



Triclosan (TCS) and Triclocarban (TCC) cause lifespan reduction and reproductive impairment through oxidative stress-mediated expression of the defensome in the monogonont rotifer (*Brachionus koreanus*)



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ABSTRACT

Triclosan (TCS) and Triclocarban (TCC) are used as antimicrobial agents and have been widely dispersed and detected in the marine environment. However, the toxicities of TCS and TCC have been poorly investigated in marine invertebrates. In this study, the effects of TCS and TCC on mortality, population growth, lifespan, and fecundity were examined in the monogonont rotifer (*Brachionus koreanus*) using cellular ROS levels, GST enzymatic activity, and gene expression of defensomes. The median lethal concentration (LC50) of TCS (393.1 µg/L) and TCC (388.1 µg/L) was also determined in the same species. In TCS- and TCC-exposed *B. koreanus*, growth retardation and reduced fecundity were observed and were shown to have a potentially deleterious effect on the life cycle of *B. koreanus*. In addition, time-dependent increases in ROS content (%) and GST enzymatic activity were shown in response to TCS and TCC exposure. Additionally, transcript levels of detoxification proteins (e.g., CYPs), antioxidant proteins (e.g., GST- σ , Cu/ZnSOD, CAT), and heat shock proteins (*Hsps*) were modulated in response to TCS and TCC exposure over a 24 h period. Our results indicate that TCS and TCC induce oxidative stress and transcriptional regulation of detoxification, antioxidant, and heat shock proteins, resulting in changes in lifespan and fecundity.

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1. Introduction

Over the last decade, Triclosan (TCS) and Triclocarban (TCC) have been widely used as antimicrobial agents in a variety of personal care products (PCPs) and are extensively used as detergents for sanitation. However, these chemicals have recently been identified as emerging pollutants in human and environment health, and both are frequently detected in high concentrations in human tissues as well as in wastewater, sediments, and aquatic environments (Table 1). Both chemicals pose environmental risks and can induce adverse effects in diverse aquatic organisms. For example, in freshwater bivalve zebra mussel (*Dreissena polymorpha*) hemocytes, genotoxic and cytotoxic effects were observed in response to TCS (Binelli et al., 2008, 2009). Also, in the aquatic insect *Chironomus riparius*, genotoxic activity of TCS was shown by comet assay (Martínez-Paz et al., 2013), while impaired swimming behavior with changes in the mRNA of excitation–contraction coupling proteins was observed in the fathead minnow *Pimephales promelas* (Fritsch et al., 2013).

In the marine environment, toxic effects of TCC are not reported as often as those of TCS. For example, in the adult male fathead minnow *P. promelas*, decreased aggression was observed in response to TCC exposure (Schultz et al., 2012). TCC has also been shown to induce DNA damage in the freshwater ciliate *Tetrahymena thermophila* (Gao et al., 2015). However, the main focus of TCS and TCC research has been on their distribution in contaminated sites and in freshwater organisms; TCS and TCC have toxic effects in marine ecosystems (Delorenzo et al., 2008; Farré et al., 2008). Therefore, it is important to understand the in vivo and in vitro molecular mechanisms of TCS and TCC exposure in marine organisms.

Rotifers are widely distributed along coastal areas and play an important role as primary consumers for energy transfer in the aquatic food chain (Isidori et al., 2005; Wallace and Snell, 2010; Dahms et al., 2011). The monogonont rotifer (*Brachionus koreanus*) is considered a suitable model species to study marine ecotoxicology and environmental genomics as it is small ($\approx 150 \mu\text{m}$) and has high fecundity, a short cycle period ($\approx 24 \text{ h}$), genetic homozygosity, and is easily maintained in the laboratory (Dahms et al., 2011; Han et al., 2013, 2014a, 2014b). Also, whole transcriptome data of *B. koreanus* were obtained using next generation sequencing (NGS) technology (Lee et al., 2015) to annotate *B. koreanus* genes from the first assembly of the *B. koreanus* whole genome (total length 110,483,901 bp, scaffold nos. 1087, and

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Table 1

Triclosan (TCS) and Triclocarban (TCC) are used in many applications and have been detected in diverse aquatic environments.

Chemical	Applications	Place	Concentration	References
TCS	Household cleaners, antibacterial mouthwash, toothpaste, shampoos, skin lotion, hand soaps, and children's toys (Reiss et al., 2009)	Tamiraparani River in India	5.16 µg/L	Ramaswamy et al. (2011)
		Lakes and river in Switzerland	74 ng/L, 2 ng/L	Lindstrom et al. (2002)
		Germany	30–90 ng/L	Wind et al. (2004)
		West Prong Little Pigeon River in east Tennessee, United States	608 ng/L	Yu and Chu (2009)
TCC	Antimicrobial soaps and body washes (Chalew and Halden, 2009)	Pearl River system of China	478 ng/L	Zhao et al. (2010)
		Urban stream in USA	6.75 µg/L	Halden and Paull (2005)
		Pearl River system of China	338 ng/L	Zhao et al. (2010)

N50 = 1,090,771 bp; unpublished data). Previously, the effects of diverse environmental stressors (e.g., B[a]P, copper, triphenyltin, and gamma radiation) were successfully measured in *B. koreanus* at a transcriptome level (e.g., glutathione S-transferases, cytochrome P450s, DNA repair, heat shock proteins) based on reproductive and physiological indices (e.g., survival rate, population growth, and reproduction rate) (Kim et al., 2013; Han et al., 2013; Yi et al., 2014).

In this study, the effects of TCS and TCC were measured on the mortality, growth, and reproduction rates and on the generation of ROS levels as well as gene expression patterns of defensomes in *B. koreanus*. This study provides a better understanding of the molecular response and toxic effects of TCS and TCC in the rotifer *B. koreanus*.

2. Materials and methods

2.1. Culture and maintenance of *B. 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 a stereomicroscope, reared, and maintained. The stock for successive culture was maintained in our lab at least for 7 years and the individuals were reared by the batch culture to reduce contamination. *B. koreanus* were incubated 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 live diet. The rotifer *B. koreanus* reproduces only through parthenogenesis and does not demonstrate a sexual cycle. Species identification was confirmed by morphological characteristics and mitochondrial genome analysis (cytochrome oxidase I; *CO1*) (Hwang et al., 2013; Mills et al., 2016).

2.2. Effects of TCS and TCC on mortality, lifespan, fecundity, and population growth

To examine the effects of TCS and TCC on mortality, life span, and fecundity, 10 *B. koreanus* (<12 h after hatching) were collected and were exposed to different concentrations (0, 50, 100, and 200 µg/L; equivalent to 0, 0.1725, 0.345 and 0.69 µM for TCS and 0, 0.1575, 0.315 and 0.63 µM for TCC) of TCS and TCC. Mortality was measured by counting the number of dead rotifers under stereomicroscopy (SZX-ILLK200, Olympus, Tokyo, Japan) at 24 h after exposure. The average lifespan and number of cumulated offspring were determined by counting deaths in response to TCS and TCC exposure.

To examine population growth in response to TCS and TCC exposure, 10 neonates were transferred into each well of a 12-well culture plate (working volume, 4 mL) as shown in previous studies (Rhee et al., 2012; Han et al., 2013, 2014a, 2014b). Then, *B. koreanus* were exposed to different concentrations of TCS or TCC. The 50% test solution was renewed every 24 h. The number of rotifers was counted over a 10-day period. During the experiment, 50% of the test solution was renewed, and the green algae *T. suecica* (approximately, 5×10^4 cells/mL) were supplied as a live diet once every 24 h.

2.3. Measurement of ROS levels and GST activity

To examine the levels of TCS- and TCC-induced oxidative stress, *B. koreanus* (about 7000 individuals) were exposed to TCS (100 µg/L) or TCC (100 µg/L) over a specified time period (0, 3, 6, 12, and 24 h). Intracellular ROS were measured as described by Kim et al. (2011). Three replicates were carried out for each treatment group. Samples were homogenized with Teflon pestle in a buffer (0.32 mM sucrose, 20 mM HEPES, 1 mM MgCl₂, and 0.4 mM PMSF at pH 7.4). The homogenized samples were centrifuged at 10,000 g for 20 min (4 °C), and the supernatants were reacted with H₂DCFDA. 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 to normalize ROS contents and GST activities using the Bradford method (Bradford, 1976).

2.4. Gene expression in *B. koreanus* exposed to TCS and TCC

To obtain the gene sequences for this study, we searched the rotifer *B. koreanus* RNA-seq information (Lee et al., 2015). To examine expression patterns of target defensome genes (*CYP3042A1*, *CYP3043A1*, *GST-a*, *GST-o*, *GST-s*, *GST-z*, *CuZn-SOD*, *MnSOD*, *Cat*, *Gpx*, and 12 isoforms of heat shock proteins), mRNA expression levels were measured for detoxification, antioxidant, and stress-related genes in response to TCS (0 (control), 25, 50, 100, and 200 µg/L) and TCC (0 (control), 25, 50, 100, and 200 µg/L) for 24 h. Total RNA was isolated from the TCS- and TCC-exposed *B. koreanus* (about 6000 individuals for each sample) 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 spectrophotometer (Ultrospec 2100pro, Amersham Bioscience, Freiburg, Germany). To synthesize cDNA for real-time RT-PCR, 2 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 RT-PCR was conducted for the following conditions: 95 °C for 4 min; 35 cycles of 95 °C for 30 s, 58 °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, 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; and 80 cycles of 55 °C for 10 s with 0.5 °C increase per cycle using real-time RT-PCR F or R primers (Suppl. Table 1). The 18S rRNA gene from *B. koreanus* was used as housekeeping gene to normalize, and expressed as relative gene expressions between samples. All analyses were done in triplicate. The relative fold change of gene expressions was calculated as suggested by Livak and Schmittgen (2001).

2.5. Statistical analysis

All results are expressed as mean value with standard error. The homogeneity of variances of data was verified by Levene's test. The significant differences in growth, antioxidant enzymes, and mRNA expression

between control and test groups were analyzed by one-way ANOVA followed by Tukey's honestly significant difference test ($P < 0.05$, different letters indicate significant differences according to exposure group). All statistical analyses were performed using SPSS® version 21 software (SPSS Inc., Chicago, IL, USA).

3. Results

3.1. Effects of TCS and TCC on mortality, lifespan, cumulative offspring, and population growth

The LC50-24h of TCS and LC50-24h of TCC in *B. koreanus* were determined as approximately 393 and 388 $\mu\text{g/L}$, respectively (Table 2).

The population growth of *B. koreanus* was significantly retarded ($P < 0.05$) after exposure to either 200 $\mu\text{g/L}$ TCS or TCC (Fig. 1). Also, cumulative offspring and lifespan were reduced in the highest concentrations of TCS and TCC compared to the control group (Figs. 2 and 3).

3.2. Measurement of ROS level and GST activity

To confirm whether oxidative stress was induced by TCS and TCC in *B. koreanus*, the intracellular ROS level and GST activities were measured. The ROS level in *B. koreanus* increased after 6 and 12 h in response to TCS (100 $\mu\text{g/L}$) and TCC (100 $\mu\text{g/L}$) exposure, respectively. Also, antioxidant GST enzymatic activity was induced after exposure to TCS and TCC (Figs. 4 and 5).

3.3. Defense gene expression in TCS- and TCC-exposed *B. koreanus*

To examine toxic effects of TCS and TCC at the molecular level, expression levels of defense genes were measured in TCS- and TCC-exposed *B. koreanus*. TCS and TCC induced expression of detoxification-related (CYP3024A2, CYP3027C2)-, antioxidant (GST-sigma, catalase), and chaperone (Heat shock proteins) genes (Figs. 6 and 7).

4. Discussion

The significant differences in lethal concentrations of TCS and TCC between species (e.g. ciliate and brine shrimp) demonstrated variable susceptibility to these chemicals over species (Gao et al., 2015; Xu et al., 2015). The difference in LC50-24h values of TCS and TCC was not statistically significant ($P > 0.05$), indicating that the toxicity of TCC was not greater than that of TCS in *B. koreanus* (Table 2). Mortality is a simple method to assess toxicity of chemicals (Amiard and Amiard-Triquet, 2015). Similar toxicity values in response to TCS and TCC indicate that those chemicals induce similar lethal effects in the rotifer *B. koreanus*; however, other aquatic organisms have shown different values. For example, in the brine shrimp *Artemia salina*, the LC50-24h value of TCS (171.1 $\mu\text{g/L}$) was much higher than that of TCC (17.8 $\mu\text{g/L}$) (Xu et al., 2015). Also, in the ciliated protist *T. thermophila*, the LC50-24h value of TCS (1063 $\mu\text{g/L}$) was higher than that of TCC (295 $\mu\text{g/L}$) (Gao et al., 2015). The differences in toxicity in other organisms can be expressed by the amount of excess toxicity in aquatic organisms using K_{ow} (Schultz, 1987).

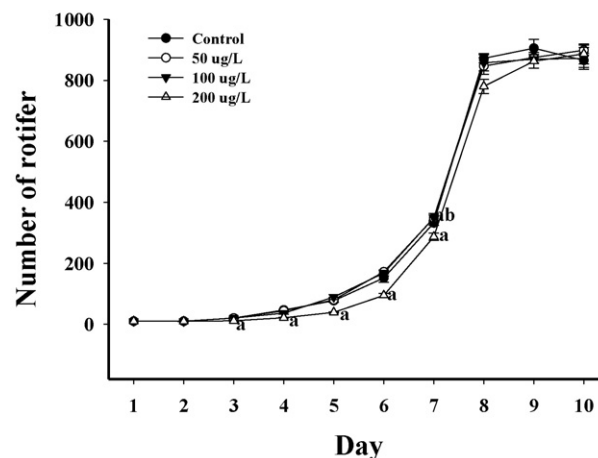
$$K_{ow} = C_o/C_w$$

Table 2

Acute toxicity tests following exposure to TCS or TCC for 24 h.

Chemical	NOEC ($\mu\text{g/L}$)	LC ₅₀ (95% CI; $\mu\text{g/L}$)
Triclosan (TCS)	100	393.051 (317.87–473.70)
Triclocarban (TCC)	100	388.09 (346.20–435.04)

A) TCS



B) TCC

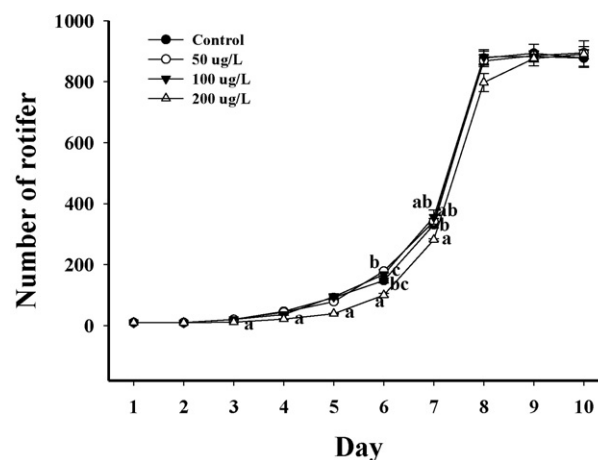


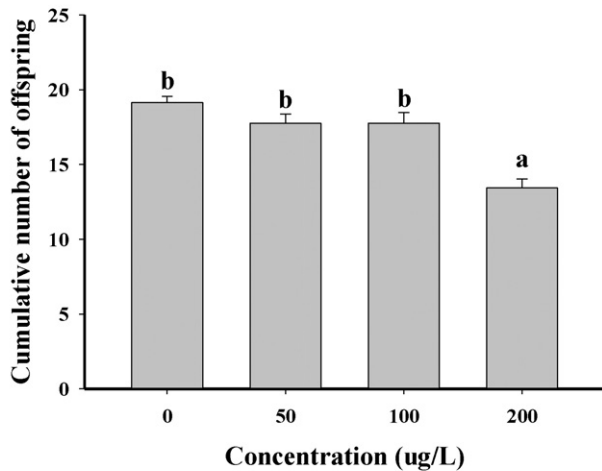
Fig. 1. Effects of different concentrations of (A) TCS and (B) TCC on population growth and fecundity after exposure for 10 days. Error bars indicate mean \pm SE.

where the subscripts o and w refer to the octanol and water phases, respectively, and C_o and C_w are the molar solute concentrations in the two phases.

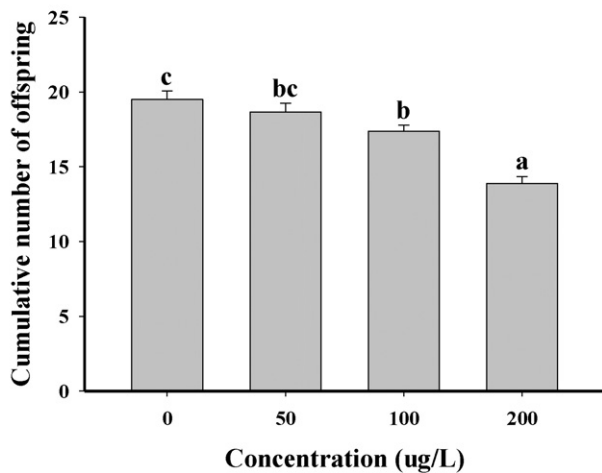
The differences in K_{ow} values of TCS and TCC indicated that TCC (log K_{ow} of 4.9) had relatively high solubility in the aquatic environment compared to TCS (log K_{ow} of 5.4). The similar susceptibility of *B. koreanus* in response to TCC and TCS suggests that *B. koreanus* may have a specific mechanism to regulate the toxicity of TCC that is not found in other species. However, more extensive studies in other aquatic organisms are needed to compare the generalized risks of these two chemicals.

In *B. koreanus*, life parameters (population growth, lifespan, and cumulative offspring) were negatively affected by exposure to TCS and TCC. In particular, the population growth of *B. koreanus* in response to TCS and TCC was retarded but only at the highest concentrations of TCS and TCC. These levels exceeded no observed effect concentration (NOEC) values, although a similar saturation density was reached after 8 days in both the control and experimental groups (Fig. 1). The sublethal toxicity test has been considered an alternative approach to evaluate the biological effects of acute toxicity (van Leeuwen and Vermeire, 2007). For example, over the past several decades, growth and reproductive success have been used as indicators of physiological health (Chandini, 1989) and population structure in ecosystems in response to environmental pollutants (Dahlhoff, 2004). In the monogonont

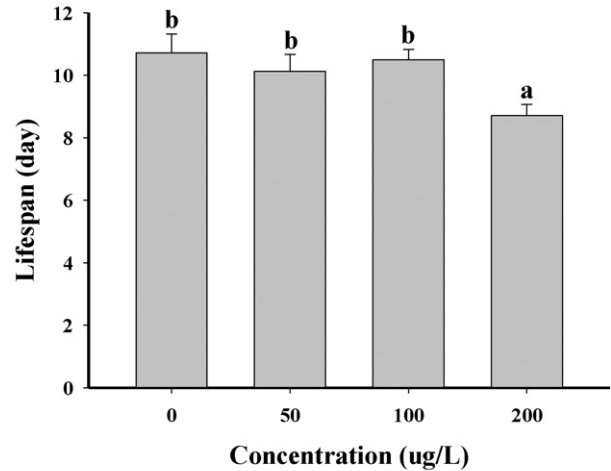
A) TCS



B) TCC



A) TCS



B) TCC

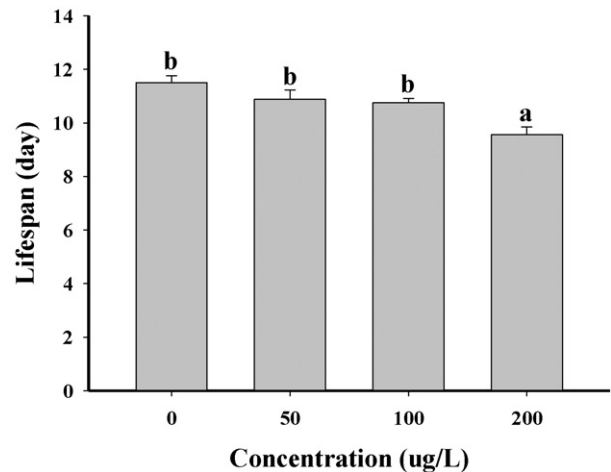


Fig. 2. Effects of different concentrations of (A) TCS and (B) TCC on the fecundity measured using cumulative number of offspring from female *B. koreanus*. Significant differences were analyzed by ANOVA (Tukey's post hoc test; $P < 0.05$) and are expressed as different letters.

rotifer *B. koreanus*, there have been many studies monitoring the sublethal responses to diverse marine pollutants (e.g., UV-B, pharmaceuticals, and Cu) that affected changes in population growth (Kim et al., 2011; Rhee et al., 2012; Han et al., 2013). Also, reductions in lifespan and fecundity and growth retardation were observed in gamma-irradiated *B. koreanus* (Han et al., 2014a, 2014b), suggesting that these in vivo parameters have great potential for evaluating effects of environmental stressors in ecotoxicological studies.

A reduced cumulative number of offspring was measured in rotifers exposed to high concentrations of TCS and TCC (Fig. 2), suggesting an effect on population growth. TCS and TCC may have potential as endocrine disrupting chemicals that affect endocrine systems such as androgen metabolism and thyroid-mediated processes (Foran et al., 2000; Matsumura et al., 2005; Veldhoen et al., 2006; Chen et al., 2007, 2008; Hinthner et al., 2011). In Japanese medaka (*Oryzias latipes*) fry (Foran et al., 2000) and North American bullfrogs (*Rana catesbeiana*) (Veldhoen et al., 2006), TCS induces abnormal traits in endocrine-associated systems including sexual development and postembryonic development. Also, the enhanced bioactivity of endogenous hormones in an in vitro assay has been observed in response to TCC exposure

Fig. 3. Effects of different concentrations of (A) TCS and (B) TCC on the lifespan of *B. koreanus*. Significant differences were analyzed by ANOVA (Tukey's post hoc test; $P < 0.05$) and are expressed as different letters.

(Chen et al., 2008). Taken together, these studies suggest that TCS and TCC disturb the internal secretion and biosynthesis of endocrine hormones that result in physiological alterations. Thus, TCS and TCC may lead to harmful effects on the endocrine system of *B. koreanus*, although this theory requires further studies.

In *B. koreanus*, the lifespan was significantly decreased in response to 200 $\mu\text{g/L}$ of either TCS or TCC (Fig. 3). In gamma-irradiated *B. koreanus*, a similar finding was observed due to the generation of ROS, oxidative stress, and DNA damage in response to gamma radiation (Han et al., 2014b). The shortening of lifespan is closely associated with regulation of normal physiology to increase cellular resources for molecular defense and DNA damage repair capacities (Larsen, 1993; Tosato et al., 2007). Thus, in oxidative stress conditions, the impairment of life cycle parameters supports the finding that the energy capacity for cellular defense and DNA repair reduces the growth, reproduction, and movement of the cells.

In TCS- and TCC-exposed *B. koreanus*, the intracellular ROS content increased (Fig. 4). ROS have important roles in initiating and catalyzing diverse radical reactions in living systems (Valko et al., 2007) and can attack various macromolecules such as DNA, proteins, and lipids, leading to mutagenesis, cellular aging, and carcinogenesis (Gniadecki et al., 2000). Oxidative stress is induced when the balance between production of ROS

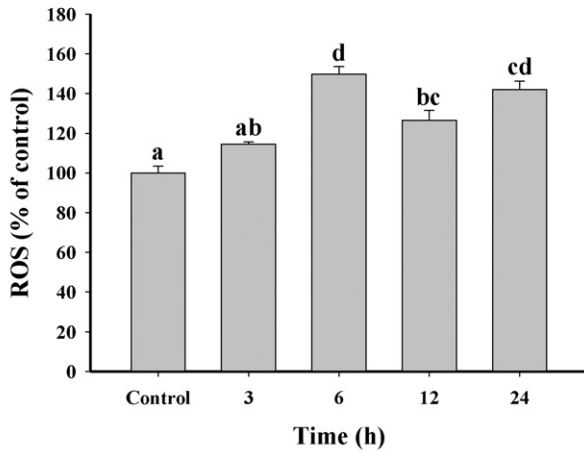
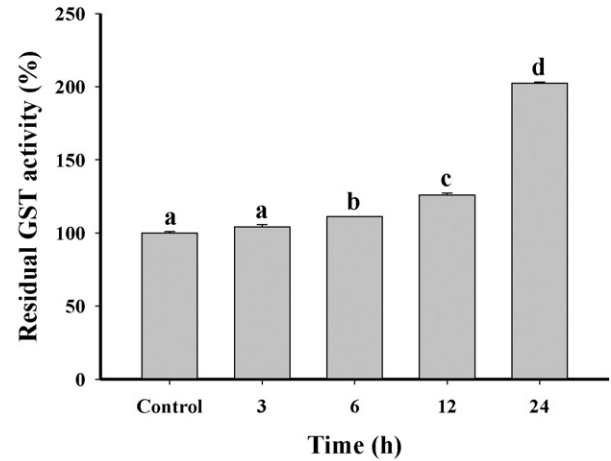
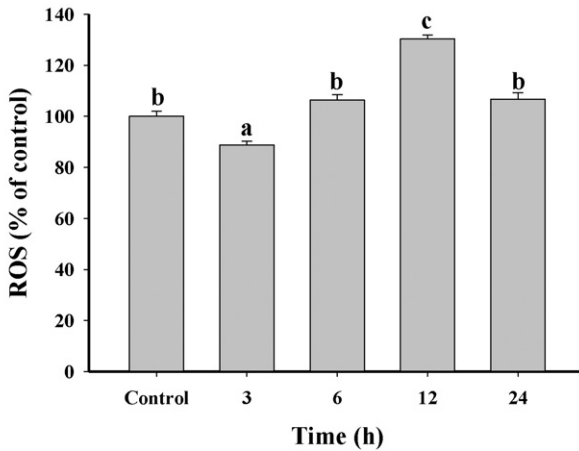
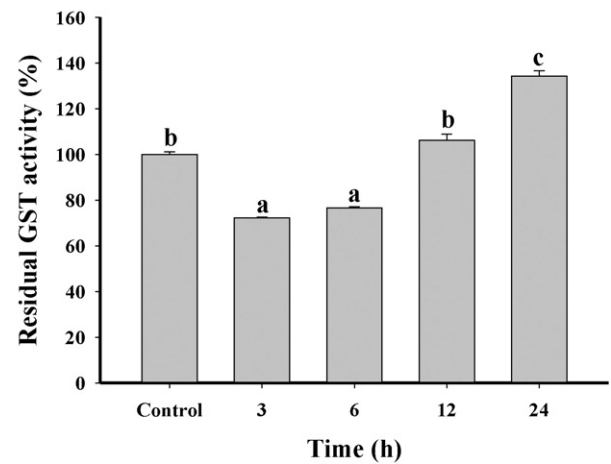
A) TCS (100 µg/L)**A) TCS (100 µg/L)****B) TCC (100 µg/L)****B) TCC (100 µg/L)**

Fig. 4. Effects of different concentrations of (A) TCS and (B) TCC on the generation of intracellular reactive oxygen species (ROS) in *B. koreanus*. Significant differences were analyzed by ANOVA (Tukey's post hoc test; $P < 0.05$) and are expressed as different letters.

Fig. 5. Effects of different concentrations of (A) TCS and (B) TCC on the enzyme activity of glutathione S-transferase (GST). Significant differences were analyzed by ANOVA (Tukey's post hoc test; $P < 0.05$) and are expressed as different letters.

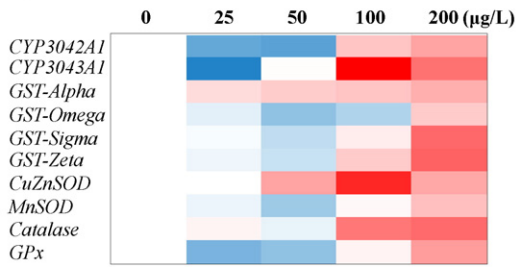
and antioxidant defense is disrupted. Several previous reports have suggested that ROS, generated by diverse environment stressors (e.g., gamma radiation, metals, and UV-B), induce harmful effects on the life cycle and reproduction in *B. koreanus* (Kim et al., 2011; Han et al., 2013, 2014a, 2014b). In another rotifer *Asplanchna brighwelli*, degenerative aging was induced by free radical reactions (Bozovic and Enesco, 1986). Similarly, in *B. koreanus*, a reduced lifespan was observed in accordance with increases in gamma radiation and generation of ROS, while the non-irradiated counterparts showed a relatively long lifespan with a high fecundity rate (Han et al., 2014a, 2014b). Together, these results suggest that ROS are directly related to retarded population growth in TCC- and TCS-exposed *B. koreanus*.

In TCS- and TCC-exposed *B. koreanus*, a significant increase in GST activity was observed (Fig. 5) with a simultaneous increase in antioxidant-related genes and heat shock protein genes (Fig. 6). After exposure to environmental stressors, adaptation and regulation of oxidative stress-induced damage occurred, as was previously shown (Kim et al., 2011; Han et al., 2013, 2014a, 2014b). GST enzymatic activity has been used as a biomarker of environmental stress as the key member of the detoxifying mechanisms through Phase II reactions (Singh et al., 2001). For example, in the zebra mussel (*D. polymorpha*), GST was activated with activation of other antioxidant enzymes (e.g., CAT and GPx) in response to TCS exposure (Binelli et al., 2010).

The mRNA expressions of two cytochrome P450 (*CYP*) genes, oxidative stress-related genes (*GST-a*, *GST-o*, *GST-s*, *GST-z*, *CuZn-SOD*, *MnSOD*, *Cat*, and *GPx*), and 12 isoforms of heat shock proteins were significantly altered in *B. koreanus* in response to TCS and TCC (Figs. 6 and 7). *CYP* enzymes are one of the multigene superfamilies participating in phase I metabolic activation of diverse xenobiotics in organisms (Guengerich, 2008). In rotifer, two *CYP* genes (*CYP3042A1* and *CYP3043A1*) previously showed the most sensitive reactions in response to B[a]P exposure (Kim et al., 2014) and were chosen as candidate molecular biomarkers to assess the effects of TCS and TCC. Conversely, in this study, we found that *B. koreanus* *CYP3042A1* and *CYP3043A1* genes were only slightly induced in response to TCS and TCC exposure. After triphenyltin (TPT) exposure, *B. koreanus* showed significantly induced expression of the *CYP3045C1* gene but not of either the *CYP3042A1* or *CYP3043A1* gene (Yi et al., 2014), suggesting that TCS and TCC have different modes of action and metabolize TPT in a phase I reaction (e.g., *CYP* gene expression).

B. koreanus GST gene expression was also increased in response to TCS and TCC exposure. Similarly, in the yellow catfish (*Pelteobagrus fulvidraco*), several isoforms of *CYP* and *GST* genes were significantly elevated in response to TCS exposure (Ku et al., 2014). In *B. koreanus*, diverse antioxidant genes (e.g., *GSTs*, *CAT*, *SOD*) were previously shown to respond to environmental stressors such as Cu, B[a]P gamma radiation,

A) TCS



B) TCC

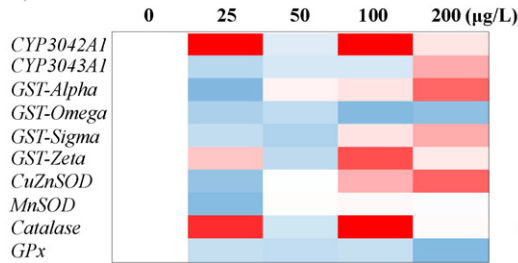


Fig. 6. Expression patterns of the detoxification and antioxidant-related genes in *B. koreanus* after exposure to (A) TCS (0, 25, 50, 100, or 200 µg/L) or (B) TCC (0, 25, 50, 100, or 200 µg/L) for 24 h.

and biocides (Han et al., 2013; Kim et al., 2013; Han et al., 2014a, 2014b; Kim et al., 2015). As expected, TCS- and TCC-exposed *B. koreanus* experienced similar cellular events in metabolism of detoxification and activation of antioxidant defense system.

The mRNA expression of the 12 heat shock proteins (*hsps*) in *B. koreanus* was differently modulated in response to TCC and TCS exposure (Fig. 7). *Hsps* are chaperone proteins associated with cellular defense

mechanisms in response to various environmental stressors (e.g., heat, xenobiotics, UV radiation) (Sarkar, 2006). Of the various *hsps*, *hsp70* is considered the major cellular protection protein that maintains cellular functions and is a useful biomarker of response to environmental stressors in diverse organisms including invertebrates (Lewis et al., 1999; Nadeau et al., 2001; Ivanina et al., 2008). In the rotifer *B. koreanus*, most *hsps* including small *hsps* were upregulated in response to TCS and TCC exposure. Particularly, the highest concentrations of TCC significantly upregulated the expression of *hsp10* and *hsp21* ($P < 0.05$), while reduced expression of *hsp10* and *hsp21* was observed at the NOEC level. In the TCS-exposed earthworm *Eisenia fetida*, expression of heat shock protein 70 (*Hsp70*) was examined as a molecular bioindicator (Lin et al., 2014). In frog tail fin biopsies and rat pituitary GH cells, mRNA expression of *Hsp30* and *Hsp70* genes was changed in response to TCS and TCC exposure (Hinthner et al., 2011). In *B. koreanus*, transcriptional modulation of all *Bk-hsps* was observed in response to environmental stresses (e.g., UV-B, gamma radiation, and triphenyltin chloride) (Kim et al., 2011; Han et al., 2014a, 2014b; Yi et al., 2014), suggesting that *Bk-hsps* would be a reliable biomarker of defense mechanisms in response to environmental stressors. TCS and TCC induced cellular stress to produce DNA damage, triggering the cellular defense system in *B. koreanus*.

In summary, TCC and TCS affect lifespan and reproductive rate in *B. koreanus* after ROS generation and regulate defensesomes associated with detoxification, antioxidant, and stress defense systems. Our study provides a better understanding of how these emerging chemicals affect sub-individual to population-levels through reduced lifespan and reproductive impairment with mechanistic aspects in the rotifer *B. koreanus*. The generation of ROS in response to TCS and TCC has adverse effects on normal physiological functions and processes (e.g., growth and reproduction) in *B. koreanus*. In particular, the potential bioavailability of TCC highlights the specific mechanisms of detoxification of TCC by *B. koreanus*.

Supplementary data to this article can be found online at <http://dx.doi.org/10.1016/j.cbpc.2016.04.002>.

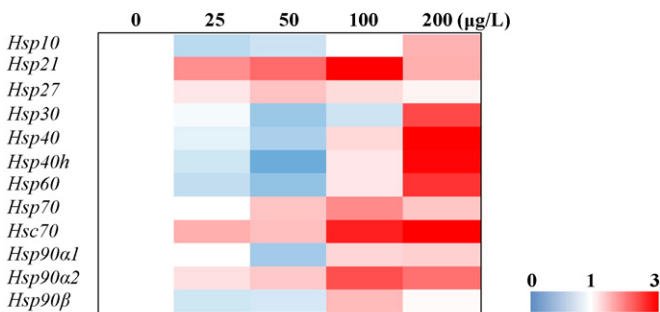
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A) TCS



B) TCC

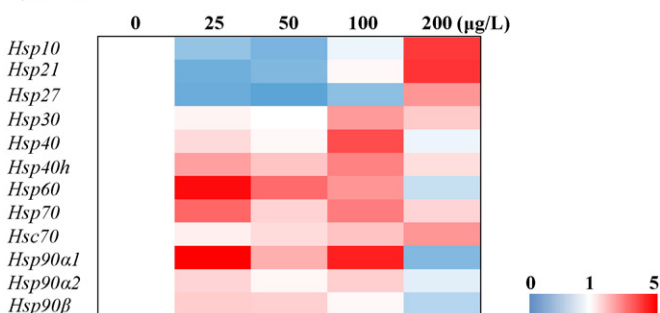


Fig. 7. Expression patterns of heat shock proteins (*hsps*) in *B. koreanus* after exposure to (A) TCS (0, 25, 50, 100, or 200 µg/L) or (B) TCC (0, 25, 50, 100, or 200 µg/L) for 24 h.

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