



## Maternal transfer, distribution, and metabolism of BDE-47 and its related hydroxylated, methoxylated analogs in zebrafish (*Danio rerio*)



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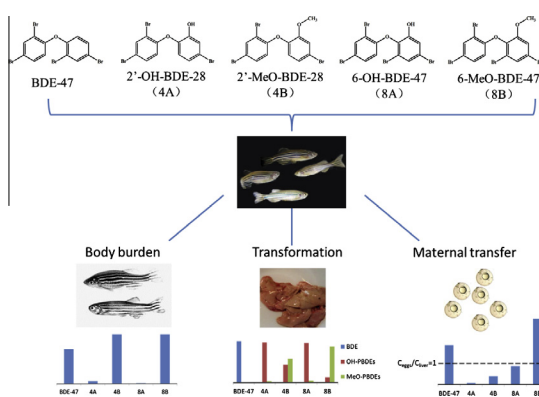
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### HIGHLIGHTS

- Concentrations of all compounds in liver of male zebrafish were greater than those in females.
- Maternal transfer of MeO-BDE-47 was greater than that of OH-BDEs.
- MeO-BDE-47 was transformed to OH-BDE-47.
- BDE-47 was not transformed into either MeO-PBDEs or OH-PBDEs.

### GRAPHICAL ABSTRACT



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### ABSTRACT

OH-PBDEs have been reported to be more potent than the postulated precursor PBDEs or corresponding MeO-PBDEs. However, there are contradictory reports for transformation of these compounds in organisms, particularly, for biotransformation of OH-PBDEs and MeO-PBDEs, only one study reported transformation of 6-OH-BDE-47 and 6-MeO-BDE-47 in Japanese medaka. In present study zebrafish (*Danio rerio*) were exposed to BDE-47, 6-OH-BDE-47, 6-MeO-BDE-47, 2'-OH-BDE-28 and 2'-MeO-BDE-28 in the diet for 20 d. Concentrations of each exposed compound were measured in eggs collected on days 0, 5, 10, 15 or 20. After 20 d exposure, concentrations of precursor and biotransformation products in liver and liver-free residual carcass were measured by use of GC-MS/MS. Total mass of the five compounds in bodies of adults were: 2'-MeO-BDE-28 ~ 6-MeO-BDE-47 > BDE-47 > 2'-OH-BDE-28 > 6-OH-BDE-47. MeO-PBDEs were also accumulated more into parental fish body than in liver, while OH-PBDEs accumulated in liver more than in liver-free residual carcass. Concentrations in liver of males were greater than those of females. This result suggests sex-related differences in accumulation. Ratios between concentration in eggs and liver (E/L) were: 2.9, 1.7, 0.8, 0.4 and 0.1 for 6-MeO-BDE-47, BDE-47, 6-OH-BDE-47, 2'-MeO-BDE-28 and 2'-OH-BDE-28, respectively. This result suggests transfer from adult females to eggs. BDE-47 was not transformed into OH-PBDEs or MeO-PBDEs. Inter-conversions of 6-OH-BDE-47 and 6-MeO-BDE-47, 2'-OH-BDE-28 and 2'-MeO-BDE-28 were observed, with metabolite/precursor concentration ratios for 6-OH-BDE-47, 6-MeO-BDE-47, 2'-OH-BDE-28 and 2'-MeO-BDE-28 being 3.8%, 14.6%, 2.9% and 76.0%, respectively. Congener-specific differences were

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observed in distributions between liver and carcass, maternal transfer and transformation. The two MeO-PBDEs were accumulated into adults, transferred to eggs, and were transformed to the structural similar OH-PBDEs, which might be more toxic. BDE-47 was accumulated into adults and transferred from females to eggs, but not transformed to MeO-PBDEs and/or OH-PBDEs. Accumulation of OH-PBDEs into adults as well as rates of transformation of OH-PBDEs to MeO-PBDEs were all several orders of magnitude less. Thus, MeO-PBDEs are likely to present more of a risk in the environment.

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## 1. Introduction

Due to their efficient performance and low-cost, polybrominated diphenyl ethers (PBDEs) have been widely used for many years as flame retardants in various commercial products, such as furniture, textiles, plastics, paints, and electronic appliances (Hites, 2004). Because of their persistence and potential to bioaccumulate (Johnson-Restrepo and Kannan, 2009), PBDEs have been reported to occur in various environmental matrices (Norén and Meironyté, 2000; Ohta et al., 2002). Analogs of PBDEs, including hydroxylated (OH-PBDEs) and methoxylated PBDEs (MeO-PBDEs), were neither manufactured nor used in industrial production, but detected extensively in the marine environment (Teuten et al., 2005; Houde et al., 2009; Rotander et al., 2012). The fact that OH-analogs were more potent than their respective PBDEs for some endpoints, raised concern about the potential toxicity of PBDEs and their analogs (He et al., 2008).

Toxic potencies of PBDEs, MeO-PBDEs and OH-PBDEs are different. PBDEs can disrupt function of hormones in adult, male consumers of Great Lakes fish (Turyk et al., 2008), the hypothalamic–pituitary–thyroid axis in fish (He et al., 2011; Chan and Chan, 2012; McClain et al., 2012; Zheng et al., 2012), motor neuron and skeletal muscle development in larvae zebrafish, gonad development in both female and male zebrafish, in particular gamete quantity and quality of male adult zebrafish (He et al., 2011). Though no statistically significant effects were observed in zebrafish embryos exposed to PBDEs and MeO-PBDEs, toxicities of OH-PBDEs were observed after exposure of zebrafish embryos to equivalent nanomolar (nM) concentrations (Boxtel et al., 2008). For some endpoints, OH-BDEs were more potent than the postulated precursor PBDEs and corresponding MeO-PBDEs (Kojima et al., 2009).

Distribution among tissues, maternal transfer to eggs and biotransformation of several PBDEs congeners has been reported previously. Correlations among concentrations of PBDEs and OH-PBDEs, OH-PBDEs and MeO-PBDEs were observed (Jakobsson et al., 2008; Zhang et al., 2010). However, there are contradictory reports for inter-conversion of the PBDE analogs in fishes. Several studies reported hydroxylation of  $^{14}\text{C}$ -BDE-47 in northern pike (Hakk and Letcher, 2003) and debromination of PBDEs both *in vivo* and *in vitro* (Hakk and Letcher, 2003; Stapleton et al., 2006; Benedict et al., 2007; Browne et al., 2009), while no transformation of BDE-47 was observed in medaka (*Oryziaslatipes*) (Wan et al., 2010). Transformation from MeO-PBDEs to OH-PBDEs in rainbow trout microsomes (*in vitro*) and transformation from 6-MeO-BDE-47 to 6-OH-BDE-47 in medaka (*in vivo*) has been observed, while transformation from 6-OH-BDE-47 to 6-MeO-BDE-47 was observed in medaka (*in vivo*) but no transformation from OH-PBDEs to MeO-PBDE was observed in rainbow trout microsomes (*in vitro*) (Wan et al., 2009, 2010). Particularly, for the metabolism of OH-PBDEs and MeO-PBDEs, only one study reported the transformation of 6-OH-BDE-47 and 6-MeO-BDE-47 in Japanese medaka as indicated above. More OH-PBDE and MeO-PBDEs should be included to redefine the transformation and a more detailed research, considering species difference, and

sex-related difference, should be conducted. In some of these studies the reported rates of transformation were very small and could be explained by the presence of contaminants in the original material used in the studies. Only the studies by Wan et al. (2009) checked the original materials for the presence of putative products of transformation.

To evaluate potential risks posed by PBDE and their analogs in aquatic environment, transformation of PBDEs, OH-PBDEs and MeO-PBDEs in whole organisms needed to be confirmed, as well as their distribution among tissues of adult fish and transfer from females to eggs. In present study, besides BDE-47, 6-OH-BDE-47 and 6-MeO-BDE-47, 2'-OH-BDE-28 and 2'-MeO-BDE-28, which is structurally similar to BDE-47, were selected to investigate the distribution, maternal transfer and metabolism in zebrafish (*Danio rerio*).

BDE-47 and its analogs have been detected in freshwater and marine species (Malmvarn et al., 2005; Valters et al., 2005; Verreault et al., 2005; Xian et al., 2008). However, information on transfer from adult females to eggs and transformation of these compounds by freshwater fish was limited, especially sex-related. In the study, the results of which are reported here zebrafish were exposed to BDE-47, 6-OH-BDE-47, 6-MeO-BDE-47, 2'-OH-BDE-28 or 2'-MeO-BDE-28 in the diet to: (1) determine distribution of these compounds in male and female zebrafish; (2) evaluate transfer of these compounds from females to eggs; (3) characterize transformation of each compound in adult zebrafish and eggs.

## 2. Materials and methods

### 2.1. Experimental design

PBDEs,  $^{13}\text{C}$ -2'-OH-BDE-99 and  $^{13}\text{C}$ -BDE-139 were purchased from Cambridge Isotope Laboratories (Andover, MA, USA), and MeO-PBDEs and OH-PBDEs were synthesized in the Department of Biology and Chemistry of City University of Hong Kong (Marsh et al., 2003). Commercial fish food (Tropical Fish Food, Jiangmen Porpoise Aquarium Co., Ltd. Guangdong, China) was weighed and spiked with one of the following: BDE-47, 2'-MeO-BDE-28, 6-MeO-BDE-47, 2'-OH-BDE-28 or 6-OH-BDE-47 stock standard solutions, dissolved in acetone. Spiked samples were mixed for an hour then dried under nitrogen. Control food was spiked acetone in the same method.

Five-month old adult zebrafish (mean mass  $0.56 \pm 0.13$  g), 8 females and 8 males were randomly assigned to 12 L tanks containing 8 L of dechlorinated tap water and allowed to acclimate for 7 d. Triplicates of each exposure group were maintained. Water temperatures were maintained at  $28 \pm 1$  °C and all water in the tanks was replaced daily with fresh water. Zebrafish were fed approximately 1% of their average body mass per day at 10:00 h. In addition brine shrimps were provided to all fish at 22:00 h, which were hatched from the embryos in our lab. All of the prepared food was consumed in each tank. Zebrafish were exposed to each target chemical for 20 d, and eggs were collected every morning (day 0–20) before feeding. Eggs collected from each

individual tank were rinsed with MilliQ water and gently dried. Eggs from each exposure were composited, weighed and stored at  $-80^{\circ}\text{C}$  until analysis on the every five days (5, 10, 15, 20th day), respectively. On the 20th day, zebrafish were not fed so as to eliminate interference from undigested food in the gut. Eggs were collected, and then five each of male and female zebrafish were collected from each treatment tank and euthanized by use of 250 mg MS-222/L. Livers were dissected from each fish, and both liver and the liver-free residual carcass were stored at  $-80^{\circ}\text{C}$ .

## 2.2. Sample extraction and cleanup

Detailed protocols for extraction, clean up, identification and quantification, and quality assurance and quality control (QA/QC) are provided elsewhere (Wen et al. 2012). Liver ( $\sim 0.03$  g), egg ( $\sim 0.3$  g), liver-free residual carcass ( $\sim 0.3$  g) and prepared food ( $\sim 0.1$  g) were homogenized and transferred into amber serum bottles, spiked surrogate recovery standards ( $^{13}\text{C}$ -2'-OH-BDE-99 and  $^{13}\text{C}$ -BDE-139 (Wellington Laboratories Inc., Guelph, Canada)), 2 mL of MilliQ water, 50  $\mu\text{L}$  of hydrochloric acid (HCl, 2 M), and 2 mL of 3-propanol, then extracted three times with 10 mL of n-hexane/methyl tert-butyl ether (MTBE) (1:1, v/v). Extractions were concentrated and dried under nitrogen. Dried residues were dissolved in 960  $\mu\text{L}$  of derivatization solvent (acetonitrile:methanol:water:pyridine = 5:2:2:1, v/v/v/v), and 100  $\mu\text{L}$  of methyl chloroformate (MCF) was added. After vortex mixing for 1 min, samples were incubated at  $25^{\circ}\text{C}$  for 1 h, after which 1.4 mL of MilliQ water was added and extracted three times with 10 mL of n-hexane. The organic solvent was concentrated and cleaned up by a silica gel column (60–100 mesh size), which was dry packed with glass-wool, 0.25 g of silica gel, 1.00 g of 44% (w/w) acid silica gel, 0.25 g of silica gel and 0.50 g of anhydrous sodium sulfate from bottom to top in a disposable Pasteur pipette. After washing with 10 mL of dichloromethane (DCM) and 10 mL of n-hexane, the sample solvent was added and the column was eluted with 15 mL of n-hexane and 15 mL of n-hexane/dichloromethane (1:1, v/v). After elute was evaporated to dryness, a known amount of  $^{13}\text{C}$ -PCB-178 (Cambridge Isotope Laboratories, USA) was added as the internal injection standard and made up to 100  $\mu\text{L}$  with n-hexane prior to GC-MS/MS analysis of PBDEs (BDE-17, BDE-28, BDE-71, BDE-47, BDE-66, BDE-100, BDE-99, BDE-85, BDE-154, BDE-138, BDE-183 and BDE-190), MeO-PBDEs (6'-MeO-BDE-17, 2'-MeO-BDE-28, 6-MeO-BDE-47, 5-MeO-BDE-47, 4'-MeO-BDE-49, 2'-MeO-BDE-68, 6-MeO-BDE-85, 6-MeO-BDE-90, 4-MeO-BDE-90, 3-MeO-BDE-100, 6'-MeO-BDE-123, 6-MeO-BDE-137) and OH-PBDEs (2'-OH-BDE-7, 3'-OH-BDE-7, 2'-OH-BDE-17, 2'-OH-BDE-25, 2'-OH-BDE-28, 6-OH-BDE-47, 4'-OH-BDE-49, 2'-OH-BDE-66, 2'-OH-BDE-68, 6-OH-BDE-85, 6-OH-BDE-90, 6-OH-BDE-137).

## 2.3. Quantification by GC-MS/MS

Concentrations of 12 PBDEs, 12 OH-PBDEs and 12 MeO-PBDEs were determined by use of a TSQ Quantum GC-MS/MS (Thermo Scientific, USA) coupled with an Agilent DB-XLB column (15 m  $\times$  0.25 mm  $\times$  0.25  $\mu\text{m}$ , J&W Scientific, USA) in 3 separate runs. The mass spectrometer detector was operated in electron impact ionization (EI) mode. Samples and standards were analyzed in both full-scan and selective reaction monitoring (SRM) modes. Precursor ions and product ions selected in SRM mode for each chemical were based on the mass spectrum of the standard solution. Detailed information about retention times, precursor ion and product ions were given in Supporting Information (Table S1 of SI). Identification of specific PBDEs, OH-PBDEs and MeO-PBDEs was performed by comparing relative retention times versus internal standard and product ions in SRM mode with standards.

## 2.4. Purity analysis

Impurities, (OH-PBDEs, MeO-PBDEs, and PBDEs) which could possibly interfere with interpretation of results were quantified in samples of food (Table S2 of SI). Concentrations of target compounds were 100–1000-fold greater than that of the other analogs of interest that occurred as impurities. Thus, impurities of interest were negligible and would not interfere with interpretation of the results of studies of transformation. Concentrations of BDE-47, 2'-OH-BDE-28, 6-OH-BDE-47, 2'-MeO-BDE-28 and 6-MeO-BDE-47 in prepared food were  $1.7 \times 10^4$ ,  $2.8 \times 10^4$ ,  $1.1 \times 10^4$ ,  $1.4 \times 10^4$  and  $1.7 \times 10^4$  ng  $\text{g}^{-1}$  dry mass (dm), respectively.

## 2.5. Quality assurance/quality control (QA/QC) and statistical analysis

Quality assurance and quality control were performed by regular analysis of procedural blanks (RSD < 22.6%, for 6 replicates, Table S3 of SI) and recoveries of standard compounds (PBDEs, MeO-PBDEs and OH-PBDEs) ranged 93–146%, 103–133% and 67–113%, respectively. Limit of detection (LOD) of each compound was defined as three times the SD of the laboratory blanks. For congeners not detected in the blanks, we set the LOD at the instrumental limit of quantification (LOQ). Method LODs ranged  $9.2 \times 10^{-2}$ – $2.9 \times 10^1$  ng  $\text{g}^{-1}$  wet mass (wm) for individual PBDEs, OH-PBDEs and MeO-PBDEs congeners. Concentrations lesser than the LOD were assumed to be not detected. PBDEs, OH-PBDEs and MeO-PBDEs were both quantified in extracts relative to  $^{13}\text{C}$ -PCB-178 internal standards. Recovery of surrogate standards  $^{13}\text{C}$ -BDE-139 and  $^{13}\text{C}$ -2-OH-BDE-99 averaged 65.9–129% and 53.8–85.6%, respectively.

Statistical analyses were performed with the SPSS 16.0 for Windows. The Kolmogorov–Smirnov test was used to determine if the data could be accurately represented by a normal probability function, and homogeneity of variances was analyzed by the Levene's test. If the data failed the Kolmogorov–Smirnov test, logarithmic transformation was performed and then checked again for homogeneity of variances. Once the data satisfied the assumptions of homogeneity of variances, one-way analysis of variance (ANOVA) followed by Least Significant Difference (LSD) test was used to evaluate the differences between the variables. Statistical significance was defined as  $p < 0.05$ .

## 3. Results and discussion

### 3.1. Distribution and maternal transfer in zebrafish

Concentrations of target compounds were detected in liver, liver-free residual carcass and eggs of zebrafish exposed to individual compounds for 20 d (Table 1). Congener-specific differences in body burden of each compound were observed. Although concentrations of BDE-47, 6-OH-BDE-47 and 6-MeO-BDE-47 were comparable in food, in zebrafish liver, liver-free residual carcass and even in eggs concentrations of BDE-47 and 6-MeO-BDE-47 were about 10–100-fold greater than concentration of 6-OH-BDE-47. The bioaccumulation factor (also called the BAF) is the ratio of contaminant concentration measured in biota and in food (Table 2). Concentrations in liver of males were greater than those of females. The bioaccumulation factor  $\text{BAF}_{(\text{liver}/\text{food})}$ , which was calculated as the ratio of concentrations in the liver divided by those in the food, was almost 2-fold greater in males than in females, which suggests sex-related differences in accumulation. Ratios between concentration in eggs and liver (E/L) were: 2.9, 1.7, 0.8, 0.4 and 0.1 for 6-MeO-BDE-47, BDE-47, 6-OH-BDE-47, 2'-MeO-BDE-28 and 2'-OH-BDE-28, respectively. This result suggests transfer from adult females to eggs. For the five compounds studied the

**Table 1**  
Concentrations of compound in food, liver, liver-free residual carcass and eggs a 20-d exposure (food: ng g<sup>-1</sup> dry mass; liver, liver-free residual carcass and eggs: ng g<sup>-1</sup>, wet mass).

Exposed compound	Food	Liver (n = 3)		Liver-free residual carcass (n = 3)		Eggs
		Female	Male	Female	Male	
BDE-47	1.7 × 10 <sup>4</sup>	(3.8 ± 0.3) × 10 <sup>3</sup>	(7.5 ± 2.4) × 10 <sup>3</sup>	(5.6 ± 2.4) × 10 <sup>3</sup>	(6.4 ± 0.6) × 10 <sup>3</sup>	6.7 × 10 <sup>3</sup>
6-OH-BDE-47	1.1 × 10 <sup>4</sup>	(4.3 ± 0.8) × 10 <sup>2</sup>	(8.7 ± 2.7) × 10 <sup>2</sup>	(4.3 ± 0.7) × 10 <sup>1</sup>	(4.9 ± 0.8) × 10 <sup>1</sup>	3.5 × 10 <sup>2</sup>
6-MeO-BDE-47	1.7 × 10 <sup>4</sup>	(2.1 ± 1.1) × 10 <sup>3</sup>	(5.1 ± 0.5) × 10 <sup>3</sup>	(1.1 ± 0.4) × 10 <sup>4</sup>	(1.4 ± 1.0) × 10 <sup>4</sup>	6.2 × 10 <sup>3</sup>
2'-OH-BDE-28	2.8 × 10 <sup>4</sup>	(8.2 ± 1.3) × 10 <sup>3</sup>	(2.1 ± 0.1) × 10 <sup>4</sup>	(2.1 ± 0.6) × 10 <sup>2</sup>	(1.9 ± 0.3) × 10 <sup>2</sup>	5.3 × 10 <sup>2</sup>
2'-MeO-BDE-28	1.4 × 10 <sup>4</sup>	(9.3 ± 3.8) × 10 <sup>3</sup>	(1.2 ± 0.6) × 10 <sup>4</sup>	(1.3 ± 0.2) × 10 <sup>4</sup>	(1.2 ± 0.2) × 10 <sup>4</sup>	3.4 × 10 <sup>3</sup>

**Table 2**  
BAFs of compound between liver and food, liver-free residual carcass and food, eggs and food after a 20-d exposure (based on wet mass concentrations).

Exposed compound	BAF <sub>(liver/food)</sub> (n = 3)		BAF <sub>(body/food)</sub> (n = 3)		BAF <sub>(eggs/food)</sub> (n = 3)
	Female	Male	Female	Male	
BDE-47	0.23	0.45	0.33	0.39	0.40
6-OH-BDE-47	0.04	0.08	0.00	0.00	0.03
6-MeO-BDE-47	0.12	0.30	0.64	0.81	0.37
2'-OH-BDE-28	0.30	0.76	0.01	0.01	0.02
2'-MeO-BDE-28	0.58	0.86	0.93	0.86	0.24

decreasing order of BAF<sub>(egg/food)</sub> was: BDE-47 > 6-MeO-BDE-47 > 2'-MeO-BDE-28 > 6-OH-BDE-47 > 2'-OH-BDE-28.

Log<sub>K<sub>ow</sub></sub> of 6-OH-BDE-47, 2'-OH-BDE-28 and 2'-MeO-BDE-28 had been shown previously to be less than the other compounds (Yu et al., 2008), which might make accumulation less and excretion of these compounds more rapid, thus resulting the observed lesser concentrations in zebrafish. Concentrations of 2'-MeO-BDE-28 and 2'-OH-BDE-28 in liver were both greater than those of the three other compounds, while in liver-free residual carcass and eggs, concentrations accumulation was observed for 2'-MeO-BDE-28 but not for 2'-OH-BDE-28. Considering of the mass proportion of liver-free residual carcass, the body burden of target compounds was 2'-MeO-BDE-28 ~ 6-MeO-BDE-47 > BDE-47 > 2'-OH-BDE-28 > 6-OH-BDE-47.

Ratios of concentrations in liver and liver-free residual carcass differed among the five chemicals. After exposure to 6-OH-BDE-47 or 2'-OH-BDE-28, concentrations of these compounds in liver were nearly 10-fold greater than those in liver-free residual carcass. Concentrations of BDE-47 in liver and liver-free residual carcass were comparable. However, for 6-MeO-BDE-47 and 2'-MeO-BDE-28, concentrations in liver were less than that in liver-free residual carcass. Thus, compared to the other analogs, OH-PBDEs might be more accumulated into liver, which is consistent with the results of other studies (Zhang et al., 2010). Furthermore, the greater concentrations of MeO-PBDEs in liver-free residual carcass also indicated their great accumulation potency in muscle and their potential exposure risk to the fisher consumer.

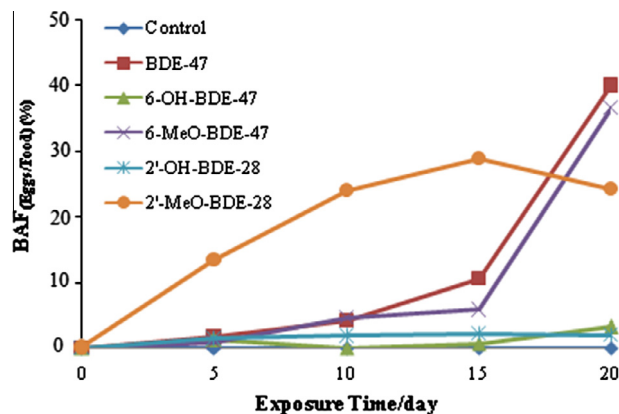
Concentrations of all compounds in liver of male zebrafish were significantly greater than those in livers of females. Previous studies indicated that chemicals might cause different toxicity to male and female fishes (He et al., 2011). Thus, greater concentrations of these compounds in livers of males might cause greater toxicity in males than females. In liver-free residual carcasses, no difference in concentrations among sexes was observed, which indicated no body burden difference between females and males. Because most processes were expected to be similar for female and male fishes, transfer of residues from adult females to eggs was thought to be one of the main factors for the sex-dependent difference in concentrations in liver.

Maternal transfer of the five target compounds from female to eggs was determined by on days 0, 5, 10, 15 and 20. After 20 d of exposure, ratios between concentrations of residues in eggs and those in liver (E/L) were 2.9, 1.7, 0.8, 0.4 and 0.1 for 6-MeO-BDE-

47, BDE-47, 6-OH-BDE-47, 2'-MeO-BDE-28 and 2'-OH-BDE-28, respectively. These results suggest significant transfer of all of these compounds from adult females to eggs. Average E/L ratios for BDE47, 6-OH-BDE47 and 6-MeO-BDE47 in Chinese Sturgeon (*Acipenser sinensis*) from the Yangtze River, which was based on the concentration (ng g<sup>-1</sup> wet mass) of compounds in eggs and liver, were 1.1, 0.93 and 4.4, respectively (Zhang et al., 2010), further indicating significant transfer of these same residues from adults to eggs of wild fish.

Early life stages of fishes are often the stages of development more sensitive to the effects of organic compounds (Tomy et al., 2004). A range of developmental defects have been observed after waterborne exposure of 25–50 nM 6-OH-BDE-47 (Bostel et al., 2008). Thus, greater maternal transfer of 6-OH-BDE-47 might affect survival and development of offspring. Another study reported maternal transfer of BDE-209 could affect motor neuron and skeletal muscle development in offspring (He et al., 2011), which is the only available literature regarding PBDEs.

Chemical-specific accumulation trends were observed over the 20 d of exposure (Fig. 1). Concentrations of BDE-47 and 6-MeO-BDE-47 did not achieve steady state during the exposure. This result is consistent with the results of a previous study, in which BDE-47 was determined to require about 60 d to achieve steady state (Tomy et al., 2004; Wan et al., 2010). Concentrations of



**Fig. 1.** Accumulation trends of BDE-47, 6-OH-BDE-47, 6-MeO-BDE-47, 2'-OH-BDE-28 and 2'-MeO-BDE-28 in eggs of zebrafish during the exposure duration (for each time point, all eggs had been analyzed in one batch).

**Table 3**Concentrations of compound detected in livers of zebrafish after exposure to BDE-47, 6-OH-BDE-47, 6-MeO-BDE-47, 2'-OH-BDE-28 or 2'-MeO-BDE-28 (ng g<sup>-1</sup> wet mass).

Compounds	BDE-47 (n = 3)		6-OH-BDE-47 (n = 3)		6-MeO-BDE-47 (n = 3)		2'-OH-BDE-28 (n = 3)		2'-MeO-BDE-28 (n = 3)	
	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male
2'-OH-BDE-28	8 ± 2	9 ± 1	18 ± 1	28 ± 6	5 ± 4	18 ± 7	8200 ± 1300	21000 ± 1000	8200 ± 3800	12000 ± 5600
6-OH-BDE-47	–	–	430 ± 78	870 ± 270	450 ± 330	420 ± 120	–	–	–	–
6-OH-BDE-85	–	–	23 ± 2	47 ± 9	–	–	–	–	–	–
2'-MeO-BDE-28	–	–	–	–	12 ± 4	25 ± 12	260 ± 71	540 ± 44	9300 ± 730	19000 ± 4200
6-MeO-BDE-47	–	–	14 ± 2	38 ± 7	2100 ± 1100	5100 ± 530	–	–	–	–
6-MeO-BDE-85	–	–	–	–	30 ± 6	57 ± 21	–	–	–	–
BDE-28	11 ± 3	15 ± 1	–	–	–	–	–	–	–	–
BDE-47	3800 ± 300	7500 ± 2400	8 ± 1	19 ± 2	17 ± 2	18 ± 13	–	–	–	–
BDE-85	14 ± 3	18 ± 2	–	–	–	–	–	–	–	–
BDE-99	15 ± 4	21 ± 3	–	–	–	–	–	–	–	–

6-OH-BDE-47, 2'-OH-BDE-28 and 2'-MeO-BDE-28 in eggs reached steady state after 5, 5 and 15 d, respectively. Concentrations of BDE-47, 6-MeO-BDE-47 and 2'-MeO-BDE-28 in eggs were greater than those of 6-OH-BDE-47 and 2'-OH-BDE-28. Also the rate of accumulation of BDE-47, 6-MeO-BDE-47 and 2'-MeO-BDE-28 into eggs was also greater than the rate of accumulation of 6-OH-BDE-47 or 2'-OH-BDE-28. Concentrations increased as a function of time and approached steady state for BDE-47, 6-MeO-BDE-47 and 2'-MeO-BDE-28 but 6-OH-BDE-47 and 2'-OH-BDE-28 did not. This result suggests that steady state concentrations of the two OH-PBDEs would be less than those of other three compounds, especially BDE-47 and 6-MeO-BDE-47.

### 3.2. Transformation in zebrafish

Inter-conversion of 6-OH-BDE-47 and 6-MeO-BDE-47, 2'-OH-BDE-28 and 2'-MeO-BDE-28 were observed, but no transformation from BDE-47 to OH-PBDEs or MeO-PBDEs, was observed in zebrafish. A minor amount of 2'-OH-BDE-28, BDE-28, BDE-85 and BDE-99 observed in liver of zebrafish exposed to BDE-47, whose concentration percentages were comparable small ratio with that in the food containing BDE-47. Therefore, observed concentrations of these residues was due to impurities in food. Thus, it was concluded that BDE-47 was not transformed into OH-PBDEs or MeO-PBDEs in zebrafish. This conclusion is consistent with those of a previous study (Wan et al., 2010). In livers of both male and female zebrafish exposed to 6-OH-BDE-47, a small amount of 2'-OH-BDE-28, 6-OH-BDE-85, 6-MeO-BDE-47 and BDE-47 were observed, too. These compounds might also be from impurities in food since concentrations of 2'-OH-BDE-28 and BDE-47 observed were the same as those in food spiked only with 6-OH-BDE-47. In livers of zebrafish exposed to 6-MeO-BDE-47, besides a large amount of the precursor, small amounts of 2'-OH-BDE-28, 2'-MeO-BDE-28 and BDE-47 were also observed, which is also due to impurities in food spiked with 6-MeO-BDE-47. In addition, the large amount of 6-OH-BDE-47 and a significant amount of 6-MeO-BDE-85 were detected in livers of zebrafish exposed to 6-MeO-BDE-47. In liver-free residual carcass of zebrafish exposed to 6-OH-BDE-47, only 6-MeO-BDE-47 was detected. While for zebrafish exposed to 6-MeO-BDE-47, both 6-OH-BDE-47 and 6-MeO-BDE-85 were observed in liver-free residual carcass, too. These results suggest inter-conversion of 6-MeO-BDE-47 and 6-OH-BDE-47 in zebrafish. This result is consistent with those of a previous study of medaka (Wan et al., 2010), but different from those of an *in vitro* study of rainbow trout microsomes (Wan et al., 2009). These different results between *in vitro* and *in vivo* studies might be due to different locations of enzymes involved in metabolism of 6-OH-BDE-47 and 6-MeO-BDE-47. The Cytochrome P4502 (CYP2) subfamily of enzymes, which catalyze the transformation of 6-MeO-BDE-47 to

6-OH-BDE-47, mainly located in microsomes (Liu et al., 2012). In control zebrafish exposed to 2'-OH-BDE-28 or 2'-MeO-BDE-28, inter-conversion of 2'-OH-BDE-28 and 2'-MeO-BDE-28 in livers of zebrafish was also observed. Here results further suggested inter-conversion of MeO-PBDEs and their same structural OH-PBDE analogs in zebrafish.

Significant amounts of 6-OH-BDE-85 and 6-MeO-BDE-85 were detected in livers of zebrafish exposed to 6-OH-BDE-47 and 6-MeO-BDE-47, respectively. Because not all possible PBDEs, OH-PBDEs and MeO-PBDEs congeners were quantified in the present study, the 6-OH-BDE-85 and 6-MeO-BDE-85, which have also been reported to occur in environment samples (Malmvarn et al., 2005; Asplund et al., 2008), might also be metabolites of other brominated OH-PBDEs and MeO-PBDEs congeners which were not analyzed in this study. Because only inter-conversions of 6-MeO-BDE-47 and 6-OH-BDE-47 were observed in the present and previous studies, other transformations pathway of OH-PBDEs and MeO-PBDEs, such as debromination, which might involve in the formation of 6-OH-BDE-85 and 6-MeO-BDE-85, were not observed in both present and previous studies. Therefore, further study would be needed to determine which BDE were the origin of these two compounds in living organisms.

Ratios of transformation products to their respective precursors (M/P) for 6-MeO-BDE-47 and 6-OH-BDE-47 in liver were 14.6% and 3.8%, respectively (Table 3). The M/P ratio for 6-MeO-BDE-47 was greater than that (2.0%) in Japanese medaka (Wan et al., 2010). The M/P concentration ratio for 6-OH-BDE-47 was lesser than that (7.3%) in Japanese medaka. Difference among species and concentrations to which fish were exposed were possible reasons for the different M/P ratio between zebrafish and medaka (Liu et al., 2012). Considering the great concentration of 6-MeO-BDE-47 in the marine environment and its great bioaccumulation in fish, the efficient metabolism of 6-MeO-BDE-47 might be the primary source of 6-OH-BDE-47 in fish. Previous studies suggested correlations between concentrations of 6-OH-BDE-47 and 6-MeO-BDE-47 in several organisms (Verreault et al., 2005; Wan et al., 2009; Zhang et al., 2010), further indicating transformation relationship between these two compounds. In zebrafish, transformation from 2'-MeO-BDE-28 to 2'-OH-BDE-28 was also more efficient than its reverse transformation (76% vs 2.9%). Particularly, in liver of zebrafish exposed to 2'-MeO-BDE-28, not only efficiency of transformation from 2'-MeO-BDE-28 to 2'-OH-BDE-28, but also concentrations of precursor and metabolites observed were all relatively great. Considering lesser bioavailability of OH-PBDEs, there is a large possibility that OH-PBDEs in aquatic organisms is the source from their same structural MeO-PBDEs. Since their toxicities in living organism were unknown, additional studies should be conducted to explore the environment risk of these two compounds in aquatic organisms.

#### 4. Conclusions

BDE-47, 6-OH-BDE-47, 6-MeO-BDE-47, 2'-OH-BDE-28 and 2'-MeO-BDE-28 were accumulated in zebrafish and transferred to eggs. Congener-specific differences existed in the accumulation and distribution of each compound. 6-MeO-BDE-47 and 2'-MeO-BDE-28 more accumulated and also more transferred to the eggs than the other chemicals studied. These two compounds were transformed into their respective OH-PBDEs, while as a postulated precursor of OH-PBDEs, BDE-47 was not transformed into either OH-PBDEs or MeO-PBDEs. Considering the abundant presence of MeO-PBDEs in fish, their great body burden, maternal transfer potency, great efficient transformation into the more toxic OH-PBDEs might raise their potential environment risk should be considered.

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#### Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.chemosphere.2014.05.050>.

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**Maternal Transfer, Distribution, and Metabolism of BDE-47 and its  
Related Hydroxylated, Methoxylated Analogs in Zebrafish (*Danio  
rerio*)**

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**Table S1.** Retention times, precursor ion and product ions of 36 screened compounds.

Compounds	Retention Time (min)	Ion Pairs		Collision Energy(eV)
		Parents	Products	
BDE-17	8.58	245.88	245.88, 138.85	20
BDE-28	9.24	245.88	245.88, 138.86	20
BDE-71	12.47	325.66	216.79, 218.94	30
BDE-47	13.27	325.66	216.79, 218.95	30
BDE-66	13.97	325.66	216.79, 218.96	30
BDE-100	16.89	405.63	296.60, 405.63	30
BDE-99	18.15	405.63	296.60, 405.64	30
BDE-85	20.52	405.63	296.60, 405.65	30
BDE-154	28.32	483.64	483.64, 402.57	30
BDE-138	21.2	483.64	483.64, 402.59	30
BDE-183	23.25	563.73	563.73, 485.15	30
BDE-190	25.86	563.73	563.73, 485.15	30
6'-MeO-BDE-17	10.95	435.57	420.89, 392.91	25
2'-MeO-BDE-28	11.87	435.57	342.12, 340.12	25
2'-MeO-BDE-68	15.07	515.45	422.14, 420.06	30
6-MeO-BDE-47	15.81	515.47	422.14, 420.06	30
6-MeO-BDE-90	20.02	433.94	420.87, 391.43, 434.01	25
3-MeO-BDE-100	20.49	433.94	420.87, 391.43, 434.01	25
4-MeO-BDE-90	21.99	593.38	499.68, 433.95	25
2-MeO-BDE-123	22.32	593.38	499.68, 433.95	25
6-MeO-BDE-85	23.1	593.38	499.68, 433.95	25
6-MeO-BDE-137	27.55	513.89	418.17, 471.39, 499.05	25
5-MeO-BDE-47	17.11	515.47	356.17, 516.26	15
4'-MeO-BDE-49	17.3	549.43	456.17, 454.13	25
3'-OH-BDE-7	9.56	263.85	155.48, 127.37	15
2'-OH-BDE-17	12.79	341.88	126.33, 235.33, 342.52	30
2'-OH-BDE-25	13.87	341.88	126.33, 235.33, 342.52	30
2'-OH-BDE-28	14.36	341.88	126.33, 235.33, 342.52	30
2'-OH-BDE-7	11.2	401.7	198.25, 183.19	15
6-OH-BDE-47	17.48	419.75	311.33, 313.33, 420.45	25
2'-OH-BDE-68	18	419.75	311.33, 313.33, 420.45	25
2'-OH-BDE-66	19.89	419.75	311.33, 313.33, 420.45	25
6-OH-BDE-90	22.61	499.64	392.58, 390.99, 340.09	25
6-OH-BDE-85	24.27	499.64	392.58, 390.99, 340.09	25
4'-OH-BDE-49	20.31	500.64	365.82, 364.24	25
6-OH-BDE-137	29.55	513.52	297.88, 470.69	25



**Table S2.** Concentrations of PBDEs, OH-PBDEs and MeO-PBDEs in Spiked Food (ng/g Dry mass)

	Control	BDE-47	6-OH-BDE-47	6-MeO-BDE-47	2'-OH-BDE-28	2'-MeO-BDE-28
BDE-17	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
BDE-28	n.d.	6	n.d.	n.d.	n.d.	n.d.
BDE-71	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
BDE-47	9± 0.45	1.7×10 <sup>4</sup> ± 0.08×10 <sup>4</sup>	7±0.35	25±1.25	n.d.	n.d.
BDE-66	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
BDE-100	n.d.	6	n.d.	n.d.	n.d.	n.d.
BDE-99	n.d.	1.9×10 <sup>2</sup> ± 0.08×10 <sup>2</sup> 1.8×10 <sup>2</sup>	n.d.	n.d.	n.d.	n.d.
BDE-85	n.d.	± 0.07*10 <sup>2</sup>	n.d.	n.d.	n.d.	n.d.
BDE-154	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
BDE-138	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
BDE-183	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
BDE-190	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
6'-MeO-BDE-17	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
2'-MeO-BDE-28	52± 2.45	30±1.5	22±1.1	1.0×10 <sup>2</sup> ± 0.05×10 <sup>2</sup>	5±0.25	1.4×10 <sup>4</sup> ± 0.07×10 <sup>4</sup>
2'-MeO-BDE-68	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
6-MeO-BDE-47	20±1	10±0.5	16±0.9	1.7×10 <sup>4</sup> ± 0.08×10 <sup>4</sup>	n.d.	n.d.
6-MeO-BDE-90	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
3-MeO-BDE-100	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
4-MeO-BDE-90	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
2-MeO-BDE-123	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
6-MeO-BDE-85	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
6-MeO-BDE-137	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
5-MeO-BDE-47	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
4'-MeO-BDE-49	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
3'-OH-BDE-7	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
2'-OH-BDE-17	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
2'-OH-BDE-25	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
2'-OH-BDE-28	27± 1.3	26±1.2	2.7×10 <sup>2</sup> ± 0.1×10 <sup>2</sup>	26±1.5	2.8×10 <sup>4</sup> ± 0.14×10 <sup>4</sup>	26±1.2
2'-OH-BDE-7	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
6-OH-BDE-47	n.d.	n.d.	1.1×10 <sup>4</sup> ± 0.08×10 <sup>4</sup>	n.d.	n.d.	n.d.

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2'-OH-BDE-68	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
2'-OH-BDE-66	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
6-OH-BDE-90	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
6-OH-BDE-85	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
4'-OH-BDE-49	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
6-OH-BDE-137	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.

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n.d. Not detected

**Table S3.** Results of repeatability, recovery and detection limit with spiked procedural blanks

Compounds	Repeatability	Recovery	Detection Limit	
	RSD		Instrument(ng/mL)	Method(ng/g)
BDE-17	15.2%	119.6%	2.7	2.7
BDE-28	14.6%	144.0%	$4.5 \times 10^{-1}$	$4.5 \times 10^{-1}$
BDE-71	10.0%	114.6%	$9.2 \times 10^{-2}$	$9.2 \times 10^{-2}$
BDE-47	9.5%	119.1%	$7.8 \times 10^{-1}$	$7.8 \times 10^{-1}$
BDE-66	9.0%	129.2%	1.2	1.2
BDE-100	10.1%	101.9%	2.3	2.3
BDE-99	10.8%	105.6%	$5.1 \times 10^{-1}$	$5.1 \times 10^{-1}$
BDE-85	11.9%	116.0%	$6.7 \times 10^{-1}$	$6.7 \times 10^{-1}$
BDE-154	12.2%	146.0%	2.6	2.6
BDE-138	12.9%	93.0%	2.9	2.9
BDE-183	13.4%	101.1%	4.1	4.1
BDE-190	12.4%	109.5%	4.2	4.2
6'-MeO-BDE-17	11.9%	113.5%	$5.4 \times 10^{-1}$	$5.4 \times 10^{-1}$
2'-MeO-BDE-28	11.7%	109.7%	$5.5 \times 10^{-1}$	$5.5 \times 10^{-1}$
2'-MeO-BDE-68	10.2%	116.4%	4.9	4.9
6-MeO-BDE-47	9.1%	119.6%	8.0	8.0
2'-MeO-BDE-47	9.9%	120.5%	6.2	6.2
4'-MeO-BDE-49	10.2%	127.3%	6.5	6.5
4'-MeO-BDE-90	9.6%	106.0%	$1.9 \times 10^1$	$1.9 \times 10^1$
6-MeO-BDE-90	11.4%	112.6%	4.5	4.5
3-MeO-BDE-100	10.5%	133.0%	9.8	9.8
2-MeO-BDE-123	11.4%	116.5%	8.9	8.9
6-MeO-BDE-85	10.1%	107.7%	$1.0 \times 10^1$	$1.0 \times 10^1$
6-MeO-BDE-137	12.1%	103.0%	$1.6 \times 10^1$	$1.6 \times 10^1$
3'-OH-BDE-7	21.2%	89%	$2.2 \times 10^1$	$2.2 \times 10^1$
2'-OH-BDE-7	21.5%	91.9%	$1.4 \times 10^1$	$1.4 \times 10^1$
2'-OH-BDE-17	18.2%	92.6%	$1.1 \times 10^1$	$1.1 \times 10^1$
2'-OH-BDE-25	22.6%	81%	$2.2 \times 10^1$	$2.2 \times 10^1$
2'-OH-BDE-28	16.7%	103.2%	9.7	9.7
2-OH-BDE-47	11.6%	88.9%	6.6	6.6
2'-OH-BDE-68	20.8%	71.6%	$1.5 \times 10^1$	$1.5 \times 10^1$
2'-OH-BDE-66	20.9%	67%	$2.9 \times 10^1$	$2.9 \times 10^1$
2-OH-BDE-90	17.0%	74.6%	9.9	9.9
2-OH-BDE-85	15.4%	112.8%	$1.7 \times 10^1$	$1.7 \times 10^1$
4'-OH-BDE-49	21.6%	87%	$2.5 \times 10^1$	$2.5 \times 10^1$
2-OH-BDE-137	17.3%	91.4%	$1.6 \times 10^1$	$1.6 \times 10^1$