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RESEARCH ARTICLE

Chronic exposure to tris (2-chloroethyl) phosphate (TCEP) induces brain structural and functional changes in zebrafish (*Danio rerio*): A comparative study on the environmental and LC50 concentrations of TCEP

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Abstract
Tris (2-chloroethyl) phosphate (TCEP) is a crucial organophosphorus flame retardant widely used in many industrial and commercial products. Available reports reported that TCEP could cause various toxicological effects on organisms, including humans. Unfortunately, toxicity data for TCEP (particularly on neurotoxicity) on aquatic organisms are lacking. In the present study, *Danio rerio* were exposed to different concentrations of TCEP for 42 days (chronic exposure), and oxidative stress, neurotoxicity, sodium, potassium-adenosine triphosphatase (Na^+, K^+-ATPase) activity, and histopathological changes were evaluated in the brain. The results showed that TCEP (100 and 1500 µg L^{-1}) induced oxidative stress and significantly decreased the activities of antioxidant enzymes (SOD, CAT and GR) in the brain tissue of zebrafish. In contrast, the lipid peroxidation (LPO) level was increased compared to the control group. Exposure to TCEP inhibited the acetylcholinesterase (AChE) and Na^+,K^+-ATPase activities in the brain tissue. Brain histopathology after 42 days of exposure to TCEP showed cytoplasmic vacuolation, inflammatory cell infiltration, degenerated neurons, degenerated purkinje cells and binucleate. Furthermore, TCEP exposure leads to significant changes in dopamine and 5-HT levels in the brain of zebrafish. The data in the present study suggest that high concentrations of TCEP might affect the fish by altering oxidative balance and inducing marked pathological changes in the brain of zebrafish. These findings indicate that chronic exposure to TCEP may cause a neurotoxic effect in zebrafish.

Keywords Organophosphate flame retardants · Antioxidants · AChE · ATPase · Brain tissue · Zebrafish

Introduction

Chlorinated organophosphate flame retardants (Cl-OPFRs) are extensively used in plastics, furniture, floor polishes, daily chemicals, etc. (Lee et al. 2016; Yang et al. 2021) due to their plasticiser and additive properties (Yang et al. 2019). Cl-OPFRs such as tris (2-cholroisopropyl) phosphate (TCPP), tris (1,3-dichloro-2-propyl) phosphate (TDCPP) and tris (2-chloroethyl) phosphate (TCEP) were widely used as alternatives for several toxic brominated flame retardants (Mihajlović and Fries 2012; Li et al. 2019a,b). As a result, these Cl-OPFRs have been detected at high levels in the aquatic environment (He et al. 2017; Yang et al. 2021) and are considered new emerging contaminants (Tang et al. 2018). In aquatic environments, the concentration of Cl-OPFRs has been detected up to 26,000 ng L^{-1} (Qi et al. 2019; Xu et al. 2019).

TCEP, one of the dominant Cl^-OPFRs (Chokwe et al. 2020), is a ubiquitous environmental contaminant due to its overuse and has a high solubility (25 ºC, 7.93 g L^{-1}) in water (Veen and Boer 2012; O'Brien et al. 2015; Arukwe et al. 2016; Lee et al. 2018; Hou et al. 2019; Hao et al. 2020; Yao et al. 2021; Wang et al. 2022). For example, the concentration of TCEP has been detected at 318 ng L^{-1} in groundwater (Marklund et al. 2005), in the range of 259–2406 ng L^{-1} in lakes (Yan et al. 2012), 85±10 ng L^{-1} in rivers (García-López

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et al. 2010), and 99 ng L^{-1} in drinking water (Stackelberg et al. 2004). TCEP is detected in higher concentrations in WWTP effluents due to their resistance to biotransformation and formation from the precursors (Kim et al. 2019; Yang et al. 2021, 2022a, b). Furthermore, TCEP has also been detected in aquatic organisms such as fish and perch (Sundkvist et al. 2010; Ma et al. 2013) and human tissues (Zhao et al. 2021).

TCEP can persist for a long time in the environment (Zhu et al. 2015; Sun et al. 2016a, b; Kim et al. 2017) and may cause adverse effects in aquatic organisms. For example, TCEP reduced the survival and growth rate of catfish (Zhao et al. 2021), thyroxine (T4) levels in zebrafish (Hu et al. 2021), growth and reproduction of protozoans (Hao et al. 2020), developmental phenotypes in zebrafish (Wu et al. 2017), changes in the AChE activity in earthworms (Yang et al. 2018), and behavioural effects in zebrafish (Jarema et al. 2015). In contrast, Li et al. (2020) have reported that TCEP at environmental concentrations promoted the growth rate of *D. magna*. Furthermore, Arukwe et al. (2016) have reported that TCEP exposure did not alter the 11-ketotestosterone (11-KT) levels in juvenile salmon. However, a few authors have reported the neurotoxicity of TCEP on aquatic organisms, especially fish (Behl et al. 2015; Sun et al. 2016a, b; Xu et al. 2017; Li et al. 2019a, b; Hu et al. 2021).

Vertebrate brain tissue is most sensitive to oxidative stress (Li and Li 2020). Most organelles and neurons in brain tissues are delicate and easily damaged by reactive oxygen species (ROS) formation due to antioxidant defence (Sachett et al. 2018; Barros et al. 2020; Leão-Buchir et al. 2021). Antioxidant enzymes such as superoxide dismutase (SOD) and catalase (CAT) play a vital role in oxidative stress in aquatic organisms. Similarly, glutathione reductase (GR) protects proteins, lipids and nucleic acids against oxidative damage (Carvalho et al. 2020). In addition, antioxidant enzymes maintain the redox status of the cell. However, high production of ROS may damage the DNA and proteins and cause lipid peroxidation (LPO). In our previous study (Sutha et al. 2020), the activities of SOD, CAT, GST, GSH and GPx and LPO levels were found to be altered in gill, liver, and kidney tissues of *Cirrhinus mrigala* exposed to TCEP (0.04, 0.2, and 1 mg L^{-1}), which indicates that TCEP may induce oxidative stress in fish. Changes in the antioxidant parameters are widely used as potential biomarkers to assess the toxicity of aquatic pollutants.

Quantification of the enzyme activity can give valuable information on the affected organs/tissues. In vertebrates, the enzyme acetylcholinesterase (AChE) is essential for the normal functioning of the neuromuscular system (Mayer et al. 1992). The alteration of acetylcholinesterase (AChE) activity is commonly used as a biomarker to assess the toxicity of organophosphorus compounds (Shi et al. 2021). Due to their organophosphorus backbone, flame retardants may induce neurotoxicity (Jarema et al. 2015). TCEP also inhibits the acetylcholinesterase (AChE) activity in aquatic organisms (World Health Organization 1998; Li et al. 2019a, b). Likewise, sodium and potassium adenosine triphosphatase (Na^+,K^+-ATPase) play a vital role in the transport of the ions across cell membranes. Changes in the Na^+,K^+-ATPase activities can be used to assess the physiological integrity of aquatic organisms exposed to aquatic toxicants (Agrahari and Gopal 2008; Ajima et al. 2021). Furthermore, during stress conditions, neurotransmitters such as dopamine (DA) and serotonin (5-HT) are released from the brain and play a vital role in mediating stress in fish (Backström and Winberg 2017). Assessment of these neurotransmitter levels indicates the toxicity of aquatic pollutants in the central nervous system (Da Rochaa et al. 2019). Similarly, histopathological anomalies can be used to assess the impact of waterborne chemicals on major organs (Ramesh et al. 2018). TCEP-induced biochemical changes and their consequences are well addressed in several organ-specific studies, including liver, gill, brain, and kidney at 0.04, 0.2, or 1 mg L^{-1}(Arukwe et al. 2016; Sutha et al. 2020), but its pathological manifestation in the brain remains elusive.

This work was intended to assess the chronic effects of TCEP on oxidative stress, acetylcholinesterase activity, and histological biomarkers in the brain tissue of zebrafish (*Danio rerio*).

Materials and methods

Ethical statement

The present study was conducted per the OECD guidelines for maintaining and handling fish (OECD/OCDE 2013) and the Committee for the Control and Supervision of Experiments on Animals (CPCSEA).

Chemicals

Tris (2-chloroethyl) phosphate (TCEP) was purchased from Sigma-Aldrich (degree of purity, 97%) (CAS number: 115-96-8) and the stock solution was prepared by dissolving 1 g L^{-1} of tris (2-chloroethyl) phosphate in Milli-Q water. All other chemicals and reagents were procured from S.D Fine Chemicals, Chennai, India.

Maintenance of zebrafish

AB strain zebrafish (*Danio rerio*) weighing 0.6 ± 0.2 g and an average length of 2.7 ± 0.4 cm were obtained from Siraco Fish Farm in Salem, Tamil Nadu. Fish were maintained in a tank of 1000 L capacity with dechlorinated water

(temperature 28 ± 1 ºC, pH 7.5 ± 0.5, dissolved oxygen 6.4 ± 0.4, total alkalinity 18.2 ± 1.5, total hardness 18. ± 1.5 with a 14:10 h (light: dark photo-cycle) and fed with commercial fish feed.

Chronic exposure

After fifteen days of acclimation, the fish were divided into three groups and maintained in a glass aquarium containing 20 L of water. Each group consisted of 20 fish. Two different concentrations of TCEP (100 and 1500 µg L^{-1}) were selected and introduced into the respective aquariums. The lowest concentration of TCEP (100 µg L^{-1}) was selected based on the reported environmental concentrations (Kawagoshi et al. 1999). In ecotoxicological studies, the LC50 value is commonly used to test the toxicity of chemicals rather than environmentally relevant levels. In this practice, animals are exposed to higher concentrations than the ecologically relevant concentrations. Hence, in the present study, a high concentration of TCEP (1500 µg L^{-1}) was selected based on the LC50 value (Sutha et al. 2022). For each concentration and control group, three replicates were maintained. After 14, 28 and 42 days of exposure, the brain tissue was dissected, washed in saline solution, and stored at -80º C for biochemical analysis.

Tissue preparation

The stored brain tissue from control and TCEP (100 and 1500 µg L^{-1}) exposed fish was homogenised with PBS buffer (pH 7.0), using a mechanical tissue homogeniser coated with Teflon. The homogenate samples were centrifuged at 15,000 rpm for 15 min, and the supernatant was used for the enzymological assay. For pathological observations, additional brain tissues were fixed in 10% neutral buffered formalin.

Antioxidant enzyme analysis

SOD activity in the brain tissue was determined following the method of Marklund and Marklund (1974). To 50 µl enzyme source, Tris-HCl buffer (50 mM, pH 8.4), EDTA (1 mM) and pyrogallol (2.64 mM) were added, and the absorbance was measured at 420 nm for 5 min in a Spectrophotometer. The enzyme activity was expressed as Units/mg protein. Catalase (CAT) activity was determined following the Aebi method (1984). The decomposition of H_2O_2 was measured at 240 nm, and the enzyme activity was expressed as micromoles of H_2O_2 utilised per minute per milligram of protein. The activity of glutathione reductase (GR) was estimated using David and Richard's (1983) method. The assay mixture consisted of phosphate buffer (1.0 ml), EDTA (0.1 mL), sodium azide (0.1 mL), oxidised glutathione (0.1 mL) and tissue homogenate (0.1 mL). The absorbance was read at 340 nm in a Spectrophotometer, and the enzyme activity was expressed as µM of NADPH/oxidised/min/mg protein.

To determine LPO level, thiobarbituric acid (TBA) reacting species for malondialdehyde (MDA) concentrations were measured using the method of Devasagayam and Terachand (1987). The assay consisted of Tris HCL buffer (0.5 mL), KH_2PO_4 (10 mM) (0.15 mL), distilled water (0.25 mL) and tissue homogenate (0.1 µL). After incubation (37 °C for 20 mins), TCA (1.0 µL) and TBA (0.75 µL) were added, and the formation of a pink-coloured complex was read at 532 nm. The LPO level was expressed as nmol of MDA formed /mg protein. Protein concentration in the brain tissue was assayed using bovine serum albumin as a standard described by Lowry et al. (1951).

Acetylcholinesterase activity quantification

AChE activity in the brain tissue was estimated using the method of Ellman et al. (1961). 0.1 mL of 0.015 M acetylthiocholine iodide and 0.1 mL of 0.01 M DTNB (5,5-dithiobis-2-dinitrobenzoic acid) were used as a substrate along with 2.55 mL of buffer (0.1 M Phosphate buffer) and 0.2 mL of tissue homogenate. The AChE activity was expressed as µ moles/min/mg protein.

Na^+/K^+-ATPase activity

Na^+, K^+-ATPase activity was estimated as described by Shiosaka et al. (1971). Tissue extract and the assay mixture (0.3 mL of Tri-HCl buffer (pH 7.5), 0.1 mL of 0.02 M ATP, 0.1 mL of 100 mM NaCl and 0.1 mL of KCl solution) were incubated at 37 °C for 15 min and 2.00 mL of 5% TCA was added and kept at 4 °C for 30 min. After centrifugation, 1 mL of ammonium molybdate and 0.4 mL of ANSA (8-Anilino-1-naphthalene-sulfonic acid) reagent were added, and the absorbance was read at 680 nm. The enzyme activity was expressed as µg/h/g.

Neurotransmitters levels

The concentrations of dopamine (DA) and serotonin (5HT) were measured using enzyme-linked immunosorbent assay (ELISA) kits (Kings Lab, India). The thawing process of the brain samples was carried out on the ice to ensure that the ambient temperature was not higher than 4 °C. Samples were weighed, homogenised in hydrochloric acid buffer, centrifuged at 4000 g for 10 min at 4 °C and the supernatant was retained and stored at -80 °C. The supernatant was used for assays according to the manufacturer's instructions.

Histological examination

The formalin-fixed brain tissue was dehydrated in ascending grades of alcohol, cleared with xylene, embedded in paraffin

wax, and sectioned at a thickness of 5 µm. Sections were stained with haematoxylin and eosin stains and observed under a light microscope (Aksm et al. 2020).

Statistical Analysis

The obtained values were expressed as mean ± S.E and analysed statistically by SPSS 19.0 software. The significant differences between the control and TCEP-treated groups were tested using one-way-ANOVA (analysis of variance) followed by Duncan's multiple range test (DMRT). The significant difference was analysed statistically at $p < 0.05$.

Results

We observed general stress-related behavioural changes (rapid swimming, movement around the wall of the tank, and spending time at the bottom) in the highest (1500 µg L^{-1}) TCEP-exposed group. The observed changes can be due to the direct neurotoxicity of TCEP or alterations in AChE activity.

Antioxidant activity induced by TCEP

SOD and CAT activity was significantly ($p < 0.05$) decreased in the brain tissue of fish exposed to high concentration (1500 µg L^{-1}) of TCEP (Fig. 1A, B). However, a significant statistical decrease ($p < 0.05$) was found in SOD and CAT activity only after 42 days of exposure to low concentration (100 µg L^{-1}) of TCEP (Fig. 1A, B). We found that TCEP caused a significant ($p < 0.05$) decrease in GR activity in brain tissue (Fig. 1C) in both treatments (except on the 14th day at 100 µg L^{-1} of TCEP-treated groups) (Fig. 1C). In the present study, a dose-dependent decrease was observed in both concentrations.

Oxidative damage induced by TCEP

The MDA content in the brain of fish at both concentrations was significantly increased when compared with the control group ($p < 0.05$) (Fig. 1D). On the 14th day, there was no significant difference in MDA content between the 100 µg L^{-1} of the TCEP-treated group and the control group.

Neurotoxicity induced by TCEP

Exposure of fish to TCEP (100 and 1500 µg L^{-1}) resulted in an inhibition of AChE activity compared to that of the control group (Fig. 2A). The AChE activity was inhibited significantly ($p < 0.05$) in treated groups, except for the 100 µg L^{-1} dose of TCEP on the 14th day. A concentration-related

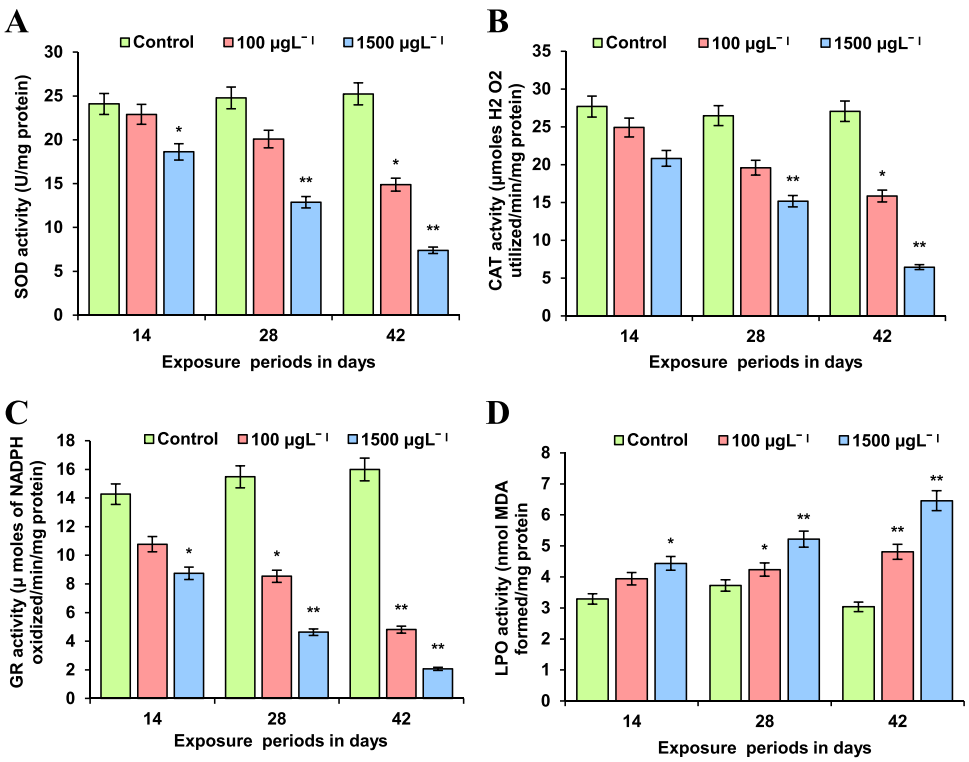

Fig. 1 Activities of (**A**) superoxide dismutase (SOD, U/mg protein), (**B**) catalase (CAT, µ moles H_2O_2 utilized/min/mg protein), (**C**) glutathione reductase (GR, µmoles of NADPH oxidized/min/mg protein) and (**D**) lipid peroxidase (LPO, nmole of MDA formed /mg protein) in the brain of *Danio rerio* exposed to tris (2-chloroethyl) phosphate (TCEP). The data are presented as mean ± SE. One-way ANOVA with Duncan's multiple range test was used; * indicates $p < 0.05$ and ** indicates $p < 0.01$

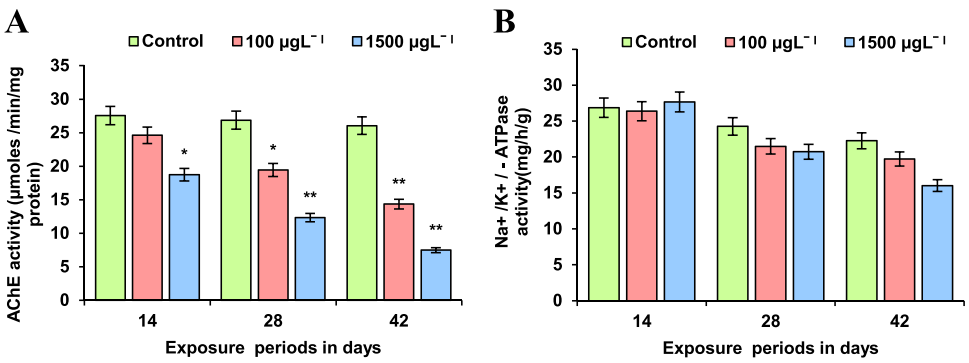

Fig. 2 Activities of (**A**) acetylcholinesterase (AChE, µmoles/min/mgprotein) and (**B**) sodium/potassium adenosine triphosphate (Na^+,$K^{\pm}$ ATPase, mg/h/g) in the brain of *Danio rerio* exposed to tris (2-chloroethyl) phosphate (TCEP). The data are presented as mean ± SE. One-way ANOVA with Duncan's multiple range test was used; * indicates $p < 0.05$ and ** indicates $p < 0.01$

inhibition of AChE activity was observed in both experimental groups.

Na^+, K^+-ATPase activity induced by TCEP

In the TCEP-exposed groups, no significant difference in Na^+,K^+-ATPase activity was found after 42 days of exposure at both concentrations (100 and 1500 µg L^{-1}) (Fig. 2B).

Neurotransmitter concentration induced by TCEP

The concentrations of DA and 5-HT (except on 14 and 28th day) levels were significantly increased in the brain of zebrafish treated with 100 and 1500 µg L^{-1} of TCEP, compared with control groups (Fig. 3A and B).

Histopathological alteration induced by TCEP

Brain tissues from the control group showed typical normal histological structures (Fig. 4A). Compared to the control group, TCEP-treated groups showed significant histopathological lesions such as cytoplasmic vacuolation, inflammatory cell infiltration, degenerated neurons, degenerated Purkinje and binucleate after 42 days (Fig. 4B, C). The severity of the histopathological changes was higher in fish exposed to the highest concentrations (1500 µg L^{-1}) of TCEP.

Discussion

TCEP, a well-known endocrine disruptor in the aquatic environment causes adverse health issues, and ecological and biological risks (Yang et al. 2022a, b; Macedo et al. 2023). The brain has high oxygen consumption, moderate antioxidant defence, and lipid-rich properties and is also susceptible to oxidative stress (Halliwell 2006; Wu et al. 2019). Therefore, oxidative stress is the primary reason for brain injury (Liu et al. 2017; Sugiyama et al. 2018). The formation of reactive oxygen species (ROS) in fish species exposed to aquatic pollutants can be prevented by antioxidant enzymes such as SOD, CAT and GR. Alterations of these enzyme activities can be used to determine the oxidative stress caused by the toxicants (Paravani et al. 2019; Qiao et al. 2019). SOD plays a vital role in maintaining the level of active electron transfer chain and free radical chain reaction processes (Capolupo et al. 2016). The cellular antioxidant mechanism used for removing and degrading H_2O_2 into H_2O and O_2 in vivo is one of the most crucial enzyme systems, CAT (Espín et al. 2014). In the antioxidant defence system, GR is an auxiliary enzyme

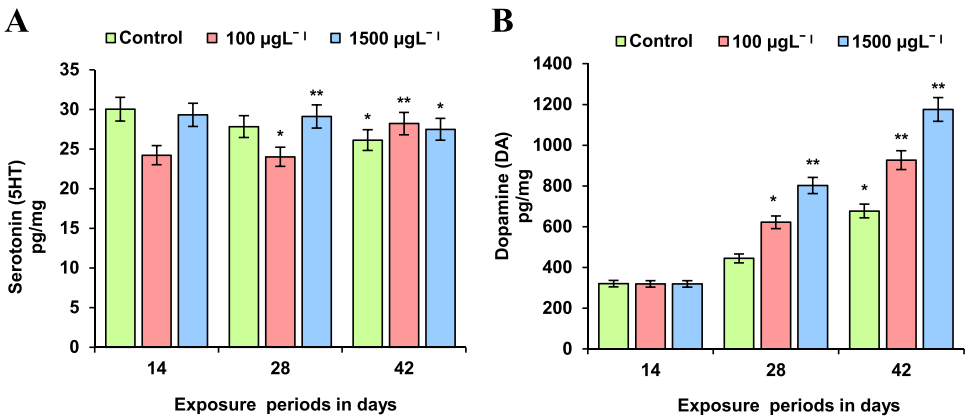

Fig. 3 (**A**) Serotonin (5HT) and (**B**) Dopamine (DA) contents in the brains zebrafish exposed to tris (2-chloroethyl) phosphate (TCEP) for 42 days. The data are presented as mean ± SE. One-way ANOVA with Duncan's multiple range test was used; * indicates $p < 0.05$ and ** indicates $p < 0.01$

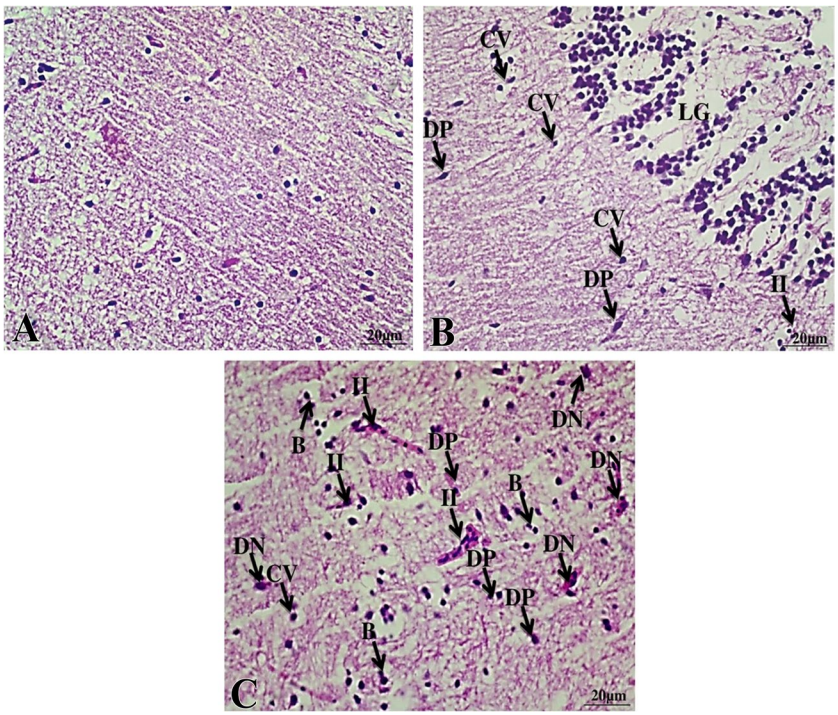

Fig. 4 Histopthology of brain of zebrafish exposed to tris (2-chloroethyl) phosphate. **A**. Control, **B**. 100 μg L^{-1}, **C**. 1500 μg L^{-1} of TCEP. CV—cytoplasmic vacuolation, II—inflammatory cell infiltration, DN—degenerated neurons, DP-degenerated purkinje, B-binucleate. Scale bar—20 μm

used to reduce oxidised glutathione (GSSG) to its active form, reduced glutathione (GSH) (Lushchak 2014). The lower values of SOD and CAT in the brain tissue of fish exposed to TCEP (1500 μg L^{-1}) might be due to oxidative stress and the depletion of antioxidant enzymes in brain tissue (Issac et al. 2021; Ran et al. 2021). Similar to our study, a decrease in CAT and SOD activities has been reported in clams exposed to tributyl phosphate (TBP) and tris (2-butoxyethyl) phosphate (TBEP) (Yan et al. 2017). Furthermore, TCEP exposure also causes inhibition of the transcription of antioxidant defence genes in adult zebrafish (Hu et al. 2021).

In the present study, a significant decrease in SOD, CAT and GR activity in the brain of fish exposed to TCEP (100 and 1500 μg L^{-1}) indicated that TCEP induced oxidative stress through the generation of ROS and damaged the antioxidant defence system of fish. The significant decrease in SOD and CAT activity may be a protection mechanism against the stress caused by the TCEP (Issac et al. 2021; Ran et al. 2021). Alterations of SOD, CAT, and GR activity in the brain tissue of *D. rerio* exposed to TCEP may be due to the failure of these enzymes to protect against the damaging action of hydrogen peroxide and hydroxyl radical (Sutha et al. 2022). The level of ROS generally increased in organisms exposed to OPFRs (Wang et al. 2019a, b). The present study observed a dose-dependent decrease in both experimental groups. The significant reduction of SOD activity in higher concentrations may be due to excess production of ROS due to TCEP toxicity. Likewise, the significant decrease in CAT activity might have resulted from its inactivation by the superoxide radical triggered by TCEP exposure. Furthermore, the observed decrease in SOD and CAT activity in low concentrations indicates prolonged exposure to TCEP might have damaged the antioxidant defence system of fish.

It has been reported that the extreme production of ROS in organisms will increase LPO levels (Wu et al. 2015). The assessment of end-product malondialdehyde (MDA) is commonly used to evaluate lipid peroxidation (Lushchak 2011). The elevation of MDA levels indicates oxidative damage to cell membranes. The significant increase in MDA content in the TCEP-exposed group indicates excessive production of ROS facilitated by TCEP. An increase in LPO levels suggests the potential of toxicants to induce a redox imbalance, which results in cellular damage (Leão-Buchir et al. 2021). In this study, liver tissue necrosis was noticed in the TCEP-exposed groups, indicating the oxidative stress caused by TCEP. Higher levels of MDA have been reported in fish species treated with organophosphate compounds (Arukwe et al. 2016; Sutha et al. 2020), and this reveals that TCEP can generate free radicals and act on the lipid profile. An increase in lipid peroxidation in TCEP-exposed salmon fish may be due to the high expression of antioxidant enzyme genes (Arukwe et al. 2016). Peng et al. (2023) reported that the mRNA expression of antioxidant-related genes in TCEP-treated zebrafish may be due to activation of the Nrf2-Keap1 pathway to protect the oxidative stress caused by TCEP. These findings suggest that chronic exposure to TCEP could cause physiological effects by disturbing the gene expression levels of the antioxidant enzyme.

AChE is a key nervous system enzyme responsible for hydrolysing acetylcholine into choline and acetic acid (O'Brien 1967). Inhibition of AChE activity is widely used to indicate exposure and effects. Inhibition of AChE activity has been reported in medaka larvae exposed to TPHP (Sun et al. 2016a, b) and in the brain tissue of Chinese rare minnows exposed to TDCPP (Yuan et al. 2016). Similarly, Shi et al. (2018) reported inhibition of AChE activity in zebrafish exposed to OPFRs. Inhibition of AChE activity in the brain tissue of fish exposed to TCEP might have resulted from the accumulation of acetylcholine in the brain due to TCEP toxicity. Accumulation of TCEP has been reported in the brain tissue of zebrafish (Wang et al. 2017). Generally, OPFRs may cause tissue damage and inhibit neurotransmitter transmission, which results in neurotoxicity (Yao et al. 2021). The potential neurotoxicity of TCEP has also been reported in many organisms (Sun et al. 2016b; Yang et al. 2018). Furthermore, the inhibition of AChE may also be due to the down-regulation of the AChE coding genes, induced by TCEP (Yang et al. 2018).

Accumulating acetylcholine at the cholinergic synapses may cause adverse effects such as behavioural and physiological abnormalities (Tilton et al. 2011). A high accumulation of OPFRs has been detected in the brain tissue of aquatic organisms (Wang et al. 2016) and in *Cyprinus carpio* exposed to organophosphorus flame retardants (OPFRs) (Tang et al. 2019). Triphenyl phosphate-induced oxidative stress and neurotoxicity in *Labeo rohita* (Umamaheswari et al. 2021). Likewise, hexabromobenzene and pentabromobenzene induced oxidative stress and neurotoxicity in zebrafish (Chen et al. 2021). ROS could inhibit AChE effects on neurotransmission in cholinergic synapses (Chen et al. 2021). Consequently, in this study, chronic exposure to TCEP can induce oxidative stress responses in fish. Oxidative stress negatively affects the nervous system and physiological development (Kim et al. 2021).

Ion-dependent ATPases are essential in intracellular functions and are widely used as biomarkers in toxicological studies (Agrahari and Gopal 2008; Ajima et al. 2021). In aquatic organisms, the transport of Na^+ and K^+ ions across the cell membrane is usually mediated by Na^+,K^+-ATPase enzymes (Li et al. 2010; Ajima et al. 2021). Na^+,K^+-ATPase are critical transmembrane enzymes that regulate the central nervous system's intracellular pH, cell volume, and calcium ion concentration (Adefegha et al. 2016). Na^+,K^+-ATPase are potential biomarkers of oxidative stress (lipid peroxidation or protein carbonylation) in organisms (zebrafish and rats) under chemical affront (Cassol et al. 2022; Gupta et al. 2023). In this study, Na^+,K^+-ATPase activity in fish brains was inhibited upon exposure to TCEP. The inhibition of Na^+,K^+-ATPase activity may be due to the direct toxicity of TCEP on the enzyme. Inhibition of Na^+,K^+-ATPase indicates neuronal damage in the brain tissue of zebrafish under TCEP insult. Excessive production of ROS due to toxicant stress may also cause inhibition of Na^+,K^+-ATPase activity (Adefegha et al. 2016; Baldissera et al. 2019). Furthermore, toxicants-induced excessive LPO levels may alter the integrity of the plasma membrane, leading to inhibition of Na^+,K^+-ATPase (Oruc et al. 2002). According to the above study, perturbations in the ATPase system and disturbances in the movement of Na^+, K^+ ions due to TCEP toxicity may cause the inhibition of Na^+,K^+-ATPase activity. It was reported that inhibition of Na^+,K^+-ATPase and elevation of lipid oxidation levels in the brain resulted in traumatic brain injury in the mammalian model (Silva et al. 2011). We also noticed a reduction in Na^+,K^+-ATPase and increased LPO levels in zebrafish treated with TCEP. Notably, the AChE activity was also inhibited. The responses of these biomarkers indicate that TCEP could affect the CNS and cause neurotoxicity. Further, Na^+,K^+-ATPase activities are potential biomarkers for oxidative stress (LPO), ion-homeostasis, and neurotoxicity in aquatic models exposed to emerging chemicals.

Neurotransmitters such as dopamine (DA) and serotonin (5HT) play an important role in the regulation and action of neurons (Tort 2011). Dopamine is a monoamine neurotransmitter primarily involved in neurochemical and hormonal actions in vertebrates (Soares 2017). Similarly, serotonin (5-hydroxytryptamine; 5-HT) is also involved in physiological and behavioural functions (Abreu et al. 2018). Aquatic pollutants may interfere with the central nervous system and disturb brain neurotransmitters (Gaworecki and Klaine 2008; Yu et al. 2021; Huang et al. 2022). In the present study, the significant increase in dopamine levels in the brain of zebrafish exposed to TCEP indicates the neurotoxicity of TCEP. Previous authors have reported that dopamine may modulate the neurotoxic effects of aquatic pollutants (Sousa and Nunes 2020). Furthermore, TCEP may affect the synthesis of dopamine through oxidative damage to dopamine neurons (Wang et al. 2019a, b). Serotonin (5-HT) generally acts as a neurotransmitter and neuromodulator, maintaining homeostasis under stressful conditions (Thilagam et al. 2014). The serotonin system in fish is also regulated by chemical substances (Prasad et al. 2015). In the present study, the observed increase in serotonin (5-HT) levels in the brains of zebrafish treated with TCEP indicates protection against TCEP toxicity or direct action of the TCEP on the brain. The observed decrease in serotonin (5-HT) level indicates neuronal dysfunction due to TCEP toxicity. Many neurotransmitters may be released from the brain during stressful conditions to avoid or cope with the stressful conditions (Sousa and Nunes 2020). We concluded that TCEP at 100 and 1500 μg L^{-1} induced neurotoxicity in fish.

In the present study, TCEP induced cytoplasmic vacuolation, inflammatory cell infiltration, degenerated neurons, degenerated Purkinje and binucleate in the brain of zebrafish. Similarly, pyknosis of the nucleolus and cavitation of cytoplasm have been reported in the brain and spinal cord

of zebrafish exposed to TCPP (Xia et al. 2021). The pathological changes in the brain tissue caused by toxicants may alter the behavioural and physiological functions of the fish (Yuan et al. 2015). These changes could further affect the individual's health and, ultimately, the population and ecosystem. Histopathological anomalies have also been reported in the gill, liver, and kidney tissues of fish *Cirrhinus mrigala* exposed to TCEP (Sutha et al. 2020) and in the gill of catfish *Pelteobagrus fulvidraco* (Zhao et al. 2021).

Conclusion

Findings reported in the present study revealed that chronic exposure to high concentrations (1500 µg L^{-1}) of TCEP caused oxidative stress, neurotoxicity, and damage to the brain tissue of zebrafish. The altered acetylcholinesterase, and Na^+,K^+-ATPase activities were strongly correlated with the increased oxidative stress in zebrafish brain. These results indicate that TCEP is neurotoxic to fish, and the other parameters may provide valuable information for assessing TCEP toxicity in aquatic organisms. Neurotoxicity could affect the organism's normal physiological, behavioural (swimming, mating, eating), and biochemical activities leading to an ecological imbalance in the ecosystem or the aquatic community. Hence, strict regulations are warranted on discharging Cl-OPFRs (TCEP) in the water systems. The biomarkers studied in this study could help evaluate the potential toxicity of Cl-OPFRs on non-target organisms.

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Authors contributions **Jesudass Sutha:** Conceptual framework, Experiments, Investigation, Data curation, Original manuscript writing.

Murugesh Gayathri: Resources, Experiments, Investigation, Formal data analysis.

Mathan Ramesh: Conceptual framework, Supervision, Resources, Writing—review and editing.

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Data availability All data generated or analysed during this study are included in this article.

Declarations

Ethical approval The present study was conducted as per the guidelines of the OECD for maintaining and handling fish (OECD/OCDE 2013) and the Committee for the Control and Supervision of Experiments on Animals (CPCSEA).

Consent to participate Not applicable.

Consent for publication Not applicable.

Competing interests The authors declare that they have no competing interests.

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