Ecotoxicological effects of mixed pollutants resulted from e-wastes recycling and bioaccumulation of polybrominated diphenyl ethers in Chinese loach (*Misgurnus anguillicaudatus*)

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Abstract

To understand potential ecotoxicological effects of electrical and electronic equipment waste (e-waste) recycling and polybrominated diphenyl ethers (PBDEs) bioaccumulation in loaches, a semi-field experiment using Chinese loach (*Misgurnus anguillicaudatus*) as experimental fish was performed. Larval loaches were kept in net-cage for three months in an e-wastes recycling site and a reference site in Southeastern China. There was significant difference of the survival rate between the loaches from the e-wastes recycling site (27%, 19/70) and from reference site (70%, 49/70). Histopathological responses were also found in all the livers examined in loaches from the e-wastes recycling site. These results showed that mixed pollutants resulted from e-wastes recycling led to ecotoxicological effects on loaches. The bioaccumulation of polybrominated diphenyl ethers (PBDEs), the main pollutants in e-waste, in loaches was also studied, the mean concentration of total PBDEs in sediment was 6726.17 ng/g wet weight and in water samples was 4.08 ng/L (dissolved phase). BDE 209 was the dominant congener in sediment and with relatively high concentration in water. Relatively low concentration of BDE 209 (less than 0.01% of total PBDEs) and high concentration of BDE47 (up to 39.34% of total PBDEs) were detected in loaches.

Key words: e-wastes; loach; ecotoxicological effects; polybrominated diphenyl ethers; semi-field experiment

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Introduction

As a consequence of electronics industry growth, electrical and electronic equipment wastes (e-wastes), such as wasted computers, mobile phones, television sets, are generated in large quantities around the world. The e-wastes throughout the world are being transported to developing countries under the name of “recycling”. It has been estimated that about 80% of computer e-waste was exported to Asia, and 90% of these exports have been sent to China (Schwarzer et al., 2005).

The recycling of e-wastes in developing countries, especially in China, becomes a serious problem due to unadvanced treatment methods. Taizhou and Guiyu are two of the most famous e-wastes recycling centers in China. In these regions, e-wastes are disassembled and shattered into powder to obtain plastics and rare heavy metals. During the treatment process, hazardous chemicals, such as heavy metals (lead, mercury, cadmium, etc.) and brominated flame retardants (BFRs), have been released and lead to severe pollution problems in air, soil, river water and sediment (Söderström and Marklund, 2002; Jang and Townsend, 2003; Luo et al., 2007; Fu et al., 2008).

Polybrominated diphenyl ethers (PBDEs) are main products of BFRs, which are used in the plastic housings of electronic equipment and in circuit boards to prevent flammability. High contamination levels of PBDEs and heavy metals have been reported in the environmental and biotic samples from e-wastes recycling sites in South China (Bi et al., 2007; Luo et al., 2007; Fu et al., 2008; Wu et al., 2008). Increasing evidences have demonstrated that PBDEs as well as PCBs has thyroid disrupting activity, neurotoxicity, reproductive toxicity, etc. (Hallgren et al., 2001; McDonald, 2002). In addition, the damages of some heavy metals to nervous system, endocrine system, and immune system are well known (Blumenthal et al., 1995; Akesson et al., 2005; Brigden et al., 2005). Therefore, the potential ecotocicological risk of mixed pollutants resulted from e-waste recycling should be paid more attention. Unfortunately, little concern has been focused on this
issue while numerous studies have reported high levels of various pollutants in e-wastes recycling sites (Luo et al., 2007; Deng et al., 2007; Liang et al., 2008; Wu et al., 2008; Zheng et al., 2008).

Laboratory experiments on toxic effects of mixed pollutants cannot simulate complete environmental exposure and reveal realistic effects of environmental exposure, although it can provide some helpful information on toxic effects of mixtures of two or more pollutants on animals (Schmidt et al., 2005). Wide environmental surveys on ecotoxicologic effects of pollutants as well as human epidemiological investigations need very large number of animal samples and cannot obtain explicit results due to some confounding factors (Volety, 2008; Randak et al., 2009). Semi-field experiment can overcome disadvantages of laboratory experiments and obtain explicit results on ecotoxicological effects of mixed pollutant-exposure in the environment (Luckenbach et al., 2001; Grinwis et al., 2000).

The aim of this study was to determine the ecotoxicological effects of e-wastes recycling treatment. A semi-field experiment using Chinese loach (Misgurnus anguillicaudatus) as test animal was conducted in Taizhou, Zhejiang, China. The bioaccumulation of PBDEs, the main pollutants in e-waste, in loaches from e-wastes recycling sites was also detected.

1 Materials and methods

1.1 Chemicals

Polychlorinated biphenyl (PCB) 209 used as surrogate was obtained from Supelco (Bellfonte, USA). A standard solution which contained 39 PBDE congeners (EPA method 1614 standard solution) (Accustandard, New Haven, USA) was used to quantify the following congeners: mono-BDEs 1, 2, and 3; di-BDEs 7, 8, 10, 11, 12, 13, and 15; tri-BDEs 17, 25, 28, 30, 32, 33, 35, and 37; tetra-BDEs 47, 49, 66, 71, 75, 77; penta-BDEs 85, 99, 100, 116, 118, 119, and 126; hexa-BDEs 138, 153, 154, 155, and 166; and hepta-BDEs 181, 183, and 190. Analytical standard of BDE 205, 206 was purchased from Supelco (Bellfonte, USA) for their quantitation. Another standard solution of PBDE congeners (EO-5278) (Cambridge Isotope Laboratories, USA), which contained BDE 28, 47, 99, 100, 153, 154, 183, and 209, was used for quantitation of BDE 209. n-Hexane, methylene dichloride, acetone, and nonane were pesticide grade and purchased from Tedia (Fairfield, USA). Anhydrous sodium sulfate, sulfuric acid, sodium hydroxide, and anhydrous ethanol were analytical grade (Beijing Chemical Factory, China). Hematoxylin and eosin were also purchased from Beijing Chemical Factory.

1.2 Sites for the semi-field experiment

Two sites in Taizhou, Zhejiang, were selected to perform a semi-field experiment in October 2007. The site of NW (121°13′21″E; 28°21′41″N) was chosen as polluted site and the site of FS (120°58′14″E; 28°35′28″N) was chosen as reference site. The selected sites are typical hill villages in South China. The altitude of FS is about 300 m higher than that of NW. In NW, the workshops to disassemble and shatter e-waste have appeared widely since last decade. Discarded e-wastes powder was stacked around the village. Under the condition, land runoffs might be considered as one of the most important pathway for PBDEs entering into the local water in NW. High contamination levels of PBDEs and heavy metals were reported in the environmental samples in this area (Fu et al., 2008; Liang et al., 2008; Shen et al., 2008; Yang et al., 2008). Except for e-waste recycling, agriculture (rice culture) was the major economic support system in NW. In FS, approximately 35 km northwest of NW, tourism and agriculture dominate the local economy, and no obvious pollution source exists.

A pond in each site was chosen to maintain loaches. Larval loaches were obtained from a piscina located in FS area. Some larval loaches were sampled to measure PBDEs contamination levels before the semi-field experiment. The concentration of PBDEs in larval loaches was 0.47 ng/g wet weight (2.39 ng/g lipid weight). This PBDE burden in larval loaches might mainly result from atmospheric transmission from pollution source area because there was not pollution source in FS. In each pond, seventy healthy loaches in net-cage were deployed in sites for three months.

1.3 Sampling and toxicological examination

In January 2008, when the semi-field experiment was over, the sediment and water samples from each pond were collected for PBDEs analysis. Sediment samples were taken from approximately the top 2 cm of the sediment and each was constituted by eight subsamples. The sediment samples were wrapped in aluminium foil and sealed in plastic bags on-site. The water samples (40 L) were filtered through glass fiber filters (0.8 μm pore size) and the filtrate was passed through a XAD-2 resins column (Supelco, Bellfonte, USA), which was pre-extracted with methanol, dichloromethane and hexane, to enrich PBDEs on-site. Then, the XAD-2 resins column and sediment samples were stored in a cool box and transported to laboratory. Each XAD-2 column was eluted with hexane, acetone and dichloromethane. The collected eluent was concentrated and solvent exchanged to hexane, and reduced the volume to approximately 0.5 mL under a gentle stream of nitrogen. The sediment samples were stored at 4°C in darkness until analysis.

The cages were dragged from the ponds at the same time to collect the water and sediment samples. All the loaches were transferred to the laboratory for examination. In the laboratory, the loaches were rinsed with clean water for three times. The survival rate, body weight and body length were measured. For each pond nine loaches were randomly sampled for the determination of PBDEs, and these samples were frozen and stored at –20°C in darkness until analysis. The other loaches were dissected. The livers were removed and fixed in Bouin’s fixative. Then, liver samples were dehydrated in a graded series of alcohol, embedded in paraffin, and sectioned in a transverse plane.
at 5 μm thickness. Serial sections were mounted on glass slides, stained with hematoxylin and eosin, and embedded with glass coverslips. Then, liver sections were examined with a light microscope.

### 1.4 Analysis of PBDEs

PBDEs in loach sample was determined according to the method of Liang et al. (2008). After samples were freeze-dried and homogenized with anhydrous sodium sulfate, 1 ng PCB 209 were spiked into the sample and equilibrated for 24 h. Then, the samples were Soxhlet extracted with hexane/dichloromethane for 24 h. After concentration, extracts were cleaned up with multilayer silica gel column. The collected eluents were concentrated and reduced the volume to 20 μL under a gentle stream of N2. All samples were analyzed by Agilent 6890 gas chromatograph coupled with Agilent 5973 mass spectrometer using electron capture negative ionization (ECNI) source in the selected ion monitoring (SIM) mode. The ion of m/z 79 and 81 were monitored for congener from mono-BDE to nona-BDE and m/z 486.7 and 488.7 for BDE 209. The identification of PBDE congeners was depended on the comparison of retention times and mass spectrum with appropriate standards. Quantification was performed by external standard method with multi-level calibration curve spanning the range of anticipated analyte concentrations in samples.

### 1.5 Quality assurance and quality control

The method precision and recovery were determined by analyzing fish tissue that was spiked with PBDEs standard. One procedural blank was run for every batch of nine samples to check the potential contamination from analysis process. Instrumental quality control was done by regular injection of solvent blanks and standard solutions. The recoveries ranged from 85% to 115% for all analytes with the exception of three mono-BDE congeners. The low recoveries of mono-BDE congeners might result from their relative high vapor pressure and volatilization loss during extraction and concentration procedure. Therefore, in this study the concentrations of mono-BDE congeners were not included in the statistical analysis. The recoveries of surrogate in all samples ranged from 85% to 110%.

### 1.6 Statistical analyses

Statistical analysis was performed using SPSS software version 13.0. The survival rates of loaches collected from two sites were compared using Chi-Square. Body weight and body length of loaches from two sample sites were compared using independent-samples t-test. A value of α= 0.05 was chosen to give a significant difference.

### 2 Results and discussion

#### 2.1 Survival, growth and histopathology of the loaches

The body length of loaches in NW ((12.25 ± 0.69) cm) showed no significant difference from that in FS ((11.85 ± 1.15) cm). However, the body weights of loaches in NW ((7.76 ± 1.32) g) showed significant difference from that in FS ((5.37 ± 1.86) g). As FS locates at relative higher altitude site than NW, the lower body weight of loaches in FS was possibly due to the relatively low temperature.

There was significant difference of the survival rate between loaches from e-wastes recycling site NW (27.14%, 19/70) and reference site FS (70%, 49/70). Relatively high mortality in controls maybe results from maladjustment of fish in the wild environment and the change of the climate from October to January. Hepatocytes exhibited a swelled shape and arranged loose in all the livers examined in loaches from NW (Fig. 1). In laboratory study, Tseng et al. (2008) reported that the exposure of pregnant mice to BDE 209 (10, 500, and 1500 mg/(kg·d)) caused cell swelling of hepatocytes in male offspring. In the present study site, pollutants resulted from e-wastes recycling were mixed pollutants which included PBDEs, PCBs, lead, mercury, cadmium, etc. (Fu et al., 2008; Liang et al., 2008; Shen et al., 2008; Yang et al., 2008). Therefore, the low survival rate and the histopathological responses in loaches from NW might be related with the mixture of contaminants including PBDEs. Raldúa et al. (2008) reported that histopathological changes (increase of the number and size of macrophage aggregates in both liver

![Fig. 1 Histology of livers of loaches collected from the control site (a) (hepatocytes with well shape arranged compact) and an e-waste recycling site (b) (hepatocytes exhibited a swelled shape and arranged loose).](image-url)
and kidney) occurred in feral barbel (*Barbus graellisi*) from downstream an industrial park, where some plants released the discharges of effluents rich in PBDEs. The authors concluded that the study provided the first evidence of PBDEs effects in feral barbel from the Ebro River basin. Our results suggested that mixed pollutants resulted from e-wastes recycling caused ecotoxicological effects on local animals, although the effects might not be completely caused by PBDEs.

### 2.2 PBDE levels in sediment and water samples

The concentrations of PBDEs in loach, sediment and water from the e-wastes recycling site were much higher than that from FS (Table 1). High PBDEs concentration in sediments (13469 ng/g dry weight (dw)) from NW was detected in the present study, and this concentration is similar with the level reported previously (Eljarrat et al., 2007; Luo et al., 2007). Total PBDEs concentration in sediment from NW was 2003 times higher than that from FS (6.02 ng/g dw) and other studies sites (Lacorte et al., 2003; Zheng et al., 2004). BDE 209 was found in sediments from NW, and the concentration was 3003 ng/g dw, which was approximately 50 times higher than that from Nanyang river bank sediment near to e-wastes open burning and dumping site (the highest concentration of BDE 209 was 62.2 ng/g dw) (Luo et al., 2007).

The concentrations of PBDEs in water samples from FS were below the limit of detection and the sum concentration of PBDEs was 4.08 ng/L in water samples from NW. Wu et al. (2008) reported 24.4 ng/L (dissolved phase) PBDEs (sum of 18 PBDE congeners: BDEs 28, 47, 66, 85, 99, 100, 138, 153, 154, 183, 196, 197, 203, 205, 206, 207, 208, and 209) in water sample from a reservoir, which was surrounded by several e-wastes recycling workshops in South China. In the present study, the sum concentration of PBDEs was 53.81 ng/L in water samples, which was higher than that reported by Streets et al. (2006) and Wu et al. (2008). High value of PBDEs detected in sediment and water samples from NW indicated that e-wastes recycling resulted in a serious PBDEs pollution in studied area.

### 2.3 PBDEs in loach samples

In this study, the average concentration of PBDEs in loaches from NW was 266.50 ng/g wet weight (ww)

### Table 1 Levels of individual PBDE congener, total PBDEs in loach, water and sediment samples collected from reference site (FS) and an e-wastes recycling site (NW) (ng/g)

<table>
<thead>
<tr>
<th>Target compounds</th>
<th>FS</th>
<th>Sediment sample</th>
<th>Water sample</th>
<th>NW</th>
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<tr>
<td></td>
<td>Loach sample</td>
<td>Water sample</td>
<td>Sediment sample</td>
<td>Water sample</td>
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<tr>
<td></td>
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<td>lw</td>
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<td>dw</td>
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<td>BDE 10</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
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<td>BDE 7</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
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<td>BDE 11/8</td>
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<td>nd</td>
<td>nd</td>
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<td>BDE 15</td>
<td>nd</td>
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<td>BDE 30</td>
<td>nd</td>
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<td>nd</td>
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<td>BDE 32</td>
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<td>nd</td>
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<td>BDE 17/25</td>
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<td>BDE 28/33</td>
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<td>BDE 37</td>
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<td>BDE 75</td>
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<td>BDE 71</td>
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<td>17.66</td>
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<td>BDE 100</td>
<td>0.07</td>
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<td>BDE 118</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td>BDE 126/155</td>
<td>0.65</td>
<td>nd</td>
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<td>nd</td>
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<tr>
<td>BDE 154</td>
<td>0.27</td>
<td>83.71</td>
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<td>BDE 190</td>
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<td>BDE 205</td>
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<td>BDE 206</td>
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<td>nd</td>
<td>nd</td>
<td>0.06</td>
<td>0.08</td>
</tr>
<tr>
<td><strong>PBDE</strong></td>
<td>1.10</td>
<td>342.57</td>
<td>4.57</td>
<td>5.94</td>
</tr>
<tr>
<td><strong>Total PBDEs</strong></td>
<td>1.10</td>
<td>342.57</td>
<td>4.63</td>
<td>6.02</td>
</tr>
</tbody>
</table>

ww: wet weight; lw: lipid weight; dw: dry weight; dp: dissolved phase; pp: particulate phase.
nd: not detected, 0 was used for the calculations.

**∑PBDE**\textsubscript{18S}: sum of quantified PBDE congeners except for BDE 209.
(16963.39 ng/g lipid weight (lw)). This total concentration of PBDEs in loach samples was almost the same level found in trout (Salmo trutta) (72–1120 ng/g ww; 2348–16753 ng/g lw) and burbot (Lota lota) (156–2265 ng/g ww; 4201–45144 ng/g lw) collected from a lake near a textile manufacturer in Norway (Mariussen et al., 2008); and was 10 times higher than that (1560 ng/g lw) collected from a lake near a textile manufacturer in Norway (Mariussen et al., 2008).

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The congener distribution patterns of PBDEs in sediment, water, and loach samples are different (Fig. 2). In samples collected from NW, the concentration order of individual PBDE congeners in sediment was BDE 209 > BDE 47 > BDE 99 > BDE 49. The concentration order of individual PBDE congeners in water was BDE 47 > BDE 209 > BDE 99 > BDE 49. However, in the particulate from water sample, BDE 209 was the dominant congener. These data showed that BDE 209 was the main pollutant in the site. However, in loach samples the concentration of BDE 209 was very low, and it contributed less than 0.01% of total PBDEs in loach samples. BDE 47 concentration among all detected congeners was the highest (up to 39.34% of total PBDEs) in loach samples, which is consistent with the results from previous studies (Law et al., 2006; Luo et al., 2007). The phenomenon may be due to that different congeners would have different uptake and excretion and debromination of higher-brominated congeners in fish (Hakk and Letcher, 2003; Stapleton et al., 2004a).

Boon et al. (2002) concluded the low bioavailability of BDE 209 in minority of biotic samples may result in a low uptake rate of the very large molecule or a relatively rapid excretion after biotransformation. Recently, however, relatively high BDE 209 values were also found in some fish species. For example, 48 ng/g lw (median concentration) BDE 209 was detected in muscle tissues of roach (Rutilus rutilus) from Lumparn estuary, Baltic Sea (Burreau et al., 2004). Eljarrat et al. (2007) reported 38–707 ng/g lw BDE 209 detected in barbel (Barbus graellsi) from downstream of an industrial park where flame retardants were used. These studies proved the bioavailability of BDE 209 for fish. Several studies have suggested that BDE 209 seems to easily debrominate in some fish species. Stapleton et al. (2004b) reported that BDE 209 debrominated in carp (Cyprinus carpio) during dietary exposure for 60 d and following depuration period within 40 d to seven apparent debrominated congeners, and the dominant congener was BDE 47. Thus, it can be concluded that debromination of BDE 209 easily occurred in carp. Therefore, we suggest that relatively low BDE 209 values in loach samples in this study may be related to reductive debromination as reported in some fish (Stapleton et al., 2004a, 2004b; 2006). In another word, the higher concentration of BDE 47 in loach samples may come from the debromination of higher-brominated congeners besides direct accumulation from sediment and water.

2.4 Potential health risks of the local residents

The total PBDEs concentration in loach samples from NW was approximately 100 times higher than that found in fish from markets of U.S. (1.12 ng/g, sum of 13 PBDE congeners: BDEs 17, 28, 47, 66, 77, 85, 99, 100, 138, 153, 154, 183, and 209) (Voorspoels et al., 2007) and that detected in fresh salmon filet in markets of Belgium (2.36 ng/g ww, sum of 7 PBDE congeners: BDEs 28, 47, 99, 100, 153, 154, and 183) (Schercter et al., 2006; Voorspoels et al., 2007). And it is more than 200 times higher than that (sum of 15 PBDE congeners: BDEs 28, 47, 66, 85, 99, 100, 138, 153, 154, 183, 203, 206, 207, 208, and 209) found in seafood products from local fishery markets in eleven coastal cities of South China (Guo et al., 2007). Thus, the PBDE-contaminated loaches or other fish species from e-wastes recycling sites could be a potential risk for the local residents who highly consume these fish as food. In addition, coexposure to PBDEs and other toxic chemicals, rather than PBDEs alone, should be considered as a health risk factor in e-wastes recycling sites because chemical mixtures possible have much greater effect than the chemical individually (Hallgren and Darnerud, 2002; Eriksson et al., 2006; Fischer et al., 2008).

3 Conclusions

The low survival rate and the histopathological responses occurred in loaches raised in e-wastes recycling site, i.e., mixed pollutants including PBDEs from e-wastes recycling had ecotoxicological effects on loaches. In addition, highly consumption of contaminated loaches or other fish from e-wastes recycling sites as food might be a potential risk to the local residents. BDE 209 was dominant congener in sediment and relative high concentration in water sample from e-wastes recycling site, while relatively low BDE 209 and high BDE 47 were detected in...
loaches. Low concentration of BDE 209 may be related to reductive debromination in some fish as reported in literature. The high concentration of BDE 47 may come from the debromination of higher-brominated congeners besides direct accumulation from sediment and water.

Acknowledgments

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