at 14 and 18 mg/L notably increased the mictic rate ($p < 0.05$). Above 6.0 mg/L BDE-47 the OF/NOF ratio was markedly decreased ($p < 0.05$).

BDE-209 also affected notably the population parameters of *B. plicatilis* ($p < 0.001$) according to one-way ANOVA analysis (Fig. 4b). Compared to the control and DMSO control, the BDE-209 decreased the population growth rate significantly ($p < 0.05$). Compared to the other test concentrations except 90 mg/L, BDE-209 at 120 mg/L increased the resting egg production ($p < 0.05$). Moreover, BDE-209 at 90 and 120 mg/L significantly increased the mictic rate and the MF/AF ratio ($p < 0.05$). The BDE-209 at concentrations above 6 mg/L decreased the OF/NOF ratio as well as the population growth rate ($p < 0.05$). As BDE-209 increased, the population growth rate and the OF/NOF ratio decreased, but the mictic rate, the MF/AF ratio and the resting egg production increased.

No observed effect concentration (NOEC) and the lowest observed effect concentration (LOEC) for inhibition of population and reproduction parameters in *B. plicatilis* exposed to BDE-47 and BDE-209 are summarized in Table 2. The NOECs for BDE-47 were between 0–3017 μg/L and 0–4641 μg/L for BDE-209. The effective nominal concentration values of BDE-47 were significantly lower than BDE-209, confirming that BDE-47 was more toxic than BDE-209 to *B. plicatilis*. However, there was no significant difference between the effective measured actual concentration values of the two PBDEs. Among them, the NOEC and LOEC for inhibition of population growth rate and OF/NOF were much lower than the values of other parameters.

2.4. Concentration of PBDEs in *B. plicatilis*

Table 3 shows the concentrations of BDE-47 and BDE-209 found in the rotifer samples. In rotifers, the concentrations of BDE-47 were over 13 times that of BDE-209 at the same nominal concentration of 6.0 mg/L. Both of the two PBDEs accumulated into the rotifers depending on the measured exposure concentrations. The relative amounts of BDE-47 were much higher than those of BDE-209 in rotifers after 7-day exposure.

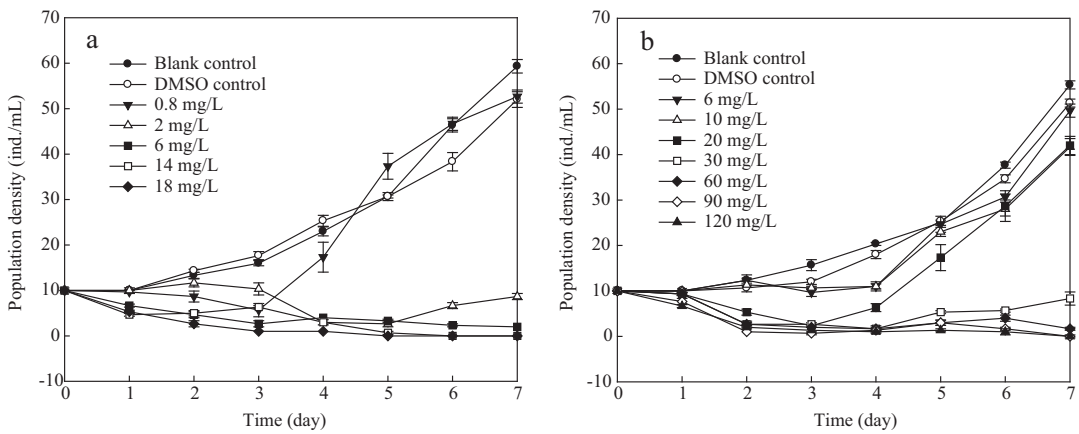


Fig. 3 – Effects of two PBDEs on the population density of *B. plicatilis*. (a) BDE-47, (b) BDE-209.

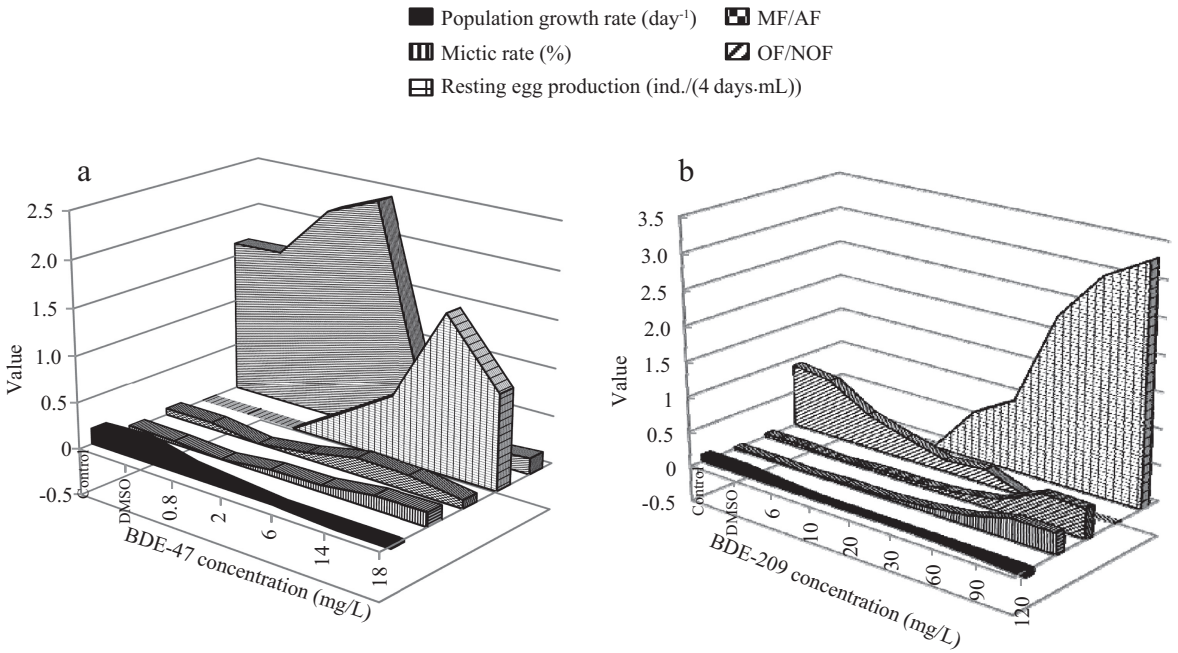


Fig. 4 – Effects of PBDEs on the population growth and reproduction of *B. plicatilis*. (a) BDE-47, (b) BDE-209. MF/AF: the ratio of mictic females/amictic females, OF/NOF: the ratio of ovigerous females/non-ovigerous females.

3. Discussion

These acute toxicity tests are in the early stage of the toxicological studies and are important for elucidating the toxic effects of the test substances. The sensitivity of rotifer *B. plicatilis* to PBDEs, especially to BDE-47 and BDE-209 with poor water solubility, is relatively unknown. Most of the effects observed in this study occurred at exposure concentrations in the units of milligrams per liter, which is much higher than those found in the environment. However, the 24-hr acute tests in this research showed that until BDE-209 reached its maximum solubility, none of the *B. plicatilis* died or were inhibited within 24 hr. Hence, the 24-hr LC_{50} and EC_{50} value of BDE-209 to *B. plicatilis* was beyond 120 mg/L, and the toxicity of its seawater solution is very low. The 24-hr LC_{50} value of BDE-47 to *B. plicatilis* was beyond 22 mg/L, while BDE-47 can inhibit the swimming capability of rotifers to some extent. Our results indicate the feasibility of *B. plicatilis* swimming behavior analysis as a sensitive sub-lethal indicator of chemical perturbations such as BDE-47 as a practical endpoint in ecotoxicological monitoring programs. Some previous work has detailed the acute toxicity of BDE-47 on marine copepods: The 96-hr LC_{50} value of BDE-47 to the harpacticoid copepod *Nitocra spinipes* was 72 μg/L and the 48-hr LC_{50} of BDE-47 to the marine copepod *Acartia tonsa* was 2.37 mg/L (Breitholtz and Wollenberger, 2003; Wollenberger et al., 2005). Compared to BDE-209 at 6 mg/L (Fig. 4b), BDE-47 at 6 mg/L had a greater impact on these population parameters of *B. plicatilis* (Fig. 4a). These results indicated that the acute toxicity of BDE-209 is lower than that of BDE-47. Similarly, Mhadhbi et al. (2012) found that BDE-47 can induce a stronger inhibition of growth *versus* BDE-99 and BDE-154, and it was clearly more toxic to algae than BDE-99 and BDE-154. This is attributed to the larger steric hindrance because there are more bromine atoms in the high-brominated PBDEs, like BDE-209. Therefore, it is more difficult for BDE-209 to enter the organism and exert its toxic effects.

Table 2 – NOEC and LOEC (measured actual concentrations) for inhibition of population and reproduction parameters in *B. plicatilis* exposed to BDE-47 and BDE-209.

Chemicals	Parameter	NOEC (μg/L)	LOEC (μg/L)
BDE-47	Population growth rate	–	405
	Mictic rate	3017	4155
	MF/AF	3017	4155
	OF/NOF	1204	3017
	Resting egg production	3017	4155
BDE-209	Population growth rate	–	555
	Mictic rate	1438	4641
	MF/AF	4641	3673
	OF/NOF	–	555
	Resting egg production	1414	1438

–: NOEC for the parameter cannot be obtained because of significant impact.

Owing to their high lipophilicity, PBDEs are scarcely soluble in water (Table 4). However, they still enter the aquatic environment and are detected in seawaters, marine organisms and sediments at many locations around the world. Recent studies indicated that BDE-47 has been detected in Hong Kong, Singapore and Holland's coastal waters, but the concentration of PBDEs in the seawaters is quite low, usually maintaining pg/L–ng/L grade (Booij et al., 2002). Hence, it is difficult to compare laboratory effect concentrations with

Table 3 – Nominal concentrations, measured concentrations and concentrations in rotifers.

	Nominal conc. (mg/L)	Measured conc. (μg/L)	Conc. in rotifers (ng/mg)
BDE-47	0.8	405	11.5
	2.0	1204	22.1
	6.0	3017	45.2
BDE-209	6.0	555	3.4
	10.0	1413	7.1
	20.0	1414	8.1

exposure levels in the environment. Actually the current PBDE levels in open coastal waters produce no obvious acute toxic effects on marine zooplankton, such as copepods and cladocerans (Breitholtz et al., 2008; Nakari and Huhtala, 2008). Nevertheless, long-term exposure to low level PBDEs may produce chronic toxic effects and PBDEs' bio-accumulation and bio-magnification through food chains may also cause toxicity expanding. In addition, The PBDE levels in marine environment and organisms has displayed a gradually increase trend. For instance, several studies in Swedish waters have indicated increasing levels of BDE-47, -99 and -100 in sediments and fish (Sellström et al., 1993, 1998). Therefore, our findings may have implications for interpretations of ecotoxicity data and for future risk assessment of very lipophilic substances like PBDEs.

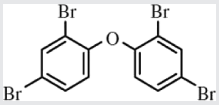
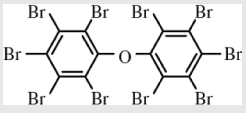
The results demonstrate that *B. plicatilis* is not very sensitive to PBDEs within a short time. However, the effects of PBDEs began to emerge with longer exposure time. It has been demonstrated by Hayton and Barron that the compound must bioaccumulate in aquatic animals to be toxic. This, however, depends on the physical and biochemical properties of the particular chemical, the anatomy and physiology of the animals and exposure time (Barron et al., 1990; Nakari and Huhtala, 2008). In this study, no acute lethal toxicity was observed, but after 3 days the toxic effects of these two PBDEs became severe. Similarly, previous research has shown that the congener-153 of PCB, PBB and PBDE were not toxic to *D. magna* according to 24-hr acute tests. Even at the highest test concentration, 210 μg/L, none of the animals died. However, in the 21-day reproductive tests, these chemicals significantly reduced the numbers of offspring produced by the parents exposed to BDE-47 at concentrations of 12.5 and 25 mg/L (Nakari and Huhtala, 2008).

The nominal concentrations used in the present study have been well corrected in accordance with the chemical analysis. Our GC–MS analysis results indicated that the solubility of BDE-47 and BDE-209 in seawater was still very low when using DMSO as a co-solvent and the solubility value of BDE-209 was lower than that of BDE-47, which agreed with the results obtained by Alcock et al. (1999) (Table 4). The highest percentage of the actual concentration of BDE-47 occurred at the nominal concentration of 2.0 mg/L. However, BDE-209 at nominal concentration of 0.2 and 120 mg/L reached the highest and the lowest percentage of the actual concentration, respectively. The reason may be that PBDEs could precipitate out and the actual solubility in the seawater began to decrease when the solution was oversaturated.

Since other routes of exposure than uptake *via* the water may be predominant in the environment, *e.g.*, by ingestion of contaminated food and particle-adsorbed PBDEs, exposure above water solubility is still relevant in ecotoxicological tests (Fliedner, 1997; Breitholtz and Wollenberger, 2003). In our study, test solutions of these two PBDEs at higher concentrations (such as BDE-47 at 18 mg/L, BDE-209 at 90 and 120 mg/L) most significantly affected the population parameters of *B. plicatilis* ($p < 0.01$) compared to the groups at lower concentrations. It might be concluded that even if not completely dissolved in the seawater phase, the PBDEs temporarily suspended in solution also influence population growth and reproduction of *B. plicatilis*.

Earlier studies have shown that the population growth rate is a sensitive indicator for monitoring the toxicity of water pollutants. Most water pollutants inhibit rotifers' population growth. The population growth rate of *Brachionus calyciflorus* increased markedly when the concentration of herbicide glyphosate was 4.0 mg/L. Similarly, the population growth rate of *B. calyciflorus* decreased significantly when the concentration of pesticide thiophanate-methyl was 0.4 mg/L (Xi and Feng, 2004). In this study, compared to the control and the co-solvent control, BDE-47 at concentrations higher than 0.8 mg/L as well as BDE-209 at concentrations higher than

Table 4 – Comparison of logK_{ow} values and solubility values for PBDE congeners, where logK_{ow} is the octanol-water partition coefficient.

PBDE congener	logK_{ow}	Solubility (mg/L)	Structural representation [a]
BDE-47	6.81 ± 0.08 [b]	0.07 (TeBDE) [c]	
BDE-209	9.98 [c]	0.02–0.03 [c]	

[a] Rayne et al., 2006.
[b] Breitholtz and Wollenberger, 2003.
[c] Alcock et al., 1999.

6 mg/L reduced the population growth rate of rotifer *B. plicatilis* significantly ($p < 0.05$). The results showed that the population growth rate of rotifers was an appropriate indicator for monitoring the toxicity of BDE-47 and BDE-209.

It has been shown that OF/NOF in a *B. calyciflorus* population was a suitable endpoint for assessing the effects of ethinylestradiol and nonylphenol (Radix et al., 2002). Similarly, in our study, a clear dose–response relationship between OF/NOF and the mictic rate and the concentration of BDE-47 and BDE-209 indicated that they are suitable endpoints for assessing the effects of BDE-47 and BDE-209. Several studies have suggested that sexual reproduction was more sensitive to toxicants than asexual reproduction (Snell and Carmona, 1995; Xi and Feng, 2004; Marcial et al., 2005), but our work found opposite results. The NOECs and LOECs for inhibition of population growth rate and OF/NOF were much lower than those of other parameters, indicating that asexual reproduction (population growth rate and OF/NOF) was more sensitive than sexual reproduction (the mictic rate, the MF/AF ratio and resting egg production) to those two PBDEs. This agreed with the effects of organochlorine pesticide lindane on the reproduction of freshwater rotifer *B. calyciflorus*. Some previous available toxicity data have shown a similar phenomenon as ours (Ke et al., 2009; Wen et al., 2011). However, the reverse was also true for the other three pesticides, dichlorodiphenyltrichloroethane (DDT), dicofol and endosulfan (Xi et al., 2007). Thus, it might be concluded that the relative sensitivity between sexual reproduction and asexual reproduction depends on the toxicant identity.

The production of resting eggs was affected by many factors including mictic rate, fertilization rate, recognition and mating between male and female individuals (Preston and Snell, 2001). Before resting eggs were produced, several generations consisting of amictic females, mictic females and males were exposed to the test solutions. Thus, the production of resting eggs was influenced throughout the entire rotifer life cycle (Zhao et al., 2007). Some researchers found that compared with the mictic rate, resting egg production was more sensitive to pollutant (Snell and Carmona, 1995; Preston and Snell, 2001; Xi and Feng, 2004). Our results confirmed the above conclusion. In this study, compared to the control, both the mictic rate and the resting egg production were increased significantly when the concentration of BDE-47 was 14 mg/L. The resting egg production of *B. plicatilis* increased significantly when the concentration of BDE-209 was 30 mg/L, but the mictic rate did not increase markedly until BDE-209 reached 60 mg/L suggesting that the resting egg production is more sensitive to BDE-209 than the mictic rate. Therefore, the resting egg production in *B. plicatilis* was a suitable and sensitive endpoint for assessing the effects of the two PBDEs.

Kierkegaard et al. (1999) found that rainbow trout can very slowly absorb BDE-209 through food. The fish bioaccumulated only 0.02%–0.13% of the available BDE-209 after 120 days. Organisms have poor absorption and rapid metabolism of BDE-209, which causes low bioconcentration. Thus, it is difficult to detect the presence of BDE-209. In the present study, the accumulated amount of BDE-209 in rotifers was much lower than that of BDE-47 and the two PBDEs affected differently the reproduction of *B. plicatilis*, which proved that BDE-47 was prone to accumulate into the body of rotifers than BDE-209. The highly accumulative BDE-47 could be used as a common simple indicator of PBDE-contamination in rotifers. However, BDE-209 at higher concentrations (90 and 120 mg/L) caused a decrease of population growth rate and significantly increased the mictic rate and resting egg production of *B. plicatilis*. After entering the body of rotifers, BDE-209 can transform into lower brominated diphenyl ethers and reach certain concentrations, which will cause stronger toxic effects. Thus, further work is needed to address the toxic mechanism and accumulation mode of PBDEs since this information is essential for accurate risk assessment of brominated compounds.

Some researchers note that in rotifers the energy gained by feeding is mainly used for body growth, reproduction and basic physiological needs such as breathing (Sarma and Rao, 1987). Reproductive energy can be used for producing larger but fewer, or more but smaller eggs, so as to maximize the population growth rate (Wen et al., 2011). Our results show that these two PBDEs at higher concentrations increased the resting egg production and decreased the population growth rate as well as the OF/NOF ratio significantly. This suggests that under the stress of BDE-47 and -209 at high concentrations, rotifers may choose the countermeasures of producing larger but fewer eggs to alleviate the damaging effects on their populations. Nevertheless, the effects of PBDEs on the rotifers' body size, egg size as well as the mechanism of reproductive strategy and energy distribution still require further research.

4. Conclusions

In summary, the results of the present study showed that the toxicity of BDE-47 was more potent than that of BDE-209 to rotifer *B. plicatilis*. The use of *B. plicatilis* swimming behavior proved to be a sensitive indicator of sub-lethal exposure to BDE-47. The results also suggested that *B. plicatilis* had a strong tolerance for BDE-209 exposure. It is concluded that PBDEs have a significant influence on the population growth and reproduction parameters of *B. plicatilis*, including population growth rate, the ratio of ovigerous females/non-ovigerous females (OF/NOF), the ratio of mictic females/amictic females (MF/AF), resting egg production and the mictic rate in *B. plicatilis* population, which were suitable for monitoring and assessing both BDE-47 and BDE-209. Also, the two PBDEs accumulate differently into rotifers. Thus, our results indicate that they are not acute lethal toxicants and a better understanding of the mechanism of their accumulation in organisms, through food chain and associated potential dangers is highly desirable.

Acknowledgments

This work was supported by the Natural Science Foundation of China (No. 41276140). We are grateful to Prof. Granville for the constructive comments and Dr. Yaoqin Hong at the University of Illinois at Urbana-Champaign for his assistance. We would like to express our thanks to all the personnel in the laboratory of marine ecotoxicology at Ocean University of China.

REFERENCES

Akutsu, K., Obana, H., Okihashi, M., Kitagawa, M., Nakazawa, H., Matsuki, Y., et al., 2001. GC/MS analysis of polybrominated diphenyl ethers in fish collected from the Inland Sea of Seto, Japan. Chemosphere 44 (6), 1325–1333.

Alcock, R.E., Sweetman, A., Jones, K.C., 1999. Assessment of organic contanhnant fate in waste water treatment plants I: selected compounds and physicochemical properties. Chemosphere 38 (10), 2247–2262.

ASTM, Reapproved, 2004. Standard Guide for Acute Toxicity Test with the Rotifer Brachionus. vol. E, pp. 1440–1491.

Barron, M.G., Stehly, G.R., Hayton, W.L., 1990. Pharmacokinetic modeling in aquatic animals I. Models and concepts. Aquat. Toxicol. 18 (2), 61–85.

Birky, C.W., Gilbert, J.J., 1971. Parthenogenesis in rotifers: the control of sexual and asexual reproduction. Integr. Comp. Biol. 11 (2), 245–266.

Booij, K., Zegers, B.N., Boon, J.P., 2002. Levels of some polybrominated diphenyl ether (PBDE) flame retardants along the Dutch coast as derived from their accumulation in SPMDs and blue mussels (*Mytilus edulis*). Chemosphere 46 (5), 683–688.

Breitholtz, M., Wollenberger, L., 2003. Effects of three PBDEs on development, reproduction and population growth rate of the harpacticoid copepod *Nitocra spinipes*. Aquat. Toxicol. 64 (1), 85–96. http://dx.doi.org/10.1016/S0166-445X(03)00025-0.

Breitholtz, M., Nyholm, J.R., Karlsson, J., Andersson, P.L., 2008. Are individual NOEC levels safe for mixtures? A study on mixture toxicity of brominated flame–retardants in the copepod *Nitocra spinipes*. Chemosphere 72 (9), 1242–1249.

Darnerud, P.O., Eriksen, G.S., Jóhannesson, T., Larsen, P.B., Viluksela, M., 2001. Polybrominated diphenyl ethers: occurrence, dietary exposure, and toxicology. Environ. Health Perspect. 109 (S1), 49.

de Wit, C.A., 2002. An overview of brominated flame retardants in the environment. Chemosphere 46 (5), 583–624.

Du, H.Y., Zhu, L., Chen, Z.Z., Li, Y., Duan, Z.H., 2008. The progress of study on the toxicological effects of deca-brominated diphenyl ether. J. Toxicol. 22 (1), 50–52.

Faimali, M., Garaventa, F., Piazza, V., Greco, G., Corra, C., Magillo, F., et al., 2006. Swimming speed alteration of larvae of *Balanus amphitrite* as a behavioural end-point for laboratory toxicological bioassays. Mar. Biol. 149 (1), 87–96.

Ferrando, M., Janssen, C., Andreu, E., Persoone, G., 1993. Ecotoxicological studies with the freshwater rotifer *Brachionus calyciflorus* II. An assessment of the chronic toxicity of lindane and 3, 4-dichloroaniline using life tables. Hydrobiologia 255 (1), 33–40.

Fliedner, A., 1997. Ecotoxicity of poorly water-soluble substances. Chemosphere 35 (1–2), 295–305.

Garaventa, F., Gambardella, C., Di Fino, A., Pittore, M., Faimali, M., 2010. Swimming speed alteration of *Artemia* sp. and *Brachionus plicatilis* as a sub-lethal behavioural end-point for ecotoxicological surveys. Ecotoxicology 19 (3), 512–519.

Hardy, M., 2002. The toxicology of the three commercial polybrominated diphenyl oxide (ether) flame retardants. Chemosphere 46 (5), 757–777.

Hardy, M., Schroeder, R., Biesemeier, J., Manor, O., 2002. Prenatal oral (gavage) developmental toxicity study of decabromodiphenyl oxide in rats. Int. J. Toxicol. 21 (2), 83–91.

Hardy, M.L., Banasik, M., Stedeford, T., 2009. Toxicology and human health assessment of decabromodiphenyl ether. Crit. Rev. Toxicol. 39 (S3), 1–44.

Janssen, C.R., Ferrando, M.D., Persoone, G., 1994. Ecotoxicological studies with the freshwater rotifer *Brachionus calyciflorus*: IV. Rotifer behavior as a sensitive and rapid sublethal test criterion. Ecotoxicol. Environ. Saf. 28 (3), 244–255.

Ke, L.X., Xi, Y.L., Zha, C.W., Dong, L.L., 2009. Effects of three organophosphorus pesticides on population growth and sexual reproduction of rotifer *Brachionus calyciflorus* Pallas. Acta Ecol. Sin. 29 (3), 182–185.

Kierkegaard, A., Balk, L., Tjärnlund, U., De Wit, C.A., Jansson, B., 1999. Dietary uptake and biological effects of decabromodiphenyl ether in rainbow trout (*Oncorhynchus mykiss*). Environ. Sci. Technol. 33 (10), 1612–1617.

Larsen, P.S., Madsen, C.V., Riisgård, H.U., 2008. Effect of temperature and viscosity on swimming velocity of the copepod *Acartia tonsa*, brine shrimp *Artemia salina* and rotifer *Brachionus plicatilis*. Aquat. Biol. 4 (1), 47–54.

Lema, S.C., Schultz, I.R., Scholz, N.L., Incardona, J.P., Swanson, P., 2007. Neural defects and cardiac arrhythmia in fish larvae following embryonic exposure to 2, 2′, 4, 4′-tetrabromodiphenyl ether (PBDE 47). Aquat. Toxicol. 82 (4), 296–307.

Letcher, R.J., Bustnes, J.O., Dietz, R., Jenssen, B.M., Jørgensen, E.H., Sonne, C., et al., 2010. Exposure and effects assessment of persistent organohalogen contaminants in arctic wildlife and fish. Sci. Total Environ. 408 (15), 2995–3043.

Marcial, H.S., Hagiwara, A., Snell, T.W., 2005. Effect of some pesticides on reproduction of rotifer *Brachionus plicatilis* Müller. Hydrobiologia 546 (1), 569–575.

Martin, M., Lam, P.K., Richardson, B.J., 2004. An Asian quandary: where have all of the PBDEs gone? Mar. Pollut. Bull. 49 (5), 375–382.

Mhadhbi, L., Fumega, J., Beiras, R., 2012. Toxicological effects of three polybromodiphenyl ethers (BDE-47, BDE-99 and BDE-154) on growth of marine algae *Isochrysis galbana*. Water Air Soil Pollut. 223 (7), 4007–4016.

Nakari, T., Huhtala, S., 2008. Comparison of toxicity of congener-153 of PCB, PBB, and PBDE to *Daphnia magna*. Ecotoxicol. Environ. Saf. 71 (2), 514–518.

Noyes, P.D., Kelly, S.M., Mitchelmore, C.L., Stapleton, H.M., 2010. Characterizing the in vitro hepatic biotransformation of the flame retardant BDE 99 by common carp. Aquat. Toxicol. 97 (2), 142–150.

Preston, B.L., Snell, T.W., 2001. Full life-cycle toxicity assessment using rotifer resting egg production: implications for ecological risk assessment. Environ. Pollut. 114 (3), 399–406. http://dx.doi.org/10.1016/S0269-7491(00)00232-3.

Radix, P., Severin, G., Schramm, K.-W., Kettrup, A., 2002. Reproduction disturbances of *Brachionus calyciflorus* (rotifer) for the screening of environmental endocrine disrupters. Chemosphere 47 (10), 1097–1101.

Rahman, F., Langford, K.H., Scrimshaw, M.D., Lester, J.N., 2001. Polybrominated diphenyl ether (PBDE) flame retardants. Sci. Total Environ. 275 (1–3), 1–17. http://dx.doi.org/10.1016/S0048-9697(01)00852-X.

Rayne, S., Wan, P., Ikonomou, M., 2006. Photochemistry of a major commercial polybrominated diphenyl ether flame retardant congener: 2,2′,4,4′,5,5′-hexabromodiphenyl ether (BDE-153). Environ. Int. 32 (5), 575–585. http://dx.doi.org/10.1016/j.envint.2006.01.009.

Roberts, S.C., Noyes, P.D., Gallagher, E.P., Stapleton, H.M., 2011. Species-specific differences and structure–activity relationships in the debromination of PBDE congeners in three fish species. Environ. Sci. Technol. 45 (5), 1999–2005.

Sarma, S.S.S., Rao, T.R., 1987. Effect of food level on body size and egg size in a growing population of the rotifer *Brachionus patulus* Muller. Archiv fur Hydrobiologie. Stuttgart. 111 (2), 245–253.

Sellström, U., Jansson, B., Kierkegaard, A., de Wit, C., Odsjö, T., Olsson, M., 1993. Polybrominated diphenyl ethers (PBDE) in biological samples from the Swedish environment. Chemosphere 26 (9), 1703–1718.

Sellström, U., Kierkegaard, A., de Wit, C., Jansson, B., 1998. Polybrominated diphenyl ethers and

hexabromocyclododecane in sediment and fish from a Swedish river. Environ. Toxicol. Chem. 17 (6), 1065–1072.
Snell, T.W., 1986. Effect of temperature, salinity and food level on sexual and asexual reproduction in *Brachionus plicatilis* (Rotifera). Mar. Biol. 92 (2), 157–162.
Snell, T.W., 1987. Sex, population dynamics and resting egg production in rotifers. Hydrobiologia 144 (2), 105–111.
Snell, T.W., Carmona, M.J., 1995. Comparative toxicant sensitivity of sexual and asexual reproduction in the rotifer *Brachionus calyciflorus*. Environ. Toxicol. Chem. 14 (3), 415–420.
Snell, T.W., Janssen, C.R., 1996. Rotifers in ecotoxicology: a review. Hydrobiologia 313–314 (1), 231–247.
Stapleton, H.M., Letcher, R.J., Li, J., Baker, J.E., 2009. Dietary accumulation and metabolism of polybrominated diphenyl ethers by juvenile carp (*Cyprinus carpio*). Environ. Toxicol. Chem. 23 (8), 1939–1946.
Tallarida, R.J., Murray, R.B., 1987. Manual of Pharmacologic Calculations with Computer Programs. Springer-Verlag, New York.
Talsness, C.E., 2008. Overview of toxicological aspects of polybrominated diphenyl ethers: a flame-retardant additive in several consumer products. Environ. Res. 108 (2), 158–167.
USEPA., 2010. DecaBDE phase-out initiative. Available at http://www.epa.gov/oppt/existingchemicals/pubs/actionplans/deccadbe.html (Date accessed: 28 January 2011.).
Viganò, L., Roscioli, C., Guzzella, L., 2011. Decabromodiphenyl ether (BDE-209) enters the food web of the River Po and is metabolically debrominated in resident cyprinid fishes. Sci. Total Environ. 409 (23), 4966–4972. http://dx.doi.org/10.1016/j.scitotenv.2011.07.062.
Wen, X.L., Xi, Y.L., Yang, Y.F., Zhang, G., Zhang, Q., Hu, Y.H., 2011. Effect of melamine on the reproductive strategy of Rotifer *Brachionus calyciflorus*. Chinese J. Zool. 46 (1), 62–67.
Wollenberger, L., Dinan, L., Breitholtz, M., 2005. Brominated flame retardants: activities in a crustacean development test and in an ecdysteroid screening assay. Environ. Toxicol. Chem. 24 (2), 400–407.
Xi, Y.L., Feng, L.K., 2004. Effects of thiophanate-methyl and glyphosate on asexual and sexual reproduction in the rotifer *Brachionus calyciflorus* Pallas. Bull. Environ. Contam. Toxicol. 73 (4), 644–651.
Xi, Y.L., Huang, X.F., 2000. Effect of culture water replacement on resting egg formation of freshwater rotifer, *Brachionus calyciflorus*. J. Lake Sci. 12 (1), 47–52.
Xi, Y.L., Chu, Z.X., Xu, X.P., 2007. Effect of four organochlorine pesticides on the reproduction of freshwater rotifer *Brachionus calyciflorus* Pallas. Environ. Toxicol. Chem. 26 (8), 1695–1699.
Xie, X., Qian, Y., Wu, Y., Yin, J., Zhai, J., 2013. Effects of decabromodiphenyl ether (BDE-209) on the avoidance response, survival, growth and reproduction of earthworms (*Eisenia fetida*). Ecotoxicol. Environ. Saf. 90, 21–27.
Zhang, X., Tang, X., Zhou, B., Wang, Y., 2013. Effects of decabromodiphenyl ether (BDE-209) on Inter-Specific competition between two species of marine Bloom-forming microalgae. PLoS One 8 (3), e56084.
Zhao, L.L., Xi, Y.L., Lin, H., Zha, C.W., 2007. Effects of phthalate acid esters on population growth and sexual reproduction of rotifers *Brachionus calyciflorus*. Acta Zool. Sin. 53 (2), 250–256.

Table 1 – Nominal and measured concentrations of BDE-47 and BDE-209.

Nominal concentration (mg/L)	Measured BDE-47 concentration (μg/L) (% nominal concentration)	Nominal concentration (mg/L)	Measured BDE-209 concentration (μg/L) (% nominal concentration)
0.1	46 (46.0)	0.2	54 (27.0)
0.2	79 (39.5)	0.6	83 (13.8)
0.6	302 (50.3)	6.0	555 (9.3)
0.8	405 (50.6)	10.0	1413 (14.1)
1.0	592 (59.2)	20.0	1414 (7.1)
1.25	724 (57.9)	30.0	1438 (4.8)
2.0	1204 (60.2)	40.0	2080 (5.2)
6.0	3017 (50.3)	60.0	4641 (7.7)
10.0	3918 (39.2)	80.0	9976 (12.5)
14.0	4155 (29.7)	90.0	3673 (4.1)
18.0	4212 (23.4)	120.0	3063 (2.6)

Editorial Board of Journal of Environmental Sciences

JOURNAL OF ENVIRONMENTAL SCIENCES

环境科学学报(英文版)

www.jesc.ac.cn

Aims and scope

Journal of Environmental Sciences is an international academic journal supervised by Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences. The journal publishes original, peer-reviewed innovative research and valuable findings in environmental sciences. The types of articles published are research article, critical review, rapid communications, and special issues.

The scope of the journal embraces the treatment processes for natural groundwater, municipal, agricultural and industrial water and wastewaters; physical and chemical methods for limitation of pollutants emission into the atmospheric environment; chemical and biological and phytoremediation of contaminated soil; fate and transport of pollutants in environments; toxicological effects of terrorist chemical release on the natural environment and human health; development of environmental catalysts and materials.

For subscription to electronic edition

Elsevier is responsible for subscription of the journal. Please subscribe to the journal via http://www.elsevier.com/locate/jes.

For subscription to print edition

China: Please contact the customer service, Science Press, 16 Donghuangchenggen North Street, Beijing 100717, China. Tel: +86-10-64017032; E-mail: journal@mail.sciencep.com, or the local post office throughout China (domestic postcode: 2-580).

Outside China: Please order the journal from the Elsevier Customer Service Department at the Regional Sales Office nearest you.

Submission declaration

Submission of the work described has not been published previously (except in the form of an abstract or as part of a published lecture or academic thesis), that it is not under consideration for publication elsewhere. The publication should be approved by all authors and tacitly or explicitly by the responsible authorities where the work was carried out. If the manuscript accepted, it will not be published elsewhere in the same form, in English or in any other language, including electronically without the written consent of the copyright-holder.

Editorial

Authors should submit manuscript online at http://www.jesc.ac.cn. In case of queries, please contact editorial office, Tel: +86-10-62920553, E-mail: jesc@rcees.ac.cn. Instruction to authors is available at http://www.jesc.ac.cn.

Journal of Environmental Sciences (Established in 1989)
Volume 28 2015

Supervised by	Chinese Academy of Sciences	**Published by**	Science Press, Beijing, China
Sponsored by	Research Center for Eco-Environmental		Elsevier Limited, The Netherlands
	Sciences, Chinese Academy of Sciences	**Distributed by**	
Edited by	Editorial Office of Journal of	Domestic	Science Press, 16 Donghuangchenggen
	Environmental Sciences		North Street, Beijing 100717, China
	P. O. Box 2871, Beijing 100085, China		Local Post Offices through China
	Tel: 86-10-62920553; http://www.jesc.ac.cn	Foreign	Elsevier Limited
	E-mail: jesc@rcees.ac.cn		http://www.elsevier.com/locate/jes
Editor-in-chief	X. Chris Le	**Printed by**	Beijing Beilin Printing House, 100083, China

CN 11-2629/X **Domestic postcode: 2-580** **Domestic price per issue RMB ¥ 110.00**

ISSN 1001-0742