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Developmental Toxicity and Cardiotoxicity Induced by PFOS and its Novel Alternative OBS in Early Life Stage of Zebrafish (*Danio rerio*)

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Abstract As a novel alternative to perfluorooctane sulfonate (PFOS), sodium *p*-perfluorous nonenoxybenzene sulfonate (OBS) has been widely applied in many industrial fields. However, there is limited information about its adverse effects on aquatic organisms. In this study, the developmental and cardiac toxicity of OBS and PFOS in early life stage of zebrafish (*Danio rerio*) were investigated. Results showed that 96 h-LC_{50} values of OBS and PFOS were 23.81 and 57.59 mg/L, respectively. Exposure to OBS and PFOS could lead to significantly inhibition of the hatching rate and embryo development. OBS and PFOS with concentrations higher than 5 mg/L induced significant malformations, such as pericardial edema and yolk sac edema. Furthermore, both OBS and PFOS exposure decreased the heart rate, stroke volume and cardiac output, indicating that the cardiac function of zebrafish was affected. Exposure to OBS and PFOS also caused oxidative stress in zebrafish embryos, resulting in significant decreases of SOD, CAT and GSH, and significant increase of the MDA content. The oxidative stress may consequently induce the cardiotoxicity by altering the expression of heart development related genes, *nkx2.5*, *tbx5*, *gata4* and *myh6*. In summary, the results revealed that OBS and PFOS exposure could induce the developmental toxicity and cardiotoxicity in early life stage of zebrafish, and OBS might not be a safety alternative to PFOS.

Keywords Developmental toxicity · Cardiotoxicity · Perfluorooctane sulfonate (PFOS) · Sodium p-perfluorous nonenoxybenzene sulfonate (OBS) · Zebrafish

1 Introduction

Due to unique high surface activity, good thermal and chemical stability, and hydro- and lipophobic properties, perfluorooctane sulfonate (PFOS) has wide-ranging industrial and commercial applications, including polishing agents, non-stick products, cleaning products, fire-fighting foams, hydraulic fluids, pesticides and insecticides, for more than 50 years (Brooke et al., 2004; Paul et al., 2009). Consequently, it has been ubiquitously detected in various environmental and biological matrices (Dasu et al., 2022; Gewurtz et al., 2014; Houde et al., 2011; Jarvis et al., 2021; Jian et al., 2017; Podder et al., 2021; Wang et al., 2018; Zhao et al., 2022). For the environmental level of PFOS, its concentrations in industrial wastewaters and receiving rivers could be μg/L to mg/L due to the

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massive use of PFOS (Lin et al., 2009; Rumsby et al., 2009). In case of accidental release, the concentration of PFOS in surface water could also be up to mg/L (Anderson et al., 2016; Moody et al., 2022). Therefore, PFOS has raised great concern and been extensively investigated. Based on plenty of evidence on its persistence, bioaccumulation, long-distance migration and toxicity, PFOS was recognized as a persistent organic pollutant in 2009, and its production and use have been restricted since then (Wang et al., 2009). Thereafter, various short-chain per- and polyfluoroalkyl Substances (PFASs) and other novel fluorinated compounds have been developed to replace PFOS in industrial applications.

Sodium *p*-perfluorous nonenoxybenzene sulfonate (OBS) is one of novel alternatives to PFOS. It has been used as an additive in the fields of film-forming fluoroprotein foams, alcohol-resistant foams and oil production surfactants (Bao et al., 2017; Chen, 1984; Xu et al., 2017). Thus, OBS has been detected in the water environment, wildlife animals and even human being (Hou et al., 2022; Li et al., 2020; Shi et al., 2020; Xu et al., 2017). In 2017, extremely high OBS contamination as 3 200 ng/L was detected in a lake near the first oil well of the Daqing oil field in China (Xu et al., 2017). Recently, the OBS contamination as high as 10 358 ng/L was reported in water collected from a drainage canal near one major fluorochemical manufacturing facility in China, which was 2 ~ 4 orders magnitude higher than those from other sampling sites (Hou et al., 2022). In addition, OBS was also detected in the blood of wild crucian carps, maternal and cord serum of pregnant women with median concentrations of 321, 0.117 and 0.249 ng/mL, respectively (Hou et al., 2022; Li et al., 2020; Shi et al., 2020). With the increasing OBS contamination in water, it is urgent to investigate its adverse effects on aquatic organisms.

To date, there are a few studies on the toxicity of OBS. Recent study reported that OBS has similar toxicity to PFOS, with 96 h LC_{50} values of 25.5 and 28.4 mg/L to adult zebrafish and tadpoles, respectively (Xu et al., 2017). It was found that OBS could affect the expression of genes involved in metabolic pathways at the transcriptional and translational levels in developing zebrafish (Tu et al., 2019). Other study revealed that OBS could not only induce oxidative stress and inflammatory responses, but also affect immune related genes expression (Huang et al., 2021b). In addition, exposure to OBS could cause the disorder of intestinal microbiota and influence the liver metabolic processes in mice and zebrafish (Huang et al., 2022; Wang et al., 2019a, 2020). Despite increasing evidence on the toxicity of OBS, information about adverse effects of OBS on the development and heart of fish is still scarce.

Heart is the first formed functional organ in the embryo development of vertebrate. Early embryonic heart development must undergo a series of complex cellular and molecular processes to form a mature organ (Stainier, 2001). During this process, any abnormality could lead to the malformation of the organ (heart defects), and worse, could result in embryo lethal. There is increasing evidence that exposure to PFASs could lead to abnormal development in organisms, such as cardiac development (Cheng et al., 2013; Huang et al., 2011; Shi et al., 2017b; Zeng et al., 2015). For instance, PFOS exposure affected the expression of cardiac development related genes, and disturbed development and function of heart in the marine medaka (Huang et al., 2011). It was also found that PFOS altered the expression of crucial genes related to normal cardiac development, reduced ATP production, induced reactive oxygen species (ROS), and stimulated apoptosis during the early stages of cardiogenesis (Cheng et al., 2013). F-53B, another PFOS alternative, could affect the embryonic heart rate in zebrafish (Shi et al., 2017b). In addition, prenatal PFOS exposure could cause mitochondria-mediated apoptosis in the hearts of weaned rats (Zeng et al., 2015). These indicate that the PFAS exposure could lead to adverse effects on the cardiac development. However, the potential effects of OBS on the development and heart of fish are not well known.

Zebrafish are an excellent vertebrate model organism, which have been widely used in developmental and toxicological studies (McGrath & Li, 2008; Sipes et al., 2011). Therefore, in the present study, zebrafish were adopted to investigate the developmental toxicity and cardiotoxicity of PFOS and OBS at the early life stage in order to provide more information for the ecological risk assessment of OBS. Their effects on survival, hatching rate, malformation, and cardiac phenotype and function were investigated, and then oxidative stress and the expression of heart development related genes were analyzed to discuss the underlying mechanism.

2 Materials and Methods

2.1 Chemicals and Reagents

OBS (≥97% purity) and PFOS potassium salt (≥98% purity) were purchased from Shanghai Futian Chemical Technology Co., Ltd. (Shanghai, China) and Sigma-Aldrich, respectively. Assay kits for enzyme activity and other biological indicators, including catalase (CAT), superoxide dismutase (SOD), glutathione (GSH), malondialdehyde (MAD) and coomassie blue, were obtained from Nanjing Jiancheng Biological Engineering Institute (Nanjing, China). Trizol reagent, reverse transcriptase kits and SYBR green reagents were purchased from Wuhan Sewell Biological Co., Ltd. (Wuhan, China). Other biochemical reagents were of analytical grade and purchased from Sangon Biotech (Shanghai, China).

2.2 Zebrafish Maintenance and Embryos Collection

Adult wild-type (AB strain) zebrafish (*Danio rerio*) were maintained at 26.5 ± 0.5 °C in a recirculating culture system with a photoperiod of 14 h light: 10 h dark. Adult zebrafish were fed freshly hatched brine shrimp (*Artemia salina*) twice daily. Preparation and collection of zebrafish embryos were performed according to OECD Test No. 236 (OECD, 2013). In the afternoon before experiments, male and female fish with a ratio of 2:1 were placed in separate compartments of a breeding box. After the onset of light on the day of experiments, the baffle was removed to allow males and females to chase freely. After mating and spawning, eggs were collected from the bottom of the breeding box. The eggs were washed with standard dilution water (294.0 mg/L $CaCl_2{\cdot}2H_2O$, 63.0 mg/L $NaHCO_3$, 123.3 mg/L $MgSO_4{\cdot}7H_2O$ and 5.5 mg/L KCl) several times. All the experiments on zebrafish embryos were conducted in accordance with the Guide for the Care and Use of Laboratory Animals of China.

2.3 Embryonic Exposure

OBS and PFOS were firstly dissolved in dimethyl sulfoxide (DMSO) as stock solutions. Working solutions were prepared by diluting the stock solutions with the standard dilution water before the experiment. The final OBS and PFOS solutions concentrations were 1, 5, 10, 20, 40, 60 and 80 mg/L. The final DMSO concentration in treatments and the solvent control was 0.01% (v/v), which showed no significant effects on developmental parameters of embryos in preliminary experiments. As results of the solvent control and the blank control had no statistically significant differences, only results of the blank control were reported and compared with those of treatments.

The zebrafish embryos toxicity test was conducted according to OECD Test No. 236 (OECD, 2013). Twenty zebrafish embryos at ~ 1.5 h post-fertilisation (hpf) were transferred to 24-well plates with 2 mL of exposure solution and one embryo per well, and additional 4 zebrafish embryos were also transferred to the 24-well plate with 2 mL of dilution water per well used as internal plate control. Three parallel plates were conducted for each concentration. Therefore, 60 embryos were tested for each concentration. During embryos toxicity test, embryos were kept at 26.5 ± 0.5 °C under a photoperiod of 14 h light: 10 h dark in an illumination incubator. Exposure solutions were renewed every 24 h. The actual concentrations of OBS and PFOS in the exposure solutions at the beginning of exposure (T_0) and before the renewal at 24 h (T_{24}), were measured following the method of Tu et al. (2019). The results were listed in Table S1. It could be seen that the concentration of OBS decreased slightly, while that of PFOS remained relatively constant after 24 h, which were consistent with the results of previous studies (Huang et al., 2010; Tu et al., 2019; Zou et al., 2021). The data suggested that PFOS and OBS were relatively stable in the exposure solutions. The state of embryonic development was observed daily under a stereomicroscope and dead embryos were removed in time.

As for the cardiac malformation of embryos, another exposure experiment was conducted. Zebrafish embryos were exposed to 1, 5, 10 and 20 mg/L PFOS or OBS in 6-well plates with 5 mL of exposure solutions and 30 embryos per well. Other conditions were the same as those used in the acute toxicity test. After 96 h of exposure, larvae were collected (60 larvae for the oxidative stress assay and 60 larvae for determination of cardiac-related gene expression) and stored at -80 °C for further analysis.

2.4 Morphological and Developmental Assessment

The development of embryos was observed at 24, 48, 72 and 96 hpf under an inverted microscope (Jiangnan Yongxin XD-202, China). Four observations, including coagulation of embryos, lack of somite formation, non-detachment of the tail bud and lack of heartbeat, were used as indicators for the death of embryos. The hatching rate was calculated at 72 and 96 hpf. Sublethal morphological characteristics of embryos were assessed from 24 to 96 hpf by using the general morphology score (GMS) system (Beekhuijzen et al., 2015; Hermsen et al., 2011). Morphological abnormalities, such as spinal curvature, tail distortion, cardiac edema and yolk sac edema, were observed and recorded at 96 hpf. For the endpoints, including the mortality rate, GMS, hatching rate and the malformation rate, a value was calculated from the results of 20 embryos in a 24-well plate. Therefore, 3 data points were obtained on these endpoints for each concentration.

2.5 Cardiac Function Assessment

Zebrafish cardiac function was assessed according to previous studies (Antkiewicz et al., 2005; Bagatto & Burggren, 2006; Li et al., 2019). Briefly, zebrafish was fixed in 3% methylcellulose on a glass depression slide in the lateral position. Then, digital videos on the heart were recorded for 15 s by using a high-speed digital camera mounted on a fluorescence stereomicroscope (MShot, China). Heart rate (beats per minute, bpm), lengths of ventricular long and short axes in both diastole and systole stages, were measured by using the image analysis functions of ImageJ (Fig. S1). Ventricle volumes at systole and diastole stages were calculated according to a prolate spheroid formula described by Bagatto and Burggren (2006):

$$\text{Volume} = 4/3\pi LS^2$$

where L and S represent the length of long axis and short axis, respectively. Stroke volume was calculated as diastolic ventricular volume minus systolic ventricular volume. Cardiac output was calculated by multiplying ventricular stroke volume with the heart rate.

2.6 Antioxidant Systems Analysis

To estimate the oxidative stress induced by the exposure of PFOS and OBS, CAT, SOD, GSH and MAD contents were measured. Briefly, zebrafish larvae (3 replicates) were homogenized in 1:9 (w/v, g/mL) chilled physiological saline solution (0.9% NaCl) and subsequently centrifuged at 3500 rpm for 10 min at 4℃. The supernatants were collected. CAT, SOD, GSH and MAD levels were measured by using commercial kits (Nanjing Jiancheng Biotechnology Institute, China) according to the standard protocols. The protein concentrations were determined by the Coomassie blue dye-binding method.

2.7 Gene Expression Analysis

Total RNA was isolated from 60 larvae (3 replicates) by using the Trizol reagent. The quality and concentration of total RNA were assessed by the OD260/OD280 ratio and the electrophoresis in 1% agarose gels. The cDNA was synthesized by using a Prime Script™ RT Reagent Kit (TaKaRa) following the manufacturer's protocols. The PCR was carried out in a total volume of 20 μL with 1,000 ng total RNA, 4 μL 5×Prime Script™ buffer, 1 μL Oligo dT Primer, 1 μL Random 6 mers and 1 μL Prime Script RT Enzyme Mix I. The reaction was incubated at 37 °C for 15 min, which was followed by a final 5 s denaturation at 85 °C. The RT-PCR was performed in triplicate according to the manufacturer's instructions using the 2×SYBR Green qPCR Master Mix (None ROX, Wuhan Sewell Biological Co., Ltd) on a LightCycler® Real-Time PCR System (Roche Diagnostics, Germany). The amplification was performed in a total volume of 20 μL containing 2 μL of 1:10 diluted original cDNA, 10 μL of 2×SYBR Green qPCR Master Mix (None ROX), 7.2 μL PCR grade water and 0.8 μL (10 mM) of each primer. The amplification protocol was as follows: a holding step at 95 °C for 30 s, followed by 40 cycles of 95 °C for 5 s and 60 °C for 32 s. The relative expression levels of the target genes were calculated followed the $2^{-\Delta\Delta ct}$ method described by Livak and Schmittgen (2001). The *β-actin* gene was selected as the reference gene. The primer sequences of heart development-related genes (*nkx2.5*, *tbx5*, *gata4* and *myh6*) and *β-actin* were summarized in Table 1.

Table 1 Primers used in the real-time quantitative PCR analysis

Gene	Sequence (5′ − 3′)	GeneBank Number
gata4	Forward—CCAGACACACACCAGCTCTACAC	NM_131236.2
	Reverse—ATCAGGCTGTTCCACACTTCACT	
nkx2.5	Forward—TAGTAATTTTCCGAGTCCAGGC	NM_131421.2
	Reverse—GCACGTTATTTAGATCCCCAAC	
tbx5	Forward—CGCTATAAATTCGCCGATAACAA	NM_001313682.1
	Reverse—AGACACCAGTTGCCTCATCCAG	
myh6	Forward—CACCAGCAGACACTGGATG	NM_198823.1
	Reverse—GCTCCAAGTCCATTCTGAC	
β-actin	Forward—AGGTCATCACCATTGGCAAT	NM_131031.1
	Reverse—AGGTCATCACCATTGGCAAT	

2.8 Statistical Analysis

All data are expressed as the mean ± standard deviation (SD). Statistical analyses were performed with SPSS 20.0. Significant differences were tested by one-way analysis of variance followed by Tukey's test. The differences were considered significant at $p < 0.05$ and highly significant at $p < 0.01$.

3 Results and Discussion

3.1 The Lethality of PFOS and OBS

Effects of PFOS and OBS exposure on the mortality rate of zebrafish embryos are shown in Fig. 1. It can be seen that the mortality rates increased with increasing exposure concentrations and prolonged exposure period. Based on the mortality curves, 72 h LC_{50} of OBS and PFOS to zebrafish embryo were calculated to be 25.00 and 62.40 mg/L, and 96 h LC_{50} values were 23.81 mg/L and 57.59 mg/L, respectively. Hagenaars et al. (2011) reported that 96 h LC_{50} value of PFOS to zebrafish embryos was 58.47 mg/L. Ding et al. (2013) demonstrated that PFOS exposure induced acute toxicity in zebrafish early life stages with 96 h LC_{50} value of 54.9 mg/L. The LC_{50} value of PFOS obtained in this study is consistent with those values reported previously. As for OBS, there are few studies on its acute toxicity to zebrafish. Xu et al. (2017) reported that 96 h LC_{50} of OBS was 25.5 mg/L for adult zebrafish, which was slightly higher than the value obtained in this study. From LC_{50} values obtained, it can be seen that the values of OBS are lower than those of PFOS, indicating that OBS shows higher toxicity to zebrafish embryos than PFOS. Therefore, OBS might not be a safety alternative to PFOS and more attention should be given to its toxicity.

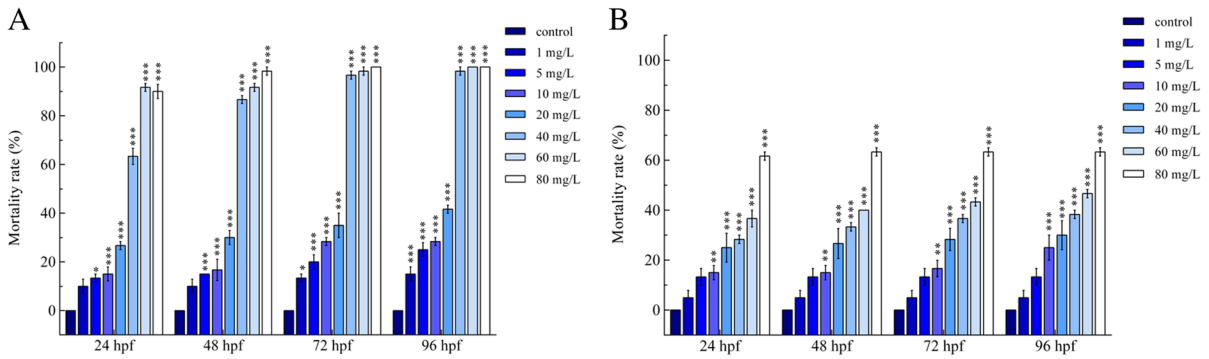

Fig. 1 Mortality rates of zebrafish embryos exposed to OBS (**A**) and PFOS (**B**). All values are expressed as the mean ± SD ($n = 3$ replicates, 20 embryos/larvae per replicate) *** for $p < 0.001$, ** for $p < 0.01$, and * for $p < 0.05$

3.2 The Developmental Toxicity of PFOS and OBS

The development of zebrafish embryos was retarded under OBS and PFOS exposure in a dose-dependent manner. Effects of OBS and PFOS on GMSs were shown in Fig. 2A. GMS values of OBS treatments with concentration greater than 5 mg/L and PFOS treatments greater than 10 mg/L were significantly lower than those of the control. The calculated 96 h EC_{50} on GMS were 23.34 and 49.52 mg/L for OBS and PFOS, respectively. It can be seen that the 96 h EC_{50} of OBS is also lower than that of PFOS, which means that OBS has higher developmental toxicity on zebrafish than PFOS.

In this study, low GMS values were mainly associated with the hatching failure. From Fig. 2B, it can be seen that the hatching rates of embryos exposed to OBS and PFOS significantly decreased with a dose-dependent manner compared to the control. 96 h EC_{50} on the hatching rate were calculated to be 23.44 and 49.77 mg/L for OBS and PFOS, respectively. Hatching is known to be a key process in the life cycle of fish, and a combination of biochemical and physical mechanisms are involved to regulate the process. During the process, the attack of hatching gland enzymes on the chorion together with spontaneous movements of embryo destroy the chorion to free the embryo (De Gaspar et al., 1999). A toxic effect of one or both of these two processes could delay the hatching or even reduce the hatching rate. Several studies suggested that the exposure to PFASs, such as PFOS and PFOA, could induce the hatching retardation of zebrafish embryos (Hagenaars et al., 2011; Huang et al., 2021a; Shi et al., 2008, 2017a). It was previously indicated that the hatching delay of zebrafish embryos induced by PFOA might be related with the interference on the chorion digestion, embryo movement or both (Hagenaars et al., 2011). In this study, the reduced hatching rates after exposure to OBS and PFOS might be also related with the interference on the chorion digestion and/or the embryo movement. However, the underlying mechanisms desire further investigation.

In addition, morphological abnormalities were observed in zebrafish exposed to OBS and PFOS at 96 hpf, including pericardial edema, yolk sac edema, spinal curvature and tail distortion (Fig. S2). The cumulative malformation rates were calculated and shown in Table S2. It can be seen that the cumulative malformation rate increased with increasing concentration of OBS and PFOS exposed. High frequencies of malformation were mainly with pericardial edema and yolk sac edema. Previous studies have also reported the developmental delay and malformation in zebrafish embryos after the exposure to PFOS and OBS. Upon the exposure to PFOS with the concentration of 1 mg/L or higher, zebrafish embryos displayed gross developmental malformations, including epiboly deformities, hypopigmentation, yolk sac edema, tail and heart malformations and spinal curvature (Shi et al., 2008). The exposure to OBS with concentrations of 20 and 30 mg/L caused hatching delays, body

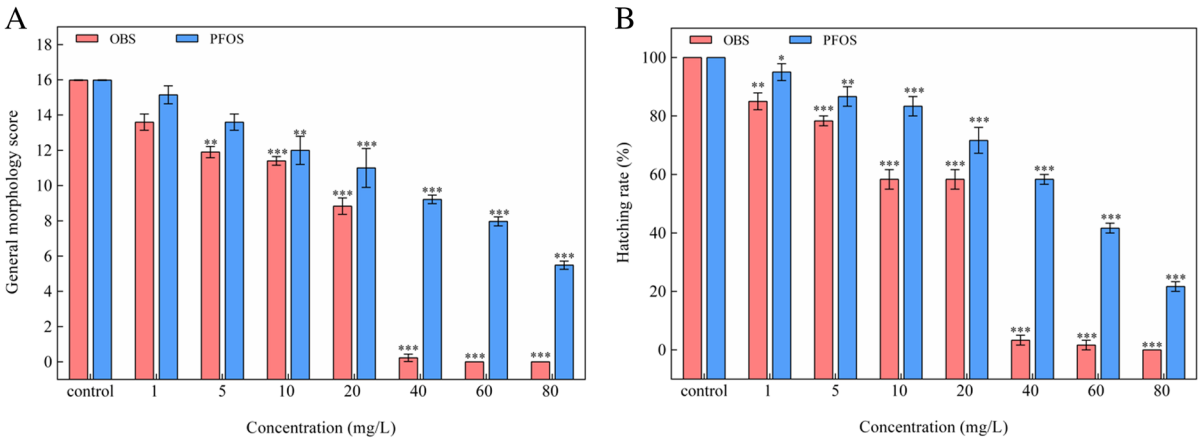

Fig. 2 Effects of OBS and PFOS on general morphological scores (**A**) and hatching rate (**B**) of zebrafish at 96 hpf. All values are expressed as the mean ± SD ($n = 3$ replicates, 20 larvae per replicate). *** for $p < 0.001$, ** for $p < 0.01$, and * for $p < 0.05$

axis curvature, neurobehavioral inhibition and abnormal cardiovascular development (Huang et al., 2021a, b). The results on the morphological development of zebrafish indicated that OBS might have similar developmental toxicity as PFOS.

3.3 The Cardiotoxicity of PFOS and OBS

Pericardial edema was found to be the most significant malformation observed after the exposure to OBS and PFOS. As shown in Fig. 3A, the pericardial edema rate presented a dose-dependent increase. For 10 mg/L of OBS and PFOS treatments, the pericardial edema rates were significantly higher than that of control. The occurrence of pericardial edema could affect the heart rate of zebrafish. From Fig. 3B, it can be seen that the heart rate decreased with increasing exposure concentrations. For the control, the heart rate was 170.80 ± 1.84 bpm at 96 h. However, the heart rate significantly decreased at 10 mg/L of OBS and 20 mg/L of PFOS. For the stroke volume, a significant reduction was observed for treatments with concentrations greater than 5 mg/L (Fig. 3C). Similarly, the cardiac output also significantly decreased for treatments greater than 5 mg/L (Fig. 3D).

In the development of zebrafish embryos, heart is the first functional organ formed, and is easier to be observed than other organs (Stainier, 2001). Early embryonic heart development is an extremely elaborate and sensitive process, which can be affected by xenobiotics, such as 6:2 chlorinated polyfluorinated ether, fenbuconazole, difenoconazole and dimethomorph (Fan et al., 2021; Shi et al., 2017b; Wu et al., 2018). The cardiotoxicity is usually determined according to changes of the cardiac morphology, heart rate, stroke volume and cardiac output (Sarmah & Marrs, 2016). Pericardial edema is a commonly observed pathology in zebrafish, which reflects abnormal cardiac development (Sarmah & Marrs, 2016). In this study, the exposure of OBS and PFOS with concentrations higher than 10 mg/L caused severe pericardial edema of zebrafish (Fig. 3A), which was consistent with results of Huang et al. (2021a). Similar results have been reported on other PFASs, such as PFOA, 6:2 FTCA and F53-B (Shi et al., 2017a, b; Zheng et al., 2012).

The exposure of OBS and PFOS on zebrafish embryos not only resulted in morphological changes but also affected the cardiac function, which suggested heart might be a target tissue of OBS and PFOS. Heart rate, stroke volume and

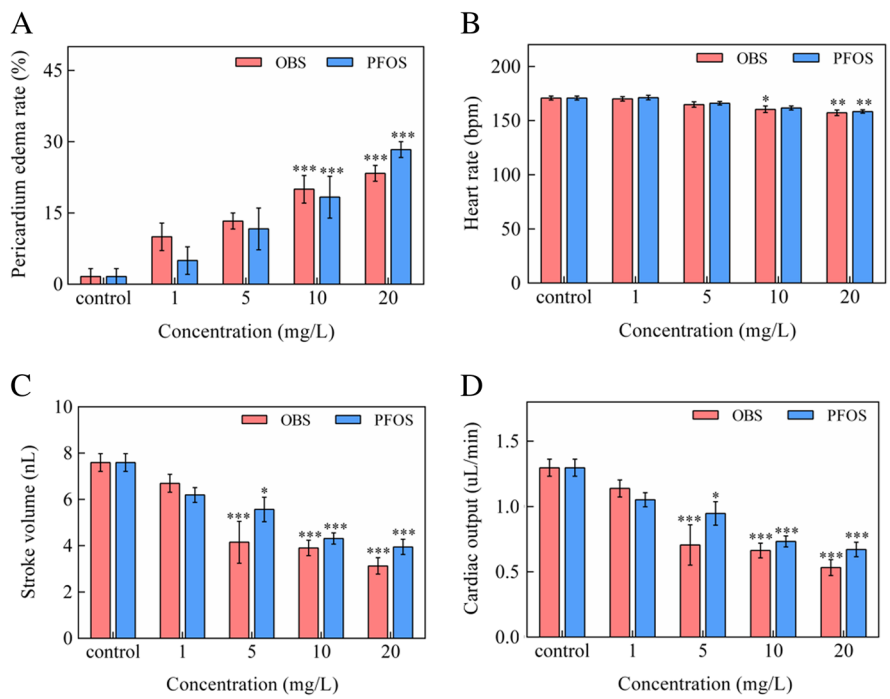

Fig. 3 Effects on the cardiac function of zebrafish after OBS and PFOS exposure. Pericardium edema rate (**A**), Heart rate (**B**), Stroke volume of ventricle (**C**), and Cardiac output of ventricle (**D**). All values are expressed as the mean ± SD ($n = 3$ replicates, 20 embryos/larvae per replicate). *** for $p < 0.001$, ** for $p < 0.01$, and * for $p < 0.05$

cardiac output are important parameters to evaluate the cardiac function (Sarmah & Marrs, 2016; Zhang et al., 2020). In this study, it was found that the exposure of OBS and PFOS decreased the heart rate, stroke volume and cardiac output of zebrafish embryos. In a previous study, it was also demonstrated that 30 mg/L of OBS could decrease the heart rate of zebrafish embryos (Huang et al., 2021a). According to previous report, the decreasing of cardiac output would lead to increase of heart rate in physiologic compensatory reaction of organism (Duan et al., 2016). However, in this study, the decreased cardiac output was in step with the decreased heart rate. This might be related with severe abnormal morphogenesis of the heart induced by OBS and PFOS. The phenomenon has also been observed in zebrafish embryos exposed to silica nanoparticles and crude oil (Duan et al., 2016; Li et al., 2019). It is known that reduction of heart rate, stroke volume and cardiac output affect the normal cardiac functions, which could further lead to the development retardation and even death (Cypher et al., 2017; Zhu et al., 2022). Thus, these results indicated that OBS and PFOS exposure could induce the cardiotoxicity in early life stage of zebrafish.

3.4 The Effect on Antioxidant Systems

As shown in Fig. 4, the activities of SOD and CAT and the GSH content decreased with increasing concentrations of OBS and PFOS, while the MDA content presented a dose-dependent increase. Compared with the control, the activity of SOD significantly decreased at 5 mg/L of OBS and 10 mg/L of PFOS, while the activities of CAT significantly decreased at 1 mg/L of OBS and PFOS. As for the GSH content, it significantly decreased at 20 mg/L of OBS and 10 mg/L of PFOS. For the MDA content, there was no significant change for the treatments of OBS, however 10 mg/L of PFOS induced significant increase.

Oxidative stress refers to the imbalance between ROS production and antioxidant action, which has become an important part of aquatic toxicology, and severe oxidative stress can result in contribute to the abnormal development of fish embryos (Domingues & Gravato, 2018; Du et al., 2017; Ge et al., 2015). In organisms, the MDA content is an important marker of oxidative stress, reflecting the extent of oxidative damage (Draper & Hadley, 1990). Compared with the control, the MDA content significantly increased at 10 mg/L of PFOS, suggesting that PFOS exposure caused oxidative damage in zebrafish embryos.

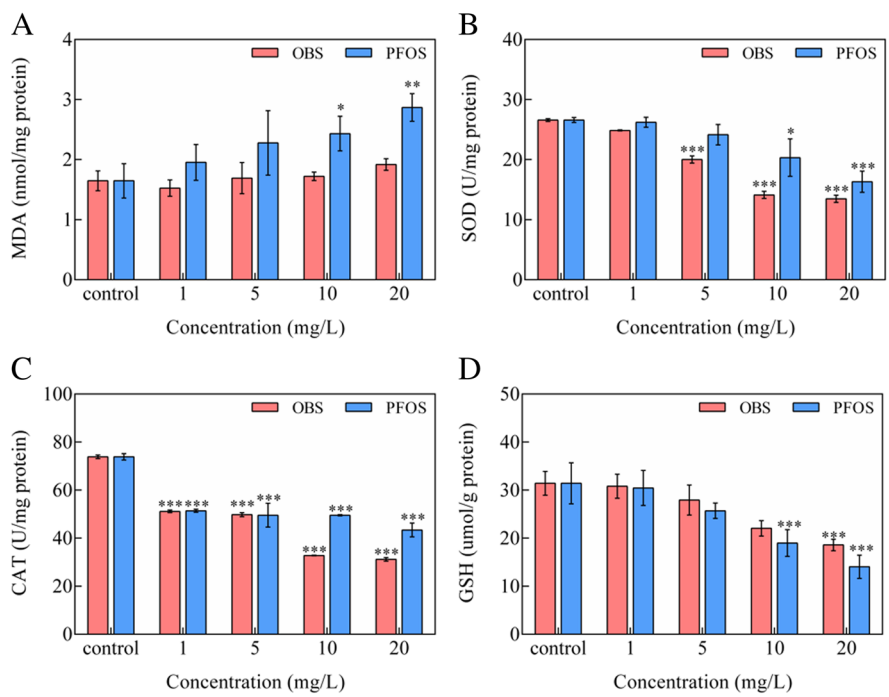

Fig. 4 Oxidative stress induced by OBS and PFOS in zebrafish embryos. MDA content (**A**), SOD activity (**B**), CAT activity (**C**), and GSH content (**D**). All values are expressed as the mean ± SD ($n = 3$ replicates, 60 larvae per replicate). *** for $p < 0.001$, ** for $p < 0.01$, and * for $p < 0.05$

Likewise, an increase of the MDA content was observed in zebrafish exposed to PFOS (Huang et al., 2021b). SOD, CAT and GSH are common biomarkers of antioxidant stress as they play an important role in the prevention of oxidative stress (Li et al., 2003; Wu et al., 2019). In present study, the activities of SOD and CAT and the GSH content were significantly decreased, which indicated that antioxidant system of zebrafish embryos was greatly influenced by OBS and PFOS exposure. These results suggested that OBS and PFOS exposure led to severe oxidative stress in zebrafish.

Previous studies reported that oxidative stress is associated with abnormal heart development and malformations of zebrafish (Cao et al., 2020; Huang et al., 2020b; Jin et al., 2020; Xu et al., 2022). For example, Jin et al. (2020) reported that trichloroethylene exposure induced oxidative stress in zebrafish embryos and consequently led to developmental defects of heart. Xu et al. (2022) showed that ticlopidine exposure resulted in imbalance of the anti-oxidative system. Subsequently excessive accumulation of ROS induced down-regulated activities of CAT and SOD together with the increase of the MDA content, and led to cardiotoxicity in zebrafish embryos. In addition, oxadiazon-Butachlor and diclofop-methyl exposure were found to cause the elevation of oxidative stress and damages cardiomyocytes, resulting in cardiotoxicity (Cao et al., 2020; Huang et al., 2020a). In this study, oxidative stress also occurred in zebrafish after exposure to OBS and PFOS, resulting in significant decrease of SOD, CAT and GSH, and increase of the MDA content. Therefore, generation of oxidative damage and reduced antioxidant enzyme activities may be key factors contributing to the developmental toxicity and cardiotoxicity in zebrafish caused by OBS and PFOS exposure.

3.5 Effects on the Gene Expression Related to the Heart Development

The transcriptional levels of heart-related genes, including master cardiac transcription factors (*nkx2.5*, *tbx5* and *gata4*) and cardiac structural development gene (*myh6*), were analyzed, the results of which were shown in Fig. 5. It can be seen that the expression of *nkx2.5*, *gata4* and *myh6* presented the dose-dependent increase, while the expressions of *tbx5*, revealed a dose-dependent decrease. The expression of *nkx2.5* was significantly up-regulated at highest concentration of OBS and all PFOS treatments, while the expression of *tbx5*

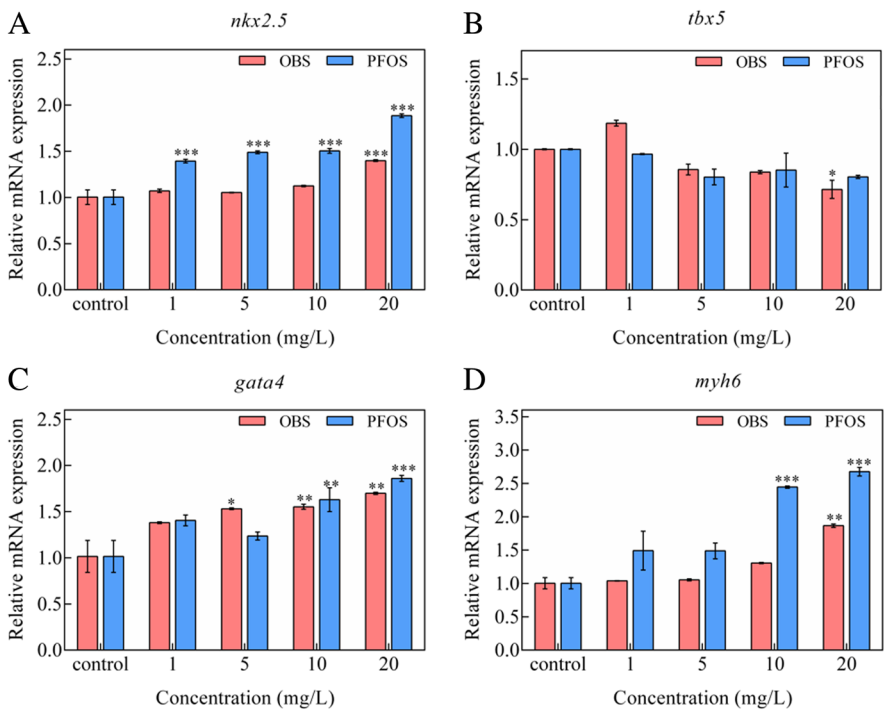

Fig. 5 The expression of *nkx2.5*, *tbx5*, *gata4* and *myh6* in zebrafish embryos at 96 hpf exposed to OBS and PFOS. All values are expressed as the mean ± SD ($n = 3$ replicates, 60 larvae per replicate). *** for $p < 0.001$, ** for $p < 0.01$, and * for $p < 0.05$

was only significantly down-regulated at 20 mg/L of OBS. The expression of *gata4* was significantly up-regulated in OBS treatments higher than 5 mg/L and PFOS treatments higher than 10 mg/L. As for *myh6*, the expression was up-regulated at highest concentration of OBS and PFOS treatments higher than 10 mg/L.

The *nkx2.5* gene is a transcription factor involved in cardiomyocyte differentiation and plays a key role in cardiac specification, proliferation and ventricular morphogenesis (Targoff et al., 2013; Tu et al., 2009; Wang et al., 2019b; Yuan et al., 2021). In this study, the expression of *nkx2.5* was up-regulated after OBS and PFOS treatments. This phenomenon was similar to previous results, in which *nkx2.5* was overexpressed and consequently induced pericardial edema and the decreased heart rate (Duan et al., 2021; Zhang et al., 2020). Therefore, OBS and PFOS exposure could affect the cardiomyocyte differentiation and then induced pericardial edema via influencing the expression of *nkx2.5*. The *gata4* gene is crucial for cardiac specific differentiation and migration of cardiac blasts, and its abnormal expression could lead to cardiac malformations (Holtzinger & Evans, 2005). The *tbx5* gene is also a transcription regulator of heart development, and its inhibition or overexpression can lead to looping failure and the decrease of the cardiac cell number (Parrie et al., 2013; Pi-Roig et al., 2014). This study showed that the expression level of *gata4* was significantly up-regulated, while expression level of *tbx5* was down-regulated in higher concentration treatments. Previous studies have shown that the disordered expression of *tbx5* and *gata4* led to cardiac septal valve defects and heart malformations (Tang et al., 2020; Xu et al., 2022). Therefore, the heart malformation and the decreased heart rate observed in this study might also be related with the disordered expression of *tbx5* and *gata4*. The *myh6* gene is related to atrial contraction and heart muscle differentiation (Singleman & Holtzman, 2012). In this study, the expression of *myh6* was up-regulated after the OBS and PFOS treatments. Abnormal expression of *myh6* may affect the heart muscle differentiation and the atrial contraction, which consequently led to the decreased heart rate. Therefore, OBS and PFOS exposure could induce structural and functional damages on the heart of zebrafish by affecting the transcriptional events of these key heart-related genes.

4 Conclusions

In this study, the developmental toxicity and cardiotoxicity of OBS and PFOS in early life stage of zebrafish were investigated. It was found that OBS and PFOS could induce adverse effects on zebrafish embryos, including reduced survival rate, delayed hatching, and increased malformations. 96 h LC_{50} values of OBS and PFOS were determined to be 23.81 and 57.59 mg/L, respectively. Their 96 h EC_{50} on the hatching rate were calculated to be 23.44 and 49.77 mg/L, respectively. These suggest that OBS has higher toxicity to zebrafish embryos than PFOS. Pericardial edema was found to be the most significant malformation observed after the exposure to OBS and PFOS, and the pericardial edema rate presented a dose-dependent increase. The exposure of OBS and PFOS also decreased the heart rate, stroke volume and cardiac output, indicating the cardiotoxicity induced in early life stage of zebrafish. In addition, exposure of OBS and PFOS resulted in significant decreases of SOD, CAT and GSH and significant increase of the MDA content, and caused aberrant expression of cardiac development-related genes. The oxidative stress might be a key factor contributing to the developmental toxicity and cardiotoxicity in zebrafish caused by OBS and PFOS. From the results, OBS might not be a safe alternative to PFOS, and the safety of OBS on aquatic organisms should be further investigated.

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Data Availability The data that support the findings of this study are available from the corresponding author upon reasonable request.

Declarations

Disclosure of Interest The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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