Chemosphere 114 (2014) 114–120

Contents lists available at ScienceDirect

Chemosphere

journal homepage: www.elsevier.com/locate/chemosphere

Chronic effects of PFOA and PFOS on sexual reproduction of freshwater rotifer *Brachionus calyciflorus*

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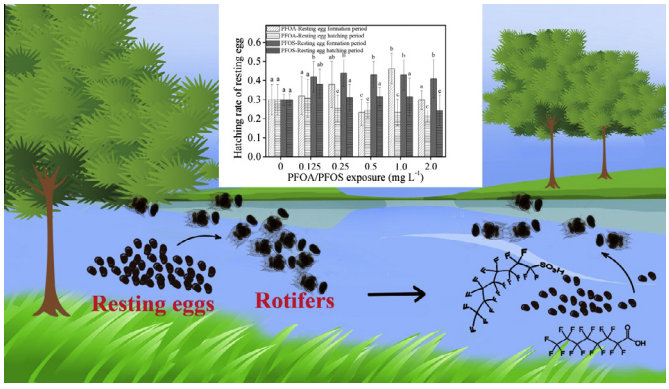
HIGHLIGHTS

- The chronic toxicity of PFOS/PFOA toward freshwater rotifers is investigated.
- PFOS/PFOA impacts the population growth rate.
- PFOS/PFOA increases the mictic ratio of *F*1-genenration rotifer.
- PFOS/PFOA shows different toxic effects on the hatching rate of resting egg.
- PFOS/PFOA exposure plays a minor role in hatching pattern of resting egg.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 15 October 2013
Received in revised form 13 March 2014
Accepted 30 March 2014

Handling Editor: H. Fiedler

Keywords:
Perfluorooctane sulfonate (PFOS)
Perfluorooctanoic acid (PFOA)
Rotifer
Reproduction toxicity

ABSTRACT

Rotifers play an important role in the dynamics of freshwater and coastal marine ecosystems, and are also important tools for assessing toxicity in aquatic environments. In this study, the effects of perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA) on the population growth rate and resting egg production of rotifer *Brachionus calyciflorus* were investigated. Reproductive bioassays indicated that PFOS increased the rotifer population growth rate at the concentration $\leqslant 2.0$ mg L^{-1}, and inhibited it at higher concentrations. For PFOA, the inhibition of population growth rate was observed when the concentration was greater than 4.0 mg L^{-1}. Exposure to PFOS (0.25 mg L^{-1}) or PFOA (2.0 mg L^{-1}) increased the mictic ratios of unexposed rotifer offspring. Population variation and increased mictic ratios were likely the two major factors leading to decline of resting egg production. The resting eggs formed under exposure to PFOA/PFOS in the range of 0.125–2.0 mg L^{-1} showed higher hatching percentages in the control medium than that without PFOA/PFOS exposure. When the resting eggs were formed in the control medium and incubated in media with different levels of PFOA/PFOS, higher hatching percentages were induced by PFOS but lower hatching percentages induced by PFOA. The effects on the hatching rate of resting eggs with PFOA/PFOS exposure during the hatching period were greater than those with exposure during resting egg formation period, and the effect of PFOS was greater than that of PFOA. Both PFOA and PFOS exhibited slight effect on the hatching pattern.

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http://dx.doi.org/10.1016/j.chemosphere.2014.03.099
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1. Introduction

Perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA) have been widely used in numerous industrial and consumer products (Paul et al., 2008). They are also the ultimate degradation products of several commercially used perfluorinated compounds (PFCs) (Parsons et al., 2008). The strong covalent C–F bonds make them resistant to hydrolysis, photolysis, biodegradation, and metabolism (Kissa, 2001), and thus they are persistent in the environment. Due to their extensive usage and non-degradable nature, PFOA and PFOS have been frequently detected in the environment, including dust (Strynar and Lindstrom, 2008), sludge (Higgins et al., 2005), various aquatic environments (Nakata et al., 2006; Bossi et al., 2008; Yamashita et al., 2005), wildlife and human (Giesy and Kannan, 2002). The widespread occurrence of PFOA and PFOS in combination with their non-biodegradable and bioaccumulative nature has caused increasing concern about their environmental risks.

The aquatic environment is often the final depository of PFOA and PFOS, and thus their potential risk to aquatic organisms is of particular concern (Rayne and Forest, 2009). The toxicity of PFOA/PFOS towards many aquatic organisms has been explored (see Table S1), including microorganisms, microalgae, aquatic macrophytes, invertebrates, amphibians and fish. PFOA and PFOS appeared to have moderate acute toxicity to these organisms, and also exhibited different chronic toxic effects on some organisms. For example, PFOA/PFOS inhibited the respiration rate of microorganisms (Beach et al., 2006) and the cell density of microalgae, and altered the zooplankton community structure (Boudreau et al., 2003; Sanderson et al., 2004), and limited the growth and development of macrophytes (e.g., length, root number and length) (Hanson et al., 2005a,b). Additionally, it had been determined that PFOA/PFOS could affect the adult survival and growth of several genera of invertebrates (MacDonald et al., 2004; Kawamoto et al., 2010; Ding et al., 2012; Hazelton et al., 2012). Interestingly, the LC_{50} or EC_{50} values differ significantly for different receptors; sometimes by several orders of magnitude (Giesy et al., 2010). To fully assess the risk of these two chemicals to the aquatic environments, more data on the toxicity of PFOA and PFOS toward aquatic organisms, especially toward abundant, widely distributed, and ecologically relevant species, are critically needed.

As basal consumers, rotifers convert a significant fraction of their food into biomass, which account for 30% of total plankton biomass in aquatic systems (e.g. lake, river) (Snell and Janssen, 1995), and thus play a significant role in maintaining the stability of the community structure and functions of water ecosystems (Dahms et al., 2011). Due to their rapid reproduction, short generation time, ease of maintenance, and sensitivity to toxicants, the monogonont rotifer *Brachionus calyciflorus* (*B. calyciflorus*) have also been included as a standard freshwater bioassay species by the American Society for Testing and Materials (ASTM). A wide range of chemicals have been assessed in previous studies using rotifers as a model receptor using their reproducible biological response as a toxic endpoint, including heavy metals (Arias-Almeida and Rico-Martínez, 2011), natural toxins (Marcial et al., 2005), and recalcitrant organic pollutants (McDaniel and Snell, 1999; Suga et al., 2007; Guo et al., 2012). Some researchers found that both PFOA and PFOS could affect species abundance and richness of rotifer rotifers in microcosm studies (Boudreau et al., 2003; Sanderson et al., 2003, 2004). Recently, we investigated the sublethal toxicity of PFOA/PFOS towards a single rotifer population, and found that PFOA/PFOS exposure had adverse effect on the asexual reproduction of *B. calyciflorus* not only at the individual level but also at the population level (Zhang et al., 2013). Because the life cycle of rotifers contains both asexual and sexual phases, asexual reproduction tests alone may underestimate the effect of pollutants (Snell and Carmona, 1995). It is thus of great importance to explore the impact of PFOA and PFOS on their sexual reproduction.

As the end-product of sexual reproduction of monogonont rotifers, resting eggs are considered as "seeds" of the rotifer to assure their permanence in the community with harsh environmental conditions (Pourriot and Snell, 1983; Nielsen et al., 2012). The process of the sexual reproduction encompasses the full-life cycle of rotifer, which can thus avoid the limitations of partial life-cycle toxicity tests and asexual toxicity tests if resting eggs are chosen as the endpoint (Preston and Snell, 2001). Indeed, resting eggs production and hatching have been shown to be sensitive to some chemicals (Marcial et al., 2005; Marcial and Hagiwara, 2007; Ke et al., 2009). It is thus an interesting and critical question to answer whether and how PFOA and PFOS can impact the resting eggs of rotifers.

The aim of the present study was to elucidate the toxicity of PFOA/PFOS towards the resting egg of rotifers. Reproductive bioassays were adopted to monitor toxic effects on the reproduction of mutigenerational rotifers exposed to PFOA/PFOS. In order to explore the inheritance effects of PFOA/PFOS, the mictic ratios (the proportion of mictic females in the total females) of unexposed *F*1-generation rotifers whose parents had been exposed to toxicants were estimated. Finally, the hatching rates and hatching pattern of resting eggs exposed to toxicants at different stages were analyzed to determine the risk of PFOA/PFOS for sexual reproduction of rotifers. The green alga *Chlorella pyrenoidosa* (*C. pyrenoidosa*) used as the food of the rotifers in this study were living on autotrophic mechanism sensu stricto with slow growth rate and weak bioaccumulation ability (Dursun et al., 2003). Furthermore, rotifers were transferred to fresh medium with fresh algae every day to minimize the effect of *C. pyrenoidosa* bioaccumulation on the rotifers. As shown in Fig. S1, the absorption of PFOS/PFOA at different concentrations was below 0.5%, which can be considered to be negligible.

2. Experimental section

2.1. Materials and chemicals

PFOA (perfluorooctanoic acid, 96%, CAS-335-67-1) and PFOS (perfluorooctane sulfonate, ⩾98%, CAS-2795-39-3) were purchased from Sigma–Aldrich Chemical Co., Ltd. (St. Louis, MO, China). The other inorganic reagents used in the culture were obtained from Sinopharm (Beijing, China). All chemicals used in the experiments were reagent grade or higher and used as received. Deionized water (resistance > 18.2 M cm) was prepared by a Milli-Q water purification system (Milli-Q Gradient-A 10, Millipore, America) at (25 ± 1) °C and was used in all the experiments.

2.2. Test organism

The test rotifers *B. calyciflorus* were originally isolated from a natural lake in Houhai Park (Beijing, China). The detailed information about this organism is shown in Fig. S2. These animals were parthenogenetically produced offspring of one individual from a single resting egg. They were cultured in an artificial inorganic medium at 20.0 ± 0.58 °C for more than six months before toxicity testing (3000 lux; light:dark, 16:8 h) to acclimate to the experimental conditions. The rotifers were provided with single-cell green alga *C. pyrenoidosa* as the diet at a density of 4.0×10^6 cells mL^{-1}. The components of rotifers and green algae media are described in detail elsewhere (Zhang et al., 2013).

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