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Sublethal or not? Responses of multiple biomarkers in *Daphnia magna* to single and joint effects of BDE-47 and BDE-209

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ABSTRACT

Polybrominated diphenyl ethers (PBDEs) are extremely incessant anthropogenic contaminants found in the environment, with dreadful risk to aquatic ecosystems. However, there is a limited amount of data concerning their impacts on freshwater organisms. 2,2′,3,3′,4,4′,5,5′,6,6′-decabromodiphenyl ether (BDE-209) and 2,2′,4,4′-tetrabromodiphenyl ether (BDE-47) are significant components of total PBDEs in water. The sublethal effects of BDE-47, BDE-209 and their binary mixtures on the aquatic organism *Daphnia magna* were investigated in acute and chronic exposure experiments. Immobilization and heartbeat were studied in daphnids after 48 h of exposure. Mortality rate, breed number, Cholinesterase (ChE), Glutathione S-transferases (GST) and Catalase (CAT) activities were evaluated after 21 days of exposure. The results showed that at 100 and 200 μg/L concentration of BDE-47, immobilization rate of daphnids were inhibited by 44.0 ± 16.7% and 88.0 ± 10.9%, respectively. The binary mixture of BDE-47 and BDE-209 had uncongenial effects on immobilization of *D. magna* under acute toxicity test. BDE-209 significantly increased the heartbeat rate of daphnids, which increased even further when combined with BDE-47. After 21 days of exposure, daphnids exposed to single BDE-47 were physiologically altered. The combination of BDE-47 with BDE-209 significantly decreased the mortality rate of daphnids. Irrespective of the concentration, higher numbers of offsprings were produced in the mixtures compared to BDE-47 treatment alone. ChE activities significantly ($p < 0.05$) decreased at concentrations of 2 and 4 μg/L in single BDE-47 treatment, while GST activity significantly ($p < 0.05$) decreased at 0.5 μg/L. CAT activities significantly increased with BDE-47 treatments in all the tested concentrations ($p < 0.05$). The mixtures significantly affect ChE ($p < 0.05$), GST ($p < 0.05$) and CAT activities ($p < 0.05$). The results illustrated that the toxicity of the mixture of PBDE congeners exposed to aquatic organisms may have antagonistic effects. The 21 days chronic test in this study suggests that acute toxicity tests, i.e. 48-h tests, using *Daphnia* may lead to underestimation of risks associated with PBDEs, especially, BDE-209. Hence, there is a necessity to re-examine PBDE congeners' environmental risk in aquatic organisms.

1. Introduction

Polybrominated Diphenyl Ethers (PBDEs) are important brominated flame retardants used in diverse consumer products such as electronics, plastics, and textiles (Johnson-Restrepo and Kannan, 2009). Concurrent with their detection in several environmental compartments, PBDEs have emerged as a new class of persistent organic pollutants with bioaccumulation and toxicity potentials in environment (Hu et al., 2010; Ma et al., 2012; Wu et al., 2013). Their presence in the aquatic environment is mainly attributed to sewage discharge and atmospheric deposition (Darnerud et al., 2001). PBDEs have become pervasive in aquatic compartments, sediments, sludge, and aquatic organisms (Hu et al., 2010). In recent years, China has been of global concern for PBDEs pollution (Wu et al., 2013). Ample of evidence shows that PBDEs are rapidly pilling up in the environment of China (Ma et al., 2012). E-waste recycling has resulted into a precise type of PBDEs emission, and 2,2′,3,3′,4,4′,5,5′,6,6′-decabromodiphenyl ether (BDE-209) was the leading congener of total PBDEs produced in China, followed by 2,2′,4,4′-tetrabromodiphenyl ether (BDE-47) (Wu et al., 2013). They are the most frequently detected and abundant PBDE congeners in

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freshwater (Darnerud et al., 2001). Consequently, BDE-47 and BDE-209 were accumulated in aquatic environments (Tomy et al., 2004) and biomagnified in the freshwater food chain through aquatic organisms (Hu et al., 2010).

Numerous scientific manifest have identified a range of adverse effects in wildlife after exposure to PBDEs. Evidence existed that PBDEs might influence reproductive activity and cause neural toxicities in variety of organisms (Darnerud et al., 2001; Viberg et al., 2002, 2004). The effects of PBDEs in fish had been reported to include: distortion in blood glucose and hematocrit, reduction in spawning success, neural defects and reproductive toxicity (Lema et al., 2007) as well as altered behavior and delayed hatching (Timme-Laragy et al., 2006). Viberg et al., (2002, 2004) found that PBDEs altered susceptibility in the cholinergic transmitter system in adult mouse. Up to date, information on the sublethal effects of BDE-209 in freshwater invertebrates is still insufficient (Xie et al., 2014). Although toxicity of single PBDEs and combined toxicity of heavy metals or organic pollutants with PBDEs in aquatic environments have been investigated (Hu et al., 2010; Nakari and Huhtala, 2008; Qiu et al., 2010; Tang et al., 2011), however, the aftermaths of combined toxicity of two PBDE congeners have not received adequate attention despite their presence in the environment. PBDE congeners are often considered as having similar toxicological profiles on organisms, however, this might be over simplified (Timme-Laragy et al., 2006). Therefore, the evaluation of combined toxicity effects of PBDE congeners is essential in order to have holistic knowledge of it ecotoxicology risk on aquatic ecosystems.

Amidst numerous biological feedbacks from contaminants, those based on biomarkers occur more rapidly and sensitively, and can be used to obtain the primal signals of environmental disturbance (Parolini et al., 2010). Among the key biomarkers, ChE is exceptionally important due to it roles in the transmission of nerve impulse that hydrolyzes acetylcholine to choline and acetic acid in the synaptic gap of cholinergic synapses and neuromuscular junctions (Chen et al., 2012; Jemec et al., 2010; Li et al., 2011). Similarly, Glutathione-S-transferase (GST) is an important biotransformation enzyme and has been extensively used as biomarker for exposure to numerous contaminants (Quesada-García et al., 2013). Amidst the antioxidant enzymes, catalase (CAT) has been frequently used as biomarkers of oxidative stress in a variety of marine and freshwater organisms reacts to various contaminants such as pesticides, heavy metals, and pharmaceuticals (Schweikert and Burritt, 2012; Ye et al., 2013).

The present study investigated the toxicity of single and combined PBDE congeners (BDE-47 and BDE-209) in acute (48-h) as well as chronic (21-d) circumstances on *Daphnia magna*, a model invertebrate zooplankton species of aquatic organism, under a well-ordered laboratory conditions. The activities of cholinesterase (ChE), Glutathione S-transferases (GST) and catalase (CAT) enzymes were assessed, since they were the key biomarker in biological processes essential for the survival of the individuals. In addition, heartbeat rates and immobilization of *D. magna* were evaluated and used as end-points of acute exposure. The total number of living offspring produced per parent animal was assessed in chronic studies. This study aimed to intensify the present understanding by generating data for the effect of combined mixture of PBDEs on one species and as such contribute to the complete evaluation of PBDE congener's occurrence and toxicity in aquatic ecosystems.

2. Materials and methods

2.1. Culture conditions

Daphnia magna were cultured in fresh water containing artificial medium M4 at pH 7 in laboratory. Animals were maintained in continuous parthenogenetic reproduction following Organization for Economic Cooperation and Development (OECD) guideline 211 (OECD, 2012, 1998). *D. magna* were grown at a density of 1 animal per 40 mL M4 medium in 1 L bottles at 20 ± 1 °C under a 16:8 h light: dark photoperiod and a light intensity of 18 $\mu Em^{-2}\ s^{-1}$. The medium was refreshed with M4 liquid twice a week. *D. magna* were fed with arsenic cultures of Unicellular *Scenedesmus obliquus* grown at 23 ± 1 °C in BG11/MBL medium under fluorescent light (Bogdan and Gilbert, 1984; Stanier et al., 1971; Stauber and Florence, 1989). Algal cultures were centrifuged, re-suspended in M4-pH7 and fed to *D. magna* at a concentration of 90,000 cells/mL which was equivalent to a daily ratio of 100 µg-200 µg Carbon/daphnid. Culture conditions met the OECD requirement of > 60 neonates produced per adult over 21 days (OECD, 1998, 2004, 2012).

2.2. Exposure chemicals and reagents

BDE-47 (CAS No. 5436–43-1; 98.0% purity) was obtained from Chem Service (West Chester, PA, USA) and BDE-209 (CAS No. 1163–19-5; 99.0% purity) was obtained from Dr. Ehrenstorfer GmbH (Augsburg, Germany). Analytical-grade dimethyl sulphoxide (DMSO, > 99.9% purity) was purchased from Alfa Aesar (MA, USA). BDE-47 and BDE-209 were dissolved in DMSO. All stock solutions were prepared prior to the tests. 1-chloro-2,4-dinitrobenzene (CDNB, > 99.9% purity), glutathione (GSH, > 99.9% purity) and 5,5′-dithiobis (2-nitrobenzoic acid) (DTNB, > 99.9% purity) and acetylthiocholine iodide (ATChI)) (> 98% purity) were purchased from Sigma Chemical Company (St. Louis, MO, USA). All other chemicals were of analytical grade and were purchased from Beijing 21st Century Biotechnology Company (Beijing, China).

2.3. Experimental design

2.3.1. Acute tests

The experiment was in accordance with OECD guidelines (OECD, 2004). A series of either single or binary mixtures PBDE congeners (BDE-47 and BDE-209) in 0.1% (V/V) DMSO and blank control (CK, M4 medium without 0.1% DMSO) and solvent control (CKs, M4 medium with 0.1% DMSO) (Davies and Zou, 2012) were prepared in glass containers (100 mL) containing 80 mL M4 medium, in five replicates of five daphnids (within 24 h after birth) for a period of 48 h. Nominal BDE-47 concentrations were 12.5, 25, 50, 100 and 200 µg/L. Nominal BDE-209 concentrations were 62.5, 125, 250, 500 and1000µg/L. The concentration of the combined PBDE congeners solution were in order of 12.5, 25, 50, 100 and 200 µg/L of BDE-47 combined with 1000 µg/L and 125 µg/L BDE-209, and 62.5,125, 250, 500 µg/L of BDE-209 combined with 50 µg/L BDE-47 respectively. Five replicates were prepared for all assays and control groups (blank and solvent control). No algal cultures were provided during the acute exposure. Immobilization as well as the heartbeat rate of *D. magna* were recorded at 48 h and compared with control values. The results were analyzed in order to calculate the EC_{50} of immobilization after 48-h.

2.3.2. Chronic experiment

In chronic test, daphnids were exposed for 21 days, in a medium and environmental conditions similar to those described for acute tests. Each treatment consist of ten 100-mL bottles with 80 mL medium containing one neonate. Daphnids were exposed to five concentrations (0.5, 1, 2, 4 and 8 µg/L) of nominal BDE-47 and five concentrations (25, 50, 100, 200 and 400 µg/L) of nominal BDE-209, respectively. Another sets of daphnids were exposed to different concentration of combined PBDE congeners solution in order of 0.5, 1, 2, 4 and 8 µg/L BDE-47 combined with 400 µg/L BDE-209 and 25, 50, 100, 200 µg/L BDE-209 combined with 50 µg/L BDE-47 respectively. Two unexposed daphnids were set aside for the control treatments (control and solvent control). Test and control media were renewed every other day before food addition. Daphnids were fed daily with *S. obliquus* (90,000 cells/mL) at the ratio of 100 µg-200 µg Carbon/daphnid (OECD, 2012). After 21 days, mortality and progeny of every individual were recorded and the