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Adverse effects of BDE-47 on life cycle parameters, antioxidant system, and activation of MAPK signaling pathway in the rotifer *Brachionus koreanus*

Jun Chul Park[a,1], Jeonghoon Han[a,1], Min-Chul Lee[a], Hye-Min Kang[a], Chang-Bum Jeong[a], Dae-Sik Hwang[a], Minghua Wang[b], Jae-Seong Lee[a,*]

[a] Department of Biological Science, College of Science, Sungkyunkwan University, Suwon 16419, South Korea
[b] Center for Marine Environmental Chemistry and Toxicology, College of the Environment & Ecology, Xiamen University, Xiamen 361102, China

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ABSTRACT

2,2′,4,4′-Tetrabromodiphenyl ether (BDE-47) is widely dispersed endocrine disrupting chemicals (EDCs) in the aquatic ecosystem. Due to its devastating effect on marine organisms and insufficient database on toxicology, we investigated the adverse effects of BDE-47 on life parameters and antioxidant defense system following the reactive oxygen species (ROS) production in the monogonont rotifer *Brachionus koreanus*. In *B. koreanus*, the reduction in life cycle, fecundity, and population growth were observed in response to BDE-47. 50 μg/L BDE-47 significantly reduced ($P < 0.05$) life expectancy and net reproductive rate. In response to 10–50 μg/L BDE-47 exposure, the oxidative stress was elicited via the generation of ROS, while the antioxidant related enzymes (e.g. glutathione *S*-transferase [GST] and glutathione reductase [GR]) have demonstrated significant activity levels ($P < 0.05$) to further alleviate the oxidative stress in a concentration dependent manner. Furthermore, transcript profiles of antioxidant function (*GST-A*, *-O*, and *-S1–S8*)-related genes have shown the significant increase over 24 h in response to BDE-47 (0, 10, 25, and 50 μg/L). As for MAPK signaling pathway analysis, up-regulation of their activities was observed at 25 μg/L BDE-47 but their activities have reduced at adult NOEC concentration of 50 μg/L. This study provides a better understanding of the effects of BDE-47 on life parameters, molecular defense system, and activation of MAPK signaling pathway against generated oxidants in the rotifer.

1. Introduction

Over the past few decades, industrial revolution has led to an increase in the use of detrimental bio-chemicals which ultimately have led to the accumulation of toxicants in our environment and living organisms (Sánchez-Bayo, 2011). In particular, endocrine disrupting chemicals (EDCs), such as tributyltin (TBT), polychlorinated biphenyls (PCBs), and dichlorodiphenyltrichloroethane (DDT), are well known as exogenous agent that interferes with the synthesis, secretion, transport, binding, action, and/or elimination of natural hormones in sustaining the homeostasis in the organisms (Crisp et al., 1998; Mills and Chichester, 2005). Recently, EDCs have frequently been detected in marine ecosystem, and developed the great concerns and regarded as priority pollutants because of their persistency and ability to bioaccumulate in marine organisms (Kelly et al., 2008). Among such EDCs, poly-brominated diphenyl ethers (PBDEs) constitute as an essential composition in household and commercial products as additive flame retardants (Alaee et al., 2003; Eriksson et al., 2001; Mazdai et al., 2003). In addition, PBDEs demonstrated molecular structure, and bio-accumulative properties which ultimately proved as potential endocrine disrupting properties (Legler and Brouwer, 2003). Since PBDEs have distinct characteristics of bio-accumulation and bio-transformation through the food chain, organisms at a high trophic level are vulnerable to its toxicity, which eventually leads to human health hazards (Hites, 2004; Hoh and Hites, 2005). Indeed, PBDEs and their unique bioaccumulation properties resulted in neurotoxicity, genotoxicity, and endocrine disruption in blue mussel (*Mytilus edulis*), zebrafish (*Danio rerio*), and zebra mussel (*Dreissena polymorpha*) (Gustafsson et al., 1999; Lema et al., 2007; Parolini et al., 2012).

Brominated flame retardant, 2,2′,4,4′-tetrabrmodiphenyl ether (BDE-47) as a congener of PBDEs, demonstrates its unique feature including high bioaccumulation and persistence. Due to its

* Corresponding author.
E-mail address: jslee2@skku.edu (J.-S. Lee).
[1] These authors contributed equally to this work.

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volatility and water solubility leading to accumulation of toxicants in marine organisms (Darnerud, 2003; Usenko et al., 2011), BDE-47 exhibits the widest distribution and highest concentrations in the environment and organisms including fish, birds, marine mammals, and humans (Noren and Meironyté, 2000; Schecter et al., 2003; Toms et al., 2007). Previously, to assess the severity of the BDE-47 accumulation, studies involving analytical measurements of accumulation have been performed. For example, two dolphin species *Sotalia guianensis* and *Steno bredanensis* were used to investigate the distribution of PBDEs and the effect in the southern hemisphere (Lavandier et al., 2015). In addition, in the polychaete *Laeonereis acuta* and the crab *Cyrtograpsus angulatus*, the accumulative property of BDE-47 led to the significant increase in oxidative stress with activation of GST enzyme (Díaz-Jaramillo et al., 2016). In the rotifer *Brachionus plicatlis*, the reproductive toxicity along with swimming behavior was examined, where BDE-47 was proved to be more toxic than BDE-209 in the rate of both growth and swimming (Sha et al., 2015). Furthermore, the correlation between ROS, GSH, and enzymatic activities (GR, GPx, and GST) were analyzed in response to the different BDE-47 exposures (Wang et al., 2015), while the transcription level (*catalase*, *superoxide dismutase*, and *calmodulin*) have been investigated in *B. plicatlis* (Zhang et al., 2016). Although toxicological assessments of BDE-47 in *B. plicatilis* are reported, the relationship explaining the in-vivo findings, physiological changes, and molecular mechanisms is still ambiguous. Therefore, we have used the rotifer *Brachionus koreanus*, under the same genus as *B. plicatilis*, to determine the interrelationship between the in-vivo findings and oxidative system along with the corresponding anti-oxidant systems in response to BDE-47.

The rotifer *B. koreanus* have been used as model species for ecotoxicological studies, as they have many advantages such as small size (≈150 μm), short generation cycle (≈24 h), simple structure, genetic homogeneity, high fecundity, and easy laboratory maintenance (Snell and Janssen, 1995). Furthermore, a recently developed and promising tool using next generation sequencing (NGS) has provided a new research impetus to mine enormous amounts of genetic information from diverse non-model organisms (Hwang et al., 2013).

In this study, we investigated the effects of BDE-47 on the life cycle parameters (e.g. mortality, growth, and reproduction), cellular ROS level with antioxidant enzymatic activities, transcriptional expressions of *GST*-isoforms, and activation of MAPK signaling pathway to determine how each factor affects one another to respond in oxidative stress condition, leading to their survivorship. This study will help a better understanding of the mechanistic toxic effects and further insight for the antioxidant systems in response to BDE-47 in the rotifer *B. koreanus*.

2. Materials and methods

2.1. Culture and maintenance of Brachionus koreanus

The monogonont rotifer *B. koreanus* was collected at Uljin (36°58′43.01″N, 129°24′28.40″E) in South Korea. For monoculture, a single individual was isolated under stereomicroscope (SZX-ILLK200, Olympus, Tokyo, Japan), reared, and maintained in 15 practical salinity units (psu) of filtered artificial seawater (Tetra Marine Salt Pro, Tetra™, Blacksburg, VA, USA) at 25 °C with a photoperiod of 12:12 h light:dark. The green algae *Tetraselmis suecica* was used as a diet (~6×10^4 cells/mL). The cultured rotifer *B. koreanus* reproduces only through parthenogenesis and does not reproduce via sexual cycle. Species identification was confirmed by morphological characteristics and mitochondrial cytochrome oxidase I (*CO1*) gene (Hwang et al., 2013; Mills et al., 2017).

2.2. Reagents

The chemicals and reagents used in this study were from Sigma-Aldrich Co. (St. Louis, MO, USA), Qiagen (Hilden, Germany), or Invitrogen (Carlsbad, CA, USA) as molecular biology grade. For exposure study, BDE-47 (molecular weight 485.79, purity >99%) was purchased from AccuStandard (New Haven, CT, USA) as analytical grade. BDE-47 dissolved in isooctane (50 μg/mL) were evaporated and re-dissolved in DMSO to prepare the concentrated stock solution (10 mg/mL).

2.3. Effects of BDE-47 on mortality, lifespan, fecundity, and population growth

To examine the effects of BDE-47 on mortality, 10 neonates *B. koreanus* (less than 2 h after hatching) were collected and were exposed to different concentrations (0, 5 [10.29 nM], 10 [20.58 nM], 50 [102.9 nM], 100 [205.8 nM], and 200 μg/L [411.6 nM]) of BDE-47 in triplicate. In addition to neonatal acute toxicity, adult rotifers were also exposed to different concentrations (0, 5, 10, 50, 100, 200, 400 [823.2 nM], and 800 μg/L [1.646 μM]) to examine the differences in tolerance between the neonate and adult rotifers.

Mortality was analyzed by counting the number of dead rotifers under stereomicroscope (Olympus) at 24 h after exposure in triplicate. In order to analyze lifespan and fecundity, neonates were collected just after hatching (<2 h) and were exposed to different concentrations of BDE-47. Newly born neonates were removed post-exposure in BDE-47 every 12 h from each well and continuously performed until death.

To investigate the effects of BDE-47 on *B. koreanus* fecundity, 10 individual rotifers were transferred into each well of a 12-well culture plate (4 mL working volume), and incubated with 5, 10, 25, and 50 μg/L BDE-47. The numbers of newborn rotifers were counted every 12 h until the matured rotifer died as a readout of fecundity. Half of the medium was renewed with 2 mL every 48 h.

To examine population growth in response to BDE-47 exposure, a single individual was transferred into each well of a three-well glass beaker (working volume, 4 mL) and were exposed to different concentrations (0, 5, 10, 25, and 50 μg/L) of BDE-47 in triplicate. The number of rotifers was counted over a 10-day period. During the experiment, a half of the test solution was renewed, and the green algae *T. suecica* were supplied as a live diet once every 48 h. All the experiments were performed in biological triplicate with temperature maintained at 25 °C.

2.4. Measurement of ROS level, GST, and GR activity

To examine the levels of ROS and BDE-47-induced oxidative stress, *B. koreanus* (approximately 6000 individuals) were exposed to BDE-47 (0, 10, 25, and 50 μg/L) over 24 h in 120 mL glass bottle (100 mL Intracellular ROS were measured as described by Bradford, 1976). Samples were homogenized in a lysis buffer (40 mM Tris–HCl [pH 8.0], 120 mM NaCl, and 0.1% Nonidet-P40) containing a complete protease inhibitor cocktail (Roche; South San Francisco, CA, USA) and used for ROS and GST and GR enzymatic activities measurement. The homogenized samples were centrifuged at 10,000 g for 20 min (4 °C) and the supernatants were reacted with H_2DCFDA. Wavelengths were measured at 485 nm for excitation and 520 nm for emission (Thermo Scientific Co., Varioscan Flash, Vantaa, Finland). The GST enzymatic activity (EC 2.5.1.18) was measured as described by Regoli et al. (1997). Total protein content of the supernatant was determined prior to remaining calculation to normalize ROS contents and GST and GR activities by the Bradford method and method provided by Foyer and Halliwell (1976), respectively. Quantification analysis was