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# Full life-cycle toxicity assessment on triclosan using rotifer *Brachionus calyciflorus*

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ABSTRACT

Triclosan (TCS) is an antimicrobial and is an aquatic contaminant. Little is known on aquatic toxicity of TCS. Rotifers are common members of freshwater zooplankton. In this study, *Brachionus calyciflorus* was chosen as a test organism to assess the acute and complete life cycle toxicity of TCS in this study. The acute toxicity results showed that the 24-h median lethal concentration ($LC_{50}$) of TCS was 345 ± 0.11 μg/L (95% confidence limits of 212–564 μg/L). Reproductive bioassays demonstrated that TCS could inhibit the population growth rate at the concentration higher than 1.0 μg/L. Resting egg production encompasses the full life-cycle of rotifer, and thus its hatching rate were explored to assess the toxicity of TCS towards rotifer population at TCS concentrations ranging from 0.1 to 200 μg/L at two different growth periods. When resting eggs were exposed to TCS during the formation period, 0.1 and 1.0 μg/L of TCS increased the hatching rate from 0.402 to 0.502, and 0.475, respectively. Exposure to 100 and 200 μg/L of TCS reduced the hatching rate to 0.309 and 0.275, respectively. When the resting eggs were formed in the control medium and hatched in medium with TCS, their hatching rates were not significantly influenced by TCS, except that 200 μg/L of TCS decreased the hatching rate from 0.402 to 0.34 significantly. The effects of TCS exposure on the hatching rate during the formation period were greater than those during the resting egg hatching period.



## 1. Introduction

Triclosan (5-chloro-2-[2,4-dichloro-phenoxy]-phenol; TCS) is a broad spectrum bactericide widely used in pharmaceutical and personal care products, such as soaps, skin creams, deodorants, toothpaste, and plastics (Bhargava and Leonard, 1996; SCCS, 2010). Over 1500 tons of TCS were utilized per year all over the world (Singer et al., 2002); just in China the total consumption was high to 100 tons per year (Zhang et al., 2015a, 2015b). The wide usage of TCS led to its extensive detection in worldwide sewage treatment plant effluents, surface water, ground and drinking water (Halden and Paull, 2005; Morrall et al., 2004; Stasinakis et al., 2008). As reported, TCS concentrations ranged from < 0.1 to 2300 ng/L in 56.8% of surface water samples, 0.001–100 ng/L in sea water, and 0.201–328.8 μg/L in pore water (Brausch and Rand, 2011; Halden, 2014; Kolpin, 2002). Moreover, TCS has been listed as one of the top 10 most commonly detected organic pollutants in the aquatic environment (Brausch and Rand, 2011). Considering its continuous input into water, inefficient removal during wastewater treatment processes, and wide detection in various water bodies (McAvoy et al., 2002), its potential negative effects on aquatic organisms, especially for its long-term exposure, are of increasing concern (Brausch and Rand, 2011).

Previous studies have evaluated the toxicity of TCS to some target organisms including algae, invertebrates, amphibians, fish, birds, and mammals (Ciniglia et al., 2005; DeLorenzo and Fleming, 2008; Fuchsman et al., 2010; Lyndall et al., 2010; Oliveria et al., 2009; Reiss et al., 2009; Tamura et al., 2013; Villa et al., 2014). The tested $LC_{50}$ values or median effective concentration ($EC_{50}$) values of TCS toward these organisms in 24, 48 or 96 h are summarized in Table S1, which displays that the environmental toxicity threshold values of TCS spanned more than six orders of magnitude in concentration (from 0.7 to 239 000 μg/L), and its acute toxicity was moderate. Water body is always the final sink of TCS, the toxicity data of TCS toward aquatic animals are necessary for establishing water quality criteria to protect aquatic life. Actually, TCS has been shown bioaccumulation ability in fatty tissues of some aquatic species like algae, freshwater snails (Coogan et al., 2007; Coogan and La Point, 2008; Dietrich and Hitzfeld, 2004). It also showed inhibition effects on the growth, development, and

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reproduction of microorganisms, microalgae, aquatic macrophytes, invertebrates, amphibians and fish (Chalew and Halden, 2009; Orvos et al., 2002; Tatarazako et al., 2004). More researches on the toxicity of TCS toward different aquatic organisms, especially those abundant, widely distributed, and ecologically relevant species, are critically needed to fully assess its aquatic toxicity.

Rotifers consume a wide array of food resource and thus they occupy several trophic levels. Their diversities are very high in aquatic environment due to the short life cycles and high turnover. They play a significant role in the conversation of organic matter into secondary production and maintaining the stability of the community structure and functions of water ecosystems (Dias et al., 2014). Rotifer also met ten criteria (e.g. represent of the other related species, owning sensitive life stages, validating testing strategies, being sensitive to potential endocrine disruptors) for the ecotoxicological test in the environment risk assessments as mentioned by Breitholtz et al. (2006). Monogonont rotifer *Brachionus calyciflorus* (*B. calyciflorus*) has been taken as a standard freshwater bioassay species by the American Society for Testing and Materials (ASTM) (ASTM - American Society for Testing and Materials, 2004). A large amount of chemicals, including heavy metals, nanoparticles, pesticides, fly ashes, and perfluorinated compounds, have been assessed using rotifers as a model receptor (Copper et al., 2013; Marcial et al., 2005; Snell and Hicks, 2011; Wang et al., 2014; Zhang et al., 2013, 2014, 2015a, 2015b). Rotifers were also found to be more sensitive to some disinfectants than other invertebrates. It is necessary to assess the effects of TCS on rotifers due to their inevitable contact.

For monogonont rotifers, resting eggs is the end-product of sexual reproduction and can survive at extreme environmental conditions such as low temperature or aridity (Pourriot and Snell, 1983; Nielsen et al., 2012; Ricci, 2001). Its production process encompasses the full-life cycle of rotifer, which can thus avoid the limitations of partial life-cycle toxicity tests if resting eggs are chosen as testing endpoint (Preston and Snell, 2001). Indeed, their production and hatching have showed to be sensitive to some chemicals (Ke et al., 2009; Marcial et al., 2005; Marcial and Hagiwara, 2007). Martinez Gomez et al. (2015) evaluated the toxicity of TCS towards rotifer *Platinus Patulus*, which found that TCS inhibited the population growth and egg production and increased egg detachment from ovigerous females. However, the effects on resting egg hatching rate and pattern have not been well documented.

The aim of this study was to evaluate the acute and chronic toxic effects of TCS. The monogonont rotifer *B. calyciflorus* was selected as the test species because of its rapid reproduction, short generation time, wide distribution, and easy maintenance in the lab. Firstly, the 24-h acute toxicity of TCS towards *B. calyciflorus* was conducted to assess its lethal effect. The time needed for producing multiple broods of the $F_0$ and $F_1$ generation of tested rotifer is four days, and thus the four-day reproductive bioassays were adopted to monitor its toxic effects on the reproduction of multigenerational rotifers. Furthermore, the hatching rates and hatching pattern of rotifer resting eggs exposed to TCS at different stages were analyzed to determine its risk on the full life-cycle of rotifer.

## 2. Experimental section

### 2.1. Materials and chemicals

TCS ( $\geq$97%, CAS-3380-34-5) was purchased from Sigma-Aldrich Chemical Co., Ltd. (St. Louis, MO). The other inorganic reagents used in the culture medium (see S2 and S3 of the Supporting information) were obtained from Sinopharm (Beijing, China). All chemicals used in the experiments were reagent grade or higher and used as received. The ultrapure water used in all experiments was produced by a Milli-Q unit (Milli-Q Gradient-A 10, Millipore, US) with the resistivity of 18 MΩ cm.

### 2.2. Test organism

The test rotifers *B. calyciflorus* were originally isolated from a natural lake in Houhai Park (Beijing, China). To reduce the potential gene and individual difference, all the test animals were parthenogenetically produced offspring of one individual from a single resting egg which is the same as reported by Sun and Niu (2012a, 2012b), and were cultured in the same artificial inorganic medium at 20.0 ± 1.0 °C for more than six months before toxicity testing (3000 lx; light:dark, 16:8-h) to acclimate to the experimental conditions. In short, the rotifers were fed with single-cell green alga *Chlorella pyrenoidosa* (*C. pyrenoidosa*) at a density of $4 \times 10^6$ cells/mL. The components of rotifers media and green algae media was described in the Supporting information (SI). The detail information on their life history and classification were described in detail elsewhere (Zhang et al., 2013).

### 2.3. Detection of TCS concentration

A concentration of 2 g/L TCS stock solution was prepared in acetonitrile, and then was diluted into 100 μg/L and 200 μg/L (50 mL) with rotifer medium. The medium dilution were kept at 20.0 ± 1.0 °C (3000 lx; light:dark, 16:8-h), and 1.5 mL of solution were taken at 0 h, 8 h, 16 h, and 24 h. TCS dissolved in DI water with the same concentrations were used as control. The concentrations of TCS in rotifer medium and DI water were determined using high performance liquid chromatography (HPLC, Dionex U3000, USA). The HPLC separation was carried out by a C18 column ($4.6 \times 250$ mm$^2$, 5 μm) and an UV–vis detector. The injection volume is 50 μL, and 89% of acetonitrile and 11% of DI water were used as the mobile phase. The flow rate was 1 mL/min. TCS was detected at 5.3 min.

### 2.4. Acute toxicity

To determine the $LC_{50}$, eleven concentrations of TCS (0, 10, 100, 200, 300, 320, 340, 360, 380, 400, and 500 μg/L) were conducted in the acute toxicity test. Firstly, about 200 rotifers with amictic eggs were randomly selected from the stock rotifer cultures and placed into a glass dish containing 10 mL of rotifer medium with *C. pyrenoidosa*. After 2 h, ten neonates of each replicate were collected and transferred into cell of 6-well culture plates (Costar, Corning Inc., USA) which contained 10 mL of rotifer media with different TCS concentration as mentioned above without *C. pyrenoidosa* at 20.0 ± 1.0 °C (3000 lx; light:dark, 16:8-h). After 24 h, the number of live rotifers was counted in each well and the survival rate was defined as the ratio of survival rotifer individual relative to the original one. The survival ratio of control experiments (0 μg/L TCS) was 100% after 24 h. Each treatment was conducted in six replicates to confirm reproducibility.

### 2.5. Reproductive bioassays

Four-day population growth studies were performed to assess the chronic toxicity of TCS on the intrinsic rate of population increase ($R$) according to the method reported by Snell and Moffat (1992) with minor modifications. Firstly, rotifer medium with $4 \times 10^6$ cells/mL *C. pyrenoidosa* was dosed with 100 μg/L TCS and no aggregation was observed after cultivating for 24 h, which meant that the effects of TCS on the food source of rotifer could be ignored (data not shown). Five toxicant concentrations (0.1, 1, 10,