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Responses of antioxidant defense system to polyfluorinated dibenzo-*p*-dioxins (PFDDs) exposure in liver of freshwater fish *Carassius auratus*

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ABSTRACT

In this study, we evaluated the toxicity of ten polyfluorinated dibenzo-*p*-dioxins (PFDDs) congeners to freshwater fish *Carassius auratus*, by determining the antioxidative responses and lipid peroxidation in the liver after the fish were injected with two different concentrations (10 and 100 μmol/kg) of individual PFDDs for 3 and 14 days. The results showed that oxidative stress was obviously induced in some PFDDs-treated groups, as implied by the significantly inhibited antioxidants levels (superoxide dismutase, catalase, reduced glutathione, and glutathione S-transferase) and elevated malondialdehyde content. In addition, the oxidative stress inducing ability was variable for different PFDDs congeners, which was related with the substitution number and position of fluorine atom. Based on the calculated integrated biomarker response (IBR) values, the toxicity was ranked as 2,3,7,8-FDD > Octa-FDD > 1,2,3,4,7-FDD > 1,3,6,8-FDD > 1,2,3,4,6,7-FDD > 1,2,6,7-FDD > 1,2,7-FDD > DD > 2,7-FDD > 2-FDD. This study can enhance the general understanding of the PFDDs induced oxidative stress in aquatic organisms.

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1. Introduction

Polyfluorinated dibenzo-*p*-dioxins (PFDDs) are a group of fluorinated aromatic compounds structurally similar to polychlorinated dibenzo-*p*-dioxins (PCDDs) and polybrominated dibenzo-*p*-dioxin (PBDDs). There are 75 congeners of PFDDs, depending on the substitution number and position of fluorine atom in the aromatic ring. PFDDs are usually found to co-occur with polyfluorinated dibenzofurans (PFDFs), and they are mainly released into the environment thorough human activities like pesticide production, metallurgical industry and waste combustion. Sakai et al. (1995) showed that total concentrations of PFDDs reach 1.0–2.1 ng/m^3 in the combustion gas when wastes containing organofluorine compounds were incinerated oxidatively in a laboratory-scale atomizing combustion plant. Weber and Hagenmaier (1997) assessed the occurrence of fluorinated dioxins in several industrial chemicals (fluorophenols, fluorobenzenes and chlorofluorophenols). They reported that the PFDD/PFDF concentration in the fluorophenols was between 0.45 and 120 ug/kg, and these substances were eventually integrated into downstream products or ended up in waste waters.

Compared with the mostly investigated polychlorinated and polybrominated congeners, few toxicological studies have been conducted on the PFDDs. Weber et al. (1995) synthesized all 75 PFDDs congeners and carried out a preliminary toxicological evaluation, showing that the elimination of 2,3,7,8-Tetra-FDD from mice was dramatically accelerated as compared to 2,3,7,8-Tetra-CDD. The author also observed that 2,3,7,8-Tetra-FDD could induce CYP4501Al-catalyzed EROD activity in a primary culture rat hepatocytes. Conrad et al. (1996) compared the tissue distribution of three PFDDs congeners (2,3,7,8-Tetra-FDD, 1,2,3,4,7,8-Hexa-FDD and 1,2,4,6,7,9-Hexa-FDD) in rats after a single intravenous application. Herzke et al. (2002) evaluated the kinetics and organ distributions of some PFDDs and PFDFs in Wistar rats after intravenous application, suggesting that the toxicity of all the PFDDs/PFDFs studied were much lower than those of the corresponding polychlorinated or polybrominated congeners. However, they pointed out that a massive exposure to huge and multiple doses of PFDDs/PFDFs might induce some effects in humans.

Many xenobiotics may exert potentially adverse effects and induce oxidative stress in organisms due to the generation of reactive oxygen species (ROS) (Tocher et al., 2002; Van der Oost et al., 2003; Almroth et al., 2008; Lushchak, 2011). Under normal conditions, ROS can be cleared by the antioxidant defense system that consists of antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT) and glutathione S-transferase (GST), and low-molecular-weight non-enzymatic antioxidants such as reduced glutathione (GSH) (Tocher et al., 2002; Song et al., 2006). If

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the generation of ROS overwhelms the antioxidant capacity, an imbalance between the ROS production and removal can produce oxidative stress, causing damages in DNA, proteins, lipids and a decrease in antioxidant protection (Mates, 2000). In addition to changes in antioxidants, lipid peroxidation (LPO), as indicated by the accumulation of malondialdehyde (MDA), is a central feature and ultimate signaling of oxidative damage (Liu et al., 2007; Modesto and Martinez, 2010). These oxidation-related biomarkers, including both enzymatic and molecular parameters, are frequently used to evaluate the effects of pollutants on native populations of fish (Palace et al., 1996; Van der Oost et al., 2003).

Bio-toxicological data are important for the environmental risk assessment of organic pollutants. Since PFDDs are present at trace levels in the environment, conventional ecotoxicological methods are insufficient to assess their impact on aquatic organisms. Enzyme bioassays provide an effective way of overcoming this problem. In this work, enzyme activities (SOD, CAT, GST), GSH level and MDA content were measured to evaluate PFDDs induced oxidative stress status in the liver of goldfish *Carassius auratus*, as liver is the most important site for biochemical processes associated with detoxification. The integrated biomarker response (IBR) index was applied for a general comparison of the potential toxicity of these PFDDs. It has been reported that the thermodynamic properties of PFDDs correlate strongly with the degree and relative position of fluorine substitutions (Yang et al., 2010). Thus, we also explored how the substitution number and position of fluorine atoms affect their oxidative stress inducing ability. The present study can enhance the general understanding of the PFDDs induced oxidative stress in aquatic organisms.

2. Materials and methods

2.1. Chemicals

2.1.1. Synthesis of PFDDs

Fig. 1 shows the structures of the parent compound dibenzo-*p*-dioxin (DD) and nine PFDDs congeners used in the study. Because the PFDDs are commercially unavailable, they were synthesized in our laboratory through the condensation reaction of the fluorinated 2-fluorophenol at 220 °C under alkaline conditions in protection of nitrogen. Potassium tert-butoxide and sulfolane was used as the alkaline agent and the solvent in the reaction, respectively. These PFDDs were all purified by column chromatography and the purities were all > 98%. The structures of the PFDDs were identified by GC/MS and nuclear magnetic resonance (^{1}H NMR). The MS spectra were presented in Fig. S1 of the Supplementary data for reference. The PFDDs were dissolved in corn oils to obtain the experimental concentrations.

2.1.2. Other reagents

DD was purchased from Wellington Laboratories (Canada). The kits for biochemical assays were acquired from Nanjing Jiancheng Bioengineering Institute (Nanjing, China).

2.2. Experimental animals

The freshwater goldfish *C. auratus* (body weight: 20.75 ± 3.25 g), commonly found in China and widely used in aquatic toxicology, was chosen as the test organism. They were purchased from a local aquatic breeding base in Nanjing Confucius Temple (Nanjing, China) and acclimated for 10 days in a 150 L

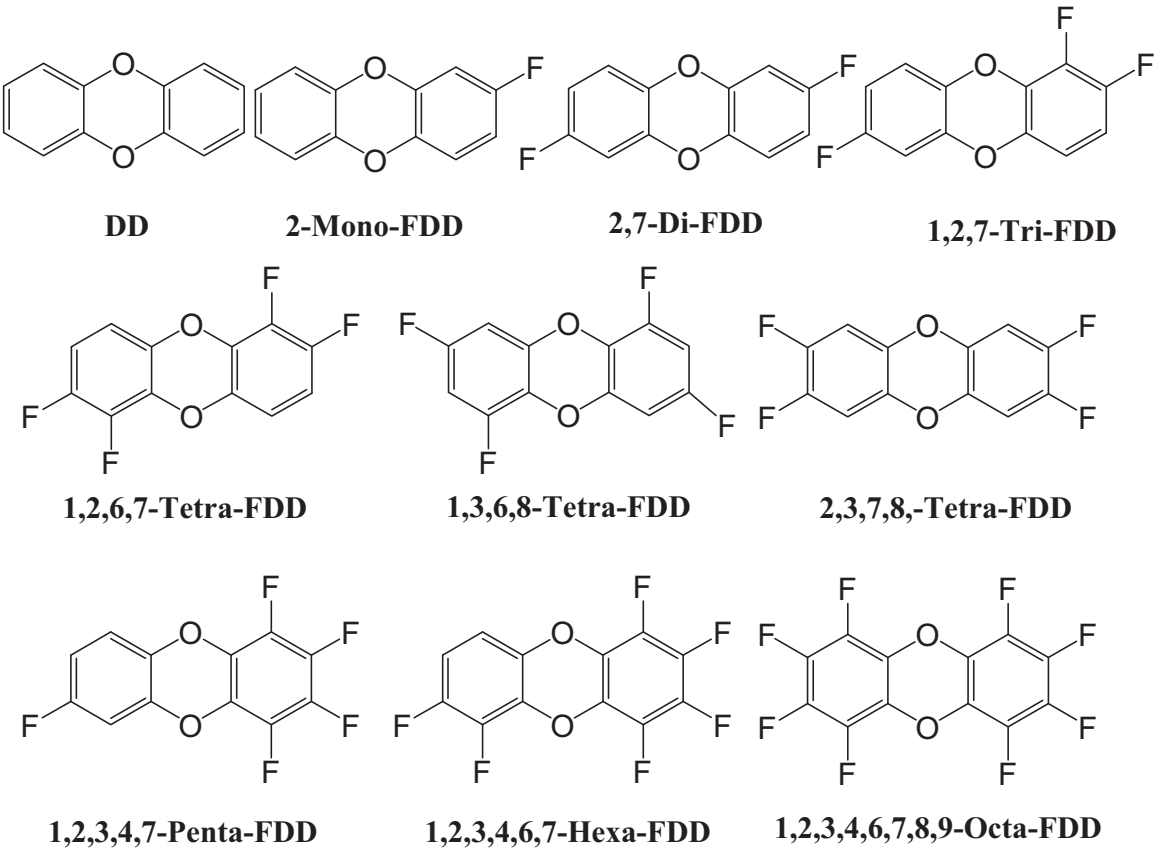

Fig. 1. The structures of the DD and nine congeners of PFDDs.

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