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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*

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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.

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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

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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

performed by comparing each result with the normalization to the control shown in percentage.

2.5. Antioxidant gene expression in B. koreanus in response to BDE-47

Expression patterns of antioxidant genes (e.g. *GST-A*, *GST-O*, and *GST-S1–S8*), mRNA expression levels were measured in response to BDE-47 (0, 10, 25, and 50 μg/L) for 24 h. Total RNA was isolated from the BDE-47-exposed *B. koreanus* using TRIZOL® reagent (Invitrogen, Paisley, Scotland, UK) according to the manufacturer's instructions. Total RNA quantity and quality were measured at 230, 260, and 280 nm using a UV/VIS spectrophotometer (QIAxpert, QIAGEN GmbH, Hilden, Germany). To synthesize cDNA for real-time RT-PCR, two μg each of total RNA and oligo(dT)$_{20}$ primer were used for reverse transcription (SuperScript™III RT kit, Invitrogen, Carlsbad, CA, USA). Real-time reverse transcriptase-polymerase chain reaction (RT-PCR) was conducted for the following conditions: 95 °C for 4 min; 40 cycles of 95 °C for 30 s, 56 °C for 30 s, 72 °C for 30 s; and 72 °C for 10 min using SYBR Green fluorescence as a probe (Molecular Probes Inc., Eugene, OR, USA) with MyIQ cycle (Bio-Rad, Foster City, CA, USA). To confirm the amplification of specific products, melting curve cycles were performed at the following conditions: 95 °C for 1 min; 55 °C for 1 min; 80 cycles of 55 °C for 10 s with 0.5 °C increase per cycle using real-time RT-PCR F or R primers (Table S1). Data from technical triplicate experiment were expressed relative to expression level of the *18S rRNA* gene to normalize expression levels between samples. The fold change in relative gene expression was calculated by the $2^{-\Delta\Delta C}$T method (Livak and Schmittgen, 2001).

2.6. Western blot

The activation of MAPK signaling pathway induced by BDE-47 were evaluated using Western blot analysis. Polyclonal analysis to phosphor-ERK1/2 (anti-mouse, Thr-202/Tyr-204), and phosphor-p38 (anti-rabbit, Tyr-182) were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Polyclonal antibodies to phosphor-SAPK/JNK (anti-rabbit, Thr-183/Tyr-185) were obtained from Cell Signaling Technology (Beverly, MA, USA) and monoclonal antibody to β-actin (control) from Sigma. Using these antibodies phosphorylation patterns of extracellular signal-regulated kinase (ERK), c-Jun-N-terminal kinase (JNK), and p38 were analyzed in *B. koreanus* by following the protocols from our previous study (Kim et al., 2016; Kang et al., 2017). Briefly, approximately 6000 rotifers were exposed to 10, 25, and 50 μg/L BDE-47 for 24 h and were homogenized in lysis buffer (40 mM Tris–HCl (pH 8.0), 120 mM NaCl, 0.1% Nonidet-P40) containing protease inhibitor cocktail (Roche, South San Francisco, CA, USA) for total protein extraction. Total protein was separated by gel electrophoresis using 10% sodium dodecyl sulfate-polyacrylamide gel and was transferred to a nitrocellulose membrane (Amersham; Arlington Heights, IL, USA), blocked with 2.5% bovine serum albumin (BSA) in Tris-buffered saline. The blocked proteins were then allowed to react with primary antibodies (diluted to 1:1000) under the humid condition for overnight at 4 °C with treatment of secondary antibody (peroxidase-conjugate, 1:1000). The developed blots were visualized by following the enhanced chemiluminescent assay procedures (Amersham; Arlington Heights, IL, USA) according to manufacturer's protocol. β-Actin was used as the internal control.

2.7. Statistical analysis

All the results were expressed as mean value with standard error. The homogeneity of variances of data was verified by Levene's test. Data were analyzed using one-way ANOVA followed by Tukey's honestly significant difference test ($P < 0.05$). All the statistical analyses were performed using SPSS® version 18 software (SPSS Inc., Chicago, IL, USA).

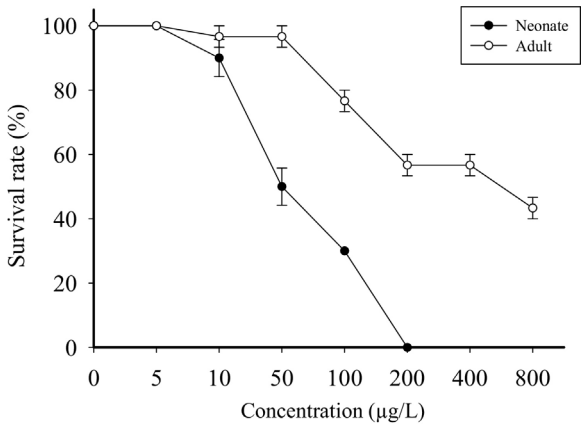

Fig. 1. Acute toxicity analysis on the effects of BDE-47 exposed to *B. koreanus* at various concentrations for neonates (0, 5, 10, 50, 100, and 200 μg/L) and for adults (0, 5, 10, 50, 100, 200, 400, and 800 μg/L). Rotifers were exposed to BDE-47 in each concentration for 24 h.

3. Results

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

The mortality was examined in the rotifer *B. koreanus* treated with different concentration of BDE-47 (0, 5, 10, 50, 100, 200, 400, and 800 μg/L) for 24 h. Each test group was carried out with 10 neonates (<2 h). The no observed effect concentration (NOEC) with neonates was determined as 5 μg/L and the LC50-24 h was 46.295 μg/L with 95% confidence intervals (CI) ranging between 35.417 μg/L to 60.515 μg/L (Fig. 1, Table S2). In addition to neonates acute toxicity, lethal dose of BDE-47 in the adult rotifer (>24 h) were approximately ten times higher than that of the neonates (NOEC-24 h, 50 μg/L and LC50-24 h, 478.849 μg/L). The experiment afterwards, were performed based on the NOEC value of the adult rotifer.

The lifespan was significantly reduced ($P < 0.05$) at 50 μg/L BDE-47 compared to the control and other BDE-47 exposed groups (Fig. 2A). Also, the fecundity experiment showed significant changes ($P < 0.05$) in offspring production in BDE-47 exposed groups at concentration 25 and 50 μg/L, which is demonstrated by slight to moderate reduction in the total numbers offspring produced (Fig. 2B). The population growth of *B. koreanus* was significantly retarded ($P < 0.05$) after exposure to 50 μg/L BDE-47 (Fig. 2C).

3.2. Measurement of ROS level, GST and GR activity

To confirm whether oxidative stress was induced by BDE-47 in *B. koreanus*, the intracellular ROS level, GST and GR activities were measured. The intracellular ROS levels were significantly increased ($P < 0.05$), compared to the control, showing the highest ROS level at 10 μg/L BDE-47 exposure (Fig. 3A). The activity of GST was significantly increased ($P < 0.05$) in the concentration dependent manner of BDE-47, whereas the GR activity was significantly increased ($P < 0.05$) only at the highest concentration (50 μg/L) (Fig. 3B and C).

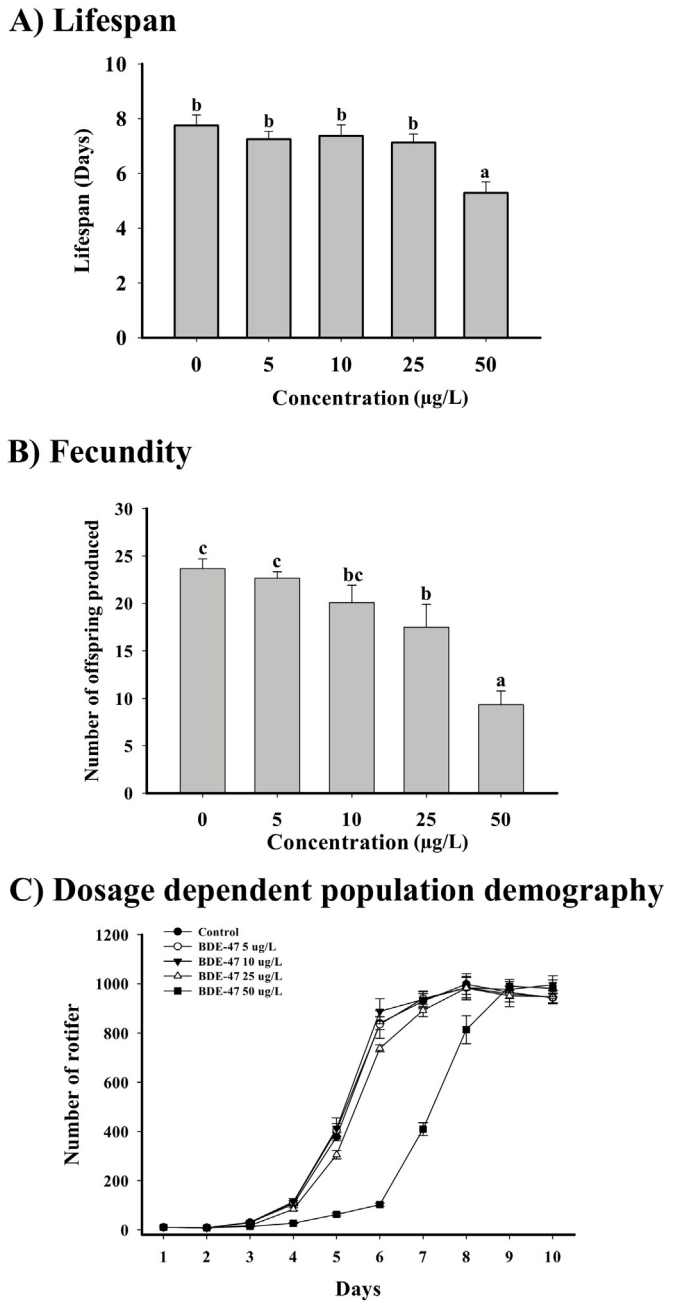

Fig. 2. Effects of BDE-47 exposed to *B. koreanus* on the life parameters, in five different concentration treatments (0, 5, 10, 25, and 50 μg/L). (A) Life span, (B) fecundity, (C) population growth rate. Differences between each test group were analyzed for significance using ANOVA (Tukey's post hoc test) ($P < 0.05$).

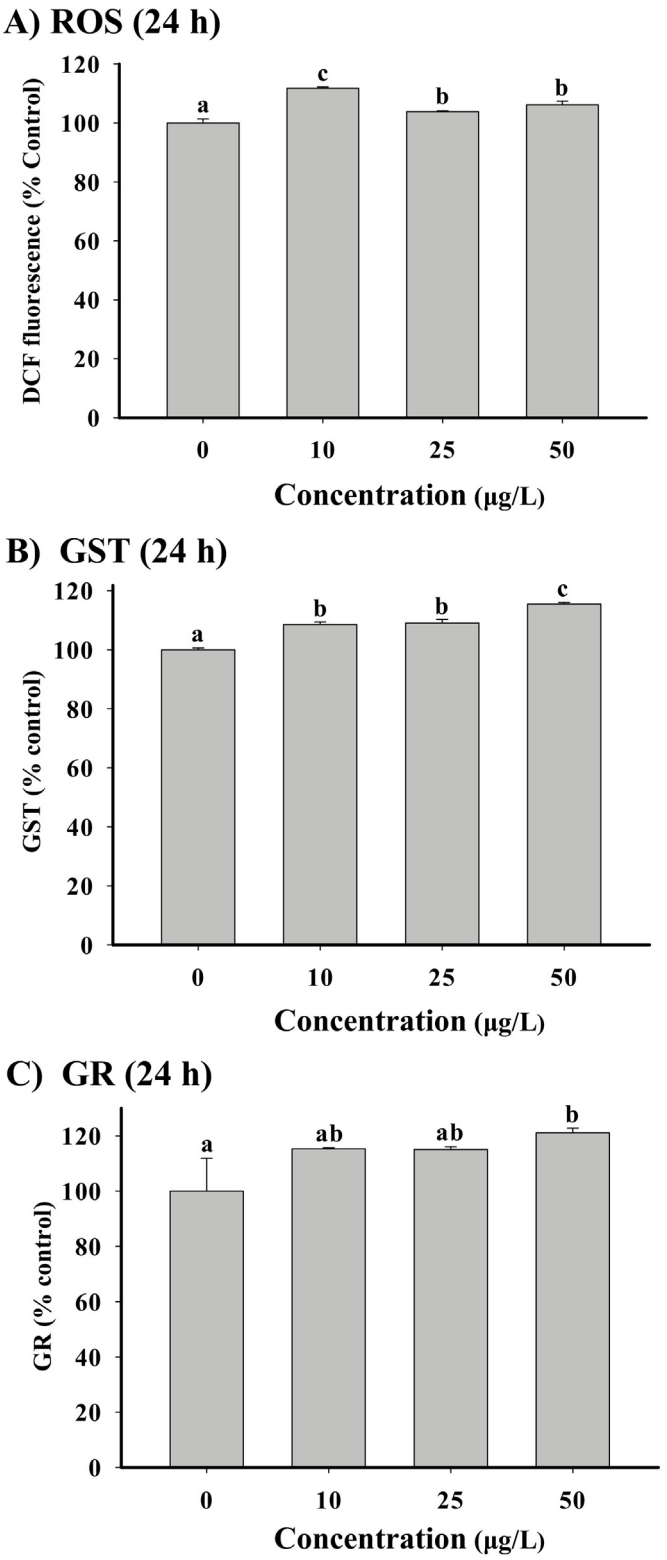

Fig. 3. Enzymatic activity analysis of BDE-47 exposed *B. koreanus* at 24 h in four different concentration treatments (0, 10, 25, and 50 μg/L). (A) DCF fluorescence (ROS), (B) glutathione *S*-transferase (GST), (C) glutathione reductase (GR). Measurements indicate the mean value compared to the control in percentage. Significant differences were analyzed by ANOVA (Tukey's post hoc test), indicated by different letters ($P < 0.05$).

3.3. *Glutathione S-transferase genes expression in BDE-47-exposed* B. koreanus

To identify the effects of BDE-47 at the molecular level in *B. koreanus*, the expressions of *GST* isoforms (*GST-A*, *GST-O*, and *GST-S1–S8*) were measured in response to different concentration (0, 10, 25, and 50 μg/L) in 24 h (Fig. 4). Four genes (*GST-A*, *S4*, *S5*, and S6) have significantly increased ($P < 0.05$) in their expressions compared to the control, with moderate increase in the expression levels of the rest of the *GST* isoforms.

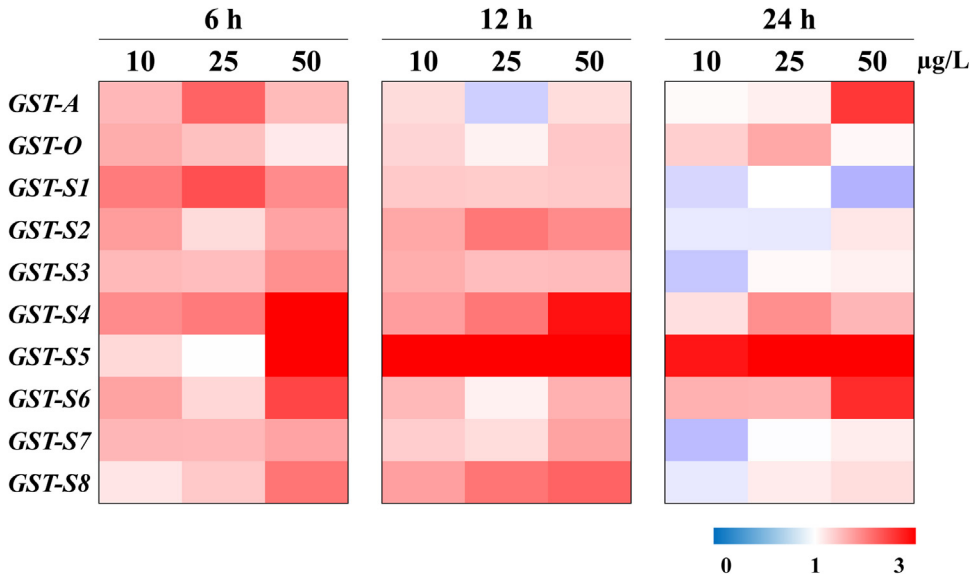

Fig. 4. Gene expression (*GST-A*, *-O*, and *-S1–S8*) after exposure to BDE-47 (0, 10, 25, and 50 µg/L) for 6, 12, and 24 h. The *B. koreanus 18S rRNA* gene was used as a reference housekeeping gene to normalize the expression level. Expression profiles were represented by a heat map. Colors represent the relative expression level compared to the control. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

3.4. Activation of MAPK signaling pathways

The phosphorylation status of ERK, JNK, and p38 in *B. koreanus* were analyzed to understand the effects on MAPK signaling pathways in response to BDE-47 exposure. p-ERK and p-JNK was activated at 25 µg/L BDE-47 exposure, compared to the control. All the MAPK signals were reduced at the highest concentration (50 µg/L BDE-47) (Fig. 5).

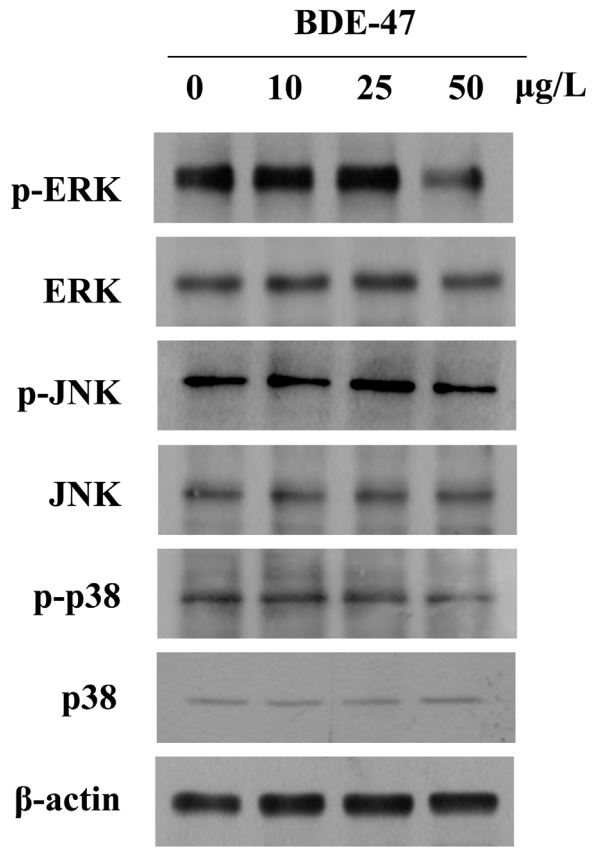

Fig. 5. Effects of BDE-47 on mitogen-activated protein kinase (MAPK) signaling pathways in *B. koreanus*. Western blot analysis provided in concentration-dependent MAPK protein expression levels in response to BDE-47 over 24 h. *B. koreanus* β-actin protein was used as the control. ERK: extracellular signal-regulated kinase, JNK: c-Jun-N-terminal kinases, p38: p38 MAPK kinase.

4. Discussion

In this study, we first investigated the difference in the tolerance and the sensitivity in response to BDE-47 in the rotifer *B. koreanus*. Different survival rate was observed in the neonates and the adults, where the NOEC value for adult was ten times higher than that of the neonates. Similar trend was also detected for LC50 with the adult compared to the neonate. The mortality or lethal concentration (LC) is one of the simplest methods to assess toxicity of chemicals (Chinedu et al., 2013), thus we have assessed the potential differences in the two different life stages of the rotifer tolerance toward BDE-47. Previous studies reported that neonates are more vulnerable than adults in terms of their toxic sensitivity when exposed to toxicants. For example, in zebrafish *Danio rerio*, exposure to BDE-47 during developmental stages has been shown to negatively alter neural development and cardiac function, exhibiting that the early life stage *D. rerio* are sensitive to BDE-47 exposure (Nyholm et al., 2008). Also, in the fathead minnow *Pimephales promelas*, when exposed to BDE-47 during early life stages, they experienced decreases in reproductive success as evidence by reductions in both fecundity and clutch size (Thornton et al., 2016). Although challenges on analysis of physiological and physical changes were inevitable, using 24 h post-hatched rotifers (i.e. adults) demonstrated higher tolerance in response to BDE-47 perhaps via energy expenditure in their survivor potential and prolonging longevity.

The life cycle parameters (e.g. cumulative offspring, population growth, and lifespan) have indeed demonstrated adverse response against at 50 µg/L BDE-47, in particular. To date, growth and reproductive success have been used as useful indicators of physiological health and population structure in ecosystem in response to environmental pollutants (Chandini, 1989; Dahlhoff, 2004). Also, the shortening of lifespan is known to be closely associated with regulation of normal physiology to increase cellular resources for molecular defense (Larsen, 1993; Tosato et al., 2007). For

example, developmental time and reproductive rate were significantly reduced in response to water accommodated fractions (WAFs) of crude oil in the copepod *Tigriopus japonicus* (Han et al., 2014) with no observed mortality at any of the treated concentration. Also, reductions in lifespan, fecundity, and growth retardation were observed in gamma-irradiated *B. koreanus* (Han et al., 2014), suggesting that alteration in lifespan and fecundity could also be due to energy allocation to maintain its homeostasis in stressful conditions. Similar finding has also been reported in the three-spined stickleback *Gasterosteus aculeatus* to fight off external stressor parasites *Schistocephalus solidus* (Heins et al., 2010). Taken together, these studies suggest that BDE-47 leads to detrimental effects and further causes the interruption on the defense mechanisms in *B. koreanus*.

To confirm whether BDE-47 induces oxidative stress and a subsequent anti-oxidative defense in *B. koreanus*, the intracellular ROS level and the activities of GST and GR were measured. Overall significant increase in their activities was found ($P < 0.05$) compared to control. Aquatic organisms have developed several cellular defense mechanisms, where regulation of the level of ROS and the protection against the deleterious effects of free radicals may take place under normal physiological conditions (Winston, 1991; Winston and Di Giulio, 1991). In response to metals (e.g. copper, nickel, and cadmium) and phenols, significant increases in ROS were observed with anti-oxidant enzyme activities in the rotifer *B. koreanus* (Han et al., 2013), the copepod *T. japonicus* (Han et al., 2014), and the carp *Cyprinus carpio* L. (Valon et al., 2013). Also, the significant increases of GST activity in polychaete *Laeonereis acuta* and in the mud crab *Cyrtograpsus angulatus* exposed to BDE-47 were found, suggesting that the GST was involved in PBDE metabolic processes in aquatic invertebrates (Roberts et al., 2011). However, in the BDE-47-exposed rotifer *B. plicatilis*, the activities of GST and GR were negatively correlated with the ROS levels (Wang et al., 2015). This may be due to the disintegration of GSH as a result of high induction of ROS from BDE-47. In case of *B. koreanus*, BDE-47 concentration below the NOEC was used and thus has not evoked severe cytotoxicity which would disrupt the anti-oxidative stress system. Therefore, we hypothesized that the defense mechanism have elicited in correspondence to the ROS generation. Taken together, despite the differences in the responses to BDE-47 exposure between the rotifers *B. koreanus* and *B. plicatilis*, *B. koreanus* has demonstrated positive correlation between the ROS and the corresponding antioxidant enzyme activities, implying that congeneric species of rotifers have proven to demonstrate different tolerance in species-specific defense mechanisms toward BDE-47. However, further investigation in discovering the differences between the two species would be necessary.

To determine the mRNA expression involved in activation of GST enzyme, we analyzed mRNA expressions of 10 *GST* isoforms exposed to different concentrations of BDE-47 (0, 10, 25, and 50 μg/L) over 6, 12, and 24 h compared to control (0 h). The mRNA expression of overall *GST* genes was significantly increased at 6 and 12 h. Particularly, *GST-S5* was significantly up-regulated ($P < 0.05$) all the times (6, 12, and 24 h) at 50 μg/L BDE-47 but in general, the rest of the *GST* isoforms tends to decrease at 24 h. Among the anti-oxidative systems in the aquatic organisms, *GST* genes in particular are known as a useful biomarker and function in a phase II detoxification mechanisms which catalyze deactivation of many harmful substances (Dourado et al., 2008). Therefore, modulations in *GST* genes expressions were studied in invertebrates in response to various stressors. For example, in response to copper, gamma radiation, and biocides, high *GST* genes expressions were demonstrated in *B. koreanus* (Han et al., 2014; Kim et al., 2011). Similarly, *GST* genes were increased in response to BDE-47 in the copepod *T. japonicus* (Han et al., 2014), indicating that mRNA expression patterns of *GST* genes in this study will help and contribute the understanding of the mode of action associated with antioxidant defense mechanisms in response to BDE-47 in rotifer. Also in the beetle *Pterostichus oblongopunctatus* exposed to multiple stressors (e.g. pesticides and starvation), the consequent life span reduced to expend extra energy costs in metabolic detoxification and other means to eliminate toxic substances (Stonem et al., 2001). Thus, deleterious effect induced by BDE-47 up-regulated the mRNA expression, which have indirectly resulted in negative impact on the life cycle parameters (e.g. population growth, fecundity, and life span) of BDE-47-exposed *B. koreanus* which, in overall, correlate with the references above and further support the deleterious induction of oxidative stress, resulting in mediation of molecular signal which might cause impairments of life cycle parameters.

In this study, exposure to BDE-47 in *B. koreanus* have demonstrated the activation of signaling proteins involved in MAPK pathway (ERK, JNK, and p38) up until 25 μg/L, but reduced at 50 μg/L BDE-47, the concentration which corresponds to the NOEC for *B. koreanus*. The three well-known subgroups of MAPKs are linked in MAPK cascade in response to growth factors, hormones, cytokines, genotoxic, and oxidative stressors (Ray et al., 2012). The ERK protein is known for its cell growth regulatory function while JNK protein is known for primary regulation in apoptosis, inflammation, and the differentiation process (Ho et al., 2007; Posser et al., 2009; Matsuoka and Igisu, 2002). Our results have revealed that both the phosphorylated ERK and JNK increased compared to the control in response to low concentration of BDE-47 (10 and 25 μg/L). Similarly, in human T-cells, the activation of ERK and JNK were demonstrated in a concentration dependent manner in response to mercury which correlates to the ROS induction in response to mercury (Haase et al., 2010). Also, p38 are involved in cell death processes (Wada and Penninger, 2004), while phosphorylated p38 is associated with reproductive impairments in the nematode *Caenorhabditis elegans* due to silver nanoparticle-induced oxidative stress (Gerke et al., 2014). In contrast, in a human non-small cell lung carcinoma cell line CL3, inhibition of p38 by SB202190 after cadmium exposure has suppressed their death, implying the role of p38 in apoptosis (Chuang et al., 2000). Taken together, dosage dependent increase in phosphorylated JNK and ERK may function in cellular differentiation and growth in order to redeem themselves from the BDE-47-induced stresses until their threshold (NOEC value, 50 μg/L). Generally, ROS evokes oxidative stress-mediated injury and mediates the activation of signaling molecules which act as a second messenger in intracellular signaling pathways that regulate the cell growth, proliferation, and apoptosis (Son et al., 2011). In normal conditions, energy budget are used for growth and reproduction, while organisms tend to allocate energy towards transcription of detoxification upon the exposure to toxicants, resulting in the inhibition of normal metabolic activities, as seen in the soft coral *Lobophytum compactum* (Michalek-Wegner and Willis, 2001). In the rotifer *B. koreanus*, reduction in both life span and fecundity at 50 μg/L BDE-47-exposed rotifer at 24 h were observed as opposed to the increase in enzymatic activities. Thus, all MAPK signals at 50 μg/L BDE-47-exposed *B. koreanus* have demonstrated reduction in the activities, possibly due to their energy expenditure in the synthesis of antioxidative enzymes in order to detoxify BDE-47 and ultimately sustain their homeostasis to maintain their life.

Our results revealed the higher the concentration of BDE-47 exposed, the more lethal impact it left on the overall life parameters (fecundity, lifespan, and population growth) in *B. koreanus*. The assessment of the adverse effect of BDE-47 in the rotifer, *B. koreanus*, provides a better understanding of how emerging chemical such as BDE-47 affect major cellular function in the rotifer *B. koreanus* at both the cellular and molecular level and at the individual level by negatively affecting life parameters and reproductive system.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.aquatox.2017.02.025.

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