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Multiple approaches to assess the effects of F-53B, a Chinese PFOS alternative, on thyroid endocrine disruption at environmentally relevant concentrations

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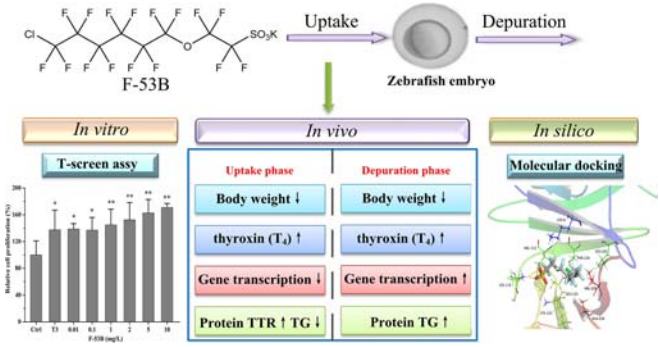
HIGHLIGHTS

- Thyroid endocrine disruption of F-53B was investigated combining *in vitro*, *in vivo* and *in silico* approaches.
- The levels of thyroxine (T_4) were significantly increased following F-53B exposure and depuration.
- Gene transcription modulation in the HPT axis was examined.

GRAPHICAL ABSTRACT



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ABSTRACT

A Chinese perfluorooctane sulfonate (PFOS) substitute frequently detected in the environment, 6:2 chlorinated polyfluorinated ether sulfonate (F-53B), has a similar structure to PFOS and it is proposed to cause thyroid dysfunction. To further confirm this hypothesis, the effects of F-53B on the thyroid endocrine system and underlying mechanisms were investigated *in vitro* and *in vivo* using rat pituitary GH3 cells and developing zebrafish, respectively. In GH3 cells, F-53B enhanced cell proliferation in a dose-dependent manner, indicative of thyroid receptor agonistic activity. In zebrafish larvae, F-53B exposure induced significant developmental inhibition and increased thyroxine (T_4) but not 3,5,3′-triiodothyronine (T_3) levels accompanied by a decrease in thyroglobulin (TG) protein and transcript levels of most genes involved in the hypothalamic-pituitary-thyroid (HPT) axis. Interestingly, T_4 levels remained significantly increased while TG protein and gene transcription levels were markedly upregulated after depuration. Molecular docking studies revealed that F-53B binds to transthyretin (TTR) by forming hydrogen bonds with Lys123 and Lys115, thereby interfering with thyroid hormone homeostasis. Our collective *in vitro*, *in vivo* and *in silico* studies provide novel evidence that F-53B disrupts the thyroid endocrine system at

Abbreviations: F-53B, 6:2 chlorinated polyfluorinated ether sulfonate; PFOS, perfluorooctane sulfonate; HPT axis, hypothalamic-pituitary-thyroid axis; TRs, thyroid receptors; THs, thyroid hormones; *CRH*, corticotropin-releasing hormone; *TSH*, thyroid-stimulating hormone; *DIO*, deiodinase; *TTR*, transthyretin; *NIS*, sodium/iodide symporter; *NKX2.1* (*TTF1*), thyroid transcription factor-1; *PAX8*, paired box-8; *TG*, thyroglobulin; *TPO*, thyroid peroxidase.

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environmentally relevant concentrations, which cannot be recovered after depuration. Given the persistence of F-53B in the environment, the long-term consequences of thyroid hormone disruption by this chemical warrant further investigation.

1. Introduction

In the electroplating industry, especially "hard chrome plating", perfluorooctane sulfonate (PFOS) is commonly used as an effective mist suppressant. However, due to stringent industrial regulations, a number of non-PFOS mist suppressants are beginning to appear in the market. In China, 6:2 chlorinated polyfluorinated ether sulfonate (6:2 Cl-PFAES, trade name F-53B) is widely accepted for use in the electroplating industry as a substitute for PFOS. A report by the China Metal Plating Association in 2009 revealed annual usage of 30–40 tons of mist suppressant (Lim et al., 2011). The lack of specific restrictions on emissions and disposal methods of F-53B lead to its eventual entry into the aquatic environment. Recently, F-53B was identified in surface water, wastewater and municipal sewage sludge at concentrations ranging from 2.0 to 44.2 ng/L (Lin et al., 2016), 43 to 112 μg/L (Wang et al., 2013) and 0.02 to 209 ng/L (Ruan et al., 2015), respectively. The reported contamination levels of F-53B were even higher than those of PFOS (Wang et al., 2013) and its removal by conventional wastewater treatment plants was generally below 20% (Gao et al., 2017).

Due to its similar structure to PFOS and highly lipophilicity (log *K*ow = 5.24) (Table 1), F-53B has strong bioaccumulation potential. The median log $BAF_{wholebody}$ (body bioaccumulation factors) values of F-53B in crucian carp (*Carassius carassius*) range from 4.124 to 4.322, which are higher than those of PFOS (3.430–3.279) (Shi et al., 2015). Recent studies have reported accumulation of F-53B in Greenland marine mammals (Gebbink et al., 2016) and even human serum (Pan et al., 2016). Given that F-53B is frequently detected in the environment and has high bioaccumulation capacity, sufficient attention must be paid to its potential health impacts on aquatic organisms.

Although F-53B has been used for over 30 years in the electroplating industry, knowledge about its potential toxic effects is limited. Wang et al. (2013) were the first group to report the acute toxicity of F-53B to zebrafish (LC_{50}-96 h value, 15.5 mg/L). A recent study revealed that F-53B causes developmental toxicity and disrupts cardiac development in zebrafish larvae (Shi et al., 2017). However, the mechanisms underlying these changes are yet to be established. Since thyroid hormones (TH) play a crucial role in the normal development of teleost fish, the observed adverse effects are possibly attributable to disruption of thyroid hormone homeostasis.

Our preliminary results indicate that F-53B enhances GH3 cell proliferation, confirming thyroid hormone receptors (TRs) agonistic activity. PFOS has been shown to alter TH and associated gene expression levels in zebrafish (Shi et al., 2009) and rat (Yu et al., 2009a). Based on the initial findings and structural similarities of F-53B and PFOS, the hypothesis that F-53B is a potential thyroid-disrupting chemical is plausible.

In this study, we employed rat pituitary GH3 cells and zebrafish larvae to investigate the thyroid disrupting potential of F-53B at environmentally relevant concentrations. The activity of F-53B towards TRs was evaluated in GH3 cells. In view of the unique susceptibility of developing animals to exogenous compounds and crucial roles of TH in the early stages of fish development, zebrafish larvae were employed as an *in vivo* model to examine developmental toxicity, thyroid hormone content, expression of thyroglobulin (TG) and transthyretin (TTR) proteins and transcript levels of the target genes involved in the HPT axis. Furthermore, the interaction patterns between F-53B and TTR were predicted *via* molecular docking analysis. Our results should aid in clarifying the effects of F-53B on the thyroid endocrine system that potentially lead to long-term consequences for animal and human health.

2. Materials and methods

2.1. Chemicals

F-53B (≥99% purity, CAS #73606-19-6) was obtained from Shanghai Maikun Chemical Co., Ltd. (Shanghai, China). A stock solution of F-53B was prepared in 100% dimethyl sulfoxide (DMSO) and diluted with culture medium to achieve the desired working solutions prior to the experiment. The final DMSO concentration in the control and exposure groups was 0.001% (v/v). Potassium $_L$-PFOS (≥98% purity, CAS #2795-39-3) as an internal standard was purchased from Aladdin Reagent Company (Shanghai, China). All other chemicals and solvents were of analytical grade.

2.2. CH3 cell culture and T-screen assay

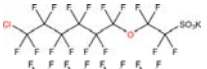
The GH3 cell line was obtained from ATCC and cultured in Dulbecco's Modified Eagle's Medium/Ham's F-12 nutrient mixture supplemented with 10% fetal bovine serum (referred to as growth medium) at 37 °C in a humidified atmosphere with 5% CO_2.

Since GH3 proliferation is affected by thyroid hormone disruptors, this cell line was used for the "T-screen" assay for identifying thyroid hormone disruption compounds, which was conducted following the procedure of Kim et al. (2015) with slight modifications. Briefly, at 24 h prior to exposure, growth medium was replaced with serum-free medium to prevent cell growth due to the presence of hormones. After 24 h, cells were collected from the culture flask, plated into 96-well plates (5000 cells/well) and treated with 0, 0.01, 0.1, 1, 2, 5 or 10 mg/L F-53B. T_3 was employed as the positive control at a concentration of 1.5 μg/L. Six replicates (n = 6) were used for each treatment and control group. After a 24 h incubation period, cell proliferation was measured with the CCK-8 assay (Yeasen Biotech Company, Shanghai, China) following the manufacturer's protocol (Zou et al., 2014).

2.3. Zebrafish maintenance and experimental design

Adult wild-type zebrafish (*Danio rerio*, AB strain) maintenance and embryo exposure were performed as described previously, with slight modifications (Tu et al., 2016a). In brief, normal embryos (2 h post-

Table 1
The structure of F-53B and PFOS.

Product name	CAS no.	Formula	Molecular weight	Structure
F-53B	73606-19-6	$C_8ClF_{16}O_4SK$	570.67	
PFOS	2795-39-3	$C_8F_{17}O_3SK$	538.22