Fish Physiol Biochem (2023) 49:1421–1433
https://doi.org/10.1007/s10695-023-01265-7

RESEARCH

Environmentally relevant concentrations of tris (2-chloroethyl) phosphate (TCEP) induce hepatotoxicity in zebrafish (*Danio rerio*): a whole life-cycle assessment

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Received: 21 September 2023 / Accepted: 5 November 2023 / Published online: 11 November 2023

Abstract Tris (2-chloroethyl) phosphate (TCEP), a typical organophosphate flame retardant, is of increasingly great concern considering their ubiquitous presence in aquatic environments and potential ecotoxicity. The present work was aimed to investigate the potential growth inhibition and hepatic stress induced by whole life-cycle exposure to TCEP (0.8, 4, 20 and 100 μg/L) in zebrafish. The results revealed that the body length, body mass and hepatic-somatic index (HSI) of zebrafish were significantly declined after exposure to TCEP for 120 days. GPx activity and GSH content were increased in the liver of zebrafish treated with low concentrations (0.8 and 4 μg/L) of TCEP, while exposure to high concentrations (20 and 100 μg/L) of TCEP reduced antioxidative capacity and elevated lipid peroxidation (LPO) levels. Gene transcription analysis demonstrated that the mRNA levels of *nrf2* were altered in a similar manner to the transcription of the downstream genes *nqo1* and *hmox1*, suggesting that Nrf2-Keap1 pathway mediated TCEP-induced oxidative stress in zebrafish liver. In addition, TCEP exposure might alleviate inflammatory response through down-regulating transcription of inflammatory cytokines (*il-1β*, *il-6* and *inos*), and induce apoptosis via activating the p53-Bax pathway. Moreover, whole life-cycle exposure to TCEP caused a series of histopathological anomalies in zebrafish liver. Overall, our results revealed that lifetime exposure to environmentally relevant concentrations of TCEP could result in growth retardation and induce significant hepatotoxicity in zebrafish.

Highlights
Whole life-cycle exposure to environmental relevant concentrations of TCEP could inhibit the growth of zebrafish.
Exposure to TCEP induced oxidative stress and led to lipid peroxidation in zebrafish liver.
Inflammatory response might be alleviated through the down-regulation of inflammatory cytokines mRNA expression.
Whole life-cycle exposure to TCEP might induce apoptosis through the activation of p53-Bax pathway.
Whole life-cycle exposure to TCEP resulted in a series of histopathological anomalies in zebrafish liver.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s10695-023-01265-7.

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Keywords Zebrafish · TCEP · Oxidative stress · Inflammatory response · Apoptosis · Histological changes

Introduction

As the primary substitutes for brominated flame retardants (BFRs), organophosphate flame retardants

(OPFRs) are widely used as additives in various products, such as plastics, electronic equipment and textiles (Zhou et al. 2020). Tris (2-chloroethyl) phosphate (TCEP) is one of the typical OPFRs, which is of increasing concern due to its widely application and ubiquitous presence in environmental media (Abdallah and Covaci 2014). Given that TCEP is hardly bound to polymers chemically, it can be transferred from synthetic products to the environment under physical effects such as volatilization, abrasion and dissolution (Bollmann et al. 2012). Additionally, TCEP has a relatively high water solubility (7820 μg/L at 20 ˚C) and cannot be eliminated effectively via the current sewage treatment technology. Thus, it is not surprising that TCEP has been frequently detected in aquatic environments such as rain, wastewater, drinking water and surface water (Marklund et al. 2005). For example, TCEP was detected in drinking water at the concentration of up to 120 ng/L in US (Benotti et al. 2009). In the Songhua River, China, the measured concentrations of TCEP ranged from 38 to 3700 ng/L (Wang et al. 2011). The highest TCEP level (87.4 μg/L) was ever reported in the raw water from a Japanese sea-based solid waste disposal site (Kawagoshi et al. 1999). The extensive existence of TCEP in environments is posing great threats to wild animals and also human beings.

A growing number of studies demonstrated that exposure to TCEP exhibited a variety of adverse effects, such as neurotoxicity, developmental toxicity, reproductive toxicity, endocrine disrupting effects, and even carcinogenicity (Sun et al. 2016; Li et al. 2019; Wang et al. 2020; Sutha et al. 2022). For instance, after exposure to TCEP, the genes and proteins associated with central nervous system (CNS) development were changed, inducing neurotoxicity during the early stages of zebrafish (Li et al. 2019). Treatment with 1250 or 6250 μg/L TCEP produced a significant inhibition on the growth of Japanese medaka (*Oryzias latipes*) (Sun et al. 2016). A recent work elucidated that TCEP exposure resulted in reproductive toxicity in zebrafish, causing variations in sexual plasma sex hormones, and gonadal damage (Sutha et al. 2022). Furthermore, TCEP exhibited carcinogenicity in mice, evidenced by the regulation of tumor-associated factors (Wang et al. 2020). Nevertheless, the exposure concentrations adopted in most previous studies were much higher than environmentally realistic levels. Besides, considering that aquatic organisms are normally exposed to environmental pollutants constantly in natural waters, life-cycle toxicity assessment may be of more realistic meaning.

Liver is the main target organ for toxic substances, performing multiple functions such as detoxification, metabolism and immunity of vertebrate body (Van den Eede et al. 2013). Several studies have so far been focused on the adverse impacts of OPFRs on fish liver (Fernandes et al. 2008; Moser et al. 2015; Chen et al. 2018; Ramesh et al. 2018). For example, exposure to TCEP significantly elevated the hepatic mRNA levels of antioxidant genes (*gst* and *gpx*) in juvenile salmon (Arukwe et al. 2016). Tris (1,3-dichloro-2-propyl) phosphate (TDCIPP), another typical OPFR, triggered inflammation in adult zebrafish liver, evidenced by the upregulation of inflammation biomarker genes and histological alterations (Liu et al. 2016). Besides, histological structure alterations such as necrosis and vacuolation were observed in the liver of *Cirrhinus mrigala* after a 21-day exposure to TCEP (Sutha et al. 2020). A recent study reported that TCEP might exert hepatotoxic effects on zebrafish by disrupting the HPT and gut-liver axes and thereafter inducing hepatic inflammation and oxidative stress (Tian et al. 2023). However, a systematic study on the hepatotoxicity resulted from whole lifetime exposure to TCEP is still required.

Due to small body size (adults reaching only 3–4 cm), high fecundity and high sensitivity to environmental stressors, zebrafish has become an important model for toxicological studies (Vliegenthart et al. 2014). The objective of this study was to investigate the antioxidant defense, inflammatory response, apoptosis and histological changes in the liver of zebrafish after lifetime exposure to environmentally relevant concentrations of TCEP. These results will broaden our understanding of the hepatotoxicity resulted from long-term exposure to TCEP in fish, and highlight the environmental hazards posed by TCEP in aquatic ecosystems.

Materials and methods

Chemicals and reagents

TCEP (CAS: 115–96–8; purity ≥ 97%), TCEP-d_{12} (purity ≥ 97%) and ethyl 3-aminobenzoate methanesulfonate (MS-222, CAS: 886–86–2; purity ≥ 98%) were purchased from Sigma-Aldrich Chemical Co. (St. Louis, USA). TCEP were dissolved in dimethyl sulfoxide

(DMSO; CAS: 67–68–5; purity ≥ 99.7%; Sigma-Aldrich, USA) as a stock solution. All other reagents used in this work were of analytical or HPLC grade.

Fish husbandry and TCEP exposure

5-month-old zebrafish (wild-type, AB strain) were selected and maintained in aquariums (40 L water and 50 individuals per tank) with water temperature 27 ± 1℃, pH 7.0 ± 0.5 and a 14-h light/10-h dark cycle. The fish were fed twice daily with newly hatched brine shrimp larvae (*Artemia salina*). After one-week acclimation, 25 males and 50 females were randomly selected and allowed to spawn. Embryos were collected and transferred to plastic culture dishes with lids (60 embryos per dish) and exposed to 0, 0.8, 4, 20 and 100 μg/L TCEP, with three replicates for each treatment. Every petri dish contained 40 mL (maximum volume 70 mL) of exposure solution. The larvae were transferred to breeding aquariums after two weeks and each aquarium contained 6 L (maximum volume was 10 L) of exposed solution and 30 individuals. Fish were fed a commercial diet (Hai Feng Feeds Co. Ltd.) 3 times daily till 120 dpf. Half of exposure medium were renewed with freshly prepared solutions daily. The final concentrations of DMSO were 0.0001% (v/v) in both solvent control and TCEP-treated groups.

Exposure solutions were sampled before and after water renewal at 119 dpf and stored at -80℃ until the quantification of TCEP. At 120 dpf, 10 fishes were randomly selected from each tank, euthanized with 0.03% MS-222. After the record of body length and body weight, liver tissues were collected, immediately frozen in liquid nitrogen and stored at -80 °C till further analysis. Another 3 individuals from each replicate were dissected to obtain liver tissues for histological analysis. The body weight and the liver weight were used for the calculation for hepatic-somatic index (HSI).

TCEP quantification

TCEP was quantified in collected water samples as previously described (Wang et al. 2022). Firstly, the internal standard TCEP-d_{12} was spiked into the water samples. After then, water samples were cleaned up using solid phase extraction (SPE) method and eluted with acetonitrile. The eluents were reduced to dryness under a gentle stream of nitrogen, and dissolved in 1 mL methanol. The quantification of TCEP was performed on a Waters ACQUITY UPLC® H-Plus Class system (UHPLC) coupled to a Waters® Xevos™ TQ-XS mass spectrometer (TQ-XS/MS) (Milford, MA, USA). Detailed protocols for the extraction, clean up and analysis was provided in Text S1 (Supporting Information).

Histological examination

Freshly dissected liver tissues were fixed in 4% paraformaldehyde (PFA) for 24 h. Then the tissues were dehydrated in ethanol, decontaminated in xylene, embedded in paraffin, and sectioned into 5 μm thick slices. Afterwards, these sections were stained with hematoxylin–eosin (H&E) staining and examined under a light microscope.

Biochemical analysis

Liver tissues were homogenized (1:9, w/v) in 0.9% physiological saline with a high-throughput tissue homogenizer (Scientz, Ningbo, China). The homogenates then were kept in ice-cold condition and finally centrifuged (3000 × g) for 10 min at 4℃ to obtain supernatants for biochemical measurements. Superoxide dismutase (SOD), hydrogenase (CAT), and glutathione peroxidase (GPX) activities, as well as glutathione (GSH) and malondialdehyde (MDA) content were determined using commercial assay kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China) according to the manufacturer's instructions.

Gene transcription analysis

Total RNA isolation was conducted with FastPure® Cell/Tissue Total RNA Isolation Kit V2 (Vazyme Biotech Co. Ltd., Nanjing, China). The cDNA was synthesized using PrimeScript® RT reagent Kit (Takara, China) according to the manufacturer's instructions. The qPCR was carried out on LightCycler® 480II (Roche, Switzerland). The primer sequences used for qPCR analysis were designed using the online Primer-BLAST tool on NCBI website and are given in Table S1 (Supporting Information). *β-actin* was chosen as an internal reference gene because the transcription level of *β-actin* did not vary significantly under different TCEP exposure concentrations. The relative expression levels of target genes were calculated by the $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen 2001).

Statistical analysis

Results were expressed as mean ± standard deviation (S.D.). Prior to statistical analysis, all data were checked for normality and homogeneity of variance using Kolmogorov–Smirnov test and Levene's test. The differences between the solvent control and treatment groups were evaluated using the one-way analysis of variance (ANOVA) and Tukey's HSD test with SPSS Statistics 19.0 (SPSS, Chicago, IL). $p < 0.05$ was considered statistically significant.

Results

TCEP concentrations in exposure media

The actual concentrations of TCEP in 0.8, 4, 20, and 100 μg/L exposure solutions were 0.85 ± 0.12, 3.79 ± 0.43, 19.24 ± 0.31 and 102.07 ± 3.66 μg/L after renewal and 0.72 ± 0.14, 3.72 ± 0.31, 18.68 ± 1.55 and 93.77 ± 6.29 μg/L before next renewal (Fig. S1, Supporting information). No TCEP was detected in the solvent control group.

Body length, body mass and HSI

At 120 dpf, the body length and body mass of zebrafish did not show significant changes in 0.8 μg/L TCEP-treated group, but were markedly declined in 4, 20 and 100 μg/L groups compared with the solvent control group (Fig. 1A, 1B). The HSI values were significantly lower in all exposure groups in comparison to the solvent control (Fig. 1C).

Histopathological changes

Normal hepatocyte structure without signs of degeneration or necrosis was observed in the control fish (Fig. 2A). In comparison to the solvent control group, exposure to 0.8 μg/L TCEP caused a mild granular degeneration and slight vacuolization (Fig. 2B). In addition, increased vacuoles, parenchyma disorganization and pyknotic nucleus occurred in 4, 20 and 100 μg/L TCEP-treated groups, appearing to be more severe with the increase of exposure concentrations (Fig. 2C, 2D, 2E). Especially, lifetime exposure to 100 μg/L TCEP resulted in extensive areas of vacuolar degeneration in the liver of zebrafish (Fig. 2E).

Antioxidant enzyme activities, GSH content and MDA level

Lifetime exposure to 20 and 100 μg/L TCEP significantly reduced the activities of SOD and GPx, while CAT activity was declined in all TCEP exposure groups (Fig. 3). The noticeable increase of GSH content was observed in 0.8, 4 and 20 μg/L TCEP exposure groups (Fig. 3). MDA contents were significantly elevated in 4, 20 and 100 μg/L TCEP-treated groups in comparison to the solvent control (Fig. 3).

mRNA levels of antioxidant genes

Exposure to 0.8 and 100 μg/L TCEP significantly increased the mRNA level of *keap1* in zebrafish liver (Fig. 4). Significant up-regulated mRNA expression of *nrf2* was only observed in 0.8 μg/L TCEP-treated group (Fig. 4). The mRNA levels of *nqo1*

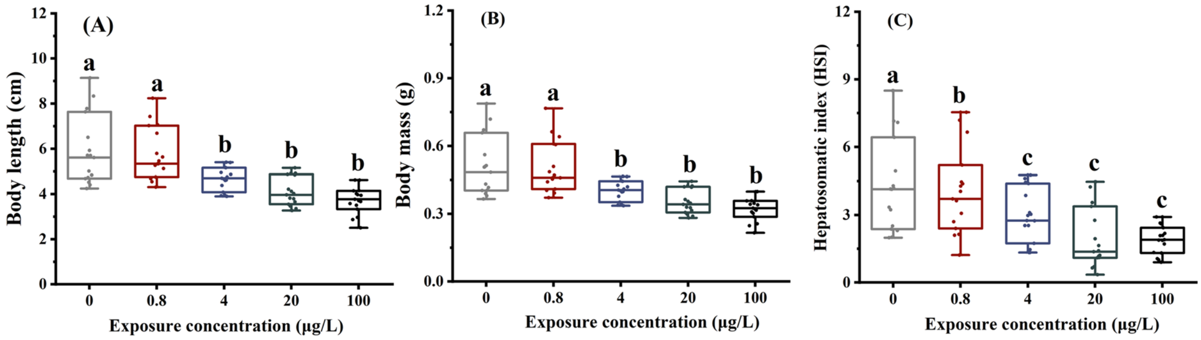

Fig. 1 Effects of lifetime exposure to TCEP on body length, body weight and HSI of zebrafish. Different letters indicate significant difference among different treatments, Tukey's HSD, $p < 0.05$

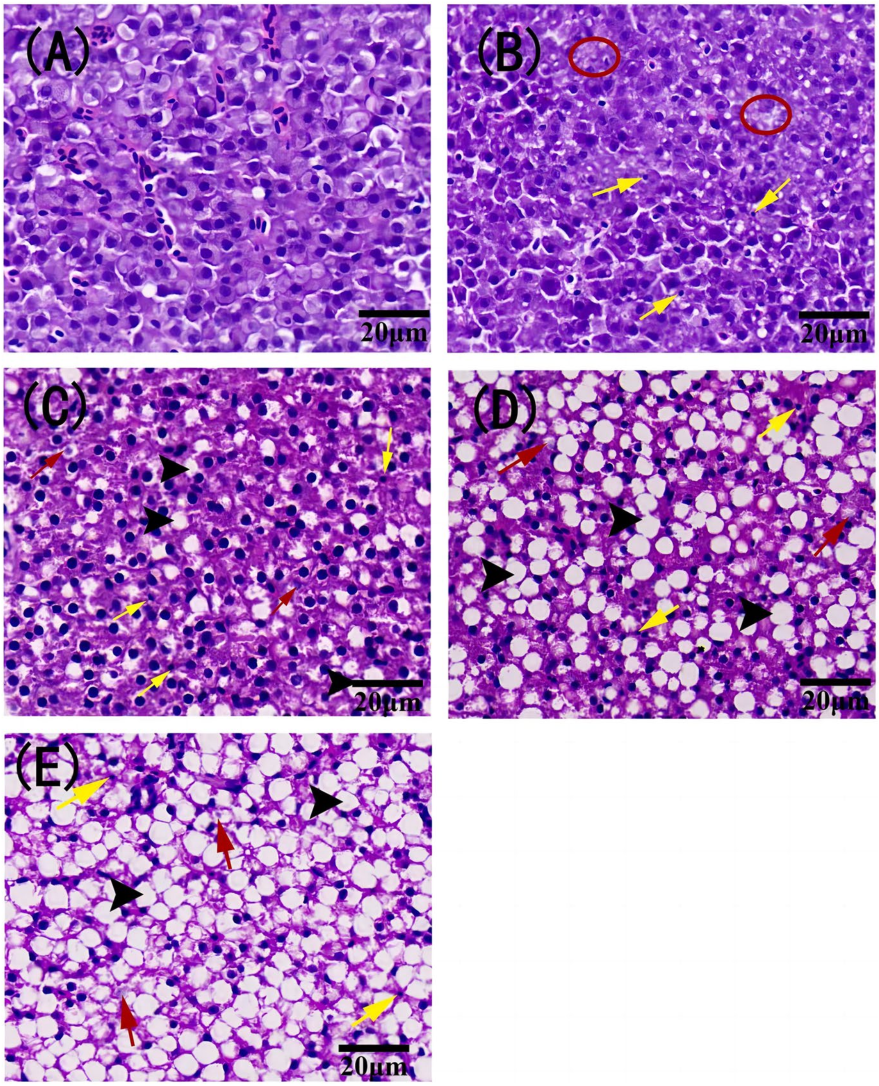

Fig. 2 Effects of lifetime exposure to TCEP on liver histology of zebrafish at 120 dpf. **A** Liver from the solvent control, showing normal hepatocytes structure; **B** Liver from 0.8 μg/L TCEP group, exhibiting parenchyma disorganization (ellipse), pyknotic nucleus (yellow arrows); **C** Liver from 4 μg/L TCEP group, pyknotic nucleus (yellow arrows), nuclear deformation (red arrows), vacuolation (black triangle); **D** Liver from 20 μg/L TCEP group, pyknotic nucleus (yellow arrows), nuclear deformation (red arrows) and showing more severe vacuolation (black triangle); **E** Liver from 100 μg/L TCEP group, pyknotic nucleus (yellow arrows), nuclear deformation (red arrows) and showing more severe vacuolation (black triangle)

were significantly increased in 0.8 and 4 μg/L TCEP-treated groups (Fig. 4). The mRNA expression of *homx-1* was up-regulated in 0.8 μg/L group, while sharply down-regulated in 4, 20 and 100 μg/L TCEP-treated groups. Besides, the transcription of *gst* were significantly suppressed in all TCEP treatments in a dose-dependent manner (Fig. 4).

mRNA levels of inflammatory genes

The mRNA level of *il-β* was significantly raised after exposure to 0.8 μg/L TCEP, while declined in 20 and 100 μg/L groups (Fig. 5). Significant down-regulation of *il-6* and *inos* expressions were observed in all TCEP treatments (Fig. 5). The transcriptional level of *il-10* was only decreased markedly in 100 μg/L TCEP-treated group (Fig. 5).

mRNA levels of apoptosis-related genes

Life-time exposure to TCEP significantly induced the up-regulation of the expression of *p53*, while down-regulated the expression of *bcl-2* (Fig. 6). The mRNA level of *bax* was only significantly increased in 0.8 μg/L TCEP-treated group (Fig. 6). The transcriptional levels of *ced-4* were remarkedly elevated in 4 and 100 μg/L exposure groups (Fig. 6). Exposure to 0.8 and 4 μg/L TCEP augmented the transcription of *cyp1a* and decreased the transcription of

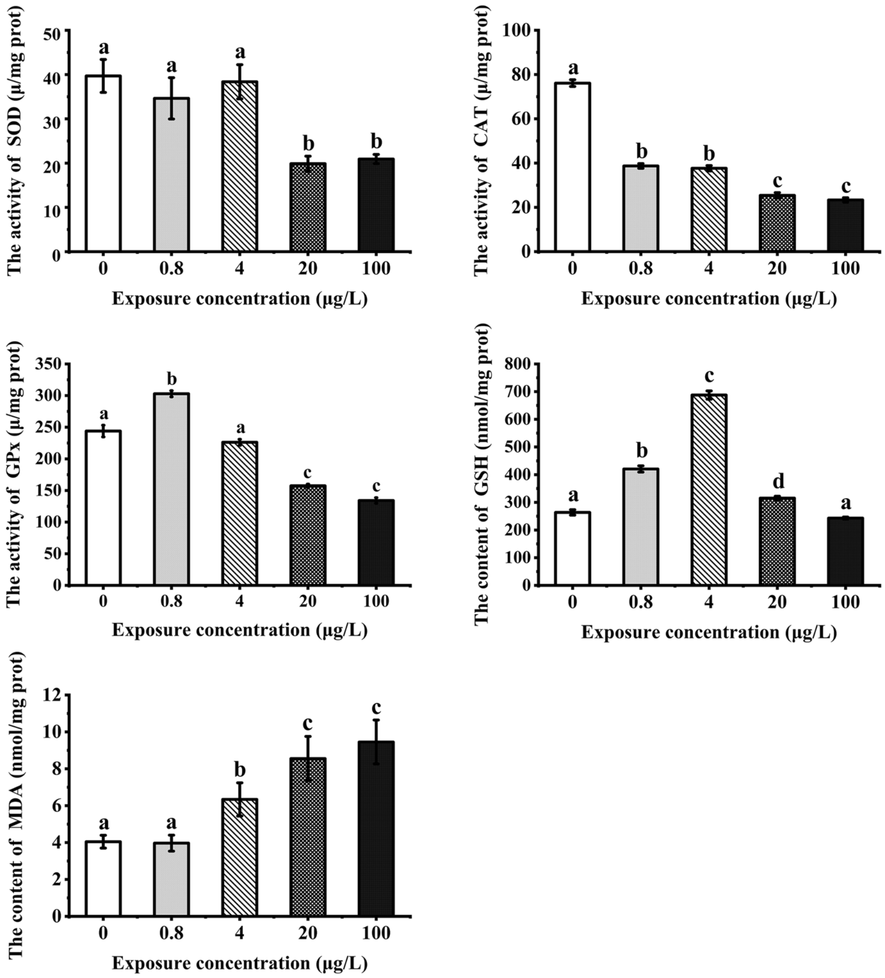

Fig. 3 Effects of lifetime exposure to TCEP on the activities of SOD, CAT, GPx, and the contents of GSH and MDA in the liver of zebrafish at 120 dpf. Different letters indicate significant difference among different treatments groups, Tukey's HSD, $p < 0.05$

cas3 compared with the solvent control (Fig. 6). The mRNA expression of *cas8* was significantly up-regulated in the 0.8 μg/L group, but down-regulated in the 4, 20 and 100 μg/L TCEP-treated groups (Fig. 6). The mRNA level of *cas9* was apparently declined in 0.8, 20 and 100 μg/L exposure groups, and was increased in 4 μg/L TCEP-treated group (Fig. 6).

Discussion

Various OPFRs such as triphenyl phosphate (TPP) and TDCIPP exhibited growth-inhibiting effects on *Daphnia magna* and zebrafish (Li et al. 2017; Yu et al. 2017). Our previous findings also demonstrated that exposure to 20 and 200 μg/L TCEP significantly decreased the body length of 5-dpf larval zebrafish (Hu et al. 2021). In this study, reduced body mass and body length were observed in zebrafish after 120-d exposure to 4, 20 and 100 μg/L TCEP, suggesting that lifetime exposure to environmentally relevant concentrations of TCEP can cause significant growth retardation in fish.

Liver is a target organ for the toxicity of numerous organic substances (Hinton et al. 2017). HSI is an indicator of the growth status of the liver in fish, which is sensitive to various environmental stressors (Larsson et al. 1984; Deng et al. 2010). Our results showed that TCEP exposure significantly reduced the HSI of zebrafish. Consistent results were found in previous studies on zebrafish exposed to TPP, TDCIPP and tris (2-butoxyethyl) phosphate (TBOEP) (Liu et al. 2013; Xu et al. 2017). This might be attributed to the hepatic TCEP accumulation after long-term exposure, which might interfere the synthesis of storage products such as glycogen and fat in liver, causing a decrease in liver weight and a reduction in

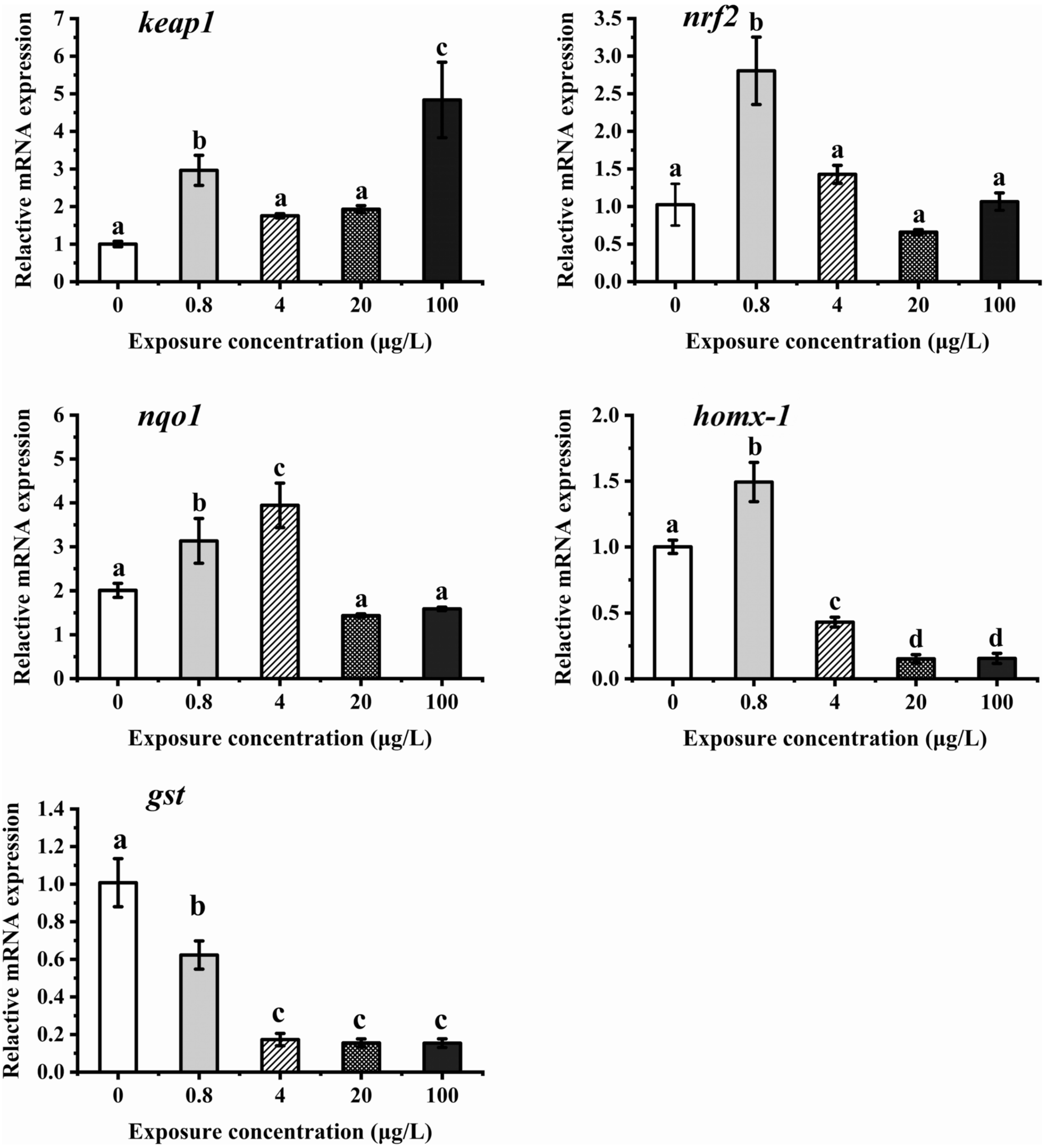

Fig. 4 Effects of lifetime exposure to TCEP on the mRNA levels of *keap1*, *nrf2*, *nqo1*, *homx-1* and *gst* in the liver of zebrafish at 120 dpf. Different letters indicate significant difference among different treatments groups, Tukey's HSD, $p < 0.05$

HSI (Kopecka and Pempkowiak 2008). Thereby, the overall decline of HSI indicated abnormal liver development and function.

In accordance with that reported in freshwater fishes *Cirrhinus mrigala* and zebrafish sub-chronic exposed to TCEP (Sutha et al. 2020; Tian et al. 2023), occurrence of severe liver injuries including vacuoles, parenchyma disorganization and pyknotic nucleus were clearly observed after whole life-cycle exposure to TCEP in this study, which were more serious with

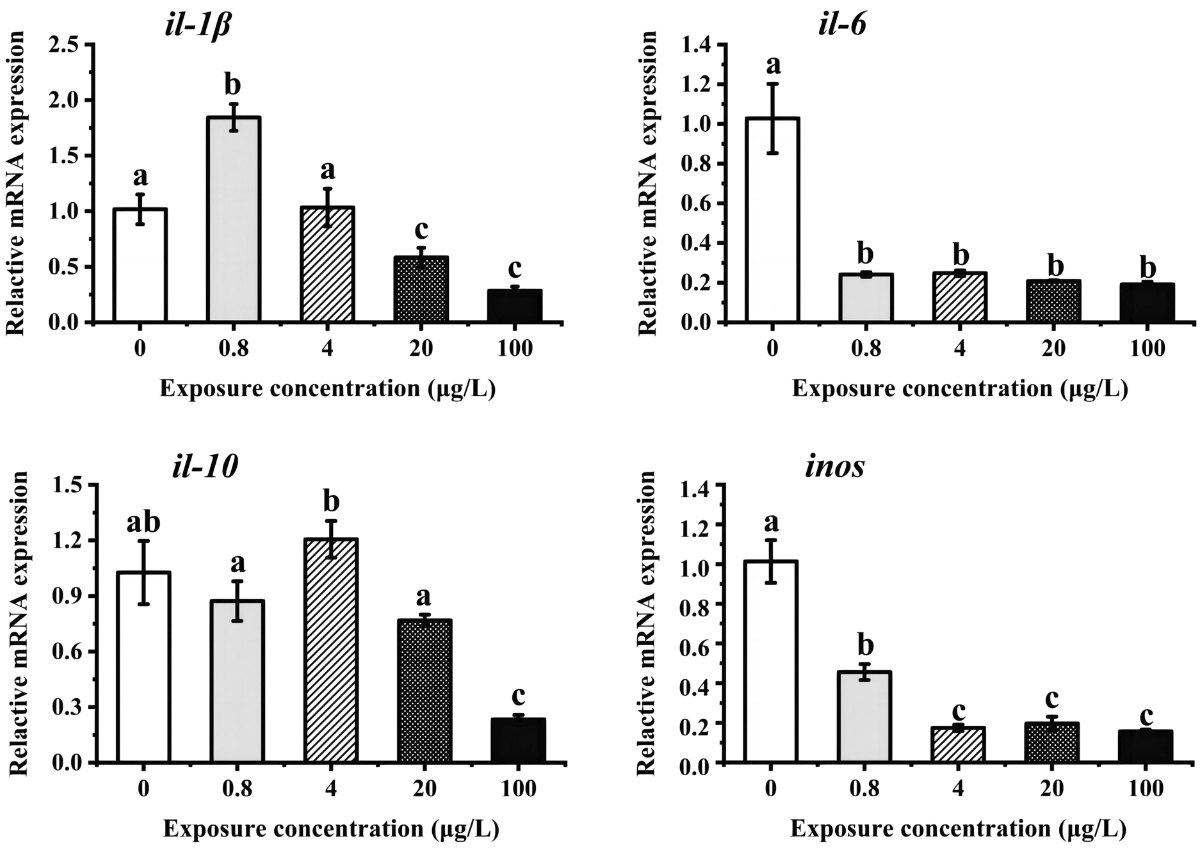

Fig. 5 Effects of lifetime exposure to TCEP on the mRNA levels of *il-1β*, *il-6*, *il-10* and *inos* in the liver of zebrafish at 120 dpf. Different letters indicate significant difference among different treatments groups, Tukey's HSD, $p < 0.05$

the increase of concentrations. Cavitation of the liver is one of the main signs of liver damage, while parenchyma disorganization and pyknotic nucleus might be indications of apoptosis and necrosis in hepatocytes (Erkmen et al. 2017; Chen et al. 2017). Therefore, these hepatic histopathological alterations provide strong evidence for TCEP-induced hepatotoxicity in zebrafish. Similarly, histological changes such as vacuolization and pyknotic nuclei were presented in the liver of juvenile yellow catfish (*Pelteobagrus fulvidraco*) (Hu et al. 2022). Moreover, previous study also pointed out that structural damage of the liver might affect the secretion of IGF, inhibiting the normal growth and development in zebrafish (Wang et al. 2019a, b). Hence, TCEP-induced growth inhibition might be attributed to these severe hepatic histological anomalies.

Oxidative damage in fish is due to excessive intracellular production of reactive oxygen species (ROS) under exposure to environmental pollutants (Jin et al. 2010). High concentrations of ROS can be countered by the action of ROS-scavenging enzymes (Arukwe et al. 2016). SOD, CAT and GPx are essential antioxidant enzymes that play crucial role in scavenging excessive ROS to maintain cellular environment dynamic balance (Lackner 1998). Glutathione (GSH) can eliminate excess ROS directly or through the ascorbate–glutathione cycle, protecting cells against oxidative damage (Polekhina et al. 1999). In the current study, the enhanced GPx activity and GSH content were observed in the liver of zebrafish treated with low doses of TCEP groups (0.8 and 4 μg/L), suggesting a defensive response or physiological adaptation to TCEP-duced oxidative stress (Moalem et al. 1999; Zhang et al. 2004). Similar results have also been reported in *C. mrigala* following exposure to TCEP (Sutha et al. 2020). Conversely, 20 and 100 μg/L TCEP remarkedly reduced the activities of SOD, CAT and GPx, indicating that exposure to high concentrations of TCEP would impair the antioxidant

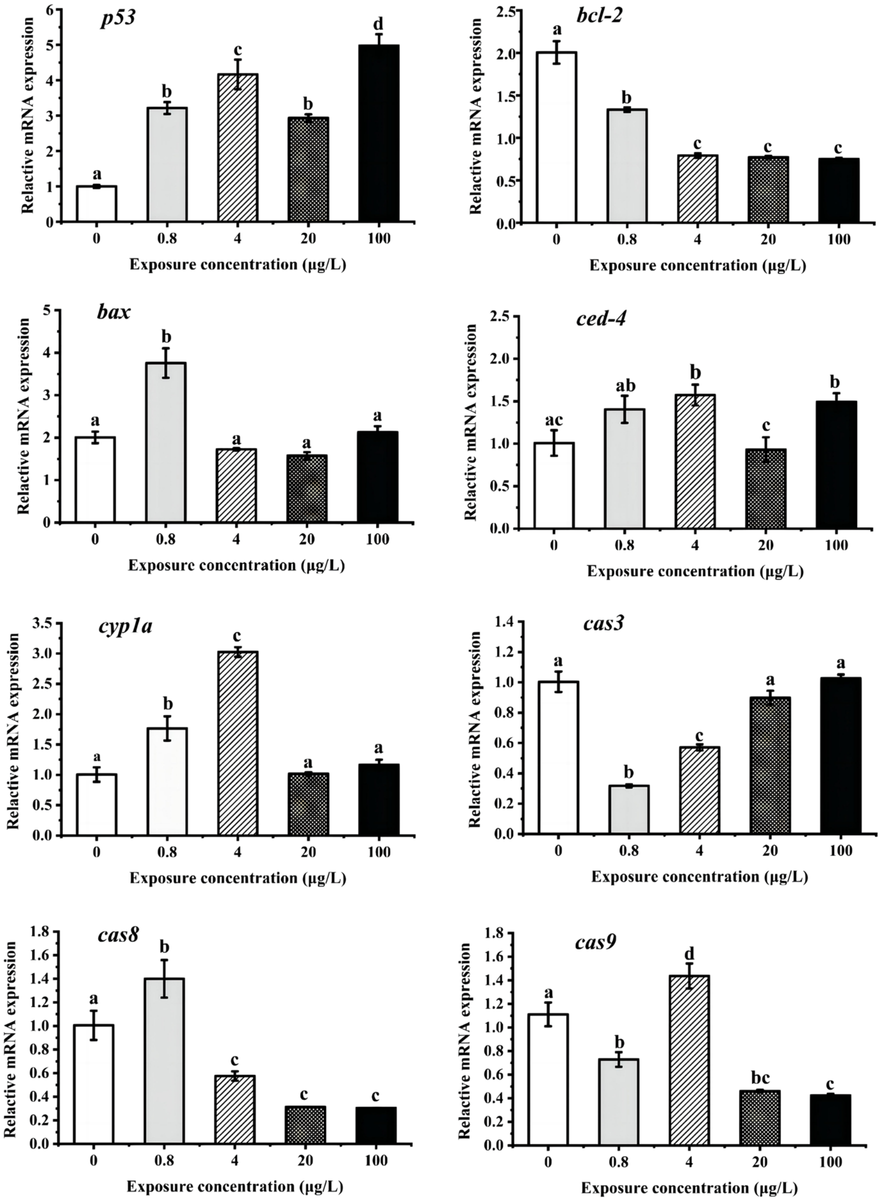

Fig. 6 Effects of lifetime exposure to TCEP exposure on the mRNA levels of *p53*, *bcl-2*, *bax*, *ced-4*, *cyp1a*, *cas3*, *cas8* and *cas9* in the liver of zebrafish at 120 dpf. Different letters indicate significant difference among different treatments groups, Tukey's HSD, $p < 0.05$

defense in the liver of zebrafish. Significant declines in SOD activity were also observed in the liver of zebrafish after a 28-day exposure to 0.5 and 5 μg/L TCEP (Tian et al. 2023). Malondialdehyde (MDA) is the end product of lipid peroxidation in living organisms, and it is usually employed as an indicator of the extent of oxidative damage in cells (Ali et al. 2012). Our results showed a significant increase of MDA content in 4, 20 and 100 μg/L TCEP treatments, demonstrating that elevated formation of ROS induced by TCEP exceeded the antioxidant capacity, and exacerbated hepatocyte oxidative damage.

To further uncover the molecular mechanisms of oxidative stress, we detected the transcriptional regulation of genes involved in the Nrf2-Keap1 pathway. Nrf2 is a crucial nuclear transcription factor and a signal pathway activator highly expressed in the liver, regulating the expressions of downstream antioxidant genes (Shaw et al. 2019). When the balance between ROS production and clearance is disrupted, Keap1 will be inactivated, which blocked the clearance of Nrf2, and ultimately lead to the excessive accumulation and the activation of Nrf2 (Ray et al. 2012). Nrf2 enters the nucleus to combine with antioxidant response elements (ARE) and transcribe a series of antioxidant response element genes, such as *gst*, *homx-1* and *nqo1* in response to oxidative stress (Sule et al. 2022). In the present work, exposure to 0.8 μg/L TCEP significantly elevated the mRNA levels of *nrf2* and its downstream genes (*nqo1* and *homx-1*) in liver, indicating that low concentration of TCEP could induce the antioxidative defense through activating the Nrf2-Keap1 pathway. However, with the increase of TCEP exposure concentrations, mRNA levels of *keap1* were significantly up-regulated, whereas the levels of downstream genes *homx-1* and *gst* were markedly down-regulated in 4, 20 and 100 μg/L TCEP groups. These results implied that high concentration of TCEP suppressed the transcriptional activation of the Nrf2-Keap1 pathway on downstream genes through up-regulating *keap1* expression, ultimately reducing the defense capacity of zebrafish.

Inflammation is a response of the immune system to tissue damage and infection, and hepatic inflammatory disorder can reflect hepatotoxicity induced by environmental pollutants (Wang et al. 2021). Cytokines are critical regulators of inflammation as well as major mediators of immune function (Hermann and Kim 2005). Among them, *il-1β* and *il-6* are two important pro-inflammatory cytokines modulating inflammatory processes (Engelsma et al. 2002; Zanotti et al. 2002). In a recent work, after 28-day TCEP exposure, higher levels of IL-6, IL-1β, and TNF-α were observed in zebrafish livers (Tian et al. 2023). Conversely, in the present study, mRNA levels of both *il-1β* and *il-6* were declined in 4, 20 and 100 μg/L TCEP-treated groups, reflecting the suppressive effect of TCEP on the immune system of zebrafish liver. It was reported that the activation of Nrf2 could negatively regulate pro-inflammatory mediators (Kim et al. 2010; Getachew et al. 2016), thus the down-regulation of inflammatory cytokines after TCEP exposure was possibly ascribed to the activation of Nrf2 in zebrafish liver. The transcription of *inos* can be promoted by interleukins, producing large amounts of toxic NO and regulating the process of inflammatory response (Saha and Pahan 2006). In this work, the mRNA expression of *inos* was significantly down-regulated, possibly contributing to alleviate inflammatory responses. *il-10* is an anti-inflammatory factor playing roles in down-regulating inflammatory response and antagonizing inflammatory mediators (Karan et al. 2016). Our study revealed that the mRNA expression of *il-10* was down-regulated only in the highest concentration exposure group, which might suppress the function of liver immune cells, resulting in an aggravated inflammatory response and liver damage.

Apoptosis is a genetically controlled cell death of self-ordered and can be regulated by multiple genes (Zhao et al. 2009). *p53* is a tumor suppressor gene responsible for mediating the apoptosis process (Calaf et al. 2009). *bcl-2* and *bax* are two members of Bcl-2 family that play critical roles in the regulation of apoptosis. *bcl-2*, an anti-apoptotic gene, prevents the release of cytochrome c from mitochondria (Bernardi et al. 2001). *bax* is a *p53* response gene, inducing the release of cytochrome to promote apoptosis (Cory and Adams 2002). In this study, TCEP exposure elevated the mRNA levels of *p53* and *bax*, while down-regulated the transcription of *bcl-2*, suggesting that TCEP might trigger apoptosis via the p53-Bax pathway in zebrafish liver. Similar to TCEP, triazophos, an organic phosphate ester, promoted apoptosis by transcriptional activation of *p53* and *bax* in zebrafish (Wang et al. 2019a, b). Caspases family is closely related with apoptosis and can be activated by external and internal pathways (McIlwain et al. 2013). Previous studies demonstrated that *caspase-8* (*cas8*) was involved in the extrinsic pathway, while *caspase-9* (*cas9*) participated in the intrinsic pathway of apoptosis, both of which induced apoptosis by activating the downstream target gene *caspase-3* (*cas3*) (D'Arcy 2019; Wang et al. 2023). CED-4 binds with cytochrome c to activate caspase cascade, and finally leads to programmed cell death (Kumar 2007). Though elevated transcriptional levels of *ced-4* were observed in the present work, the transcription of *cas3*, *cas8* and *cas9* mainly exhibited downward tendency, indicating that the caspase-dependent apoptotic pathway might be negatively involved in the TCEP-induced apoptosis in zebrafish. CYP1A is a member of the cytochrome P450 superfamily, and the induction of CYP1A by environmental pollutants might cause apoptosis (Tsuchiya et al. 2005; Özdemir et al. 2018). Previous studies have revealed a positive correlation between the induction of *cyp1a* and cell apoptosis in zebrafish and medaka (Cantrell et al. 1996; Xu et al. 2015). In our study, the transcription of *cyp1a* was only significantly increased in 0.8 and 4 μg/L TCEP exposure groups, suggesting that TCEP at low concentrations might induce apoptosis through the activation of *cyp1a* expression.

Inflammation and apoptosis may lead to irreversible damage and structural changes in liver tissue. In this study, alterations including vacuoles, parenchyma disorganization and pyknotic nucleus were clearly observed in the liver of zebrafish after whole life-cycle exposure to TCEP, which were more severe with the increasing concentrations. Cavitation of the liver is one of the main signs of liver damage, while parenchyma disorganization and pyknotic nucleus might be indications of apoptosis and necrosis in hepatocytes (Erkmen et al. 2017; Chen et al. 2017). Therefore, these hepatic histopathological alterations provide strong evidence for TCEP-induced hepatotoxicity in zebrafish. Similarly, histological changes such as vacuolization and pyknotic nuclei were presented in the liver of juvenile yellow catfish (*Pelteobagrus fulvidraco*) (Hu et al. 2022). Moreover, previous study also pointed out that structural damage of the liver might affect the secretion of IGF, inhibiting the normal growth and development in zebrafish (Wang et al. 2019a, b). Hence, TCEP-induced growth inhibition might be attributed to these severe hepatic histological anomalies.

Conclusion

In summary, our findings suggested that life-cycle exposure to TCEP at environmental relevant concentrations could lead to growth inhibition in zebrafish and exerted significant hepatotoxicity via inducing oxidative stress, inflammatory disorder, apoptosis and histological alterations. These data provide insight into the toxicological effects of TCEP in target organ of fish and highlight the environmental hazard of TCEP in aquatic environments.

Author contributions FH: Writing -Writing - Review & Editing, Investigation, Supervision, Project administration. WL: Conceptualization, Methodology, Validation, Investigation, Writing - original draft & Review, Funding acquisition. HW: Conceptualization, Methodology, Formal analysis, Investigation. HP: Validation, Visualization. JH: Investigation. JD: Validation. WZ: Validation. All the authors revised and approved the ms.

Funding This work was supported by National College Students Innovation and Entrepreneurship Training Program (China, 202310389025).

Data availability Data and materials will be made available on request.

Declarations

Competing interests The authors declare no competing interests.

Ethical approval Our experimental protocols were approved by Laboratory Animals Ethics and Welfare Committee of College of Animal Science, Fujian Agriculture and Forestry University (PZCASFAFU22039).

Conflict 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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