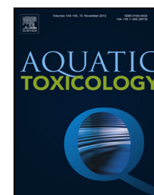
Aquatic Toxicology 175 (2016) 269–276

Contents lists available at ScienceDirect

Aquatic Toxicology

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



# Zebrafish reproductive toxicity induced by chronic perfluorononanoate exposure

Wei Zhang [1], Nan Sheng [1], Minhui Wang, Hongxia Zhang, Jiayin Dai *

*Key Laboratory of Animal Ecology and Conservation Biology, Institute of Zoology, Chinese Academy of Sciences, Beijing 100101, PR China*

ARTICLE INFO

*Article history:*
Received 19 January 2016
Received in revised form 4 April 2016
Accepted 4 April 2016
Available online 5 April 2016

*Keywords:*
Perfluoroalkyl acids
Zebrafish
Reproductive toxicity
VTG

ABSTRACT

Perfluoroalkyl acids (PFAAs) are a group of anthropogenic compounds that have been widely used in consumer products for over 50 years. One of the most dominant PFAAs is perfluorononanoate (PFNA), a compound detected ubiquitously in aquatic ecosystems. While PFNA is suspected of being an endocrine disruptor, the mechanisms behind PFNA-induced reproductive disorders are poorly understood. The aim of this study was to investigate the reproduction-related effects and possible mechanisms of PFNA on adult zebrafish (*Danio rerio*) following 180 days of exposure at different concentrations (0.01, 0.1, 1 mg/L). PFNA concentration in the gonads of zebrafish was tested by HPLC–MS/MS after chronic exposure to study possible inconsistent accumulation between the genders. The results showed that the accumulation of PFNA in the male gonads was almost one-fold higher than that in the female gonads, indicating a possible higher PFAA gonad burden for male zebrafish. Significant reductions in the male gonadosomatic index (GSI) and female egg production were observed. In addition, the decreased 72 h hatching rate displayed an evident dosage effect, indicating that maternal exposure to PFNA might impair offspring developmental success. To investigate how PFNA exposure affects the hypothalamic-pituitary-gonadal-liver axis (HPGL axis), the transcriptional levels of genes were measured by real-time PCR. The disrupted expression of genes, such as ERα, ERβ, FSHR, LHR, StAR, and 17βHSD, indicated the possible interference of PFNA on the HPGL axis function and sex hormone synthesis. Furthermore, testosterone (T) and estradiol ($E_2$) levels in serum and VTG content in the liver were detected to clarify the influences of PFNA on sex hormone levels. Except for the increase in serum estrogen levels, as an estrogen analogue, PFNA also induced the synthesis of biomarker protein vitellogenin (VTG) in the adult male liver. The results of this study indicate that chronic exposure to PFNA can lead to dysfunction in the HPGL axis and sex hormone synthesis and cause adverse effects on fish reproduction.

© 2016 Elsevier B.V. All rights reserved.

## 1. Introduction

Perfluoroalkyl acids (PFAAs) are a family of perfluorinated chemicals consisting of high-energy carbon-fluorine (C—F) bonds (Hekster et al., 2003). With their unique characteristics, PFAAs have been widely used over the past several decades in industrial and domestic products, ranging from surfactants and emulsifiers to textiles and paper products (Lemal, 2004; Renner, 2001, 2004). This has led to the continuous global detection of PFAAs in environmental matrices, including aquatic systems (Giesy and Kannan, 2002). These chemicals, which do not exist naturally, are non-biodegradable and persistent, resulting in widespread environmental detection and pollution (Yakata et al., 2003). Recently, concerns have arisen about the possible health impacts of exposure to perfluorononanoate (PFNA), a perfluorinated alkyl acid containing nine carbon atoms, due to the detection of increased PFNA levels in the environment as well as in human and wildlife tissue (Keller et al., 2005). PFNA concentrations have been found at higher levels than perfluorooctanoate (PFOA) in a variety of fish species (Houde et al., 2006; Kallenborn, 2006; Li et al., 2008; Martin et al., 2004a; Martin et al., 2004b). For example, in the serum of fish from Gaobeidian Lake in China, the concentration of PFNA ranged from 0.114 to 1.18 ng/mL, while PFOA ranged from 0.108 to 0.669 ng/mL (Li et al., 2008). As a result, PFNA has become a dominant PFAA in fish-eating marine mammals (PFNA: 236 ± 25 ng/g; PFOA: 10 ± 2 ng/g; perfluorodecanoic acid (PFDA): 89 ± 9 ng/g in polar bear liver) (Hart et al., 2009; Houde et al., 2005; Ishibashi et al., 2008; Smithwick et al., 2005).

* Corresponding author.
*E-mail address:* daijy@ioz.ac.cn (J. Dai).
[1] These authors contributed to this work equally.

http://dx.doi.org/10.1016/j.aquatox.2016.04.005
0166-445X/© 2016 Elsevier B.V. All rights reserved.

Studies on model organisms support the hypothesis that PFAAs may exhibit estrogen-like activity. For teleosts, PFAA exposure can affect sex steroid hormone levels, increase hepatic vitellogenin (VTG) expression, disrupt gonad development and even lead to bisexual gonads (Ankley et al., 2005a,b; Benninghoff et al., 2011; Du et al., 2009; Oakes et al., 2005; Wei et al., 2007). Evidence for estrogen-like activity of some PFAAs has also been shown *in vitro* (Ishibashi et al., 2007; Liu et al., 2007; Maras et al., 2006). However, although these studies examined PFOS and PFOA, little research has been done on PFNA.

Evidences in vertebrates have shown that PFAAs can affect the hypothalamic-pituitary-gonadal (HPG) axis (Liu et al., 2009b; Liu et al., 2011). In fish, the HPG axis is commonly referred to as the hypothalamic-pituitary-gonadal-liver axis (HPGL axis) because many egg-yolk and chorionic proteins (*e.g.* VTG and choriogenin) are synthesized heterologously in fish livers, which are necessary for oocyte growth and development in females (Arukwe and Goksoyr, 2003). In addition, previous studies have shown that genes regulating steroidogenesis are important target sites for endocrine disrupting chemicals (EDCs). For example, after exposure to EDCs, such as bisphenol A (BPA), ketoconazole, and vinclozolin, genes in the HPG axis, including ERα, ERβ, FSHR, LHR, StAR, CYP11A, and 17βHSD, were found to be significantly up or down regulated (Rhee et al., 2011; Villeneuve et al., 2007; Walker and Gore, 2011). Due to their short reproductive cycle and facile material, zebrafish are considered appropriate models for testing EDCs, and have been used previously for investigating the effects of EDCs, such as PFOA and PFOS (Ankley and Johnson, 2004; Liu et al., 2011). However, existing studies on the reproductive toxicity of PFNA are limited and no study has assessed the potential endocrine disrupting effect of PFNA on fish following long-term exposure.

The objectives of this study were to investigate the adverse effects of chronic PFNA exposure on zebrafish. Male and female adult zebrafish were exposed to PFNA at concentrations of 0.01, 0.1 and 1.0 mg/L for 180 days under a flow-through system. PFNA concentrations in gonadal tissue were measured to assess accumulative levels. Toxicological endpoints were tested, including histological alterations of gonads as well as reproductive and developmental success (*i.e.* gonadosomatic index (GSI) of both genders, female egg production, fertilization rate, hatching rate, and abnormality rate of the F1 generation). The VTG content in liver and sex steroid hormones (testosterone (T) and estradiol ($E_2$)) in serum were measured. Finally, transcriptional profiles of a suite of functionally relevant genes associated with the synthesis of sex hormones and the HPGL axis were investigated. Based on previous research on long-chain PFAAs (Liu et al., 2011), significant interference on VTG content, sex steroid hormone levels, and HPGL axis-related gene expressions were expected, and could lead to adverse effects on reproduction. The results obtained in this study will be helpful in clarifying the mechanism of PFNA estrogenic activity and evaluating the potential long-term ecological risks of PFNA on aquatic organisms.

## 2. Materials and methods

### 2.1. Materials

The PFNA was purchased from Sigma Aldrich (CAS number 375-95-1, 97% purity, St. Louis, MO, USA). The PFNA physiochemical data are given in Table S1 (Supplementary material). Solvent-free stock solutions of PFNA were prepared by dissolving crystals in water with stirring. Three stock solutions of 30, 300, and 3000 mg/L were used to span the desired range of target solutions in exposure water.

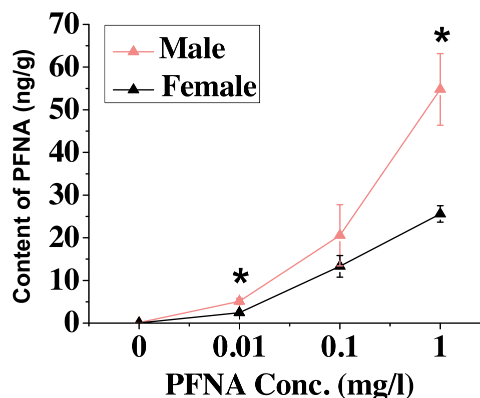



**Fig. 1.** PFNA content in gonads of zebrafish (n = 6 for each group). Error bars indicate standard errors, and * $p < 0.05$ indicates significant differences between sexes.

### 2.2. Animals and treatment

Five-month-old zebrafish ($n$ = 480) (wild-type, *Tuebingen* strain) were separated by sex and randomly assigned to nominal PFNA concentrations of 0 (control), 0.01, 0.1, and 1.0 mg/L (0, 22, 215, and 2150 nM, respectively) for 180 days using a flow-through exposure system (ISO7346-3) with a flow velocity of 30 mL/min. All zebrafish were fed twice a day with live brine shrimp. During the 180 days of exposure, all fish were held under the same photoperiodic conditions of 16-h light: 8-h dark, and water temperature of 25–27 °C (pH 8.1–8.3). Six pairs of male and female fish from the same dose groups were selected randomly to count egg production, fertilization rate, hatching rate, and abnormality rate every week. After exposure, all fish were ice-bath anesthetized for sampling. The body weight and gonad weight of fish were measured. Gonads were surgically removed after blood was taken from the tail fin using a glass capillary; a portion was accurately weighed to analyze PFNA accumulation in the gonad and the remainder was immediately frozen in liquid nitrogen and stored at −80 °C for RNA extraction. The GSI was calculated according to the formula (organosomatic index = organ weight × 100/body weight) (Bharti and Banerjee, 2013).

### 2.3. PFNA accumulation in gonads

Concentrations of PFNA in gonad samples from males ($n$ = 12) and females ($n$ = 6) of each group were quantified using high-performance liquid chromatography-tandem mass spectrometry (HPLC–MS/MS) following the process described in our previous study (Zhang et al., 2012). Briefly, gonads were extracted with 5 mL of acetonitrile (ACN) in a 15 mL polypropylene (PP) tube. All tubes were then placed on a mechanical shaker for 20 min followed by centrifugation at 3000*g* for 10 min. The top layers, which contained PFNA (analytes and internal standards), were transferred into new PP tubes. The extraction procedure was repeated and a final solution of 10 mL of acetonitrile was combined and concentrated to 0.5 mL under nitrogen gas at 40 °C. After the addition of 0.5 mL of MeOH, the final solution was diluted into 10 mL Milli-Q water for SPE cleanup. All samples were then extracted using Oasis WAX cartridges (Oasis1HLB; 150 mg, 6 cc; Waters, USA). The cartridges were pre-equilibrated by the addition of a sequence of 4 mL of 0.1% $NH_4OH$ in MeOH, 4 mL of MeOH, and 4 mL of water at a rate of 1 drop per second. Samples (11 mL) were then passed through the cartridges at a rate of 1 drop per second. After loading all samples, cartridges were rinsed with 5 mL of Milli-Q water and then washed with 4 mL of 25 mM acetate buffer solution (pH 4). Any water remaining in the cartridges was removed by centrifugation

Download English Version:

https://daneshyari.com/en/article/4528856

Download Persian Version:

https://daneshyari.com/article/4528856

Daneshyari.com