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Evaluating the sub-lethal toxicity of PFOS and PFOA using rotifer *Brachionus calyciflorus*

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ABSTRACT

The acute and chronic effects of perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA) on the rotifer *Brachionus calyciflorus* (*B. calyciflorus*) were investigated at the organismal and the population level. The acute toxicity of PFOS to rotifers was approximately 2.5-fold greater than that of PFOA. From 0.25 to 2.0 mg L^{-1}, PFOS exhibited higher toxicity than PFOA on the *F*0-generation of *B. calyciflorus*, including effects on body size, juvenile periods, net reproductive rate, and generation time. Both PFOS and PFOA exposure induced a smaller egg size in *B. calyciflorus*, suggesting that these risks can be transferred from mother to offspring. The 28-day population growth studies showed that PFOS and PFOA reduced the population density and increased the mictic ratio. Our results demonstrated that both PFOS and PFOA had adverse effects on *B. calyciflorus*, not only at the individual level but also at the population level.

1. Introduction

Polyfluorinated compounds (PFCs) have been widely used in materials such as wetting agents, lubricants, stain resistant agents, and foam in fire extinguishers since the 1950s because of their resistance to hydrolysis, photolysis, biodegradation, and metabolism (Niu et al., 2012; Prevedouros et al., 2005). Among them, perfluorooctane sulfonate (PFOS) and perfluorooctanoate (PFOA) are the most commonly used PFCs in the industry. Due to their widespread and non-biodegradable nature, PFOS and PFOA have been detected in dust (Murakami and Takada, 2008), sediment, domestic sludge, the air of the remote Arctic region (Shoeib et al., 2006), humans (Völkel et al., 2008), biota (Houde et al., 2011), and water (up to 6574 μg PFOS L^{-1} and 2300 μg PFOA L^{-1}) (Hu et al., 2011; Kwadijk et al., 2010; Schultz et al., 2004). Because of their global distribution, environmental persistence, and potential risk to human beings, PFOS has been categorized as one of the new persistent organic pollutants (POPs) at the 2009 Stockholm Convention (Wang et al., 2009). Therefore, investigation of their potential impacts on natural aquatic environments is imperative.

Many studies have explored the toxicity of PFOS and PFOA in rats (Wang et al., 2012), mice (Johansson et al., 2008; Vanden Heuvel et al., 2006; Wolf et al., 2007), fish (Ji et al., 2008), monkeys (Renner, 2001), birds (Yoo et al., 2008), and humans (Vanden Heuvel et al., 2006; Wolf et al., 2012). Exposure of these animals to PFOS and PFOA has resulted in reductions of body weight and serum cholesterol, an increase in live weight (Olsen et al., 2003; Strömqvist et al., 2012), or developmental toxicity (Lau et al., 2004; Wolf et al., 2007). Because bodies of water are the final sinks for PFOS and PFOA, aquatic animal toxicity data for PFOS and PFOA are of great importance for establishing water quality criteria for the protection of aquatic life. More information on their toxicity to aquatic fauna, particularly to freshwater invertebrates, is needed (Giesy et al., 2010). To the best of our knowledge, toxicological data on PFOS contamination are only available for seven genera of freshwater invertebrates: *Unio* (Hazelton et al., 2012), *Daphnia* (Boudreau et al., 2003; Ding and Peijnenburg, 2012; Ding et al., 2012; Ji et al., 2008; Li, 2009), *Chironomus* (MacDonald et al., 2004), *Dugesia*, *Physa*, *Neocaridina*, *Paramecium caudatum* (Kawamoto et al., 2010), and *Moina* (Li, 2009).

Rotifers, as basal consumers, are widely distributed in freshwater as well as in estuaries and coastal waters. Their high assimilation efficiencies allow rotifers to convert a significant fraction of their food into biomass, making it available to higher trophic levels (Snell and Janssen, 1995). With their rapid population turnover rate, rotifers contribute significantly to nutrient recycling in aquatic habitats, implying that the function of freshwater ecosystems can be altered if rotifer populations are adversely affected by a toxin

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(Dahms et al., 2011). In addition to being of ecological significance, rotifers meet ten criteria for the ecotoxicological test in the environmental risk assessments of Breitholtz et al. (2006). These characteristics, including enzyme activity, life-history variables, the rate of population increase, and the maximum population density, have been employed and quantified in rotifers responding to toxic stressors (Arnold et al., 2011; Dahms et al., 2011; Snell and Hicks, 2011; Snell and Janssen, 1995). However, investigation of the toxicity of PFOS and PFOA that are widely and persistently distributed in water that contains rotifers, is quite limited. We performed a literature survey and found only two relevant studies, which reported that PFOS was more toxic to a zooplankton community consisting of total *Rotifera* sp. than was PFOA using zooplankton species abundance and richness as endpoints (Sanderson et al., 2004). In addition, in a freshwater community, PFOA had lower toxicity to rotifers than to other zooplankton, such as *Daphnia magna*, *Cyclops canthocamptus staphylinus*, and *Cyclops diaptomus* (Sanderson et al., 2003). However, acute and chronic toxicity to the organism and to population of single rotifer species have not been reported.

Safe concentrations of chemicals are calculated based on single-species toxicity experiments, which can be extrapolated to population, community, and ecosystem effects (Chapmen, 1995). Most relevant studies evaluating the toxic effects of PFCs on aquatic animals have focused on acute tests at the individual level and only a few responses of a limited part of the life cycle (Ding et al., 2012; Hazelton et al., 2012; MacDonald et al., 2004). Generally, the risks posed by PFOS and PFOA are chronic and long-term in the aquatic ecosystem (Hekster et al., 2003). It is difficult to accurately predict the chronic toxicity of PFCs towards rotifers from the acute test and partial life-cycle effect. Therefore, more data related to their long-term toxicity towards rotifer populations and chronic effects on the full life cycle of rotifers are necessary to provide comprehensive information for the ecological risks of PFOS and PFOA to aquatic systems.

The aim of this study was to investigate the acute and chronic toxicity of PFOS/PFOA towards rotifers at the organismal level (body and egg size, life-history variables) and the population level (population density, population structure, and growth curves). The monogonont rotifer *Brachionus calyciflorus* (*B. calyciflorus*) was selected as a target because of its rapid reproduction, easy maintenance in the lab, short generation time, and cosmopolitan distribution. It has also been included as a standard freshwater bioassay species by the American Society for Testing and Materials (ASTM). The aims of this study were: (1) to assess the acute and chronic toxicity of PFOS and PFOA on parent rotifers, (2) to compare the toxicity effect of PFOS and PFOA on rotifers, and (3) to evaluate the long-term effects of PFOS and PFOA on *B. calyciflorus* population. Knowledge of the toxicity of PFOS and PFOA towards rotifers can enhance the potential of rotifers as suitable aquatic models for ecotoxicology tests in natural marine and freshwater systems.

2. Experimental section

2.1. Chemicals

PFOA (perfluorooctanoic acid, 96%, CAS-335-67-1) and PFOS (perfluorooctane sulfonate, ≥98%, CAS-2795-39-3) were supplied by Sigma–Aldrich Chemical Co., Ltd. (St. Louis, MO, China). Solvent-free stock solutions of PFOS (1000.0 mg L^{-1}) and PFOA (1000.0 mg L^{-1}) were prepared by dissolving the solid in deionized (DI) water via sonication. PFOS may aggregate in aqueous media beyond its critical micelle concentration. However, PFOS has critical micelle concentrations of 370.0 mg L^{-1} in freshwater and 570.0 mg L^{-1} in pure water, which are much higher than the maximum concentration (16.0 mg L^{-1}) in our experiments. Chemical measurements were taken to determine the concentrations of these compounds in the medium used in the experiments, and the methods and results are shown in the Supporting Information. The other inorganic reagents were obtained from Sinopharm (Beijing, China). All chemicals used in the experiments were reagent grade or higher and were used as received. DI 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 experiments.

2.2. Test organism

The monogonont rotifers species are cyclical parthenogens (see Supporting Information) living in limnic habitats with considerable seasonal variation and often with island-like features. Its typical species *B. calyciflorus* was used as the test species in our experiments. All animals were parthenogenetically produced offspring of one individual from a single resting egg collected from a natural lake in Houhai Park (Beijing, China). The rotifers were cultured in an artificial inorganic medium at 20 °C for more than six months before toxicity testing (3000 lux; light:dark, 16:8 h) to acclimate to the experimental conditions. The animals were transferred daily to new US Environmental Protection Agency (USEPA) medium (USEPA, 1985) containing fresh green algae *Chlorella pyrenoidosa* (*C. pyrenoidosa*) at a density of 4×10^6 cells mL^{-1}. The specific information is shown in Supporting Information. In stock populations, the rotifers were cultured at a low population density (<0.4 individual mL^{-1}, ind. mL^{-1}) to inhibit sexual reproduction (for this clone, the proportion of mictic females which is one female producing mictic egg was 10% under these culture conditions). All the toxicity experiments were carried out in the same culture media and under the same conditions (i.e., pH, temperature, illumination).

The common rotifer food, fresh green algae *C. pyrenoidosa*, survives by autotrophic mechanisms sensu stricto, with a slow growth rate. Algal bioaccumulation ability was negatively related to metabolic activity (Dursun et al., 2003). Additionally, rotifers were removed to fresh media containing new algae every day in our experiments. Thus, the effect of bioaccumulation of *C. pyrenoidosa* on the rotifers was considered to be minimal and was therefore not included in analyses. The media components for the rotifers and green algae may be found in the Supporting Information.

2.3. Acute toxicity

To determine the median lethal concentration (LC_{50}), seven concentrations of PFOS (i.e., 40.0, 50.0, 60.0, 70.0, 80.0, 90.0, and 100.0 mg L^{-1}) and PFOA (i.e., 60.0, 80.0, 100.0, 120.0, 140.0, 160.0 and 180.0 mg L^{-1}) were used in the acute toxicity test. Rotifers with amictic eggs were randomly selected from the stock rotifer cultures and placed into a glass dish containing 10 mL of medium with *C. pyrenoidosa*. After 2 h, ten neonates (<2 h old) for each replicate were collected and transferred into 15 mL 6-well cell culture plates (Costar, Corning Inc., USA) containing 10 mL of test solution without *C. pyrenoidosa*. After 24 h, the number of live rotifers was counted in each well. Control experiments without PFOS or PFOA were also performed; survival was nearly 100% after 24 h. Each treatment was repeated six times to confirm reproducibility.

2.4. PFCs-induced body size and egg size plasticity

To determine the PFCs-induced change in body size and egg size for *B. calyciflorus*, the size at first reproduction (SFR) and the egg size were measured. SFR was used as an indicator of mature body size because a very slow increase in body size after maturation has been observed in rotifers. Approximately 50 neonates were randomly sampled from each acclimated population at different concentrations of PFC treatment (0.25, 0.5, 1.0, 2.0, and 4.0 mg L^{-1}). They were checked every 2–4 h and were selected from each culture after carrying the first egg. The animals were fixed with 5% formaldehyde for later measurements. To reduce the effect of dehydration and contraction of rotifers in 5% formaldehyde, all measurements on SFR and egg size were conducted within 3 h after fixation.

Measurements of SFR and egg size were conducted with an image analysis system (AxioVision 4.6.3.0, Carl Zeiss Inc., Germany), connected to a microphotography system (AxioCam MRc5 and Axioskop 2 plus, Carl Zeiss Inc., Germany). The long/short axes of bodies and eggs were determined, and volume was calculated using the equation for the volume of a regular oblate spheroid.

2.5. Life-history table

To assess the effect of PFOS and PFOA on the survival and reproduction of the *B. calyciflorus*, four concentrations (i.e., 0.25, 0.50, 1.0, 2.0 mg L^{-1}), which are far lower than the minimal lethal doses of PFOS/PFOA and have been detected in some contaminated aquatic environment (Ahrens, 2011; Schultz et al., 2004), were selected for the life-history table experiments. Neonates (<2 h) were selected at random and transferred individually into 24-well culture plates. Each well contained 2 mL of EPA medium with different level of PFOS or PFOA. Each animal was checked every 2 h until mature to record accurate juvenile periods (*JP*s) which related to the time from newborn to carrying first egg, and the amictic rotifers were used to create the cohort for different treatment groups. Fifteen rotifers of each cohort were individually cultured in 24-well cell culture plates with 2 mL of fresh medium; the medium was refreshed daily. The life-history table experiments were conducted at 20 °C (3000 lux, light:dark, 16:8 h) until each individual in every cohort died. The