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Chronic neurotoxicity of Tetrabromobisphenol A: Induction of oxidative stress and damage to neurons in *Caenorhabditis elegans*

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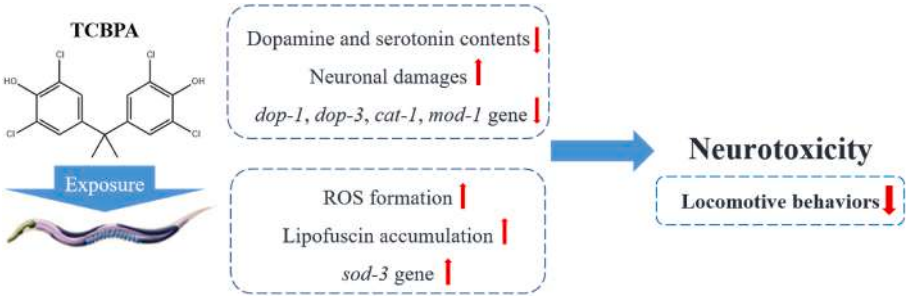
HIGHLIGHTS

- Low-dose TCBPA caused neurotoxicity on locomotive behaviors in *C. elegans*.
- TCBPA induced oxidative damage, accompanied by upregulating *sod-3* gene.
- Ascorbic acid attenuated the damage of mitochondrial dysfunction.
- TCBPA at 100 μg/L triggered damages in dopaminergic and serotonergic neurons.

GRAPHICAL ABSTRACT



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ABSTRACT

Tetrachlorobisphenol A (TCBPA) has been used as an alternative flame retardant in various fields. However, the long-term effects of TCBPA on the nervous system remain unclear. Thus, *Caenorhabditis elegans* (L4 larvae) were selected as a model animal to investigate the neurotoxic effects and underlying mechanisms after 10 d of TCBPA exposure. Exposure to TCBPA (0.01–100 μg/L) decreased locomotive behavior in a concentration-dependent manner. In addition, reactive oxygen species (ROS) formation and lipofuscin accumulation were significantly increased, and the expression of *sod-3* was upregulated in the exposed nematodes, indicating that TCBPA exposure induced oxidative damage. Furthermore, 100 μg/L TCBPA exposure caused a reduction in dopamine and serotonin levels, and damage in dopaminergic and serotoninergic neurons, which was further confirmed by the downregulated expression of related genes (e.g., *dop-1*, *dop-3*, *cat-1*, and *mod-1*). Molecular docking analysis demonstrated the potential of TCBPA to bind to the neurotransmitter receptor proteins DOP-1, DOP-3, and MOD-1. These results indicate that chronic exposure to TCBPA induces neurotoxic effects on locomotive behavior, which is associated with oxidative stress and damage to dopaminergic and serotoninergic neurons.

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1. Introduction

Tetrabromobisphenol A (TBBPA), one of the currently occurring traditional brominated flame retardants (BFRs), is widely used for electronic equipment, plastics, building materials, and textiles (Covaci et al., 2011). Owing to its large demand in the global market, TBBPA yield accounts for 60% of the total brominated flame retardants and is expected to increase at an annual rate of 3.8% (Liu et al., 2016). Having a similar chemical structure, TCBPA is used as an alternative flame retardant to TBBPA (Covaci et al., 2009; Ye et al., 2016). Because of its extensive utilization, TCBPA is released into various environmental matrices, such as sediment, soil, water, and house dust. The concentration of TCBPA in wastewater has been reported to be 2 μg/L (Fukazawa et al., 2001). The content of TCBPA in river sediment is 542.6 ng/g dry weight (Yuan et al., 2010). Notably, TCBPA was detected in the human body at a concentration of 1.53 ng/g (Chen et al., 2016). Owing to its high lipophilicity and persistence, TCBPA is subsequently absorbed by wildlife or human beings through air inhalation, dermal contact, and diet, and accumulates in the human body and other organisms, raising concerns about potential health risks (Liu et al., 2016). Therefore, it is important to investigate the toxicity of TCBPA.

The toxic effects of TCBPA on growth and development (Liu et al., 2023), and the hepatic (Jia et al., 2022), endocrine (Wang et al., 2021), and reproductive system (Lei et al., 2024) have been reported. A previous study revealed that TCBPA inhibits the neural differentiation of mouse embryonic stem cells (Yin et al., 2018). Exposure to TBBPA has been shown to result in abnormal swimming behavior in *Danio rerio* (Liu et al., 2023). The expression of more neuro-related genes has been demonstrated to be altered by TCBPA than by TBBPA, indicating that it may not be a safe alternative to TBBPA (Liang et al., 2019). Although neurotoxicity induced by TCBPA has been characterized, relatively little is known about its toxic effects and underlying mechanisms.

Caenorhabditis elegans is highly suitable for assessing behavioral effects and neurodegeneration because of its simple nervous system, which contains the same neurotransmission pathways as those in humans (Bargmann, 1998; Yu et al., 2022). Previous studies have shown that locomotive behavior is associated with neurological systems and related pathways in nematodes (Negga et al., 2012; Li et al., 2017). In addition, toxins spread easily in the nervous system owing to the lack of a functional blood-brain barrier in *C. elegans* (Shaye and Greenwald, 2011). In the present study, *C. elegans* were exposed to TCBPA at environmentally relevant concentrations to investigate its neurotoxicity and potential mechanisms through a multi-level approach comprising locomotive behavior, oxidative stress, and neurotransmitters. These results will contribute to a deeper understanding of the neurotoxicity of TCBPA and the need to take action to prevent the risk of TCBPA to the environment and human beings.

2. Materials and methods

2.1. Chemicals and experimental solutions

2,2′,6,6′-Tetrachlorobisphenol A (TCBPA, CAS NO: 79-95-8, purity >98.0%) was purchased from J & K Scientific (Beijing, China). Dimethyl sulfoxide (DMSO, purity >99.0%), 5′,6′-chloromethyl-2′,7′ dichlorodihydrofluorescein diacetate (CM-H_2DCFDA), L-ascorbic acid (purity >99.5%), and other chemicals were purchased from Aladdin Corporation (Shanghai, China). The final DMSO concentration was no more than 0.01%.

2.2. Culture of C. elegans and TCBPA chronic exposure

Wild-type N2 and transgenic strains were obtained from the *Caenorhabditis* Genetics Center. The transgenic strains included BZ555 [*dat-1p::GFP*] and GR1366 [*tph-1:GFP + rol-6 (su1006)*]. Nematodes were cultured on nematode growth medium agar plates, seeded with adequate *Escherichia coli OP50* and maintained at 20 °C in a sterile incubator. Before exposure, the adult nematodes were synchronized by treatment with sodium hypochlorite and sodium hydroxide. The eggs were incubated with adequate food for 48 h to obtain the experimental nematodes (Williams and Dusenbery, 1990). The exposure concentration of TCBPA was set at 0.1–100 μg/L based on the concentrations of TCBPA in the environment (Fukazawa et al., 2001). Synchronized nematodes were exposed to different experimental concentrations of TCBPA in 6-well plates for 10 d at 20 °C in an incubator (Boxun, China) in the presence of food (Zhou et al., 2016). An oviposition inhibitor was added to reduce progeny interference. Each plate contained approximately 300 nematodes. After chronic exposure, *C. elegans* were collected and washed with K medium for subsequent assessment.

2.3. Measurement of lethality and locomotive behaviors

Lethality was assayed using the total number of living nematodes as previously described (Williams and Dusenbery, 1990). Following chronic exposure to TCBPA, the inactive nematodes were scored under a dissecting microscope. *Caenorhabditis elegans* were judged dead if they could not respond to the stimulus of a metal wire. Locomotive behavior was assayed using head thrashes and body bends. A head thrash is defined as one swing of the nematode body, and a body bend refers to crawling of one wavelength (Tsalik and Hobert, 2003). After exposure, at least 20 nematodes from each concentration assay were placed on fresh nematode growth medium agar without food. After a minute of recovery, the frequency of locomotive behavior was counted within 1 min (head thrashes) or 20 s (body bends) by using a dissection microscope. Three independent experiments were performed.

2.4. Measurement of oxidative stress

Oxidative stress was evaluated based on the generation of ROS and accumulation of lipofuscin in *C. elegans*. To measure ROS generation, 40 nematodes per concentration were labeled with 1 μM CM-H_2DCFDA solution for 2 h. Lipofuscin accumulation is promoted by oxidative stress in aging cells (Brunk and Terman, 2002). Fluorescence images of nematodes were captured at a suitable wavelength for fluorescence microscopy (Olympus BX51; Olympus Tokyo, Japan). Three independent replicates were used.

2.5. Pharmacological assay

Pharmacological analysis was performed to confirm the direct role of oxidative stress in nematode toxicity. Based on the adverse physiological effects of TCBPA, 100 μg/L TCBPA was used in subsequent experiments. After exposure to TCBPA, the nematodes were transferred to 24-well plates with or without 10 mM ascorbic acid solution. Treatment with 10 mM ascorbate does not influence locomotive behavior and induces oxidative stress in nematodes (Li et al., 2012). Subsequently, nematodes were examined for ROS generation and lipofuscin accumulation.

2.6. Measurement of neurotransmitter content

To explore the relationship between neurotransmitters and TCBPA, the dopamine (DA), glutamic acid (GLU), and serotonin levels were determined in TCBPA-exposed nematodes. After exposure to TCBPA for 10 d, the nematodes were collected and ground with normal saline. The supernatants were collected and stored at −20 °C for subsequent tests. Neurotransmitters were extracted using an ELISA kit according to the manufacturer's instructions (Jiangsu Meimian Industrial Co., Ltd., China). The OD value was measured at 450 nm. Concentrations were calculated using a standard curve. Detailed information is provided in Text S1.

2.7. Dopaminergic and serotoninergic neurons analysis

Changes in neuronal structure and morphology are observed when the cell bodies of the BZ555 and GR1366 strains are affected by chemicals. After chronic exposure to TCBPA, the nematodes were washed several times and placed on agar pads under anesthesia for 30 min. Fluorescence images were captured at the same magnification using an Olympus BX51 microscope. The fluorescence intensity of GFP was calculated and the percentage of abnormal neurons was counted in the exposed nematodes. At least 40 nematodes were examined in each group.

2.8. Gene expression analysis

Caenorhabditis elegans exposed to TCBPA were pretreated with K medium. Total RNA was extracted using the RNAsimple Total RNA Kit (Tiangen, China). Synthesis of cDNA was performed using the FastKing gDNA Dispelling RT SuperMix (Tiangen, China). Quantitative real-time PCR was performed to test the mRNA levels using the SYBR Green master mix. Gene expression was normalized to that of the *tba-1* gene. RT-qPCR parameters were as follows: 95 °C for 30 s, 40 cycles of 95 °C for 5s, and 60 °C for 30 s. For analysis of each selected gene, three replicates were measured per concentration. The designed primers are listed in Supplementary Data Table S1.

2.9. Molecular docking

The crystal structures of the proteins DOP-1, DOP-3, and MOD-1 were obtained from the UniProt database. The three-dimensional structure of TCBPA was downloaded from the PubChem database and defined as the ligand. To analyze the relationship between TCBPA and DOP-1, DOP-3, and MOD-1, the AutoDock software was used for molecular docking, and binding affinities were calculated based on the AMBER force field using a previously reported method (David et al., 2016; Forli et al., 2016). Finally, images were generated using PyMol software.

2.10. Statistical analysis

All data are expressed as the mean ± standard error of the mean against the control group, and graphs were plotted using Origin 8.0 (USA). The normality and homogeneity of the data were analyzed using the Shapiro–Wilk and Levene tests. One-way analysis of variance (ANOVA) with Tukey's test (SPSS 24, USA) was used to analyze significant differences.

3. Results and discussion

3.1. Effects of TCBPA on lethality and locomotive behaviors in C. elegans

Brominated flame retardants can cause neurotoxicity related to locomotive behavior (head thrashes and body bends). Therefore, we investigated the link between locomotive behavior and TCBPA in *C. elegans*. Chronic exposure to 1 μg/L TCBPA did not significantly affect the survival rate of nematodes (Supplementary Data Fig. S2). However, 10–100 μg/L caused a significant reduction, indicating the lethal effects on nematodes. Head thrashes significantly declined in nematodes at concentrations of 1 μg/L and above (Fig. 1A). At 10 μg/L and 100 μg/L TCBPA, head thrashes were significantly reduced by 11.7% and 15.3%, respectively, compared to that in the control. Additionally, exposure to TCBPA at more than 0.01 μg/L significantly inhibited body bends, and the reduction rate showed a concentration-effect relationship (Fig. 1B). Compared to the control group, the frequency of body bends decreased by 12% (0.1 μg/L), 24% (1 μg/L), 28% (10 μg/L), and 36% (100 μg/L) in the different treatment groups.

In contrast to the results for TBBPA exposure to TCBPA at environmentally relevant concentrations influenced the death of nematodes and survival rates declined in a dose-dependent manner. In addition, following chronic exposure to TCBPA body bends and head thrashes significantly decreased to lower levels. Reduction in body bends was more severe than in head thrashes in *C. elegans*, indicating that TCBPA causes neurotoxic effects on locomotive behavior. There was a marked decrease in body bends, head thrashes, and crawling movements in exposed nematodes (10–200 μg/L TBBPA), and the effective concentration of TBBPA was higher than that of TCBPA (Liu et al., 2019). A previous study has demonstrated that the toxicity of TCBPA is more severe than that of TBBPA (Zhang et al., 2018). Therefore, more attention should be paid to the neurotoxicity of TCBPA at a range of environmentally relevant concentrations, and the mechanisms by which TCBPA acts on *C. elegans* warrant further study.

3.2. Effects of TCBPA on oxidative stress in C. elegans

Oxidative stress is a major cause of toxicity and behavioral deficits, and ROS production and lipofuscin accumulation are common indicators of oxidative stress (Kim et al., 2013). As shown in Fig. 2B, chronic exposure to TCBPA at 1–100 μg/L significantly increased ROS generation compared to that in the control group. Chronic exposure to TCBPA (1–100 μg/L) significantly increased lipofuscin accumulation in nematodes (Fig. 2D), which is consistent with the results for ROS generation. Reactive oxygen species production and lipofuscin accumulation was significantly increased by 59% and 53%, respectively, after exposure to 100 μg/L TCBPA compared with that in the control group. Moreover, a pharmacological analysis was performed using ascorbic acid treatment to confirm the direct role of oxidative stress in nematodes. After treatment with 10 mM of ascorbate, ROS generation and lipofuscin accumulation were effectively suppressed in nematodes chronically exposed to 100 μg/L TCBPA (Fig. 3A and B).

These results suggested that chronic exposure to TCBPA induces oxidative stress in *C. elegans*. Exposure to TCBPA at higher concentrations (1–100 μg/L) both increased ROS generation and lipofuscin accumulation and damaged the redox balance in nematodes. A previous study reported that exposure of *Rana nigromaculata* to TCBPA significantly increased ROS content, whereas the activity of superoxide dismutase was inhibited (Jia et al., 2022). Another study detected ROS generation after rats were exposed to TCBPA (Nakagawa et al., 2007). Exposure to TCBPA may also induce oxidative stress in *Saccharomyces cerevisiae* cells (Zhang et al., 2020a). Oxidative stress induced by TCBPA may contribute to liver damage in *Pelophylax nigromaculatus* (Han et al., 2023). Moreover, treatment with ascorbate, an antioxidant, suppressed ROS generation and lipofuscin accumulation, further confirming the induction of oxidative stress. Ascorbate treatment has been reported to inhibit oxidative stress in nematodes (Wang et al., 2018). Similarly, treatment with antioxidants prevents neurotoxic effects on locomotive behavior and the function of AFD neurons (Li et al., 2012; Wu et al., 2012). These results confirm that oxidative stress plays a vital role in the neurotoxicity in TCBPA-exposed nematodes.

3.3. Effects of TCBPA on neurotransmitter content in C. elegans

Neurons are the most vulnerable to oxidative stress, which further influences the generation of neurotransmitters between neurons (Naziroglu, 2011). Therefore, the potential effects of TCBPA on neurotransmitters were investigated to explore its neurotoxicity in *C. elegans*. The concentrations of DA at 0 μg/L and 100 μg/L TCBPA were 0.99 ng/mL and 0.71 ng/mL, respectively, whereas those of serotonin were 0.24 ng/mL and 0.19 ng/mL, respectively (Supplementary Data Table S2). These results demonstrate that DA and serotonin levels were significantly decreased in TCBPA-exposed nematodes (Fig. 4). However, chronic exposure to TCBPA at 100 μg/L did not change GLU concentration in neurons.

Dopamine, serotonin, and GLU act as essential transmitters that

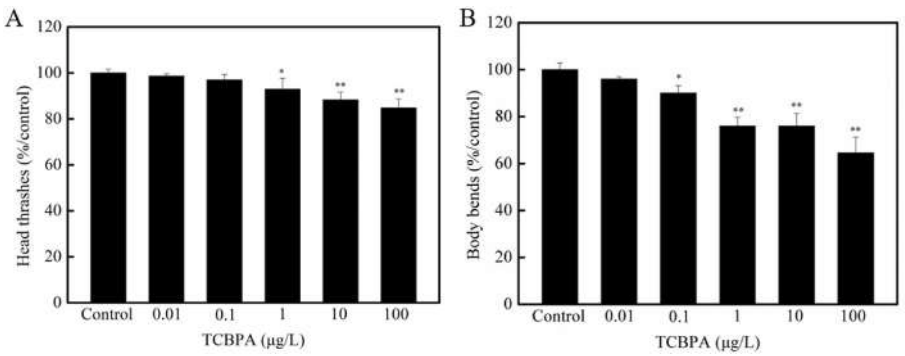

Fig. 1. Effects of TCBPA on locomotion in exposed nematodes. (A) The frequency of head thrashes compared to the control group (B) The frequency of body bends compared to the control group. Data are presented as mean ± standard error of the mean, and the asterisks indicate significant differences between the exposure (0.01–100 μg/L) and control (0 μg/L) groups. *$p < 0.05$ or **$p < 0.01$ indicate significant.

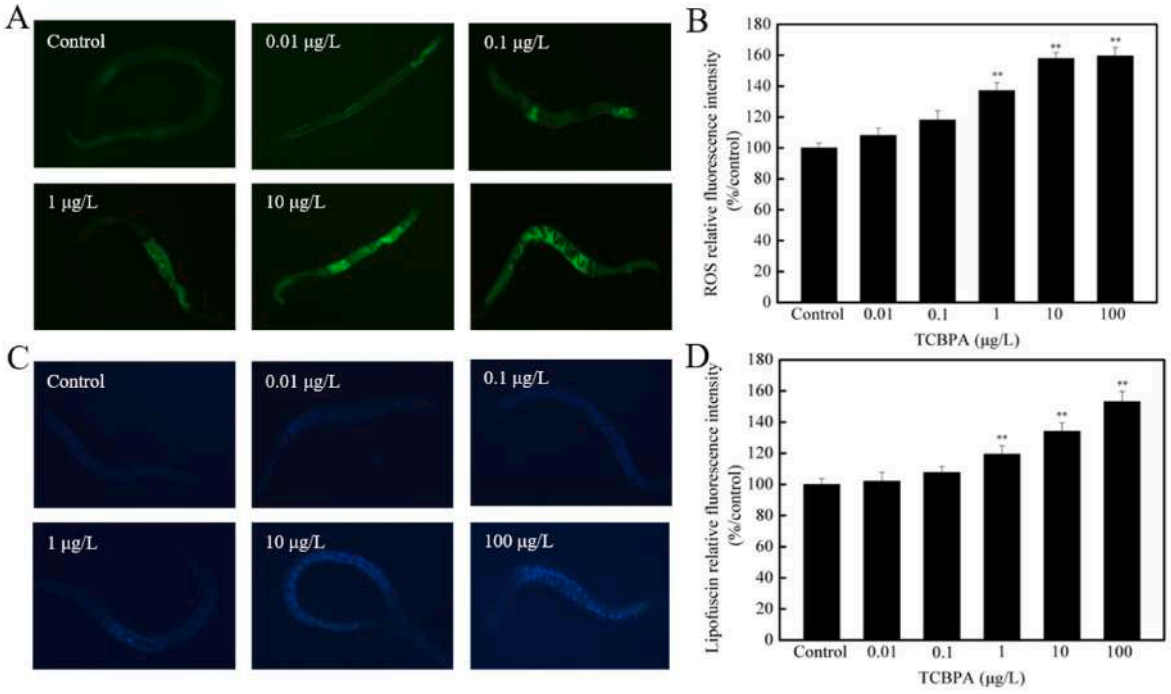

Fig. 2. Effects of TCBPA on oxidative stress in exposed nematodes. (A) The representative images of ROS. (B) The fluorescence intensity of ROS compared to the control group. (C) The representative images of lipofuscin accumulation. (D) The fluorescence intensity of lipofuscin compared to the control group. Relative fluorescence intensity was analyzed using ImageJ software. Data are presented as mean ± standard error of the mean, and the asterisks indicate significant differences between the exposure (0.01–100 μg/L) and control (0 μg/L) groups. *$p < 0.05$ or **$p < 0.01$ indicate significant.

modulate locomotive behavior. Serotonin and DA levels were significantly reduced in nematodes exposed to TCBPA at 100 μg/L, whereas GLU did not change compared to levels in the control, implying that DAergic and serotoninergic neurons were affected by TCBPA exposure. This was similar to the effect of graphene oxide on DA and serotonin neurons in exposed nematodes (Kim et al., 2019). Furthermore, TBBPA exposure induces damage to DA neurons and further impairs locomotion (Yu et al., 2021). Therefore, damage to DA and serotonin neurons may be involved in TCBPA-induced neurotoxicity.

3.4. *Effects of TCBPA on dopaminergic and serotoninergic neurons in C. elegans*

To further validate the roles of neurotransmitters in nematodes, transgenic strains BZ555 and GR1366 were investigated after chronic exposure to 100 μg/L TCBPA. After exposure to 100 μg/L TCBPA, the fluorescence intensity was significantly decreased by 23% in DAergic neurons compared to that in the control. The loss of dendrites and soma was observed in the head and tail of the nematodes, and the percentage of neuronal loss ranged from 15% to 30% (Fig. 5). The serotonin system is closely related to the tryptophan hydroxylase (TPH) promoter, *tph-1*, which limits the rate of serotonin synthesis (Lin et al., 2020). Compared to the control, after chronic exposure to TCBPA, GR1366 showed a significant decrease (28%) in the fluorescence intensity of TPH neurons, and the percentage of abnormal neurons in the nematodes increased to 25% (Fig. 5).

Exposure to exogenous pollutants can impair neurotransmitter levels. For example, damaged DAergic neurons have been observed in nematodes after exposure to 5 mM arsenic, accounting for 35% of total DAergic neurons (Zhang et al., 2020b). In the present study, the relative fluorescence intensity of the BZ555 and GR1366 strains decreased and abnormal neurons were observed in the exposed nematodes, suggesting that TCBPA exposure damaged DAergic and serotoninergic neurons. Neurotransmitters play important roles in physiological functions and

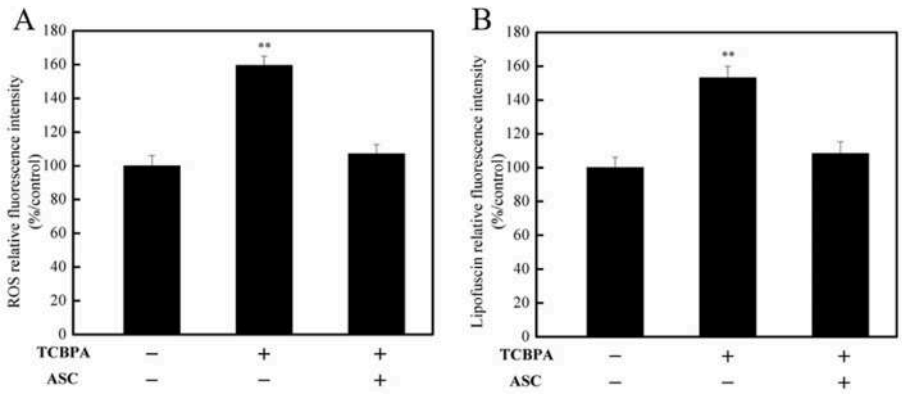

Fig. 3. Effects of TCBPA on mitochondrial function in exposed nematodes. (A) Effects of antioxidant treatment on ROS production of nematodes chronically exposed to 100 μg/L TCBPA. (B) Effects of antioxidant treatment on lipofuscin accumulation of nematodes chronically exposed to 100 μg/L TCBPA. Three independent experiments and forty nematodes per treatment were conducted. Statistical significance was analyzed using one-way ANOVA and Tukey's post hoc test. The asterisks indicate significant differences between the exposure and control groups. $^{**}p < 0.01$ indicate significant.

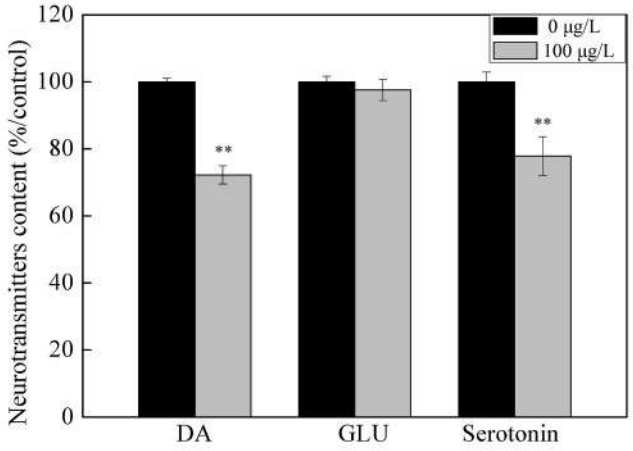

Fig. 4. Effects of TCBPA on neurotransmitters content in exposed nematodes. The contents of DA, GLU and serotonin at 100 μg/L TCBPA compared to the control group. Data from three independent experiments are expressed as means ± standard error of the mean and percentage values compared to the control group. Statistical significance was analyzed using one-way ANOVA and Tukey's post hoc test. The asterisks indicate significant differences between the exposure and control groups. $^{**}p < 0.01$ indicate significant.

could be a reason for the development of neurodegenerative diseases, such as Alzheimer's and Parkinson's diseases (Li et al., 2017; Kim et al., 2019). Similarly, tris(2-chloroethyl) phosphate or tris(2-chloropropyl) phosphate at concentrations of 750 mg/L and higher caused a marked loss of neurons in the tail of BZ555 nematodes, indicating locomotor defects (Xu et al., 2017). The DAergic neurons were impaired after nematode exposure to silver nanoparticles, which further reduced the learning ability of *C. elegans* (Zhang et al., 2021). These results confirmed that TCBPA exposure caused neurotoxicity by damaging DAergic and serotoninergic neurons in *C. elegans*.

3.5. Effects of TCBPA on related gene in C. elegans

Considering the above results, 100 μg/L TCBPA was selected to further explore the mechanism of neurotoxicity following exposure to TCBPA. In the present study, genes related to oxidative stress and neurotransmitters, *sod-3*, *ctl-1*, *mev-1*, *cat-1*, *mod-1*, *tph-1*, *dop-1*, *dop-3,* and *dat-1*, were measured in exposed nematodes. Exposure to TCBPA did not significantly regulate the expression of *ctl-1* and *mev-1* but upregulated *sod-3* (Fig. 6). Moreover, after exposure to TCBPA for 10 d, the expression of *cat-1*, *mod-1*, *dop-1,* and *dop-3* was significantly decreased ($p < 0.05$), whereas *tph-1* and *dat-1* showed no distinct changes compared to that in the control group.

The specific binding sites of TCBPA in DOP-1, DOP-3, and MOD-1 were further analyzed by molecular docking. For DOP-1, the oxygen atom of TCBPA was bound to the nitrogen atom in residue ARG-197 via hydrogen bonds, and hydrogen atoms were bound to the oxygen atoms on residue GLN-323 through hydrogen bonds (Fig. 7A). For the DOP-3 protein, the oxygen atom in TCBPA was bound to the oxygen atom of the residue PHE-86 by hydrogen bonds, and the chlorine atom was bound to the oxygen atom in the residue ASP-85 through halogen bonds, and the aromatic rings in the two structures also had π-π stacking interactions. For MOD-1, the oxygen atom at position 17 in TCBPA was bound to the side chain in GLN-85 via hydrogen bonds, and the hydrogen atom was bound to the oxygen atom in residue GLN-85 via hydrogen bonds. The calculated binding energies of TCBPA against DOP-1, DOP-3, and MOD-1 were −6.8, −7.7, and −6.2 kcal/mol, respectively (Fig. 7B). Molecular docking analysis demonstrated the potential of TCBPA to bind to the neurotransmitter receptor proteins DOP-1, DOP-3, and MOD-1.

The superoxide dismutase *sod-3* encodes Mn-SODs that defend nematodes against oxidative damage (Wu et al., 2017). A previous study detected a distinct increase in *sod-3* expression after chronic exposure to Al_2O_3-nanoparticles, demonstrating that oxidative stress is involved in the neurotoxicity of Al_2O_3-nanoparticles (Li et al., 2012). In *C. elegans*, both DA and serotonin play important roles in locomotor behavior. The synthesis of both DA and serotonin involves *cat-1*, and *mod-1* encodes an ionotropic serotonin transporter in the serotonin system (Ranganathan et al., 2000; Wu et al., 2015). Similarly, exposure to 10 μg/L nonylphenol for 10 d downregulated the expression of *mod-1* and *cat-1* and upregulated *sod-3,* suggesting that nonylphenol could inhibit the function of serotonin and induce oxidative stress (Cao et al., 2019). In addition, *dop-1* and *dop-3* are D1/2-like receptors that act on the ventral motor neurons (Jayanthi et al., 1998; Chase et al., 2004). A previous study reported that *dop-1*, *dop-3,* and *dat-1* decreased after acute exposure to MPA-capped CdTe quantum dots, resulting in severe neurotoxicity in nematodes (Wu et al., 2015). Taken together, the combination of DA and serotonin contributes to the neurotoxicity of TCBPA on locomotive behavior.

4. Conclusion

We investigated oxidative stress, neurotransmitters, and their association with neurotoxicity in TCBPA-exposed nematodes. Chronic exposure to TCBPA reduced locomotive behavior in a concentration-

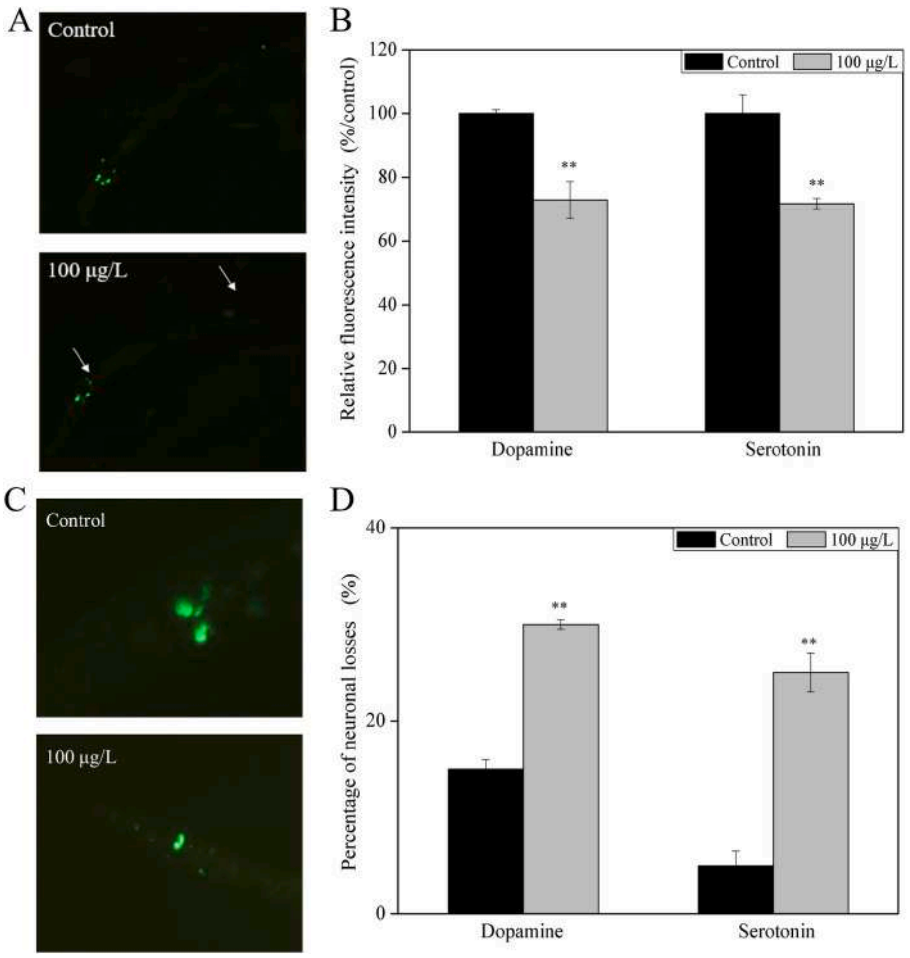

Fig. 5. Effects of TCBPA on dopaminergic and serotoninergic neurons in exposed nematodes. (A) Morphology changes of DAergic neurons in nematodes. (B) Comparison of relative fluorescent intensities in BZ555 (dopamine) and GR1366 (serotonin) strains. (C) Morphology changes of serotoninergic neurons in head of nematodes. (D) Percentage of neuronal loss in BZ555 (dopamine) and GR1366 (serotonin) strains. Relative fluorescence intensity was analyzed using ImageJ software. White arrows indicate the loss of dendrite and soma. Data are presented as mean ± standard error of the mean, and the asterisks indicate significant differences between the exposure (0.01–100 μg/L) and control (0 μg/L) groups. $^{**}p < 0.01$ indicate significant.

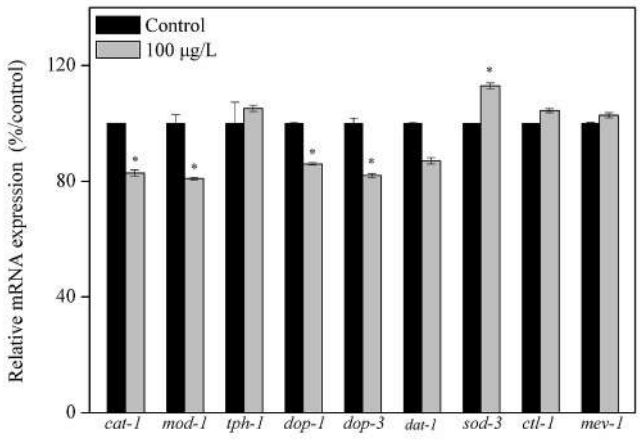

Fig. 6. Gene expression required for oxidative stress and neurotransmitters in nematodes exposed to 100 μg/L of TCBPA. Values of gene expressions were normalized using *tba*-1 mRNA and represented means (n = 3) relative to the control. Statistically significant differences were analyzed using analysis of variance with Tukey's test. $^{*}p < 0.05$ indicate significant.

dependent manner, which was accompanied by increased ROS formation and lipofuscin accumulation in exposed nematodes. Treatment with antioxidants repaired the oxidative damage induced by TCBPA exposure and *sod-3* played a vital role in neurotoxicity. In addition, TCBPA exposure caused a reduction in DA and serotonin levels, damage to DAergic and serotoninergic neurons, and downregulation of the expression of *dop-1*, *dop-3*, *cat-1*, and *mod-1*. Molecular docking confirmed that the mechanism of action of TCBPA was via the binding sites of DOP-1, DOP-3, and MOD-1. Furthermore, it was revealed that five amino acid residues, ARG-197, GLN-323, PHE-86, ASP-85, and GLN-85, played important roles in the stabilization of the interaction of TCBPA with target receptors. Therefore, our findings indicate that a combination of oxidative stress and damage to serotoninergic and dopaminergic neurons contributes to TCBPA neurotoxicity.

CRediT authorship contribution statement

Yunjiang Yu: Conceptualization, Supervision. **Shihui Tan:** Data curation, Investigation, Writing – original draft, Writing – review & editing. **Hongzhi Guo:** Data curation, Investigation, Writing – original draft. **Xin Hua:** Investigation, Writing – original draft. **Haibo Chen:** Data curation, Investigation, Writing – review & editing. **Yue Yang:** Data curation, Investigation, Writing – review & editing. **Dongli Xie:** Investigation. **Chuan Yi:** Validation. **Haibo Ling:** Validation. **Mingdeng Xiang:** Validation.

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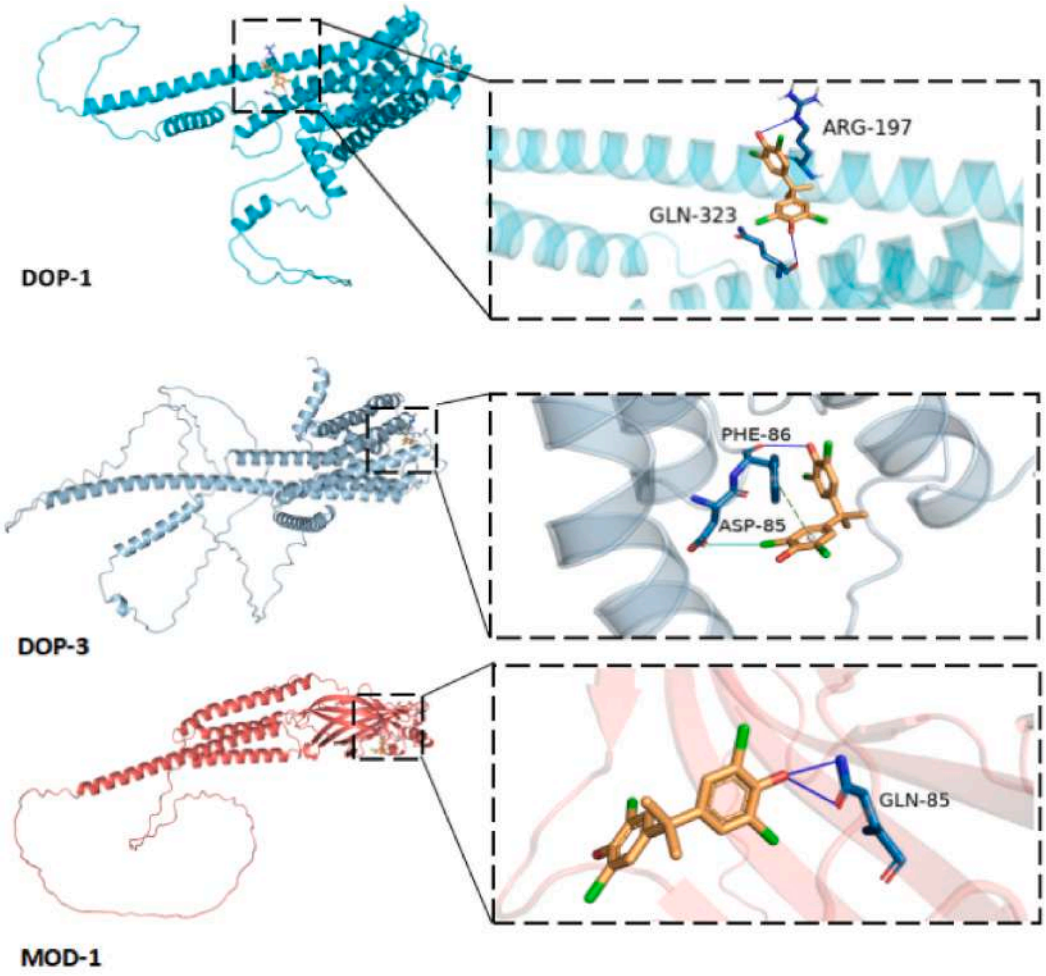

B

pollutant	Neurotransmitter receptor proteins	binding energy (kcal/mol)	binding sites
TCBPA	DOP-1	-6.8	ARG-197 GLN-323
	DOP-3	-7.7	PHE-86 ASP-85
	MOD-1	-6.2	GLN-85

Fig. 7. Effect of TCBPA exposure on the molecular basis of neurotoxicity. (A) Molecular docking between TCBPA and Neurotransmitter receptor proteins of DOP-1, DOP-3 and MOD-1. The proteins were depicted as cyan, gray, deepsalmon cartons, respectively. TCBPA was depicted as yellow sticks. The surrounding residues in the binding pockets are depicted as blue. The hydrogen bond is depicted as solid blue lines. (B) The lowest binding energy of TCBPA bound to the active sites of DOP-1, DOP-3, and MOD-1.

Declaration of competing 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.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.chemosphere.2024.141142.

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