Effect of polybrominated diphenyl ether exposure on Gadus macrocephalus Tilesius

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ABSTRACT
This study aimed to analyze the changes in the activity of superoxide dismutase and catalase in Gadus macrocephalus Tilesius exposed to different concentrations of dibromodiphenyl ether, tetrabromodiphenyl ether, and decabromodiphenyl ether. Comet assay was used to explore the degree of DNA damage caused by tetrabromodiphenyl ether. The results revealed that different concentrations of these compounds and different exposure times could inhibit the antioxidant enzyme activity. Dibromodiphenyl ether had the highest inhibitory effect, followed by tetrabromodiphenyl ether and decabromodiphenyl ether. The results of the comet assay showed that high concentrations of tetrabromodiphenyl ether can cause DNA damage to G. macrocephalus. Furthermore, the DNA damage caused by tetrabromodiphenyl ether showed a significant concentration- and time-dependent effect.

1. Introduction

The Pacific cod (Gadus macrocephalus Tilesius, Gadiformes, Gadidae, Gadus) is a typical cold-water fish located in the northern Pacific coast waters. In China, G. macrocephalus is mainly native to the Yellow Sea, which generally inhabits sand or ooze sediments with water depths of 50–80 m.[1] These fish are of high economic value, and they are mainly fished for the meat, which is tender, white, tasty, and non-greasy. However, resources of the pacific cod have been severely damaged due to overfishing and pollution of coastal waters in recent years, and the demand for Pacific cod cannot be met. As an excellent new object of mariculture, the prospect of development and utilization of G. macrocephalus is very broad.[2] Thus far, no breakthroughs have been achieved in artificial breeding of Pacific cod, and studies on Pacific cod, including ecotoxicology, are lacking.

Polybrominated diphenyl ethers (PBDEs; C 12 H(0–9)Br(1–10)O) are brominated compounds containing 209 types of homologues. PBDEs are fire-retardant compounds with very efficient fire retardation capacity and are used widely for this purpose.[3] PBDEs can enter the environment through several ways including evaporation and infiltration, and can cause widespread pollution of the biosphere by migration.[4] In 1979, PBDEs were detected for the first time in the soil near a manufacturing plant of the United States; thereafter, they have been found as pollutants in the environment and water, as well as in humans and animals.[5] Due to the refractory nature, stability, and biomagnification of PBDEs, they can diffuse and gather in the environment by migration and food chain transfer, causing toxicity to higher organisms and presenting a hazard to human health.[6–14] In recent years, with the concern of society for the safety of the marine ecosystem, more and more studies are focusing on the accumulation and toxic effects of PBDEs in marine organisms in vivo.[8,15,16] PBDEs are a persistent organic pollutant that have been shown to interfere with the nervous system, thyroid, and endocrine systems and have a carcinogenic effect.[17] Studies have shown that such toxic bromides can hinder the normal development of the human brain, central nervous system, and reproductive system.[15] Eriksson et al. [18] found that the effect of PBDEs on the nervous system in mice and rats manifested as abnormal motor behavior, and decreased memory and learning ability when entering adulthood in male rats.

The antioxidant system is one of the most important defense systems in vivo, and it plays an important role in the healthy growth of organisms. Superoxide dismutase (SOD) and catalase (CAT) are important enzymes that are widespread in the body, and their concentrations fluctuate in response to stresses, such as exposure to environmental pollutants; therefore, the concentrations of these enzymes can reflect the presence of organic pollutants in the environment and can serve as an indicator of environmental pollution stress.[19]
far, many studies have reported the effects of marine pollution on the biological antioxidant defense system. [20,21] Thus far, however, there have been no studies on the effects of PBDEs on the antioxidant defense system of *G. macrocephalus*.

Single cell gel electrophoresis (SCGE) or comet assay and micro gel electrophoresis (MGE) are rapid, simple, and sensitive techniques to detect DNA fragmentation of a single cell.[22] Comet assay can be used to visually detect DNA damage to a single cell. Compared with the conventional methods for detecting DNA damage, SCGE is advantageous due to its wide scope, short experimental procedure, security, accuracy, and high sensitivity.[23] Currently, SCGE is widely used in experiments studying genetic toxicity, DNA damage, repair mechanisms, and biological monitoring.[24–27] No studies have yet used SCGE to investigate the effect of PBDE exposure on *G. macrocephalus*. Therefore, in this study, we investigated the toxic effects and mechanisms of PBDEs on *G. macrocephalus* by determining the impact of PBDEs on its antioxidant enzyme system and DNA in order to understand the toxic effects of PBDEs on marine fish.

### 2. Materials and methods

#### 2.1. Reagents

Dibromodiphenyl ether, tetrabromodiphenyl ether, and decabromodiphenyl ether were purchased from J & K Reagent Company. Dimethyl sulfoxide (DMSO) was purchased from Shanghai Sangon Co. ltd.. The SOD and CAT assay kits were purchased from Nanjing Jiancheng Bioengineering Institute. The SCGE kit was purchased from Beijing Seajet Science and Technology Co. Ltd., and other chemical reagents were purchased from Shenlian Reagent Company (analytical grade).

#### 2.2. Test fish

Test fish were collected from Heroitte and Lushun wharf in the Dalian city. Fish with intact bodies and good vitality (body length, 45 ± 6.8 cm and body weight, 1225 ± 395 g) were selected to temporarily feed in the key laboratory of northern mariculture, Ministry of Agriculture, Dalian Ocean University. The fish were fed once daily and the tank water was changed once daily. The holding time was 10 days.

### 2.3. Experimental methods

#### 2.3.1. Grouping

The fish were sorted into one of 11 different groups (*n* = 6 each): a control group; a DMSO control group; and three groups each with low, middle, and high-concentration gradients with three parallels per concentration group (Table 1). Fish of each group were placed in separate 300-L water tanks (*n* = 6 per tank) with the same conditions and did not fed.

#### 2.3.2. Measurement of enzymatic activity

Muscle samples were placed in 2-ml refrigerated centrifuge tubes after exposing for 24, 48, 72, and 96 h, storing at −20 °C in refrigerator until further testing. Determination of SOD and CAT activity was conducted according to the assay method of kits.

#### 2.3.3. Comet assay

Blood samples were obtained from the tails in a 1-ml syringe, immediately suspended in ice-cold phosphate-buffered saline (PBS) at 4 °C, and the cell density of the suspension was adjusted to 10^4–10^5 cells/mL under a microscope for the standby. SCGE was used according to the assay method of the SCGE kit.

#### 2.3.4. Data processing

In this study, the median lethal concentration was calculated by measuring the straight line difference. The activity of three types of antioxidant enzymes between groups was statistically analyzed, and multiple comparisons of the average values that were significant in the F test were analyzed by SPSS19 single-factor analysis of variance (*p* < 0.05) using the least significant differences (LSD) test. The plots were analyzed using Microsoft Excel.

CASP software was used to analyze image data and to obtain the percentage of tail DNA content. In each concentration group, 100 cells were randomly measured, calculating the rate of DNA tailing and the specific index of DNA damage. Tables were used to tabulate the data. The following equation was used:

### Table 1. The condition of grouping in the experiment.

| Group No. | PBDEs               | Concentration |
|-----------|---------------------|---------------|
| 1         | Dibromodiphenyl ether | 5 ng/L        |
| 2         | Dibromodiphenyl ether | 500 ng/L      |
| 3         | Dibromodiphenyl ether | 50 μg/L       |
| 4         | Tetrabromodiphenyl ether | 5 ng/L       |
| 5         | Tetrabromodiphenyl ether | 500 ng/L     |
| 6         | Tetrabromodiphenyl ether | 50 μg/L      |
| 7         | Decabromodiphenyl ether | 5 ng/L       |
| 8         | Decabromodiphenyl ether | 500 ng/L     |
| 9         | Decabromodiphenyl ether | 50 μg/L      |
| Control   | –                   | –             |
| DMSO control | Dimethyl sulfoxide | 25 μg/L       |
3. Results

3.1. Antioxidant enzyme activity of Pacific cod exposed to dibromodiphenyl ether

3.1.1. SOD activity
As shown in Figure 1, SOD activity was significantly higher in the low and middle-concentration group exposed to dibromodiphenyl ether for 24 h than in the control and DMSO control groups ($p < 0.05$). However, the SOD activity was significantly lower in the high-concentration group than in the control group ($p < 0.05$). The SOD activity continued to decrease with time in the high-concentration group, while SOD activity in the low and middle-concentration group also decreased gradually with time.

3.1.2. CAT activity
As shown in Figure 2, CAT activity was slightly higher in the group exposed to low concentration of dibromodiphenyl ether stress for 24 h than in the control group, but this difference was not statistically significant ($p > 0.05$). However, CAT activity was significantly lower in the group exposed to high concentration of dibromodiphenyl ether for 48–96 h than in the control group ($p < 0.05$).

3.2. Antioxidant enzyme activity of Pacific cod exposed to tetrabromodiphenyl ether

3.2.1. SOD activity
As shown in Figure 3, SOD activity was slightly higher in the group exposed to low concentration of tetrabromodiphenyl ether for 24 h than in the control group, but this difference was not statistically significant ($p > 0.05$). Furthermore, the SOD activity was significantly lower in the high-concentration group than in the control group ($p < 0.05$). After exposure to tetrabromodiphenyl ether for 48 h, SOD activity was significantly higher in the medium- and low-concentration groups than in the high-concentration group ($p < 0.05$), and the SOD activity was significantly lower in the high-concentration group than in the control group ($p > 0.05$).

3.2.2. CAT activity
As shown in Figure 4, tetrabromodiphenyl ether had the same impact on CAT and SOD activity in the fish. Medium and low concentrations of tetrabromodiphenyl ether resulted in an increase in the CAT activity, but high concentrations of tetrabromodiphenyl ether inhibited CAT activity. The difference in the CAT activity between the three exposure groups and the control group started to show a decreasing trend after 96 h of exposure.

3.3. Antioxidant enzyme activity of Pacific cod exposed to decabromodiphenyl ether

3.3.1. SOD activity
As shown in Figure 5, exposure to decabromodiphenyl ether affected the SOD activity in G. macrocephalus. There were no significant differences in the SOD activity between groups at 24 h. After 48 h of exposure, however, the SOD activity was significantly higher in the medium- and low-concentration groups than in the high-concentration group ($p < 0.05$), and the SOD activity was slightly lower in the high-concentration group than in the control group ($p > 0.05$).

3.3.2. CAT activity
As shown in Figure 6, there was no significant difference in the CAT activity between the three exposure groups and the control group at 24 h ($p > 0.05$). After 48 h, the CAT activity in each exposure group was significantly higher than the corresponding level in the control group ($p < 0.05$), and the CAT activity in the high-concentration group was slightly lower than that in the control group ($p > 0.05$). The CAT activity was stabilized after 96 h.

3.4. Effect of tetrabromodiphenyl ether on DNA damage

Tables 2–5 present the results of the DNA damage analysis in blood cells following exposure to different concentrations of tetrabromodiphenyl ether for different times. The results indicated that during weeks 1 and 2, tetrabromodiphenyl ether did not cause any significant DNA damage to the blood cells of G. macrocephalus Tilesius ($p > 0.05$). At week three, the blood cells showed significant DNA damage in the middle- and high-concentration groups with trailing rates of 8 and 16%. At week four, the trailing rates of the middle- and high-concentration groups were 9 and 18%, and the difference was significant, and the group exposed to the high concentration of tetrabromodiphenyl ether revealed the presence of grade 4 cells.

4. Discussion

4.1. Effect of PBDEs on antioxidant enzyme activity

Many studies have shown that pollutants affect antioxidant enzyme activities of fish to different extents due to different concentrations and time of exposure. Fish adapt to the toxic reactions caused by contamination and have a certain threshold as a result of this adaptation. When fish are exposed to contaminants at concentrations and...
4.1.1. Effect of PBDEs on SOD activity  

The experimental results showed that the SOD activity of *G. macrocephalus* was affected when the fish were exposed to polybrominated diphenyl ethers. Of these compounds, the effect of dibromodiphenyl ether seemed to be the most obvious. The SOD activity in the three groups exposed to different concentrations of dibromodiphenyl ether for 24 h was significantly different compared to the control group (*p* < 0.05), and these results were consistent with the results of Wu [28] who durations of time that are lower than this threshold, they produce an adaptive response and eliminate the damage caused by active oxygen-free radicals by enhancing the antioxidant enzyme activity; therefore, it can be said that pollutants produce an induction on the fish body; when it is greater than the threshold, the adaptive response of the fish body to the pollutants is exceeded, and beyond this threshold, the cells are damaged and the antioxidant enzyme activity decreases; thus, pollutants can be thought to produce inhibition in fish.

**Figure 1.** SOD activity of Pacific cod exposed to dibromodiphenyl ether.

**Figure 2.** CAT activity of Pacific cod exposed to dibromodiphenyl ether.

**Figure 3.** SOD activity of Pacific cod exposed to tetrabromodiphenyl ether.

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found that tetrabromodiphenyl ether had a relatively strong toxic effect when compared to decabromodiphenyl ether. In the present experiment, dibromodiphenyl ethers showed a high biological toxicity in terms of SOD activity, which was consistent with the results of Ruma Ghosh[29]. Different concentrations of PBDEs showed different impacts on the SOD activity. Three different high-concentration groups of PBDEs had a strong inhibitory effect on SOD activity throughout the duration of exposure, indicating that the high concentrations of PBDEs had crossed the threshold at which *G. macrocephalus* could tolerate the PBDEs, and at this concentration of PBDEs, it could completely destroy the antioxidant system. Therefore, the SOD activity was inhibited in exposure experiments exceeding this threshold value. However, at low concentrations, PBDEs resulted in increased SOD activity during the early stage of exposure, and the SOD activity gradually approached the level of the control group with time. This phenomenon was consistent with the findings of Ruma Ghosh [29] and Zhengxin Xie[30].

### 4.1.2. Effect of PBDEs on CAT activity

The results of this study revealed that the three types of PBDEs affected the CAT activity of *G. macrocephalus*, showing a trend that was consistent with the effect on SOD activity. The CAT activity of the three exposure groups at different concentrations did not significantly differ from that of the control group at 24 h, and the difference between the exposure and control groups reduced after 96 h, indicating that CAT was slightly more sensitive to SOD, and the overall reaction was relatively lagging behind. Thus, although SOD and CAT can be used as biological markers, SOD may be more sensitive for biological monitoring of persistent organic pollutants in the environment, based on the results of the current study on the effect of PBDEs on *G. macrocephalus*. This result was consistent with the results of Zhengxin Xie [30] obtained by studying *Carassius auratus*.

In summary, three PBDEs were found to inhibit antioxidant enzyme activity in *G. macrocephalus* Tilesius, but they were found to affect SOD activity more significantly and in a more time- and concentration-dependent manner than CAT activity, for which there is no regularity in terms of concentration and time of exposure. This also proves that SOD is the only antioxidant enzyme that uses oxygen-free radicals (O2⁻) as a substrate.[21] When contaminants enter the water environment, the SOD activity of the organisms is first affected. SOD then combines with the oxygen-free radicals to generate H₂O₂, and when the concentration of H₂O₂ in the body increases, the CAT combines with excess H₂O₂ to produce water and oxygen in order to prevent oxidative damage.[31] These
confirmed in the experimental results of the LC₅₀ of the three PBDEs to G. macrocephalus. Dibromodiphenyl ether had the highest LC₅₀ value, followed by tetrabromodiphenyl ether, and decabromodiphenyl ether.

Table 3. The second week of effect of tetrabromodiphenyl ether for the DNA damage in cod.

| Group No. | Number of cell | 0 | 1 | 2 | 3 | 4 | Special indicators of DNA damage | Smear rate (%) |
|-----------|----------------|---|---|---|---|---|---------------------------------|---------------|
| Control   | 100            | 100| 0 | 0 | 0 | 0 | 0                               | 0             |
| DMSO      | 100            | 100| 0 | 0 | 0 | 0 | 0                               | 0             |
| 4         | 100            | 99 | 1 | 0 | 0 | 0 | 0.01                           | 1             |
| 5         | 100            | 96 | 1 | 1 | 1 | 0 | 0.08                           | 1             |
| 6         | 100            | 84 | 3 | 6 | 7 | 0 | 0.36                           | 16*           |

Note: Means with the indicator (*) in the same column indicate a significant difference (P<0.05).

Table 4. The third week of effect of tetrabromodiphenyl ether for the DNA damage in cod.

| Group No. | Number of cell | 0 | 1 | 2 | 3 | 4 | Special indicators of DNA damage | Smear rate (%) |
|-----------|----------------|---|---|---|---|---|---------------------------------|---------------|
| Control   | 100            | 99 | 1 | 0 | 0 | 0 | 0.01                           | 1             |
| DMSO      | 100            | 95 | 1 | 2 | 1 | 0 | 0.08                           | 1             |
| 5         | 100            | 96 | 1 | 3 | 1 | 0 | 0.10                           | 4             |
| 6         | 100            | 82 | 5 | 6 | 6 | 1 | 0.39                           | 18*           |

Note: Means with the indicator (*) in the same column indicate a significant difference (P<0.05).

Table 5. The fourth week of effect of tetrabromodiphenyl ether for the DNA damage in cod.

| Group No. | Number of cell | 0 | 1 | 2 | 3 | 4 | Special indicators of DNA damage | Smear rate (%) |
|-----------|----------------|---|---|---|---|---|---------------------------------|---------------|
| Control   | 100            | 100| 0 | 0 | 0 | 0 | 0                               | 0             |
| DMSO      | 100            | 100| 0 | 0 | 0 | 0 | 0                               | 0             |
| 4         | 100            | 95 | 5 | 0 | 0 | 0 | 0.05                           | 5             |
| 5         | 100            | 91 | 4 | 5 | 0 | 0 | 0.14                           | 9*            |
| 6         | 100            | 82 | 5 | 6 | 6 | 1 | 0.39                           | 18*           |

Note: Means with the indicator (*) in the same column indicate a significant difference (P<0.05).

experiments also demonstrate that dibromodiphenyl ether is the most toxic of the three PBDEs, followed by tetrabromodiphenyl ether, and decabromodiphenyl ether, which has the least toxicity. This was further

Figure 6. CAT activity of Pacific cod exposed to decabromodiphenyl ether.
4.2. Analysis of the extent of DNA damage caused by tetrabromodiphenyl ether exposure

Tetrabromodiphenyl ether is currently one of the most extensively studied PBDEs. Therefore, we selected tetrabromodiphenyl ether to study the extent of DNA damage caused by this compound. The results showed that the tailing rate of the cells gradually increased with increase in the concentration of tetrabromodiphenyl ether, indicating that tetrabromodiphenyl ether had a concentration-dependent effect on DNA damage of blood cells. Furthermore, the tailing rate gradually increased with time in different concentration groups, showing the time-dependent effect of tetrabromodiphenyl ether on DNA damage. Some cells in the control group also exhibited tailing, and it was considered that living cells were susceptible to outside factors and may have been altered during the experiment, resulting in the emergence of a slight migration of trace DNA during electrophoresis.[32]

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Disclosure statement

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