Neurological responses of embryo-larval zebrafish to short-term sediment exposure to decabromodiphenylethane*

Mei-qing JIN¹, Dong ZHANG¹, Ying ZHANG², Shan-shan ZHOU³, Xian-ting LU¹, Hong-ting ZHAO^{†‡1}

¹College of Materials Science and Environmental Engineering, Hangzhou Dianzi University, Hangzhou 310018, China

²Shanghai Key Laboratory for Urban Ecological Processes and Eco-Restoration, School of Ecological and Environmental Science, East China Normal University, Shanghai 200062, China

³College of Environment, Zhejiang University of Technology, Hangzhou 310014, China

[†]E-mail: info-iem@hdu.edu.cn

Received Jan. 18, 2018; Revision accepted Mar. 20, 2018; Crosschecked Apr. 18, 2018

Abstract: Decabromodiphenylethane (DBDPE) has been widely used as an alternative flame retardant due to the restriction or phase-out of traditional polybrominated diphenyl ethers (PBDEs), and is of increasing concern regarding its ubiquity, persistence, and potential adverse effects. In the present study, the toxicological effects of DBDPE were evaluated using zebrafish as an in vivo model. Upon being exposed to DBDPE-polluted sediments for a short term, it was found that the mortality and malformation of zebrafish (including edema, bent notochord, and bent tail) were not affected even at the highest concentration tested (1000.0 µg/kg dry sediment). Regarding behavioral responses, it was found that zebrafish larvae of 48 hours post fertilization (hpf) in all groups escaped successfully with a touch to the dorsal fin. However, when exposed to the highest DBDPE concentration, the larvae of 120 hpf exhibited significantly smaller distances as compared to the control. Moreover, the results of the acetylcholinesterase (AChE) activity, the expression levels of two important nerve-related genes, and the cell apoptosis all indicated that DBDPE posed low neurotoxicity in embryo-larval zebrafish. The results in this study shed some light on the potential risks of DBDPE in the real environment and highlight the application of the sediment exposure route in the future.

Key words: Decabromodiphenylethane; Flame retardant; *Danio rerio*; Neurotoxicity

<https://doi.org/10.1631/jzus.B1800033>

CLC number: X131

1 Introduction

With the worldwide restriction on the use and production of polybrominated diphenyl ethers (PBDEs) due to their environmental and human health concerns, novel non-PBDE-halogenated flame retardants have been introduced into the market and used as replacements (Chen et al., 2013). For instance,

decabromodiphenylethane (DBDPE) is reported to be used as an alternative to decabromodiphenyl ether (deca-BDE). In China, DBDPE has been put into production since 2005 and the annual production was estimated to be as high as 12000 t in 2006 and the domestic demand for DBDPE is expected to increase at a rate of 80% per year (Hong et al., 2015).

As an additive flame retardant, DBDPE can be readily released into the environment during its production, use, recycle, and the disposal of its related products (Lee et al., 2014). DBDPE was first reported to be detected in the environment in 2003 and ever since it has been widely detected in various abiotic and biotic matrices (Kierkegaard and Bjorklund, 2003). For instance, in surface sediments collected

[‡] Corresponding author

* Project supported by the National Natural Science Foundation of China (Nos. 21407038, 21407037, 21407051, 21307023, and 21377117) and the Zhejiang Provincial Natural Science Foundation of China (No. LY18B070008)

ORCID: Hong-ting ZHAO, <https://orcid.org/0000-0002-4562-4576>
© Zhejiang University and Springer-Verlag GmbH Germany, part of Springer Nature 2018

during 2009–2010 from the Pearl River Delta, China, the levels of DBDPE ranged from non-detectable to 1728 ng/g dry weight and were higher than those from most other areas (Chen et al., 2013). The levels have exceeded those of deca-BDE in most sediments and are supposed to increase in the future. The presence of novel brominated flame retardants in remote regions suggests that DBDPE is an emerging environmental contaminant and it has attracted increasing attention from the public (Moller et al., 2011).

Previous field studies have revealed a wide occurrence of DBDPE in biotic media, e.g. fish, birds, mammals, and even human hair and blood, indicating that DBDPE has the potential to biomagnify through the food webs and to exert harmful effects on the ecosystem and human health (He et al., 2012, 2013; She et al., 2013). However, to the best of our knowledge, previous research attention has mostly been focused on DBDPE's environmental fate, while little study has been reported regarding its potential toxicological effects. Generally, it has been reported that DBDPE could exhibit low acute toxicity to mammals (Wang et al., 2010), soil organisms (Hardy et al., 2011), and aquatic species (Hardy et al., 2011). As regards to the aquatic system, few studies have been reported and the existing results were controversial. Hardy et al. (2012) found that the tested species (e.g. rainbow trout, freshwater alga, daphnia, midge, and oligochaetes) were unaffected by exposure to DBDPE, while Nakari and Huhtala (2010) found that DBDPE was acutely toxic to water fleas and zebrafish. These controversial results might be attributed to different exposure methods used in their studies. Knudsen et al. (2017) examined the disposition of DBDPE in female Sprague Dawley rats treated by oral, topical, or intravenous administration. The results indicated that various administration routes may affect the accumulation of DBDPE, which may contribute to the biological response in organisms. Due to its large molecular weight and high $\log K_{ow}$ (K_{ow} is the octanol-water partition coefficient), sediment has been considered as a major potential sink that may serve as a major exposure route of DBDPE for aquatic organisms. Therefore, it is crucial to evaluate the potential toxic effects of DBDPE based on sediment exposure routes.

According to previous studies, deca-BDE could exert neurological effects, for example, behavior

changes in zebrafish (Garcia-Reyero et al., 2014) and impaired neurological development in humans (Gascon et al., 2012). As an alternative to deca-BDE, DBDPE has a similar structure and may behave similarly. However, to our knowledge, no information on the neurological effects of DBDPE has been reported based on sediment exposure so far. Given their small size, visible embryo developmental phases, and their high genetic similarity to humans, zebrafish (*Danio rerio*) has proven to be a reliable and sensitive model (Liu et al., 2017) and is widely used in the evaluation of the neurotoxicity of various xenochemicals (Bailey et al., 2013).

Therefore, the main objectives of this study were: (1) to investigate neurological responses to DBDPE by using zebrafish as an in vivo model; (2) to elucidate the potential mechanisms of DBDPE-induced neurotoxicity in embryo-larval zebrafish.

2 Materials and methods

2.1 Preparation of the contaminated sediments

The artificial sediments were prepared according to previous described methods (EPA, 2000) with slight modifications. Briefly, the sediments were comprised of quartz sand (69%), kaolin clay (20%), peat (10%), and CaCO_3 (1%). DBDPE (purity >96%), purchased from Aladdin Biochemical Co., Ltd. (Shanghai, China), was dissolved in tetrahydrofuran to prepare the stock solution (50 $\mu\text{g}/\text{ml}$) and stored at 4 °C in darkness. Test solutions were prepared by dilution of the stock solution with tetrahydrofuran. For spiking sediments, DBDPE solutions of the selected concentrations (3.125, 6.25, 12.5, 25, 50 $\mu\text{g}/\text{ml}$) were mixed with quartz sands and placed in a ventilation cabinet overnight to evaporate the solvent. Then they were mixed thoroughly with the sediments through tumbling for two days in dark.

2.2 Embryo collection and exposure

Embryos were obtained by natural mating of healthy adult zebrafish (wild type, AB strain) and maintained in petri dishes at 28.0 °C with a 12 h:12 h (light:dark) photoperiod in embryo water until chemical treatment. The conditions of the embryo water were as follows: 200 $\mu\text{g}/\text{ml}$ of Instant Ocean (Aquarium Systems, Sarrebourg, France) was dissolved in reverse

osmosis purified water, and the conductivity, pH, and hardness were kept in the range of 480–510 $\mu\text{S}/\text{cm}$, 6.9–7.2, and 53.7–71.6 mg/L CaCO_3 , respectively. All procedures were approved by the Association for Assessment and Accreditation of Laboratory Animal Care (Frederick, USA).

The petri dishes were used as the exposure vessels and 10 g spiked sediments were placed in each vessel, topped with oxygen saturated embryo water (4 ml/g). The vessels were then incubated at 28 °C in the darkness overnight for equilibrium before adding the embryos. On Day 2, 30 fertilized zebrafish embryos at 4 hours post fertilization (hpf) per vessel were transferred to the spiked sediments (or the control) and exposed from 4 to 120 hpf under semi-static conditions at 28.0 °C in the dark. Replacement of the topped embryo water was done daily, while the sediments remained unchanged.

2.3 Embryo toxicity assay and microscopic observation

Observations of zebrafish development were made during the exposure period using a dissecting microscope (SZX7, Olympus, Japan). Mortality and malformations were recorded in the control and spiked sediment groups every 24 h, and embryos and larvae were considered dead when no heartbeat was observed. Dead embryos or larvae were removed immediately at each observation time. From 48 hpf, embryos started to come out of the chorion asynchronously and the number of hatched embryos was recorded every 24 h.

2.4 Behavior assays

To assess the effects of DBDPE on the motor behavior of zebrafish larvae, two types of behavior assay including touch-escape response and free-swimming activity, were conducted. For touch-escape response, 20 embryos at 48 hpf per group were randomly chosen and dechorionated. The embryos were touched dorsally near the tail fin area with a dissecting needle, and the numbers of the embryos that have

escaping responses were recorded. For the free-swimming activity assessment, 10 normal larvae at 120 hpf per group were randomly chosen and individually transferred to a 24-well plate (one fish per well). The distances travelled during 1 h were achieved using the VideoTrack for Zebrafish™ (V3, ViewPoint Life Sciences, France) and analyzed based on previous studies with some modifications (Winter et al., 2008; Jin et al., 2009; Chen et al., 2017).

2.5 Measurement of acetylcholinesterase activity

A total of 90 zebrafish embryos per treatment were exposed to DBDPE (62.5, 125.0, 250.0, 500.0, 1000.0 $\mu\text{g}/\text{kg}$) and the control from 4 to 96 hpf. At the end of each exposure period (72, 96 hpf), the organisms from various concentration groups were collected for measurement of acetylcholinesterase (AChE) activity using an Amplite™ Fluorimetric Acetylcholinesterase Assay kit (AAT Bioquest®, Inc., USA) according to the manufacturer's instructions. Five replicates were set for all the treatments.

2.6 Gene expression analysis

At different sampling time points (48, 72, or 96 hpf), 30 fish per treatment were pooled together for the analyses of the selected gene expression levels by polymerase chain reaction (PCR). Total RNA in the embryos or larvae was extracted using the TRIzol reagent (Invitrogen, USA) according to the manufacturer's protocol. The banding patterns on a 2% (0.02 g/ml) agarose gel were checked for the integrity of the total RNA and the purity was also analyzed using the Microvolume Spectrophotometers (Nanodrop 2000, Thermo, USA). Reverse transcription (RT) was carried out with a ReverTra Ace qPCR RT kit (Toyobo, Osaka, Japan). PCR was performed with the listed primers as shown in Table 1, and the products were separated by a 2% (0.02 g/ml) agarose gel electrophoresis. The relative quantification of target gene expression was analyzed according to the optical density (OD) of the corresponding bands normalized by β -actin.

Table 1 PCR primer sequences for the selected genes

Gene	Forward primer	Reverse primer
β -actin	CATCAGCATGGCTTCTGCTCTGTATGG	GACTTGTCAGTGACAGAGACACCCT
gap43	GCAGCAGGAAGTGGAGAAGCCA	GGATTCTCAGCAGCGTCTGGT
α 1-tubulin	AATCACCAATGCTTGCTTCGAGCC	TTCACGTCTTTGGGTACCACGTCA

2.7 Apoptotic cell death analysis

To estimate cell death due to apoptosis, the terminal deoxynucleotidyl transferase (TdT)-mediated dUTP nick end labeling (TUNEL) assay was conducted on whole-mounted zebrafish larvae at 96 hpf with a One Step TUNEL Apoptosis Assay kit (Beyotime Biotechnology Co., Ltd., Shanghai, China). After exposure to various concentrations of DBDPE from 4 to 96 hpf, the larvae were fixed with 4% (w/v) paraformaldehyde, permeabilized, and incubated with the reaction mixture according to the assay protocol provided by the manufacturer's instructions. The images of the apoptotic cells were taken under a fluorescent microscope (AZ100, Nikon, Japan).

2.8 Statistical analysis

All data were expressed as the mean $\pm$ standard deviation (SD). Statistical analyses of the data were performed in the program package SPSS 16.0 (SPSS Inc., Chicago, IL, USA). Data were analyzed for statistical significance with a one-way analysis of variance (ANOVA) followed by least-significant difference (LSD) (equal variances assumed) or Dunnett's T3 (equal variances not assumed) test. In all cases, values were considered statistically different when $P < 0.05$.

3 Results

3.1 Effects of DBDPE in sediment on zebrafish survival and non-lethal malformations

It was found that no zebrafish embryo or larva died even at the highest concentration tested (1000.0 $\mu\text{g/kg}$) during the exposure period. All the treatment groups were also examined from 4 to 120 hpf on the malformation effects of DBDPE on the zebrafish embryo-larval development, including edema, bent notochord, and bent tail. As shown in Fig. 1, at 120 hpf, no significant effects were induced by exposure to the DBDPE in sediment. The results indicated that no observed effect concentration (NOEC) of DBDPE on zebrafish survival and malformations was higher than 1000.0 $\mu\text{g/kg}$.

The hatching rates of the tested groups were also recorded from 48 hpf, when the embryos started to come out from the chorions asynchronously. At 48 hpf, only a small portion of the embryos have hatched, with

Fig. 1 Microscopic observations of zebrafish exposed to DBDPE-contaminated sediments from 4 to 120 hpf

(a) Control; (b) 62.5 $\mu\text{g/kg}$; (c) 125.0 $\mu\text{g/kg}$; (d) 250.0 $\mu\text{g/kg}$; (e) 500.0 $\mu\text{g/kg}$; (f) 1000.0 $\mu\text{g/kg}$

the hatching rates ranging from 3.3% to 16.7% (Table 2). At 72 hpf, most of the embryos have hatched into larvae, and the percentages of the hatched embryos increased to 96.7%, 100.0%, 93.3%, 96.7%, 100.0%, and 80.0% for the control, 62.5, 125.0, 250.0, 500.0, and 1000.0 $\mu\text{g/kg}$ groups, respectively. Exposure to the lowest concentration of DBDPE (62.5 $\mu\text{g/kg}$) seemed to slightly accelerate the hatching process, while the highest concentration of DBDPE (1000.0 $\mu\text{g/kg}$) inhibited the process to some degree at 72 hpf. However, the hatchability seemed not to be influenced by sediment exposure to DBDPE at all tested concentrations and the hatching rates of all the treated groups were 100.0% until 96 hpf.

Table 2 Hatching rates of the embryo-larval zebrafish exposed to DBDPE-treated sediment ($n=30$) at various periods

Concentration ($\mu\text{g/kg}$)	Hatching rate (%)		
	48 hpf	72 hpf	96 hpf
Control	6.7	96.7	100.0
62.5	16.7	100.0	100.0
125.0	6.7	93.3	100.0
250.0	6.7	96.7	100.0
500.0	3.3	100.0	100.0
1000.0	6.7	80.0	100.0

3.2 Effect of DBDPE in sediment on zebrafish behavior

Two types of motor behavior, touch-escape response and free-swimming activity, were chosen to evaluate the effect of DBDPE exposure at different time points. At 48 hpf, the touch responses were examined and all the treated groups escaped immediately with the touch on the dorsal fin. At 120 hpf, free-swimming activity during a period of 1 h was detected, and the total distances travelled as well as

the distances travelled at different speeds (low, <4 mm/s; medium, 4–20 mm/s; high, >20 mm/s) were recorded (Figs. 2 and 3). A significant decrease was observed in the total distance of free swimming with the highest concentration treatment of DBDPE (1000.0 $\mu\text{g/kg}$) ($P<0.05$), while no significant differences were found for the other groups compared to the control (Fig. 2). For all the treatment groups (including the control), a similar pattern was observed, with the major part travelling at medium speed, and the rest small part at high or low speed. For those swimming at medium speed, larvae in the 62.5 $\mu\text{g/kg}$ exposure group exhibited significantly larger distances as compared to the control ($P<0.05$), while no significant differences were observed for the remaining groups (Fig. 3).

3.3 Effect of DBDPE in sediment on zebrafish acetylcholinesterase activity

The effects of different DBDPE concentrations and time of exposure on AChE activity of zebrafish larvae were shown in Fig. 4. Generally, over time, AChE activity decreased slightly. Take the control for example, at 72 hpf, AChE activity was (2.37 ± 0.23) mU/ml, while at 96 hpf, the value decreased to (1.84 ± 0.24) mU/ml. However, at a specific time point (72 or 96 hpf), no significant alteration of AChE activity was observed when the larvae were exposed to DBDPE even at the highest concentration compared to the control.

3.4 Effects of DBDPE in sediment on zebrafish gene expression levels and cell apoptosis

In order to elucidate the potential mechanisms of the behavior alteration, the expression levels of two important nerve-related genes, *$\alpha 1$ -tubulin* and *gap43*, were also detected in zebrafish larvae by RT-PCR.

Fig. 2 Effects on the total distances of zebrafish by exposure to DBDPE-contaminated sediments from 4 to 120 hpf CTRL: control. ** Significant difference compared to the control at $P\leq0.01$

Fig. 3 Effects on the distances travelled under different speeds

(a) Low, <4 mm/s; (b) Medium, 4–20 mm/s; (c) High, >20 mm/s; CTRL: control. * Significant difference compared to the control at $P\leq0.05$

Fig. 4 Effects on the AChE activity of zebrafish (a) 72 hpf; (b) 96 hpf. AChE: acetylcholinesterase; CTRL: control

However, as shown in Fig. 5, no significant difference was observed in all DBDPE treatment groups when compared to the control at all sampling time points (48, 72, and 96 hpf).

Furthermore, in order to confirm whether exposure to DBDPE induces cell apoptosis, the TUNEL assay was then conducted in whole-mounted zebrafish larvae at 96 hpf. Nevertheless, as shown in Fig. 6, no significant apoptotic cell death was observed in all DBDPE treatment groups when compared to the control.

Fig. 5 Expression levels of two nerve-related genes and β -actin detected by RT-PCR

The results for zebrafish sampled at 48, 72, and 96 hpf were listed from top to bottom; for each band, the control, 62.5, 125.0, 250.0, 500.0, and 1000.0 $\mu\text{g/kg}$ groups were listed from left to right

4 Discussion

In the present study, the embryo-larval zebrafish was used as a model for investigating the

Fig. 6 Effects on the apoptotic cell death of zebrafish by exposure to DBDPE-contaminated sediments from 4 to 96 hpf

(a) Control; (b) 62.5.0 $\mu\text{g/kg}$; (c) 125.0 $\mu\text{g/kg}$; (d) 250.0 $\mu\text{g/kg}$; (e) 500.0 $\mu\text{g/kg}$; (f) 1000.0 $\mu\text{g/kg}$

effect of DBDPE-contaminated sediment exposure on neurotoxicology, including teratology, motor behavior, AChE activity, and expression levels of nerve-related genes. As demonstrated in the results, exposure to DBDPE at the levels up to 1000.0 $\mu\text{g/kg}$ of dry sediment during the embryo-larval stages did not cause any adverse effects on survival or malformation (including edema, bent notochord, and bent tail). The hatching process seemed to be slightly accelerated by exposure to the lowest concentration of DBDPE (62.5 $\mu\text{g/kg}$) and inhibited by the highest concentration of DBDPE (1000.0 $\mu\text{g/kg}$) at 72 hpf. Hatching is a process regulated by both the choriolytic enzyme and the physical movement of the embryo (Jin et al., 2009). The hypoactivity induced by the highest concentration of DBDPE may partly explain the inhibition effect on the hatching process. However, it remained unclear whether the hyperactivity caused by the lowest concentration of DBDPE (62.5 $\mu\text{g/kg}$) at medium speed was correlated with the acceleration of the hatching process and awaits further studies. As free-swimming behavior is mainly regulated by the motor neuron in the early stages (Chen et al., 2017), two important genes related to axonal growth, *gap43* and *al-tubulin*, were quantified. Nevertheless, no significant effects were found on the expression levels of the two genes. Further studies on the cell apoptosis also showed that all DBDPE treatment groups were negative on apoptotic cell death. Given the complex interaction between neuronal function, integrity of muscle structure, and motor behavior, further investigations are needed to provide insight into the molecular mechanisms of DBDPE-induced neurotoxicity (e.g. hypoactivity).

Generally, low toxicity of DBDPE in our findings was consistent with those of previously reported studies (Hardy et al., 2002, 2010; Wang et al., 2010),

especially those on mammalian toxicology. Several studies have been recently reported on the mammalian toxicology of DBDPE. Hardy et al. (2010) found no evidence of prenatal developmental toxicity when treated with DBDPE at dosage levels up to 1250 mg/(kg·d) from gestation Day 6 through Day 15 for rats and Day 6 through Day 18 for rabbits. When extending the exposure duration to 28 or 90 d, still no overt toxicity was indicated but hepatotoxicity was induced in rats at similar or lower concentrations (Hardy et al., 2002; Wang et al., 2010; Sun et al., 2014). Moreover, the results from the Ames and chromosome aberration tests in Chinese hamster lung cells showed negative genotoxicity (Putman and Morris, 1991; San and Wagner, 1991). Low toxicity of DBDPE was also reported in the terrestrial and sediment compartments (Hardy et al., 2012). The NOECs of DBDPE identified for sewage sludge respiration, soil nitrification, and earthworm survival were >10 mg/kg dry soil, >2500 mg/kg dry soil, and >3720 mg/kg dry soil, respectively (Hardy et al., 2011). Hardy et al. (2012) explored the potential toxicity of DBDPE in two sediment species and found that the NOECs were higher than 5000 mg/kg. All these studies demonstrated that DBDPE presents little risk to organisms, presumably due to its low bioavailability. Considering DBDPE's large molecule and low water solubility, along with the large logarithmic organic carbon-water partitioning coefficient and soil-water partitioning coefficient, it could presumably exhibit strong binding to soils and sediments, thereby limiting its transfer from water into aquatic organisms. Hardy (2004) tested the bioconcentration factor of DBDPE and found that the contaminant did not bioconcentrate in Japanese carp (*Cyprinus carpio*).

The above data, however, were inconsistent with those reported by Nakari and Huhtala (2010), in which obvious responses including reduced hatching rates and increased mortality were reported by water-borne exposure in embryo-larval zebrafish. Hardy et al. (2012) questioned the above findings and suggested that the residue of toluene as well as the dispersant dimethyl sulfoxide (amount not stated) might have contributed to the effects of DBDPE, considering the negligibly low solubility and the low bioavailability. In our study, though used as a solvent, tetrahydrofuran was evaporated by being placed in a ventilation cabinet overnight before introducing

zebrafish into the exposure systems. Furthermore, exposure to DBDPE-contaminated sediment, rather than to the water, more closely resembles the real environment scenario and can better evaluate the ecological risks of the hydrophobic contaminants.

Many studies have found that BDE-209 can exert neurological effects, including changes in spontaneous behavior in the adult rat (Viberg et al., 2007), free-swimming activity deficits in larval zebrafish (Garcia-Reyero et al., 2014), and even impaired neurological development in humans (Gascon et al., 2012). PBDEs including BDE-209 were also reported to cause oxidative stress in neurons and to lead to apoptotic neuronal death (He et al., 2008; Huang et al., 2010). More obvious responses were observed for BDE-209 in those studies, compared to the results of DBDPE in our study. This may be possible given that the bioavailability of DBDPE was lower than that of BDE-209. Wang et al. (2010) investigated the bioavailability of DBDPE and BDE-209 in male rats by oral administration, and the concentrations of BDE-209 were 3–5 orders of magnitude higher than the DBDPE concentrations. Garcia-Reyero et al. (2014) studied the effects of BDE-209 on zebrafish development by using the similar sediment exposure route as we used in this study. No effects on gross morphology or structures of the nervous system were observed in both studies. However, it is interesting to note that BDE-209 could induce hyperactivity at a low dose (13.67 mg/kg) and hypoactivity at a high dose (20 mg/kg) (Garcia-Reyero et al., 2014), while hypoactivity was also induced by a much lower concentration (1 mg/kg) of DBDPE in our study. The discrepancy may be attributed to the sediments used. Surface sediment from a pristine area was collected for BDE-209, while artificial sediment was applied in our study. Other factors, such as exposure time and concentrations as well as metabolism of the contaminants, may also contribute to the differences.

5 Conclusions

In this study, results indicated that a short-term exposure to DBDPE-polluted sediments could pose little risk to the gross morphology of embryo-larval zebrafish. The hatching process and the free-swimming activity seemed to be two sensitive endpoints, and

responded positively at the highest concentration tested. Moreover, we tried to elucidate the potential mechanisms of DBDPE-induced neurotoxicity by examining the expression levels of two nerve-related genes, AChE activity, and the cell apoptosis. However, the mechanisms remained unclear and need to be explored in more details in the future. Furthermore, regarding the low concentration and short duration of exposure combined with the more realistic scenario we mimicked, the application of the sediment exposure route in the present study should be taken into consideration, especially for those hydrophobic contaminants.

Compliance with ethics guidelines

Mei-qing JIN, Dong ZHANG, Ying ZHANG, Shan-shan ZHOU, Xian-ting LU, and Hong-ting ZHAO declare that they have no conflict of interest.

All institutional and national guidelines for the care and use of laboratory animals were followed.

References

- Bailey J, Oliveri A, Levin ED, 2013. Zebrafish model systems for developmental neurobehavioral toxicology. *Birth Defects Res C Embryo Today*, 99(1):14-23.
<https://doi.org/10.1002/bdrc.21027>
- Chen SJ, Feng AH, He MJ, et al., 2013. Current levels and composition profiles of PBDEs and alternative flame retardants in surface sediments from the Pearl River Delta, southern China: comparison with historical data. *Sci Total Environ*, 444:205-211.
<https://doi.org/10.1016/j.scitotenv.2012.11.104>
- Chen XP, Dong QX, Chen YH, et al., 2017. Effects of Dechlorane Plus exposure on axonal growth, musculature and motor behavior in embryo-larval zebrafish. *Environ Pollut*, 224:7-15.
<https://doi.org/10.1016/j.envpol.2017.03.011>
- EPA (Environmental Protection Agency), 2000. Methods for Measuring the Toxicity and Bioaccumulation of Sediment-associated Contaminants with Freshwater Invertebrates. EPA, Washington DC.
- Garcia-Reyero N, Escalon BL, Prats E, et al., 2014. Effects of BDE-209 contaminated sediments on zebrafish development and potential implications to human health. *Environ Int*, 63:216-223.
<https://doi.org/10.1016/j.envint.2013.11.012>
- Gascon M, Fort M, Martinez D, et al., 2012. Polybrominated diphenyl ethers (PBDEs) in breast milk and neuropsychological development in infants. *Environ Health Perspect*, 120(12):1760-1765.
<https://doi.org/10.1289/ehp.1205266>
- Hardy ML, 2004. A comparison of the fish bioconcentration factors for brominated flame retardants with their non-brominated analogues. *Environ Toxicol Chem*, 23(3):656-661.
<https://doi.org/10.1897/03-03>
- Hardy ML, Margitich D, Ackerman L, et al., 2002. The sub-chronic oral toxicity of ethane, 1,2-bis(pentabromophenyl) (Saytex 8010) in rats. *Int J Toxicol*, 21(3):165-170.
<https://doi.org/10.1080/10915810290096298>
- Hardy ML, Mercieca MD, Rodwell DE, et al., 2010. Prenatal developmental toxicity of decabromodiphenyl ethane in the rat and rabbit. *Birth Defects Res B Dev Reprod Toxicol*, 89(2):139-146.
<https://doi.org/10.1002/bdrb.20237>
- Hardy ML, Aufderheide J, Krueger HO, et al., 2011. Terrestrial toxicity evaluation of decabromodiphenyl ethane on organisms from three trophic levels. *Ecotoxicol Environ Saf*, 74(4):703-710.
<https://doi.org/10.1016/j.ecoenv.2010.10.027>
- Hardy ML, Krueger HO, Blankinship AS, et al., 2012. Studies and evaluation of the potential toxicity of decabromodiphenyl ethane to five aquatic and sediment organisms. *Ecotoxicol Environ Saf*, 75(1):73-79.
<https://doi.org/10.1016/j.ecoenv.2011.08.005>
- He MJ, Luo XJ, Chen MY, et al., 2012. Bioaccumulation of polybrominated diphenyl ethers and decabromodiphenyl ethane in fish from a river system in a highly industrialized area, South China. *Sci Total Environ*, 419:109-115.
<https://doi.org/10.1016/j.scitotenv.2011.12.035>
- He P, He W, Wang A, et al., 2008. PBDE-47-induced oxidative stress, DNA damage and apoptosis in primary cultured rat hippocampal neurons. *Neurotoxicology*, 29(1):124-129.
<https://doi.org/10.1016/j.neuro.2007.10.002>
- He SJ, Li MY, Jin J, et al., 2013. Concentrations and trends of halogenated flame retardants in the pooled serum of residents of Laizhou Bay, China. *Environ Toxicol Chem*, 32(6):1242-1247.
<https://doi.org/10.1002/etc.2172>
- Hong B, Wu T, Zhao GC, et al., 2015. Occurrence of decabromodiphenyl ethane in captive Chinese alligators (*Alligator sinensis*) from China. *Bull Environ Contam Toxicol*, 94(1):12-16.
<https://doi.org/10.1007/s00128-014-1353-z>
- Huang SC, Giordano G, Costa LG, 2010. Comparative cytotoxicity and intracellular accumulation of five polybrominated diphenyl ether congeners in mouse cerebellar granule neurons. *Toxicol Sci*, 114(1):124-132.
<https://doi.org/10.1093/toxsci/kfp296>
- Jin MQ, Zhang XF, Wang LJ, et al., 2009. Developmental toxicity of bifenthrin in embryo-larval stages of zebrafish. *Aquat Toxicol*, 95(4):347-354.
<https://doi.org/10.1016/j.aquatox.2009.10.003>
- Kierkegaard A, Bjorklund J, 2003. The presence of a "new" flame retardant, decabromodiphenyl ethane, in environmental samples. *Organohalogen Compound*, 61:183-186.
- Knudsen GA, Sanders JM, Hughes MF, et al., 2017. The biological fate of decabromodiphenyl ethane following oral, dermal or intravenous administration. *Xenobiotica*, 47(10):1242-1254.

- 894-902.
<https://doi.org/10.1080/00498254.2016.1250180>
- Lee S, Song GJ, Kannan K, et al., 2014. Occurrence of PBDEs and other alternative brominated flame retardants in sludge from wastewater treatment plants in Korea. *Sci Total Environ*, 470-471:1422-1429.
<https://doi.org/10.1016/j.scitotenv.2013.07.118>
- Liu HC, Zhu XY, Chen JH, et al., 2017. Toxicity comparison of different active fractions extracted from radix *Sophora tonkinensis* in zebrafish. *J Zhejiang Univ-Sci B (Biomed & Biotechnol)*, 18(9):757-769.
<https://doi.org/10.1631/jzus.B1600158>
- Moller A, Xie ZY, Cai MH, et al., 2011. Polybrominated diphenyl ethers vs alternate brominated flame retardants and dechloranes from East Asia to the Arctic. *Environ Sci Technol*, 45(16):6793-6799.
<https://doi.org/10.1021/es201850n>
- Nakari T, Huhtala S, 2010. In vivo and in vitro toxicity of decabromodiphenyl ethane, a flame retardant. *Environ Toxicol*, 25(4):333-338.
<https://doi.org/10.1002/tox.20499>
- Putman DL, Morris MJ, 1991. Chromosome aberrations in Chinese Hamster Lung (CHL) cells. Test article Saytex 402. Study Number: T9499.337025. Microbiological Associates, Inc., Rockville, MD, p.1-55.
- San RHC, Wagner VO, 1991. Salmonella/mammalian-microsome plate incorporation mutagenicity assay (Ames test) and *Escherichia coli* WP2 uvrA reverse mutation assay. Test article Saytex 402. Study Number: T9499.501038. Microbiological Associates, Inc., Rockville, MD, p.1-68.
- She YZ, Wu JP, Zhang Y, et al., 2013. Bioaccumulation of polybrominated diphenyl ethers and several alternative halogenated flame retardants in a small herbivorous food chain. *Environ Pollut*, 174:164-170.
<https://doi.org/10.1016/j.envpol.2012.11.024>
- Sun RB, Xi ZG, Zhang HS, et al., 2014. Subacute effect of decabromodiphenyl ethane on hepatotoxicity and hepatic enzyme activity in rats. *Biomed Environ Sci*, 27(2):122-125.
<https://doi.org/10.3967/bes2014.026>
- Viberg H, Fredriksson A, Eriksson P, 2007. Changes in spontaneous behavior and altered response to nicotine in the adult rat, after neonatal exposure to the brominated flame retardant, decabrominated diphenyl ether (PBDE 209). *Neurotoxicology*, 28(1):136-142.
<https://doi.org/10.1016/j.neuro.2006.08.006>
- Wang FX, Wang J, Dai JY, et al., 2010. Comparative tissue distribution, biotransformation and associated biological effects by decabromodiphenyl ethane and decabrominated diphenyl ether in male rats after a 90-day oral exposure study. *Environ Sci Technol*, 44(14):5655-5660.
<https://doi.org/10.1021/es101158e>
- Winter MJ, Redfern WS, Hayfield AJ, et al., 2008. Validation of a larval zebrafish locomotor assay for assessing the seizure liability of early-stage development drugs. *J Pharmacol Toxicol Methods*, 57(3):176-187.
<https://doi.org/10.1016/j.vascn.2008.01.004>

中文概要

题目: 底泥中的十溴二苯乙烷的短期暴露对斑马鱼胚胎和幼鱼神经毒性的研究

目的: 评估底泥中的十溴二苯乙烷 (DBDPE) 对斑马鱼早期发育阶段的胚胎毒性和神经行为毒性, 并探索其潜在影响机制。

创新点: 底泥暴露能更真实地反应 DBDPE 等强疏水性污染物在实际环境中的暴露情况, 有利于污染物生态风险评估的科学性和准确性。

方法: 将受精后 4 小时 (4 hpf) 的斑马鱼胚胎置于对照底泥和染毒底泥 (DBDPE 系列浓度) 中进行短期暴露, 观察不同发育阶段的存活率、孵化率、畸形率以及行为 (包括触碰反应和自由泳动) 效应; 并通过斑马鱼幼鱼的乙酰胆碱酶活性、神经系统的相关基因 ($\alpha 1$ -tubulin 和 gap43) 的转录水平以及斑马鱼整体组织的细胞凋亡情况的检测探讨其神经毒性的潜在机制。

结论: DBDPE 从 4 hpf 处理至 120 hpf, 各浓度组的斑马鱼均未出现明显的畸形和死亡。在 72 hpf 时, 最低浓度组 (62.5 $\mu\text{g/kg}$) DBDPE 轻微加快了斑马鱼的孵化, 而最高浓度组 (1000.0 $\mu\text{g/kg}$) DBDPE 轻微延迟斑马鱼的孵化。所有浓度组的 DBDPE 对 48 hpf 时斑马鱼的触碰反应没有任何影响, 最高浓度组 (1000.0 $\mu\text{g/kg}$) DBDPE 对 120 hpf 时斑马鱼的自由泳动总距离有显著的抑制作用 ($P < 0.05$)。但是, 斑马鱼的乙酰胆碱酶活性、 $\alpha 1$ -tubulin 和 gap43 的转录水平未发生显著变化, 所有浓度组的 DBDPE 亦均未诱发斑马鱼整体组织的细胞凋亡。

关键词: 十溴二苯乙烷; 阻燃剂; 斑马鱼; 神经毒性