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6:2 Chlorinated polyfluorinated ether sulfonate, a PFOS alternative, induces embryotoxicity and disrupts cardiac development in zebrafish embryos

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ABSTRACT

As an alternative to perfluorooctanesulfonate (PFOS), 6:2 chlorinated polyfluorinated ether sulfonate (commercial name: F-53B) has been used as a mist suppressant in Chinese electroplating industries for over 30 years. It has been found in the environment and fish, and one acute assay indicated F–53B was moderately toxic. However, the toxicological information on this compound was incomplete and insufficient for assessment of their environment impact. The object of this study was to examine the developmental toxicity of F–53B using zebrafish embryos. Zebrafish embryos were incubated in 6-well plates with various concentrations of F–53B (1.5, 3, 6, and 12 mg/L) from 6 to 132 h post fertilization (hpf). Results showed that F–53B exposure induced developmental toxicity, including delayed hatching, increased occurrence of malformations, and reduced survival. Malformations, including pericardial and yolk sac edemas, abnormal spines, bent tails, and uninflated swim bladders, appeared at 84 hpf, and increased with time course and dose. A decrease in survival percentages was noted in the 6 and 12 mg/L F-53B-treated groups at 132 hpf. Continuous exposure to 3 mg/L F–53B resulted in high accumulation levels in zebrafish embryos, suggesting an inability for embryos to eliminate this compound and a high cumulative risk to fish. We also examined the cardiac function of embryos at specific developmental stages following exposure to different concentrations, and found that F–53B induced cardiac toxicity and reduced heart rate. Even under low F–53B concentration, o-dianisidine staining results showed significant decrease of relative erythrocyte number at 72 hpf before the appearance of observed effects of F–53B on the heart. To elucidate the underlying molecular changes, genes involved in normal cardiac development were analyzed using real-time qPCR in the whole-body of zebrafish embryos. F–53B inhibited the mRNA expression of *β-catenin* (*ctnnb2*) and *wnt3a*. The mRNA levels of β-catenin targeted genes (*nkx2.5* and *sox9b*), which play critical roles in cardiogenesis, were also reduced after exposure. Thus, exposure to F–53B impaired the development of zebrafish embryos and disrupted cardiac development, which might be mediated by effects on the Wnt signaling pathway and decrease of erythrocyte numbers.

1. Introduction

As persistent contaminants of anthropogenic origin, perfluorooctane sulfonate acid and its salts (PFOS, $C_8F_{17}SO_3^-$) are regulated under the Stockholm Convention (Fujii et al., 2007; UNEP, 2009). Given the regulatory pressure to reduce the production of PFOS and its importance in modern society, the demand for fluorinated alternatives has increased in recent years. To date, fluorinated alternatives are short-chain homologues with four or six atoms or functionalized perfluoropolyethers (PFPEs). The chemical structure of PFPEs is similar to that of per- and polyfluoroalkyl substances (PFASs), with ether linkage(s) between perfluoroalkyl chains or fluorine atoms replaced by chlorine and hydrogen (Wang et al., 2013a, 2013b, 2015). While some alternative substances, such as 6:2 fluorotelomer sulfonic acid salts (6:2 FTSA, $C_6F_{13}C_2H_4SO_3H$), are being tested at trial phase (UNEP, 2012), some perfluoroether sulfonic acids (PFESAs) have a relatively longer production history. One important example is chlorinated polyfluorinated ether sulfonate (commercial name F-53B, Cl-$C_6F_{12}OCF_2CF_2SO_3K$, CAS No. 73606-19-6), which has been widely used in decorative and hard metal plating since the late 1970s (Wang et al., 2013a, 2013b; Wang et al., 2014a, 2014b).

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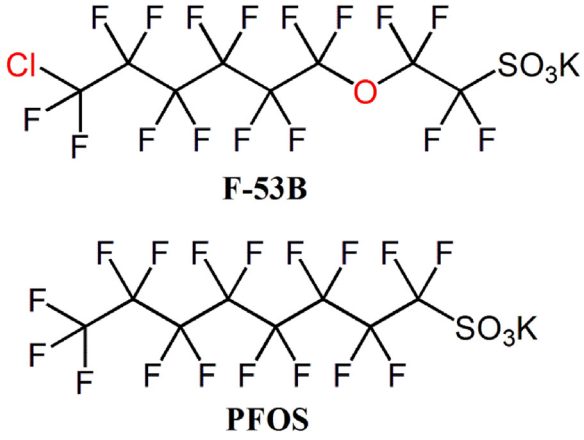

Fig. 1. The molecular structural differences between F–53B and PFOS, red color indicates atoms different from PFOS. (The reader is referred to the web version of this article to view the figure in color.)

Owing to lower production costs, F–53B has quickly dominated the Chinese mist suppressant market (Huang et al., 2010a, 2010b; Ruan et al., 2015; UNEP, 2012). Compared with PFOS, studies on the emissions, environmental occurrence, bioaccumulation, and toxic effects of F–53B are scarce. Wang et al. (2013a, 2013b) first studied the potential impacts of F–53B on the environment. The 96-h LC_{50} value (15.5 mg/L) indicates that F–53B is moderately toxic to zebrafish (*Brachydanio rerio*). Importantly, LC_{50} values within the range of 10–100 mg/L belong to Category III chemicals (harmful to aquatic life) (GSH, 2015). In addition, wastewater treatment has not yet successfully eliminated F-53B, with concentrations from wastewater effluent following treatment still found to be above 40 μg/L (Wang et al., 2013a, 2013b). Furthermore, F–53 B is widely distributed in China, and has been found ubiquitously in water and municipal sewage sludge at concentrations ranging from 2.0 to 44.2 ng/L and 0.02 to 209 ng/L, respectively (Ruan et al., 2015; Lin et al., 2016). F–53B also shows strong bioaccumulation propensity in fish (Shi et al., 2015). A recent study found that the median Log $BAF_{wholebody}$ (body bioaccumulation factors) values for F–53B (4.124–4.322) in low trophic level fish (*Carassius carassius*) exceeded regulatory bioaccumulation criteria, and were even higher than those of PFOS (3.430–3.279) (Shi et al., 2015). In 2009, F–53B production in the Chinese decorative and hard metal plating industry was estimated to be 20–30 tons, and increasing demand for F–53B as a substitute for PFOS in other sectors was foreseeable (Huang et al., 2010a, 2010b). Thus, there is urgent need for environmental hazard assessment of F-53B.

A decade of research has proven that PFOS is harmful to organisms, including to their reproduction, development, and nervous, endocrine, and immune systems (Abbott et al., 2009; Ankley et al., 2004, 2005; Arukwe and Mortensen, 2011; Austin et al., 2003; Chen et al., 2013; Shi et al., 2009; Wan et al., 2012; Wang et al., 2014a, 2014b; Wang et al., 2011; Zushi et al., 2012). Structurally similar chemicals often have similar effects on human and environmental health, and thus it is reasonable to hypothesize that F–53B might create similar biotic toxicity (Fig. 1). Except for an acute toxicity assay that determined the LC_{50} (96 h), no other data demonstrating F–53B toxicity currently exists.

Due to their high fecundity, rapid embryonic development, and optical transparency, zebrafish (*Danio rerio*) are widely used for investigating the developmental toxicity of compounds. To analyze the effects of F–53B on development, zebrafish embryos were exposed to different concentrations of F–53B (0, 1.5, 3, 6 and 12 mg/L) from 6 h post-fertilization (hpf). The percentages of hatching, survival, and malformation, heart rates, and occurrence of erythrocytes were measured to assess F–53B toxicity. To further explore the underlying mechanisms of F–53B exposure-induced toxicity, we also analyzed the expression of cardiac-related genes. Data from our study will help clarify the toxicity of F–53B and its ecological risks on aquatic organisms.

2. Materials and methods

2.1. Zebrafish maintenance and embryo collection

Adult wild-type (Tüebingen strain) zebrafish were housed in automatic flow-through feeding aquariums (ESEN, EnvironScience, China) in an environmentally controlled room (28.5 °C, 14 h/10 h light/dark cycle). The parameters of fish water were 3.5 g/L NaCl, 0.05 g/L KCl, 0.1 g/L $CaCl_2$ and 0.025 g/L $NaHCO_3$, pH: 7.2–8.0, and conductivity 500–800 μS. Adult fish were fed with live brine shrimp twice a day. Zebrafish embryos were obtained by natural breeding of adults at a sex ratio of 1:1 in breeding tanks, with spawning occurring after the light was turned on the next morning. Embryos were collected from different spawning boxes and washed three times with fish water. An optical microscope (LEICA DFC290, Germany) was used to examine embryo stages, and healthy embryos that had developed normally and reached the blastula stage were selected for subsequent experiments. All embryos were raised at similar densities. In all experiments, the solution was renewed daily with fresh test medium, and dead embryos were removed from the 6-well plates every 12 h.

2.2. Chemicals and reagents

The F–53B ($C_8ClF_{16}O_4SK$; CAS #73606-19-6, purity >96%) was provided by Dr. Guo Yong from the Shanghai Institute of Organic Chemistry, Chinese Academy of Sciences, and was dissolved in 100% dimethyl sulfoxide (DMSO). The stock solution was 64 mg/mL, and the working solution was obtained by serial dilution with fish water. The final DMSO concentration in the control and each exposure group was 0.1% (v/v).

2.3. Experiment design

According to previous PFOS studies, zebrafish embryos were exposed to 0, 1, 2, 4, 8, 16, 32 and 64 mg/L F–53B from 6 to 96 h post-fertilization (hpf) to determine the value of LC_{50}. Based on the value of LC_{50}, five concentrations of F–53B (0, 2, 4, 8, and 16 mg/L) that induced clear phenotypic effects during early zebrafish development were chosen for the further experiments.

Thirty normal 6 hpf embryos were randomly distributed in each well of a 6-well plate containing 5 mL of different concentrations of F–53B (0, 2, 4, 8, 16 mg/L). All test concentrations and the control were replicated three times, and the exposure solution was renewed daily. The developmental stage of zebrafish embryos was monitored via optical microscope (LEICA DFC290, Germany) at specific time points. Acute endpoints, such as hatching, survival, occurrence of malformations, and heart rate, were used for assessing the developmental toxic effects of F-53B. The embryos were identified as dead when coagulation of embryos, failure to develop somites, lack of heartbeat, or non-detachment of the tail from the yolk sac were observed. Abnormal morphological structures, including heart, head, eye, muscle, tail, and swim bladder, were recognized as malformations. Hatching percentages were recorded at 48, 60, and 72 hpf. We recorded the heart beat for 10 s, using 30 embryos per treatment group at 72 hpf. The mortality and malformation results were recorded every 12 h.