Ecotoxicology and Environmental Safety 132 (2016) 339–344

Contents lists available at ScienceDirect

Ecotoxicology and Environmental Safety

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

Uptake, depuration and bioconcentration of bisphenol AF (BPAF) in whole-body and tissues of zebrafish (*Danio rerio*)

Jiachen Shi, Yunjia Yang, Jing Zhang, Yixing Feng, Bing Shao *

Beijing Key Laboratory of Diagnostic and Traceability Technologies for Food Poisoning, Beijing Center for Disease Control and Prevention, Beijing 100013, China

ARTICLE INFO

Article history:
Received 17 March 2016
Received in revised form
25 May 2016
Accepted 26 May 2016
Available online 27 June 2016

Keywords:
Bisphenol AF
Bioconcentration
Uptake
Depuration
Zebrafish

ABSTRACT

Bisphenol AF (BPAF) is an analog of Bisphenol A (BPA) and is widely used as a raw material in the plastics industry. However, an understanding of the potential risks posed by BPAF in the aquatic environment is lacking. The bioconcentration factor (BCF) is a measure used to assess the secondary poisoning potential as well as risks to human health. In this work we measured the accumulation and elimination of BPAF in the whole-body and in liver, muscle and gonad tissues of zebrafish. BPAF uptake was relatively rapid with equilibrium concentrations reached after 24–72 h of exposure. We observed gender differences both in whole-body and in tissue accumulation. Muscle was the primary BPAF storage tissue during the uptake phase in this study. In the elimination phase, BPAF concentrations declined rapidly during depuration, especially during the initial 2 h, and the rate of elimination in males was faster than females from the whole-body and from tissues. The appearance of BPAF glucuronide (BPAF-G) at the start of the uptake phase indicated the rapid biotransformation of BPAF to BPAF-G *in vivo*. The high lipid content of female gonad could act to delay the diffusion of the xenobiotic within the body in a contaminated environment, but it also acts to delay xenobiotic elimination from the body.

1. Introduction

Bisphenol AF (BPAF) is a fluorinated derivative of bisphenol A (BPA) and is used widely as both a crosslinking agent and as a monomer in the plastics industry (Feng et al., 2012). It is used in the manufacture of fluoroelastomers such as gaskets and hoses in food processing equipment and with polycarbonate copolymers used in high temperature composites, electronic materials and other plastics in contact with the environment.

With the widely used of BPAF in industrial, the occurrences of BPAF in the environmental matrices have raised the concerns of researchers. About 46% of sewage sludge samples from United States were detected with BPAF in the survey of U.S. Environmental Protection Agency (EPA) and the sediments from industrialized areas in Korea were also detected with BPAF (Liao et al., 2012; Yu et al., 2015). In China, the concentrations of BPAF in sediments and soils near ecosystems were also up to several micrograms per kilogram and in river water in the micrograms per liter range (Song et al., 2012; Yang et al., 2014b). Hydrophobicity at the methylene bridge of BPA derivatives is an important factor for their estrogenic activity. Hydrophobic substituents in place of the 1-methyl group of the propane moiety, as seen in BPAF, increase its hormonal activity (Kitamura et al., 2005). The binding affinity of BPAF was approximately 20 times stronger and 48 times stronger than that of BPA as a ligand for ERα and ERβ, respectively (Matsushima et al., 2010).

In addition to the *in vitro* toxicity of BPAF, there are numerous reports of its potential ecotoxicity. In studies with zebrafish, embryo-larvae treated with 2.0 mg/L of BPAF showed 100% mortality after 120 h post-fertilization (Song et al., 2014). A 28-day exposure to 2-month-old zebrafish with 1 mg/L BPAF caused liver damage and disrupted sex hormone levels as well as vitellogenin expression in males (Yang et al., 2016). We recently reported that BPAF exposure to zebrafish decreased their fertilization success and the survival rates of offspring (Shi et al., 2015). However, an understanding of the potential risks posed by BPAF in the aquatic environment is lacking.

The bioconcentration factor (BCF) is the ratio of the concentration of a substance in an organism to the concentration of the substance in the surrounding water. BCF can be a measure for considering secondary poisoning potential and assessing risks to human health (Regoli et al., 2012). The BCF of BPA was reported to be as high as 144 in the freshwater clam *Pisidium amnicum* and even higher levels (2800 – 13,000) have been found in algae

Abbreviations: BPAF, Bisphenol AF; BPA, Bisphenol A; BCF, Bioconcentration factor; BPAF-G, BPAF glucuronide; OECD, Organization for Economic Cooperation and Development; PFOA, Perfluorooctanoic Acid

* Corresponding author.
E-mail address: shaobingch@sina.com (B. Shao).

http://dx.doi.org/10.1016/j.ecoenv.2016.05.025
0147-6513/

(Heinonen et al., 2002; Yang et al., 2014a). Lee et al. (2015) detected low amounts of BPA from wild freshwater fish with concentrations varying from non-detectable to 25.2 μg/kg and BCF values ranging from 1 to 274. However, to our far knowledge, information concerning the bioconcentration of BPA and its analogs are limited and there is a complete lack of information on the bioconcentration of BPAF in fish.

Zebrafish (*Danio rerio*) is a commonly used model for the analysis of sublethal effects of toxicants in vertebrates (Segner, 2009; Tokarz et al., 2013). In this study we measured BPAF bioconcentration, tissue uptake, distribution and elimination in zebrafish exposed to different concentrations of the chemical. Since our previous research indicated that BPAF glucuronide (BPAF-G) was a major metabolite, we also measured BPAF-G in this study (Li et al., 2013).

2. Materials and methods

2.1. Chemicals

BPAF (CAS No. 1478-61-1, 99% purity) was purchased from Sigma-Aldrich (St. Louis, MO, USA) and dissolved in ethanol (Sigma-Aldrich, St. Louis, MO, USA, ≥ 99.8% purity) to obtain a concentration of 10 mg/mL. Deuterated BPAF (98 atom D) was purchased from CDN Isotopes Inc. Quebec, Canada. BPAF-G was isolated and purified from the urine of Sprague-Dawley rats by semi-preparative HPLC as described in a previous report (Li et al., 2013).

2.2. Instrumentation and analytical procedures

Analyte identification and quantitation were performed using an Acquity ultra performance liquid chromatography system (UPLC) coupled to a Xevo TQ-S triple quadrupole mass spectrometer (Waters, Milford, MA, USA). UPLC separation was conducted on an Acquity BEH C18 column (2.1 mm × 100 mm; 1.7 μm; Waters). The mobile phases were LC-MS grade methanol and water. The flow rate was set at 0.4 mL/min, and the injection volume was 5 μL. The initial gradient conditions were 40% methanol for 1 min, followed by a linear increase to 80% methanol over 5 min. Methanol was increased to 100% at 6.1 min, held for 2.0 min, and finally returned to the initial state to equilibrate for 2 min before the next injection. MS/MS acquisition was operated in negative-ion mode with multiple reaction monitoring (MRM). The capillary voltage was 2.9 kV. The source temperature and desolvation temperatures were 150 °C and 400 °C, respectively. Nitrogen gas (purity 99%) was used as the cone and desolvation gas at flow rates of 150 L/h and 1000 L/h, respectively. For each analyte, two transitions were selected for identification and the corresponding cone voltage and collision energy were optimized for maximum intensity. This data is shown in Table S1 (Supplementary Information). To ensure that the samples could be accurately analyzed, an isotopic internal standard curve using deuterated BPAF at 0.05, 0.1, 0.2, 0.5, 1.0, 2.0 and 5.0 ng/mL were prepared for the determination of BPAF. The standard addition method was applied for the analyzing of BPAF-G because an isotopic version was not commercially available.

2.3. Zebrafish maintenance

Zebrafish (*Danio rerio*, AB strain) were obtained from the Department of Biological Sciences and Biotechnology, Tsinghua University (Beijing, China). The fish were cultured in a flow-through system (Esen, Beijing, China) with conditioned water at 27 ± 1 °C with a photoperiod of 14:10 h light/dark cycles. To generate conditioned water, 75 g $NaHCO_3$, 18 g sea salt and 8.4 g $CaSO_4$ were added to 1000 L reverse osmosis-generated water. 10–20% of the total fish water volume was changed with conditioned water on a daily basis. All the fish were fed with freshly hatched *Artemia nauplii* (Fengnian Aquaculture Co., Ltd. Tianjin, China) twice and flake food (Tetra, Germany) once daily. In order to ensure high water quality, food remains and debris were removed daily.

2.4. Experimental overview

Zebrafish were grown until 6 months old and divided into groups based on sex and kept in separate tanks for use in two different formal experimental studies. The experiments were totally conducted under the guidance of the Organization for Economic Cooperation and Development (OECD) test number 305 (http://www.oecd-ilibrary.org/environment/test-no-305-bioaccumulation-in-fish-aqueous-and-dietary-exposure_9789264185296-en). The procedures were performed in compliance with the guidelines of Institutional Animal and Care and Use Committees (IACUC) of Beijing Center for Disease Control and Prevention, Beijing of China.

In the first experiment, male and female zebrafish were exposed separately to five different exposure concentrations of BPAF for studies of bioconcentration and the metabolite BPAF-G levels in whole-body fish. In the second experiment, males and females were exposed to a single concentration of BPAF in aquariums to study uptake, distribution and elimination. However, before the formal experiments, preliminary experiments were conducted to investigate the water exchange rate and the duration of the uptake phase.

To determine the optimal water exchange rate, approximately 30 fish were distributed into one tank containing 10 L exposure solution with 20 μg/L BPAF. According to the guidance of OECD 305 (Paragraph 51), the 20 μg/L was selected as the highest concentration in this study based on the 144 h half-lethal-concentration (LC_{50}) of BPAF for zebrafish larvae for long-term exposure (Song et al., 2014). The water sample of each tank was collected and measured every 12 h for 48 h without any renewal. To investigate of the duration of the uptake phase, 16 male and 16 female fish were distributed into one tank containing 10 L exposure solution with 20 μg/L BPAF. Every seven days, 4 male and 4 female fish were sampled from the tank and plunged into ice-water for euthanasia and then slightly dried on absorbent paper and weighed (wet weight). The whole-body fish were suspended in 1 mL acetonitrile and homogenized at a vertical velocity of 6 m/s for 40 s or longer using a FastPrep-24 Tissue and Cell Homogenizer (MP Biomedicals, Santa Ana, California, USA) (Bellamy et al., 2014). This procedure resulted in complete homogenization of the whole-body fish. After sonication for 10 min and centrifugation at 12,000 × *g* for 10 min, the supernatants were collected and the analytes measured. The exposure experiment was run 28 days and the fish were sampled four times.

In the first formal experiment, male and female zebrafish were exposed separately to different BPAF concentrations for 168 h in 10 L aquariums. Control fish were kept in clean water. The aquariums used were one piece glass tanks with a capacity of 15 L (18 cm × 30 cm × 30 cm). Whole-body BPAF concentrations were then determined from six males and six females from each exposure group. BPAF concentrations of 1, 2, 5, 10 and 20 μg/L used in this study were based on the guidance of OECD 305 (Paragraph 51). The 1 μg/L as the lowest concentration in this study was based on the determined concentrations in aquatic systems (ranged from ND (not detected) to 15 μg/L with a median value of 3 μg/L) (Song et al., 2012). The water was renewed every 12 h and the exposure time was prolonged to 168 h based on our preliminary studies.

In the second formal experiment, males and females were exposed to a single concentration of 20 μg/L of BPAF in aquariums to