# Interconversion of Hydroxylated and Methoxylated Polybrominated Diphenyl Ethers in Japanese Medaka

YI WAN,[†] FENGYAN LIU,[†] STEVE WISEMAN,[*,†] XIAOWEI ZHANG,[*,†] HONG CHANG,[†] MARKUS HECKER,[†,‡] PAUL D. JONES,[†,§] MICHAEL H. W. LAM,[∥] AND JOHN P. GIESY[†,∥,⊥,#,▽,○]

*Toxicology Centre, University of Saskatchewan, Saskatoon, Saskatchewan S7N 5B3, Canada, ENTRIX, Inc., Saskatoon, Saskatchewan S7N 5B3, Canada, School of Environment and Sustainability, University of Saskatchewan, Saskatoon, Saskatchewan, S7N 5C8, Canada, State Key Laboratory in Marine Pollution and Department of Biology and Chemistry, City University of Hong Kong, Kowloon, Hong Kong, SAR China, Department Biomedical Veterinary Sciences, University of Saskatchewan, Saskatoon, Saskatchewan S7N 5B3, Canada, Department Zoology and Center for Integrative Toxicology, Michigan State University, East Lansing, Michigan, United States, Zoology Department, College of Science, King Saud University, P.O. Box 2455, Riyadh 11451, Saudi Arabia, and School of Biological Sciences, University of Hong Kong, Hong Kong, SAR, China*

*Received July 7, 2010. Revised manuscript received September 23, 2010. Accepted September 28, 2010.*

Polybrominated diphenyl ethers (PBDEs), hydroxylated (OH) and methoxylated (MeO), have been widely detected in aquatic environments. However, relationships among these structurally related compounds in exposed organisms are unclear. To elucidate biotransformation relationships among BDE-47, 6-OH-BDE-47, and 6-MeO-BDE-47, dietary accumulation, maternal transfer, and tissue distribution of these compounds and their transformation products were investigated in sexually mature Japanese medaka (*Oryzias latipes*). In addition, transformation of each compound was determined *in vitro* using liver microsomes of medaka. OH-PBDEs and MeO-PBDEs were not detected in fish exposed to BDE-47. However, significant concentrations of 6-OH-BDE-47 were detected in medaka or microsomes exposed to 6-MeO-BDE-47. Significant concentrations of 6-MeO-BDE-47 were also measured in fish exposed to 6-OH-BDE-47, but 6-MeO-BDE-47 was not detected in microsomes exposed to 6-OH-BDE-47. Similar patterns of transformation products were observed in medaka eggs from adult fish during exposure. This study presents direct *in vivo* evidence of biotransformation of 6-MeO-BDE-47 to 6-OH-BDE-47. In addition, this is the first study to demonstrate biotransformation of 6-OH-BDE-47 to 6-MeO-BDE-47. Demethylation of 6-MeO-BDE-47 was the primary transformation pathway leading to formation of 6-OH-BDE-47 in medaka, while the previously hypothesized formation of OH-PBDEs from synthetic BDE-47 did not occur. Biotransformation products formed in adult female medaka were transferred to eggs.

## Introduction

Over the course of the past decade, brominated flame retardants (BFRs) have emerged as persistent organic pollutants of concern (*1*). Among the different classes of BFRs, polybrominated diphenyl ethers (PBDEs) have received the greatest attention, mostly due to their widespread use, ubiquitous environmental distribution, and bioaccumulation potential (*1*). Recently, focus has shifted to structural analogues of PBDEs, such as hydroxylated (OH) and methoxylated (MeO) PBDEs, concentrations of which in marine sponges, algae, and mussels in some marine systems can exceed those of PBDEs (*2*−*4*). The occurrence of OH-PBDEs is of particular interest since, for some endpoints, they are more potent than PBDEs or MeO-PBDEs (*5, 6*). Effects of OH-PBDEs on organisms include disruption of thyroid hormone homeostasis, disruption of oxidative phosphorylation, altered estradiol synthesis, and neurotoxicity (*5, 7*−*10*). Relatively great ratios of transfer of OH-PBDEs from parent to offspring have been reported for pregnant women (*11*) and wild fish, such as the Chinese sturgeon (*Acipenser sinensis*) (*12*).

Several origins of OH-PBDEs and MeO-PBDEs have been postulated (*13, 14*). It has been suggested that ortho-substituted OH-PBDEs and MeO-PBDEs are formed from naturally occurring compounds in marine ecosystems (*2, 3*). Two abundant congeners of MeO-PBDEs, 6-MeO-BDE-47 and 2′-MeO-BDE-68, have been reported to be natural products of marine organisms (*3*). Similarly, ortho-substituted OH-PBDEs are produced naturally by marine algae or associated microorganisms (*2, 4*). It has been suggested that MeO-PBDEs are formed via methylation of OH-PBDEs. This pathway was hypothesized on the basis of knowledge of bacterial methylation of phenols in the environment (*3, 15, 16*). Alternatively, the similarity in structure between these compounds and synthetic PBDEs has led to suggestions that meta-/para-substituted OH-PBDEs and MeO-PBDEs could originate from biotransformation of synthetic PBDEs (*17*−*19*). Several *in vitro* studies have demonstrated that OH-PBDEs can be biotransformation products of PBDEs in fish, rat, and human cells (*17*−*19*). However, concentrations of OH-PBDEs detected in laboratory studies were extremely small compared to the ratios of concentrations of OH-PBDEs and MeO-PBDEs to PBDEs in marine organisms (*12, 13, 17*−*19*). On the basis of these results, the occurrence of OH- and MeO-PBDEs in wild organisms cannot be explained by formation from synthetic PBDEs alone. This information is consistent with the existence of sources of both OH-PBDEs and MeO-PBDEs other than synthetic PBDEs. On the basis of *in vitro* exposures using rainbow trout, chicken, and rat microsomes, it was reported that demethylation of naturally occurring MeO-PBDEs is a contributor of OH-PBDEs in wildlife from remote areas (*13*).

While the results of *in vitro* studies provided insight into biotransformation relationship(s) between PBDEs, MeO-

* Address correspondence to either author. Address: Toxicology Centre, University of Saskatchewan, Saskatoon, Saskatchewan S7N 5B3, Canada (S.W. and X.Z.). E-mail: stw870@mail.usask.ca (S.W.); howard50003250@yahoo.com (X.Z.).
[†] Toxicology Centre, University of Saskatchewan.
[‡] ENTRIX, Inc.
[§] School of Environment and Sustainability, University of Saskatchewan.
[∥] City University of Hong Kong.
[⊥] Department Biomedical Veterinary Sciences, University of Saskatchewan.
[#] Michigan State University.
[▽] King Saud University.
[○] University of Hong Kong.

10.1021/es102287q 
Published on Web 10/25/2010

**TABLE 1. Concentrations of 6-OH-BDE-47, 6-MeO-BDE-47, and BDE-47 in Spiked Food (ng/g Dry Weight) and Stock Standard Solutions (ng/mL)[a]**

| | 6-OH-BDE-47 | 6-MeO-BDE-47 | BDE-47 |
|---|---|---|---|
| control food | <0.02 | 0.1 | <1.6 |
| 6-OH-BDE-47 spiked food | 900 | 0.2 | 15 |
| 6-MeO-BDE-47 spiked food | <0.02 | 8000 | 28.3 |
| BDE-47 spiked food | <0.02 | 0.2 | 21 000 |
| 6-OH-BDE-47 stock solutions | 1 500 000 | 4300 | 1900 |
| 6-MeO-BDE-47 stock solutions | <0.8 | 1 300 000 | 4800 |
| BDE-47 stock solutions | <0.8 | <2.0 | 50 000 |

[a] Underlined numbers are concentrations of parent compounds. Numbers that are not underlined are the concentrations of impurities.

PBDEs, and OH-PBDEs, these relationships were still to be confirmed *in vivo*. In this study, relationships among BDE-47, 6-MeO-BDE-47, and 6-OH-BDE-47 were explored in sexually mature Japanese medaka (*Oryzias latipes*). Medaka were exposed to each chemical through their diet. Relationships among the target chemicals were assessed in liver, residual carcass (body without liver), and eggs. Furthermore, accumulation into embryos and maternal transfer of the three target compounds and their transformation products were investigated by measuring their concentrations in eggs of exposed adult medaka. Finally, on the basis of the data generated during this study and a thorough review of the available literature, origin and plausible transformation pathways of these brominated compounds are proposed and discussed. Sources of the various PBDEs as well as OH- and MeO-PBDEs have important implications for risk assessment and how exposures can be controlled.

## Materials and Methods

**Preparation of Diet.** Commercial fish food (Nutrafin Basix Staple Food, Rolf C. Hagen Inc. Canada) was ground with a mortar and pestle, weighed into a glass flask, and spiked with known amounts of each target compound (BDE-47, 6-MeO-BDE-47, or 6-OH-BDE-47) dissolved in 150 mL of acetone. The flask was shaken for 30 min to ensure thorough mixing of the food and chemicals. Following mixing, the contents of the flasks were concentrated to dryness in a rotary-evaporator. The resulting spiked food was air-dried for approximately 3 h in a fume hood. An identical protocol was used to prepare the acetone-spiked "control" food. Concentrations of BDE-47, 6-MeO-BDE-47, and 6-OH-BDE-47 in the stock standards and food samples were analyzed (Table 1) using methods described below.

**Feeding Study.** Prior to initiation of exposure, 5-month-old medaka (mean weight: 0.60 ± 0.08 g, 8 females and 4 males per tank) were randomly assigned to 10 L tanks containing 6 L of dechlorinated tap water. Medaka were allowed to acclimate to the experimental tanks for 3 days prior to the initiation of exposure. Two replicate tanks were used for each exposure. Each day, approximately 50% of the water volume in the tanks was replaced. Water temperatures were maintained at 23−24 °C during the course of the experiment. Other details of the animals are provided in the Supporting Information.

Medaka were fed diets of food spiked with BDE-47, 6-MeO-BDE-47, 6-OH-BDE-47, or the carrier solvent acetone alone (vehicle control). This method provided an effective non-invasive means of administering the chemicals without stressing the fish. Dietary exposure was deemed a more realistic route of exposure than intraperitoneal injections (*20*). Fish were fed approximately 2% of their average body weight per day, half the daily food was provided in the morning and the other half in the afternoon. All fish displayed vigorous eating behavior, and the food was completely consumed in each tank.

Medaka were exposed to each target chemical for 14 days, and eggs were collected each morning (day 0−14) during the exposure period. After collected eggs from each individual tank were rinsed with Nanopure (Barnsted) water and gently dried on Kimwipes, the mass of each egg was determined, and then, eggs from each exposure were composited and stored separately at −20 °C until analysis. On day 14, following egg collection, six female fish were sampled from each treatment tank and sacrificed for the determination of target chemicals. The liver was dissected from each fish, and both liver and the liver-free residual carcass were weighed and stored at −20 °C until analysis.

To confirm the results of the *in vivo* feeding study, transformation of BDE-47, 6-MeO-BDE47, and 6-OH-BDE-47 was investigated using microsomes isolated from livers of female medaka obtained from the same breeding colony as those animals used in the feeding study, according to the method of Kennedy and Jones (*21*). The details of the methods used for microsomal incubations are described elsewhere (*13*) and provided in the Supporting Information.

**Sample Extraction and Cleanup.** The methods used to quantify 6-OH-BDE-47 have been published previously (*22*), and details of the chemicals, instrumental analysis, quality assurance, and quality control are provided in the Supporting Information. For this study, quantifications of BDE-47 and 6-MeO-BDE-47 were incorporated in the same method. Liver (∼0.02 g), egg (∼0.2 g), fish food (∼0.2 g), and a microsomal incubation mixture (250 μL) were homogenized and transferred into amber tubes. After spiking with surrogate recovery standards, 2 mL of Nanopure water (18 MΩ), 50 μL of hydrochloric acid (HCl, 37%), and 3 mL of 3-propanol were added to the samples. Samples were extracted three times with 3 mL of hexane/methyl *tert*-butyl ether (MTBE; 1:1; v/v). To remove residual acid, extracts were washed four times with 4 mL aliquots of pure water. Samples were concentrated and dried under nitrogen. For whole fish (about 0.6 g), the extraction process was the same as described above, except that the amount of each solvent used was 10-fold greater to accommodate the greater mass.

Dried residues were dissolved in 200 μL of aqueous sodium bicarbonate (100 mmol/L, pH adjusted to 10.5 with sodium hydroxide), and 200 μL of dansyl chloride (1 mg/mL in acetone) was added. After vortex mixing for 1 min, the samples were incubated at 60 °C for 5 min. Once the samples cooled, 1 mL of pure water and 3 × 3 mL of hexane were added, and the organic solvent layer was removed and transferred onto a silica gel column (60−100 mesh size) for fractionation. The silica gel column was wet packed with 4 g of silica gel and 4 g of sodium sulfate. After application of the extract, the column was eluted with 15 mL of hexane/dichloromethane (DCM; 1:1, v/v) and, then, 20 mL of DCM. The first fraction was evaporated to dryness and reconstituted with 50 μL of nonane for high-resolution gas chromatography combined with high-resolution mass spectrometry (HRGC-HRMS) analysis of PBDEs (BDE-28, BDE-47, BDE-99, BDE-100, BDE-119, BDE-154, and BDE-153) and MeO-PBDEs (6-MeO-BDE-17, 4-MeO-BDE-17, 2′ -MeO-BDE-68, 6-MeO-BDE-47, 5-MeO-BDE-47, and 4′ -MeO-BDE-49). The second fraction was evaporated to dryness and reconstituted with 50 μL of acetonitrile/water (60:40) for liquid chromatography-tandem mass spectrometry (LC-MS/MS) analysis of OH-PBDEs (3-OH-BDE-47, 5-OH-BDE-47, 6-OH-BDE-47, 4′−OH-BDE-49, and OH-triBDEs).

## Results

**Purity of Stock Solutions and Food Preparations.** 6-OH-BDE-47 was not present as an impurity in stock standard solutions of BDE-47 or 6-MeO-BDE-47. A negligible con-

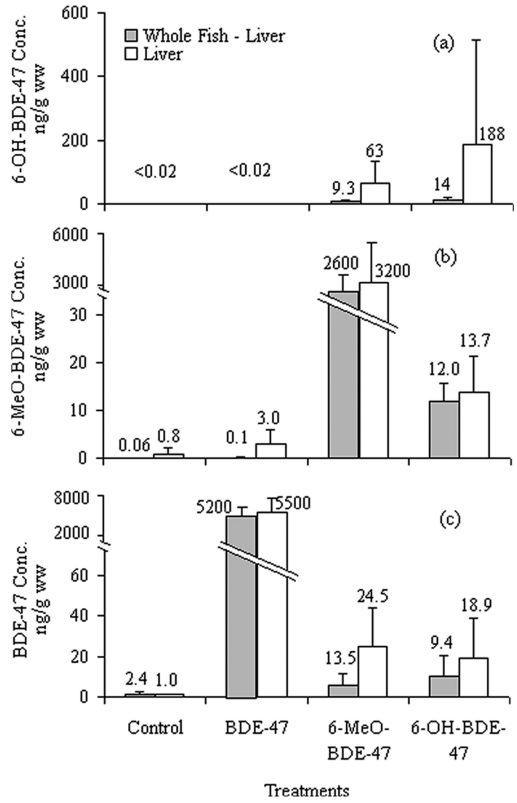


**FIGURE 1. Concentrations of BDE-47, 6-MeO-BDE-47, and 6-OH-BDE-47 in liver (Liver) and liver-free residual carcass (Whole Fish-Liver) tissue from female Japanese medaka after 14 days of dietary exposure to feed spiked with solvent (control), BDE-47, 6-MeO-BDE-47, or 6-OH-BDE-47. The numbers above the error bars are the average concentrations of target compounds (ng/g ww).**

centration of 6-MeO-BDE-47 (4.3 ng/g) was detected as an impurity (0.002%) in the 6-OH-BDE-47 stock standard solution (Table 1). BDE-47 was detected as an impurity in both stock solutions of 6-OH-BDE-47 (0.12%) and 6-MeO-BDE-47 (0.36%). Concentrations of the parent compounds in prepared food were 900, 8000, and 2100 ng/g dry weight for 6-OH-BDE-47, 6-MeO-BDE-47, and BDE-47, respectively. 6-OH-BDE-47 was not present as an impurity in either the BDE-47 or the 6-MeO-BDE-47 spiked food. Concentrations of BDE-47 in the 6-MeO-BDE-47 and 6-OH-BDE-47 food preparations were 28.3 and 15 ng/g, respectively. Trace amounts of 6-MeO-BDE-47 (0.1−0.2 ng/g) were detected in the control (acetone spiked), 6-OH-BDE-47, and BDE-47 food preparations. The presence of 6-MeO-BDE-47 and BDE-47 as impurities in stock solutions and prepared food did not affect conclusions drawn from the studies.

**Concentrations and Distributions of Target Compounds in Medaka.** Of all the screened compounds, only BDE-47, 6-MeO-BDE-47, and 6-OH-BDE-47 were detected in tissues from medaka exposed separately to individual chemicals for 2 weeks (Figure 1, Figure S1 in Supporting Information). Concentrations of BDE-47, 6-MeO-BDE-47, and 6-OH-BDE-47 were 5500 ± 2200, 3200 ± 2300, and 190 ± 330 ng/g ww, respectively, in livers of exposed medaka. Concentrations of BDE-47, 6-MeO-BDE-47, and 6-OH-BDE-47 were 5200 ± 1400, 2600 ± 970, and 14 ± 5.9 ng/g ww, respectively, in liver-free residual carcass of medaka. 6-OH-BDE-47 was only detected as a transformation product in medaka (liver: 63 ± 71 ng/g ww; liver-free residual carcass: 9.3 ± 4.2 ng/g ww) exposed to 6-MeO-BDE-47 (Figure 1a). Concentrations of 6-MeO-BDE-47 (liver: 14 ± 7.6 ng/g ww; liver-free residual carcass: 12 ± 3.6 ng/g ww) detected in female medaka exposed

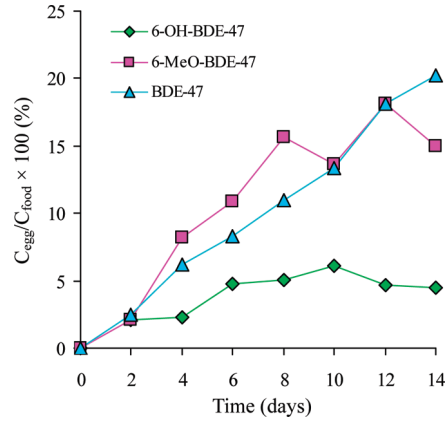


**FIGURE 2. Accumulation of BDE-47, 6-OH-BDE-47, and 6-MeO-BDE-47 in eggs of female Japanese medaka during the course of a 14-day dietary exposure to feed spiked with BDE-47, 6-MeO-BDE-47, or 6-OH-BDE-47. $C_{egg}$ is the concentration of target compounds in fish eggs, and $C_{food}$ is the concentration in dosing food. At each of the sampling time points, all eggs from the individual experimental tanks were collected for analysis.**

to 6-OH-BDE-47 were significantly greater than concentrations in medaka that were unexposed or exposed to BDE-47 ($p < 0.01$, Figure 1 b). Comparable concentrations of BDE-47 were observed in female medaka exposed to 6-MeO-BDE-47 (liver: 25 ± 19 ng/g ww; liver-free residual carcass: 14 ± 5.6 ng/g ww) and 6-OH-BDE-47 (liver: 19 ± 20 ng/g ww; liver-free residual carcass: 9.4 ± 10 ng/g ww; Figure 1c).

Distributions of each of the three chemicals either as exposed compound or transformation product were different among liver, egg, and whole fish (Table S2 in Supporting Information). Concentration ratios of 6-OH-BDE-47 between liver and whole fish (L/F) were 6.1−6.9, which was greater than those of 6-MeO-BDE-47 (1.2) or BDE-47 (1.1). Concentration ratios between egg and liver (E/L) were 0.74−0.76 and 0.93 for 6-MeO-BDE-47 and BDE-47, respectively, which were comparable to those between egg and whole fish (E/F) of 6-MeO-BDE-47 (0.62 and 0.72) and BDE-47 (0.92). However, greater E/F ratios (0.34 and 1.8) compared to E/L ratios (0.23 and 0.59) were observed for 6-OH-BDE-47. Significantly greater maternal transfer ratios were observed for 6-OH-BDE-47 (0.59−1.8) as a parent compound than as a transformation product (0.23−0.34).

**Accumulation in Eggs.** Each of the exposure compounds and their tranformation products were detected in eggs collected during the exposure period. Daily rates of egg mass production were 0.17 ± 0.05, 0.14 ± 0.04, 0.19 ± 0.03, and 0.18 ± 0.06 g ww for control, BDE-47-exposed, 6-MeO-BDE-47-exposed, and 6-OH-BDE-47-exposed during the exposure period, respectively. The slopes of the accumulation trend lines, as a function of exposure duration during the initial 6 days of the exposure, were 1.94, 1.44, and 0.73 for 6-MeO-BDE-47, BDE-47, and 6-OH-BDE-47, respectively (Figure 2). Accumulation of 6-OH-BDE-47 and 6-MeO-BDE-47 in eggs reached steady state on days 6 and 12, respectively, but steady state was not achieved for BDE-47 (Figure 2). 6-OH-BDE-47, as a transformation product, was only detected in eggs of medaka exposed to 6-MeO-BDE-47 with concentrations ranging from 0.5 to 4.1 ng/g ww during the exposure period (Figure 3a). Concentrations of 6-MeO-BDE-47, as a transformation product of 6-OH-BDE-47, increased in a time-dependent manner over the entire course of the study (Figure 3b). Concentration ratios between transformation products and their parent compounds (M/P) in eggs of medaka exposed to 6-MeO-BDE-47 (6-OH-BDE47/6-MeO-BDE47: 0.003 ± 0.002) were less than those of fish exposed to 6-OH-BDE-47 (6-MeO-BDE47/6-OH-BDE47: 0.057 ± 0.030). The

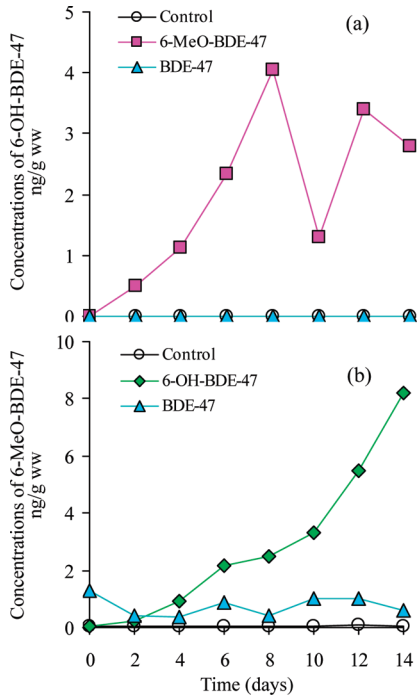


**FIGURE 3. Accumulation trend of (a) 6-OH-BDE-47 and (b) 6-MeO-BDE-47 as metabolites in eggs of Japanese medaka during a 14-day dietary exposure to feed spiked with solvent (control, panel a and b), BDE-47 (panel a and b), 6-MeO-BDE-47 (panel a), or 6-OH-BDE-47 (panel b). At each of the sampling time points, all eggs from the individual experimental tanks were collected for analysis.**

M/P ratios in medaka eggs were significantly less than those in liver and liver-free residual carcass in each treatment (6-OH-BDE47/6-MeO-BDE47: liver = 0.021 ± 0.022, liver-free residual carcass = 0.005 ± 0.006; 6-MeO-BDE47/6-OH-BDE47: liver = 0.273 ± 0.249, liver-free residual carcass = 0.998 ± 0.466).

## Discussion

**Relationships among Exposed Compounds and Their Transformation Products.** Significant concentrations of 6-OH-BDE-47 were measured in medaka exposed to 6-MeO-BDE-47 but not BDE-47 (Figures 1a and S1 and Table S1 in Supporting Information). Previous studies have indicated that an important prerequisite for exposure studies is ensuring standards and prepared exposure media are free of impurities that could lead to false results (*23, 24*). The analysis of purity suggested that 6-OH-BDE-47 was not a trace contaminant in any of the stock solutions or media employed in this study. Thus, 6-OH-BDE-47 measured in fish and eggs from these treatment groups originated from biotransformation of exposed parent compounds. This is consistent with results from previous *in vitro* studies (*13*), further supporting the hypothesis that 6-MeO-BDE-47 is a contributor to formation of 6-OH-BDE-47.

6-MeO-BDE-47 was also observed to be formed from 6-OH-BDE-47 in medaka. Detection of trace concentrations of 6-MeO-BDE-47 in all prepared food was not surprising (Table 1), since the ingredients of the commercial fish food include fish, plankton, and shrimp, which could contain MeO-PBDEs of natural origin (*3*). Assuming that the dosing level did not influence the food accumulation factors, the accumulation factors of fish exposed to parent 6-MeO-BDE-47 were used to calculate concentrations of impurity (6-MeO-BDE-47) accumulated in fish in other treatments. Concentrations of 6-MeO-BDE-47 in liver-free residual carcass were calculated to be 0.06 ± 0.02 ng/g ww with dosing concentrations of 0.2 ng/g in food, which is similar to those of fish exposed to BDE-47 (0.1 ± 0.03 ng/g ww) and in controls (0.06 ± 0.02 ng/g ww). In addition, concentrations of 6-MeO-BDE-47 in medaka exposed to 6-OH-BDE-47 (liver-free residual carcass: 12.01 ± 3.58 ng/g ww) were significantly greater than those in unexposed and medaka exposed to BDE-47 ($p < 0.01$, Figure 1b). A second feeding study was performed with 6-OH-BDE-47 in order to verify this observation. Consistent with the first study, significant concentrations of 6-MeO-BDE-47 compared to the control group were observed. This result confirmed that 6-MeO-BDE-47 is formed as a biotransformation product of 6-OH-BDE-47. Previous studies have suggested that some MeO-PBDEs are formed via methylation of OH-PBDEs (*3, 16*). The results presented here are the first experimental evidence of *in vivo* formation of a MeO-PBDE from an OH-PBDE. To further confirm this observation, *in vitro* transformation of 6-OH-BDE-47 by microsomes isolated from female medaka was assessed (Table S1, Supporting Information). Consistent with previous *in vitro* studies (*13*), biotransformation of 6-OH-BDE-47 to 6-MeO-BDE-47 was not observed (Table S1, Supporting Information). Taken together, these results suggest that 6-OH-BDE-47 can be transformed to 6-MeO-BDE-47 in Medaka, but the conversion does not occur in the hepatic microsomal fraction under the conditions of this experiment.

Comparable concentrations of BDE-47 were observed in female medaka exposed to 6-MeO-BDE-47 and 6-OH-BDE-47 (Figure 1c), which is likely due to BDE-47 impurities in the stock standard solutions. Using the method described above, concentrations of BDE-47 in liver-free residual carcass were calculated to be 7.0 ± 1.9 and 2.7 ± 1.0 ng/g ww for dosing concentrations of 28.3 and 15 ng/g ww in food, respectively. The calculated concentrations were comparable to those in fish exposed to 6-MeO-BDE-47 (liver-free residual carcass: 13.5 ± 5.6 ng/g ww) and 6-OH-BDE-47 (9.4 ± 10.0 ng/g ww). The fact that significant concentrations of neither 6-OH-BDE-47 nor 6-MeO-BDE-47 were detected in female medaka exposed to BDE-47 is consistent with some previous *in vitro* observations that some PBDE congeners are not biotransfromed into either OH-PBDEs or MeO-PBDEs in rainbow trout, Chinese sturgeon, salmon, chicken, beluga whale, or rat microsomes (*12, 13, 25, 26*).

Significant differences in distributions of parent compounds and their biotransformation products were observed among tissues. The relatively large L/F ratios for 6-OH-BDE-47 are consistent with its preferential accumulation in liver compared to the other compounds studied. This result is also consistent with the tissue distribution of each target compound examined in Chinese green sturgeon, in which the greatest concentrations of 6-OH-BDE-47 were found in liver, while 6-MeO-BDE-47 and BDE-47 preferentially accumulated in adipose tissue (*12*). The fact that the E/F ratio for 6-OH-BDE-47 was greater than the E/L ratio is also mainly due to the preferential accumulation of 6-OH-BDE-47 in liver. The greater maternal transfer ratios (E/L and E/F ratios) for 6-OH-BDE-47 as a parent compound than that of 6-OH-BDE-47 as a transformation product could be a result of the more than 10-fold lesser concentrations of 6-OH-BDE-47 formed as a transformation product. These results are consistent with reports that maternal transfer ratios of brominated flame retardants in zebrafish are dose dependent, with greater ratios observed in individuals exposed to greater concentrations of the parent material (*27*).

**Accumulation of Exposed Compounds and Biotransformation Products in Eggs.** Chemical-specific accumulation trends were observed for each exposed compound over the course of the exposure period (Figure 2). In contrast to 6-OH-BDE-47, relatively great assimilation efficiencies were observed for 6-MeO-BDE-47 and BDE-47 as indicated by the

**TABLE 2. Reported Concentrations of MeO-PBDEs and OH-PBDEs in Blood and Livers of Various Organisms Worldwide[a]**

| specie | sample type | location | ref | sex | MeO-PBDEs mean | MeO-PBDEs 47(%)[b] | OH-PBDEs mean | OH-PBDEs 47(%)[c] | PBDEs mean | PBDEs 47(%)[d] |
|---|---|---|---|---|---|---|---|---|---|---|
| glaucous gulls | plasma | Norwegian Arctic | *32* | M | 0.95 | 5 | 0.43 | 33 | 20.2 | 44 |
| | | | | F | 0.69 | 6 | 0.37 | 38 | 19.8 | 54 |
| | | | *33* | M | 1 | | 0.44 | | 21.3 | |
| | | | | F | 0.67 | | 0.33 | | 19.3 | |
| | | | *34* | | 2.78 | | 3.54 | | 51.5 | |
| bald eaglet | plasma | British Columbia, Canada | *35* | | <0.01 | | 0.31−0.92 | 40−100 | 1.78−8.49 | 43−53 |
| polar bear | blood | East Greenland | *36* | | 0.16 | 70 | 2.9 | <20 | 1.2 | <40 |
| glaucous gulls | liver | Norwegian Arctic | *34* | | 32.2 | | 3.57 | | 522 | |
| albatross | liver | Indian Ocean, South Atlantic Ocean, South Pacific Ocean | *13* | | 1.1 | 56 | 0.5 | 92 | 0.3 | 29 |
| polar bear | liver | Arctic Ocean | *13* | | 0.02 | 9 | 0.01 | 63 | 0.7 | 83 |
| beluga whale | liver | St. Lawrence River and Hudson Bay, Canada | *37* | | 25<br>43 | 60−80 | <0.5<br><0.5 | >50 | 53<br>2210 | 40 |
| tuna | liver | North Pacific Ocean | *13* | | 0.5 | 69 | 0.03 | 84 | 0.2 | 38 |
| Chinese sturgeon | liver | Yangtze River | *12* | F | 0.03 | 74 | 0.2 | 83 | 22.7 | 57 |

[a] Concentrations are reported as ng/g. Glaucous gulls (*larus hyperboreus*), polar bear (*Ursus maritimus*), bald eaglet (*Haliaeetus ieucocephalus*), beluga whale (*Delphinapterus leucas*), tuna (*Katsuwonus pelamis*), five albatross species (*Thalassarche chlororhynchos*, *Phoebetria palpebrata*, *Thalassarche chrysostoma*, *Thalassarche cauta*, and *Thalassarche melanophrys*), and Chinese sturgeon (*Acipenser sinensis*). [b] Average percentage of 6-MeO-BDE-47/ΣMeO-PBDEs. [c] Average percentage of 6-OH-BDE-47/ΣOH-PBDEs. [d] Average percentage of BDE-47/ΣPBDEs.

steep slopes for accumulation during the initial 6 days of exposure. Accumulation of BDE-47 did not reach steady state, and previous studies have reported that, when zebrafish (*Danio rerio*) were exposed to BDE-47, eggs required 60 days to reach steady state (*27*). On the basis of the slow assimilation rate and large concentration ratios between fish and feed, it can be inferred that the depuration rate of BDE-47 is likely less than that of 6-MeO-BDE-47 (*28*).

The observation that both 6-OH-BDE-47 and 6-MeO-BDE-47 occurred in eggs as biotransformation products of 6-MeO-BDE-47 and 6-OH-BDE-47, respectively, while neither transformation product was detected in eggs collected from medaka exposed to BDE-47 is consistent with profiles of transformation products in liver and liver-free carcass (Figure 1). This observation is consistent with the hypothesis that OH-PBDEs are formed from MeO-PBDEs but not from PBDEs. It is also consistent with OH-PBDEs being precursors of MeO-PBDEs *in vivo*. However, the times required to reach steady state for 6-MeO-BDE-47 and 6-OH-BDE-47 as biotransformation products in eggs were different (Figure 3). The linear increase in concentration of 6-MeO-BDE-47 in eggs through biotransformation could be due to larger biotransformation ratios (M/P ratio) and/or maternal transfer of 6-MeO-BDE-47 as a transformation product (Table S2 in Supporting Information). Biotransformation products detected in eggs were likely generated in female medaka and subsequently transferred to the eggs. This conclusion is based on the fact that M/P ratios in medaka eggs were significantly less than those in liver and liver-free carcass. This result is consistent with the results of previous studies that have also demonstrated that the biotransformation capacity of fish eggs is less than that of later life stages (*27, 29*).

**Naturally Occurring Concentrations and Relationships.** On the basis of the results of the controlled laboratory study, the relationship among PBDEs, OH-PBDEs, and MeO-PBDEs was further investigated by comparing concentrations in organisms collected worldwide (Table 2). When all the compounds were quantified, PBDEs, OH-PBDEs, and MeO-PBDEs were almost always codetected in aquatic animals with BDE-47, 6-MeO-BDE-47, and 6-OH-BDE-47 as the predominant congeners. Concentrations of PBDEs have a wider range (0.2−2210 ng/g ww), and similar concentration variations were observed for MeO-PBDEs and OH-PBDEs. Concentrations of OH-PBDEs were generally greater than those of MeO-PBDEs in blood. This could be due to binding of OH-PBDEs to plasma transport proteins, including the thyroxine transport proteins (e.g., transthyretin (TTR), thyroxine binding globulin (TBG)) (*11, 30*). The fact that both MeO-PBDEs and OH-PBDEs were detected with relatively great concentrations in liver suggested that liver would be the suitable tissue for investigations of the two groups of chemicals.

Relationships among PBDEs, OH-PBDEs, and MeO-PBDEs in environmental samples are of interest in the context of the relative risk assessment of these compounds, especially the origin of the most toxic of these three classes of compounds, OH-PBDEs. Biotransformation of PBDEs to OH-PBDEs has been reported with quantified concentrations in previous *in vitro* (rat microsome and human-cell culture *18, 19, 31*) and *in vivo* (rat (*17*)) studies; however, none of those studies reported the purities of the standards used. In the *in vivo* exposure study, the concentration ratios of OH-PBDEs/PBDEs (M/P ratio) were small (rat: 0.0002 and 0.004, from ref *17*) compared to those of the current study (6-OH-BDE-47/6-MeO-BDE-47 ratio in liver of medaka: 0.021), and concentrations of detected OH-PBDEs remained constant even though concentrations of PBDEs decreased by more than 10-fold after five days of exposure (*17*). The possible contribution of impurities of MeO-PBDEs in commercial rat food cannot be neglected, since 6-MeO-BDE-47 has been detected in the fish food in the current study. In the *in vitro* studies, the percentage of OH-PBDEs relative to PBDE exposure concentrations were <0.06%, 0.022−0.84%, and 0.1−3% in rat microsomes exposed to BDE-99 (*31*), rat microsomes exposed to BDE-47 (*18*), and human cells exposed to BDE-99 (*19*), respectively. Relatively greater percentages of 6-OH-BDE-47/6-MeO-BDE-47 were found in chicken (9%), rainbow trout (7%), rat (3%), and medaka (3%) liver microsome exposed to 6-MeO-BDE-47 ((*13*), Table S1 in Supporting Information). In a previous study, OH-PBDEs were not detected in microsomes exposed to BDE-99, BDE-47, or PBDE mixtures (*13*). In the current study, no OH-BDEs were detected in medaka exposed to BDE-47 *in vivo* or medaka microsomes exposed to BDE-47 *in vitro*. This is consistent with some previous *in vitro* studies in which some PBDE congeners are not biotransfromed into OH-PBDEs in salmon, beluga whale, or rat microsomes (*25, 26*). Therefore, demethylation of MeO-PBDEs could be the primary source of ortho-substituted OH-PBDEs rather than hydroxylation of PBDEs. The results of a recent study with Chinese sturgeon

also suggested that natural accumulation in the aquatic environment could be another important source of 6-OH-BDE-47 (*12*). In addition, herein, we report for the first time the *in vivo* biotransformation of 6-OH-BDE-47 to 6-MeO-BDE-47. The interconversion of 6-OH-BDE-47 and 6-MeO-BDE-47 in Japanese medaka was consistent with the codetection of the two groups of chemicals in aquatic organisms (Table 2).


## Acknowledgments

The research was supported by a Discovery Grant from the Natural Sciences and Engineering Research Council of Canada (Project 326415-07) and grants from Western Economic Diversification Canada (Project # 6971 and 6807). Prof. Giesy was supported by the Canada Research Chair program and an at large Chair Professorship at the Department of Biology and Chemistry and State Key Laboratory for Marine Pollution, City University of Hong Kong. The authors wish to acknowledge the support of an instrumentation grant from the Canada Foundation for Innovation.


## Supporting Information Available

Detailed information on chemical, animals, *in vitro* biotransformation, instrumental analysis, and data analysis. Concentration ratios (liver/whole fish, egg/whole fish, and egg/liver) of exposed chemicals and metabolites in female Japanese medaka after a 14 days dietary exposure. Chromatographic profiles of OH-Tetra-BDEs detected in prepared food and medaka livers after 14-day exposure. This material is available free of charge via the Internet at http://pubs.acs.org.

## Literature Cited


(1) Hites, R. A. Polybrominated diphenyl ethers in the environment and in people: A meta-analysis of concentrations. *Environ. Sci. Technol.* **2004**, *38*, 945–956.

(2) Unson, M. D.; Holland, N. D.; Faulkner, D. J. A brominated secondary metabolite synthesized by the cyanobacterial symbiont of a marine sponge and accumulation of the crystalline metabolite in the sponge tissue. *Mar. Biol.* **1994**, *119*, 1–11.

(3) Teuten, E. L.; Xu, L.; Reddy, C. M. Two abundant bioaccumulated halogenated compounds are natural products. *Science* **2005**, *307*, 917–920.

(4) Malmvarn, A.; Marsh, G.; Kautsky, L.; Athanasiadou, M.; Bergman, A.; Asplund, L. Hydroxylated and methoxylated brominated diphenyl ethers in the red algae *Ceramium tenuicorne* and blue mussels from the Baltic Sea. *Environ. Sci. Technol.* **2005**, *39*, 2990–2997.

(5) Boxtel, A. L. V.; Kamstra, J. H.; Cenijn, P. H.; Pieterse, B.; Wagner, M. J.; Antink, M.; Krab, K.; Van Der Burg, B.; Marsh, G.; Brouwer, A.; Legler, J. Microarray analysis reveals a mechanism of phenolic polybrominated diphenylether toxicity in zebrafish. *Environ. Sci. Technol.* **2008**, *42*, 1773–1779.

(6) Marchesini, G. R.; Meimaridou, A.; Haasnoot, W.; Meulenberg, E.; Albertus, F.; Mizuguchi, M.; Takeuchi, M.; Irth, H.; Murk, A. J. Biosensor discovery of thyroxine transport disrupting chemicals. *Toxicol. Appl. Pharmacol.* **2008**, *232*, 150–160.

(7) Meerts, I. A. T. M.; Letcher, R. J.; Hoving, S.; Marsh, G.; Bergman, Å.; Lemmen, J. G.; van der Burg, B.; Brouwer, A. In vitro estrogenicity of polybrominated diphenyl ethers, hydroxylated PBDEs, and polybrominated bisphenol A compounds. *Environ. Health Perspect.* **2001**, *109*, 399–407.

(8) Canton, R. F.; Sanderson, J. T.; Letcher, R. J.; Bergman, A.; van den Berg, M. Inhibition and induction of aromatase (CYP19) activity by brominated flame retardants in H295R human adrenocortical carcinoma cells. *Toxicol. Sci.* **2005**, *88* (2), 447–455.

(9) Dingemans, M. M. L.; de Groot, A.; van Kleef, R. G. D. M.; Berman, Å.; van den Berg, M.; Vijverberg, H. P. M.; Westerink, R. H. S. Hydroxylation increase the neurotoxic potential of BDE-47 to affect exocytosis and calcium homeostasis in PC12 cells. *Environ. Health Perspect.* **2008**, *116*, 637–643.

(10) Morgado, I.; Hamers, T.; Vander Ven, L.; Power, D. M. Disruption of thyroid hormone binding to sea bream recombinant transthyretin by ioxinyl and polybrominated diphenyl ethers. *Chemosphere* **2007**, *69*, 155–163.

(11) Wan, Y.; Choi, K.; Kim, S.; Ji, K.; Chang, H.; Wiseman, S.; Jones, P. D.; Khim, J. S.; Park, S.; Park, J.; Lam, M. H. W.; Giesy, J. P. Hydroxylated polybrominated diphenyl ethers and bisphenol A in pregnant women and their matching fetuses: placental transfer and potential risks. *Environ. Sci. Technol.* **2010**, *44*, 5233–5239.

(12) Zhang, K.; Wan, Y.; Giesy, J. P.; Lam, M. H. W.; Jones, P. D.; Hu, J. Y. Tissue distribution of polybrominated compounds in Chinese sturgeon (*Acipenser sinensis*): origin, hepatic sequestration, and maternal transfer. *Environ. Sci. Technol.* **2010**, *44*, 5781–5786.

(13) Wan, Y.; Wiseman, S.; Chang, H.; Zhang, X. W.; Jones, P. D.; Hecker, M.; Kannan, K.; Tanabe, S.; Hu, J. Y.; Lam, M. H. W.; Giesy, J. P. Origin of hydroxylated brominated diphenyl ethers: natural compounds or man-made flame retardants. *Environ. Sci. Technol.* **2009**, *43*, 7536–7542.

(14) Fernie, K. J.; Letcher, R. J. Historical contaminants, flame retardants, and halogenated phenolic compounds in peregrine falcon (*Falco peregrinus*) nestling in the Canadian great lakes basin. *Environ. Sci. Technol.* **2010**, *44*, 3520–3526.

(15) Allard, A. S.; Remberger, M.; Neilson, A. H. Bacterial O-methylation of halogen-substituted phenols. *Appl. Environ. Microbiol.* **1987**, *53*, 839–845.

(16) Haglund, P. S.; Zook, D. R.; Buser, H. R.; Hu, J. Identification and quantification of polybrominated diphenyl ethers and methoxy-polybrominated diphenyl ethers in Baltic biota. *Environ. Sci. Technol.* **1997**, *31*, 3281–3287.

(17) Malmberg, T.; Athanasiadou, M.; Marsh, G.; Brandt, I.; Bergman, A. Identification of hydroxylated pobrominated diphenyl metabolites in blood plasma from polybrominated diphenyl ether exposed rat. *Environ. Sci. Technol.* **2005**, *39*, 5342–5348.

(18) Hamers, T.; Kamstra, J. H.; Sonneveld, E.; Murk, A. J.; Visser, T. J.; Van Velzen, M. J. M.; Brouwer, A.; Bergman, A. Biotransformation of brominated flame retardants into potentially endocrine-disrupting metabolites, with special attention to 2,2′,4,4′-tetrabromodiphenyl ether (BDE-47). *Mol. Nutr. Food Res.* **2008**, *52*, 284–298.

(19) Stapleton, H. M.; Kelly, S. M.; Pei, R.; Letcher, R. J.; Gunsch, C. Metabolism of polybrominated diphenyl ethers (PBDEs) by human hepatocytes in vitro. *Environ. Health Perspect.* **2009**, *117*, 197–202.

(20) Gamperl, A. K.; Vijayan, M. M.; Boutilier, R. G. Experimental control of stress hormone levels in fishes-techniques and applications. *Rev. Fish. Biol. Fish.* **1994**, *4*, 215–255.

(21) Kennedy, S. W.; Jones, S. P. Simultaneous measurement of cytochrome P4501A catalytic activity and total protein-concentration with a fluorescence plate reader. *Anal. Biochem.* **1994**, *222*, 217–233.

(22) Chang, H.; Wan, Y.; Naile, J.; Zhang, X. W.; Wiseman, S.; Hecker, M.; Lam, M. H. W.; Giesy, J. P.; Jones, P. D. Simultaneous quantification of multiple classes of phenolic compounds in blood plasma by liquid chromatography-electrospray tandem mass spectrometry. *J. Chromatogr., A* **2010**, *1217*, 506–513.

(23) Koistinen, J.; Sanderson, J. T.; Nevalainen, T.; Paasivirta, J.; Giesy, J. P. Ethoxyresorufin O-deethylase (EROD) Induction Potency of Polychlorinated Diphenyl Ethers (PCDE) in H4IIE Rat Hepatoma Cells. *Environ. Toxicol. Chem.* **1996**, *15*, 2028–2034.

(24) Sanders, J. M.; Burka, L. T.; Smith, C. S.; Black, W.; James, R.; Cunningham, M. L. Differential expression of CYP1A, 2B, and 3A genes in the F344 rat following exposure to a polybrominated diphenyl ether mixture or individual compounds. *Toxicol. Sci.* **2005**, *88*, 127–135.

(25) Browne, E. P.; Stapleton, H. M.; Kelly, S. M.; Tilton, S. C.; Gallagher, E. P. In vitro hepatic metabolism of 2,2′,4,4′,5-pentabromodiphenyl ether (BDE 99) in Chinook Salmon (*Onchorhynchus tshawytscha*). *Aquat. Toxicol.* **2009**, *92*, 281–287.

(26) McKinney, M. A.; De Guise, S.; Martineau, D.; Beland, P.; Arukwe, A.; Letcher, R. J. Biotransformation of polybrominated diphenyl ethers and polychlorinated bipheyls in beluga whale (*Delphinapterus leucas*) and rat mammalian model using an in vitro hepatic microsomal assay. *Aquat. Toxicol.* **2006**, *77*, 87–97.

(27) Nyholm, J. R.; Norman, A.; Norrgren, L.; Haglund, P.; Andersson, P. L. Maternal transfer of brominated flame retardants in zebrafish (*Danio rerio*). *Chemosphere* **2008**, *73*, 203–208.

(28) Tomy, G. T.; Palace, V. P.; Halldorson, T.; Braekevelt, E.; Danell, R.; Wautier, K.; Evans, B.; Brinkworth, L.; Fish, A. T. Bioaccumulation, biotransformation, and biochemical effects of brominated diphenyl ethers in juvenile lake trout (*Savelinus namaycush*). *Environ. Sci. Technol.* **2004**, *38*, 1496–1504.

(29) Petersen, G. I.; Kristensen, P. Bioaccumulation of lipophilic substances in fish early life stages. *Environ. Toxicol. Chem.* **1998**, *17*, 1385–1395.
(30) Meerts, I.; van Zanden, J. J.; Luijks, E.; van Leeuwen-Bol, I.; Marsh, G.; Jakobsson, E.; Bergman, A.; Brouwer, A. Potent competitive interactions of some brominated flame retardants and related compounds with human transthyretin in vitro. *Toxicol. Sci.* **2000**, *56*, 95–104.
(31) Erratico, C. A.; Szeitz, A.; Bandiera, S. M. Validation of a novel in vitro assay using ultra performance liquid chromatography-mass spectrometry (UPLC/MS) to detect and quantify hydroxylated metabolites of BDE-99 in rat liver microsomes. *J. Chromatogr., B* **2010**, *878*, 1562–1568.
(32) Verreault, J.; Gabrielsen, G. W.; Chu, S.; Muir, D. C.; Andersen, M.; Hamaed, A.; Letcher, R. J. Flame retardants and methoxylated and hydroxylated polybrominated diphenyl ethers in two Norwegian Arctic top predators: glaucous gulls and polar bears. *Environ. Sci. Technol.* **2005**, *39*, 6021–6028.
(33) Verreault, J.; Bech, C.; Letcher, R. J.; Ropstad, E.; Dahl, E.; Gabrielsen, G. W. Organohalogen contamination in breeding glaucous gulls from the Norwegian Arctic: Associations with basal metabolism and circulating thyroid hormones. *Environ. Pollut.* **2007**, *145*, 138–145.
(34) Verreault, J.; Shahmiri, S.; Gabrielsen, G. W.; Letcher, R. J. Organohalogen and metabolically-derived contaminants and associations with whole body constituents in Norwegian Arctic glaucous gulls. *Environ. Int.* **2007**, *33*, 823–830.
(35) McKinney, M. A.; Cesh, L. S.; Elliott, J. E.; Williams, T. D.; Garcelon, D. K.; Letcher, R. J. Brominated flame retardants and halogenated phenolic compounds in North American west coast bald eaglet (*Haliaeetus Ieucocephalus*) plasma. *Environ. Sci. Technol.* **2006**, *40*, 6275–6281.
(36) Gebbink, W. A.; Sonne, C.; Dietz, R.; Kirkegaard, M.; Riget, F. F.; Born, E. W.; Muir, D. C. G. Letcher. Tissue-specific congener composition of organohalogen and metabolic contaminants in East Greenland polar bears (*Ursus maritimus*). *Environ. Pollut.* **2008**, *152*, 621–629.
(37) McKinney, M. A.; De Guise, S.; Martineau, D.; Beland, P.; Lebeuf, M.; Letcher, R. J. Organohalogen contaminants and metabolites in beluga whale (*Delphinapterus leucas*) liver from two Canadian populations. *Environ. Toxicol. Chem.* **2006**, *25*, 1246–1257.

ES102287Q

**SUPPORTING INFORMATION**

# Interconversion of Hydroxylated and Methoxylated Polybrominated Diphenyl Ethers in Japanese Medaka

Yi Wan, Fengyan Liu, Steve Wiseman*, Xiaowei Zhang*, Hong Chang, Markus Hecker, Paul D. Jones, Michael H. W. Lam, John P. Giesy

* corresponding author

**This supporting information includes:**

- **Chemicals and Standards**
- **Animals**
- ***In vitro* Biotransformation**
- **Instrumental Analysis**
- **Quality Assurance and Quality Control (QA/QC)**
- **Data Analysis**
- **Table S1**
- **Table S2**
- **Figure S1**

**Summary of the number of pages, figures, and tables**

- **Number of pages: 5**
- **Number of figures: 1**
- **Number of tables: 2**

## MATERIALS AND METHODS

### Chemicals and Standards

MeO-PBDEs (6-MeO-BDE-17, 4-MeO-BDE-17, 2'-MeO-BDE-68, 6-MeO-BDE-47, 5-MeO-BDE-47 and 4'-MeO-BDE-49) and OH-PBDEs (3-OH-BDE-47, 5-OH-BDE-47, 6-OH-BDE-47, 4'-OH-BDE-49 and 6'-OH-BDE-17) were synthesized in the Department of Biology and Chemistry, City University of Hong Kong, Hong Kong, China. PBDEs (BDE-28, BDE-47, BDE-99, BDE-100, BDE-119, BDE-154 and BDE-153) and $^{13}$C-PBDEs were obtained from AccuStandard (New Haven, Connecticut, USA). All compounds were determined to be >98% pure by high-resolution gas chromatograph interfaced to a high-resolution mass spectrometer (HRGC-HRMS). Dichloromethane (DCM), n-hexane, nonane, methyl *tert*-butyl ether (MTBE), acetone, acetonitrile and methanol were pesticide residue grade and were obtained from OmniSolv (EM Science, Lawrence, KS, USA). Silica gel (60-100 mesh size), formic acid, hydrochloric acid (37%, A.C.S. reagent), 2-propanol and dansyl chloride were obtained from Sigma-Aldrich (St. Louis, MO, USA).

### Animals

Male and female wild-type *O. latipes* were obtained from a stock maintained at the aquatic culture unit at the US Environmental Protection Agency Mid-Continent Ecology Division (Duluth, MN, USA). Individuals were maintained at the Aquatic Toxicology Research Facility (ATRF) in the Toxicology Centre, University of Saskatchewan (Saskatoon, SK, Canada), by use of previously described methods. Medaka were cultured in flow-through tanks under conditions that facilitated breeding (23-24 °C; 16:8 light/dark cycle)

and were fed once daily to satiety. All protocols were approved by the University of Saskatchewan Animal Research Ethics Board (U of S-AREB).

### *In vitro* Biotransformation

To confirm the results of the *in vivo* feeding study, transformation of BDE-47, 6-MeO-BDE47, and 6-OH-BDE-47 was investigated using microsomes isolated from female medaka liver according to the method of Kennedy and Jones (*19*). The details of the methods used for microsomal incubations are described elsewhere (*11*). Briefly, reactions were performed in 0.1 M $NaH_2PO_4$ buffer (pH 7.4) containing 1 mM ethylenediaminetetraacetic acid (EDTA), 10 mM dithiothreitol (DTT), and 100 μM NADPH. The final reaction volume was 250 μL and contained 125 μL of microsomes and 10 μL of exposure chemicals. The concentration of the chemical in the final reaction mixture was 2 μg/mL. Incubations without chemicals and without microsomes were used as negative controls to assess the presence of background contaminants and the possibility of non-enzyme mediated changes in chemical structure. After incubation, the samples were extracted immediately for quantification of residues and their transformation products.

### Instrumental Analysis

Identification and quantification of BDE-47 and 6-MeO-BDE-47 were performed using a Hewlett-Packard 5890 series II high-resolution gas chromatography (HRGC) interfaced to a Micromass® Autospec® high-resolution mass spectrometry (HRMS) (Micromass®, Beverly, MD). Chromatographic separation was achieved by use of a DB-5MS fused silica capillary

column for all target compounds (30 m length, 0.25 mm ID, 0.1 μm film, Agilent, Carlsbad, CA) and helium was used as the carrier gas. The mass spectrometer was operated in a Selective Ion-Monitoring (SIM) mode. Resolution of all reference peaks in all time windows was greater than 7,000. The injector temperature was held at 285 °C and the ion source was kept at 285 °C. The electron ionization energy was 37 eV and the ion current was 750 μA. The temperature program was from 110 °C (10 min) to 250 °C at a rate of 25 °C/min, then increased to 260 °C at a rate of 1.5 °C/min, and then to 323 °C (15 min) at a rate of 25 °C/min.

Quantification of 6-OH-BDE-47 was conducted using an Agilent 1200 series high performance liquid chromatography system (Santa Clara, CA, USA) connected to an API 3000 triple-quadrupole tandem mass spectrometry (MS/MS) system (PE Sciex, Concord, ON, Canada). An XBridge C18 column (100 × 2.1 mm, 3.5 μm particle size) from Waters (Milford, MA, USA) was used for chromatographic separation at room temperature. Injection volume was 20 μL. The mobile phase, consisting of acetonitrile (Solvent A) and 0.1% formic acid in water (Solvent B), was used with a gradient elution of A:B = 60:40 (0-1 min) to 95:5 (1-15 min) and 95:5 (15-22 min) at a flow rate of 0.25 mL/min. Analytes were detected using a mass spectrometer equipped with a turbo ion spray source operated in the positive multi-reaction monitoring (MRM) mode. All the source and instrument parameters were optimized by infusing the purified dansyl derivatives of analytes into the mass spectrometer. Optimal MS conditions were: ion spray voltage 3750 V, curtain gas ($N_2$) 8, nebulizer gas ($N_2$) 12, collision gas ($N_2$) 10, turbo ion spray probe temperature 475 °C.

**Quality Assurance and Quality Control (QA/QC).**

To avoid cross contamination among treatments, equipment was changed after processing each treatment. To confirm the purities of the chemicals used in this study, concentrations of target compounds were analyzed in spiked food and stock standard solutions (Table 1). To avoid sample contamination during chemical analysis, all equipment was rinsed repeatedly with acetone and hexane. A laboratory blank was incorporated in the analytical procedures for every batch of 10 samples. Concentrations of all congeners were quantified by the internal standard isotope-dilution method using mean relative response factors determined from standard calibration runs. BDE-47 and 6-MeO-BDE-47 were quantified in sample extracts relative to $^{13}$C-BDE-47, and 6-OH-BDE-47 was quantified relative to 6'-OH-BDE-17. Recoveries of $^{13}$C-BDE-47 and 6'-OH-BDE-17 were 82±26% and 72±28%, respectively, in all samples, and the concentrations of the analytes were recovery-corrected. The method detection limits (MDL) were defined as three times of the standard deviation in the blank samples, in which BDE-47 was detected. The MDLs for the other compounds, which were not detected in blank samples, were set to the instrumental minimum detectable amounts. Detection limits were 0.02 ng/g ww, 0.05 ng/g ww, and 1.6 ng/g ww for 6-OH-BDE-47, 6-MeO-BDE-47 and BDE-47, respectively, in analyzed samples. For those result less than the MDL, half of the MDL was assigned to avoid missing values in statistical analyses, and details of the data analysis were provided in the supporting information.

## Data Analysis

All statistical analyses were performed using SPSS 11.0 (SPSS Inc., Chicago, IL, USA). Results are reported as mean ± standard deviation (SD). Differences between two groups were analyzed by the non-parametric Wilcoxon signed rank sum test. Differences in concentrations of target compounds among treatments were compared using one-way analysis of variance (ANOVA). Levene's test was used to check the equality of variances (the value of significance is less than 0.05). Where variances were equal, data were analyzed by the F test. Where the equality of variances could not be assumed, Welch's and Brown-Forsythe's robust tests were used to perform one-way ANOVA analysis. Multiple paired comparisons were used to determine which means differed from one another. Tukey's Honestly Significant Differences (HSD) was used where variances were presumed to be equal, and the Games-Howell test was used where equality of variances could not be assumed. Results were considered significant when $p$ values were less than 0.05.

Table S1. Concentrations of target compounds after metabolism with medaka microsomes exposed to BDE-47, 6-MeO-BDE-47, and 6-OH-BDE-47 (ng/mL). The dosing concentrations for all chemicals were 2 μg/mL.

| Exposed Chemicals | Analyzed Chemicals | | |
|---|---|---|---|
| | BDE-47 | 6-MeO-BDE-47 | 6-OH-BDE-47 |
| 6-MeO-BDE-47 | <1.6 | 710±72 | 62.8±9.9 |
| 6-OH-BDE-47 | <1.6 | <0.05 | 680±110 |
| BDE-47 | 620±185 | <0.05 | <0.02 |

Table S2. Concentration ratios (liver/whole fish, egg/whole fish and egg/liver) of exposed chemicals and metabolites in female Japanese medaka after a 14 d dietary exposure to feed spiked with either BDE-47, 6-MeO-BDE-47 or 6-OH-BDE-47.

| Exposure groups | 6-OH-BDE-47 | | 6-MeO-BDE-47 | | BDE-47 | |
|---|---|---|---|---|---|---|
| | Exposed chemical | Metabolite | Exposed chemical | Metabolite | Exposed chemical | Metabolite |
| | 6-OH-BDE-47 | 6-MeO-BDE-47 | 6-MeO-BDE-47 | 6-OH-BDE-47 | BDE-47 | - |
| Liver/Whole fish (L/F) | 6.9±7.6 | 1.2±0.5 | 1.2±0.7 | 6.1±5.9 | 1.1±0.5 | - |
| Egg/ Whole fish (E/F) | 1.8±0.93 | 0.72±0.22 | 0.62±0.41 | 0.34±0.12 | 0.92±0.23 | - |
| Egg/ Liver (E/L) | 0.59±0.44 | 0.76±0.40 | 0.74±0.71 | 0.23±0.35 | 0.93±0.31 | - |

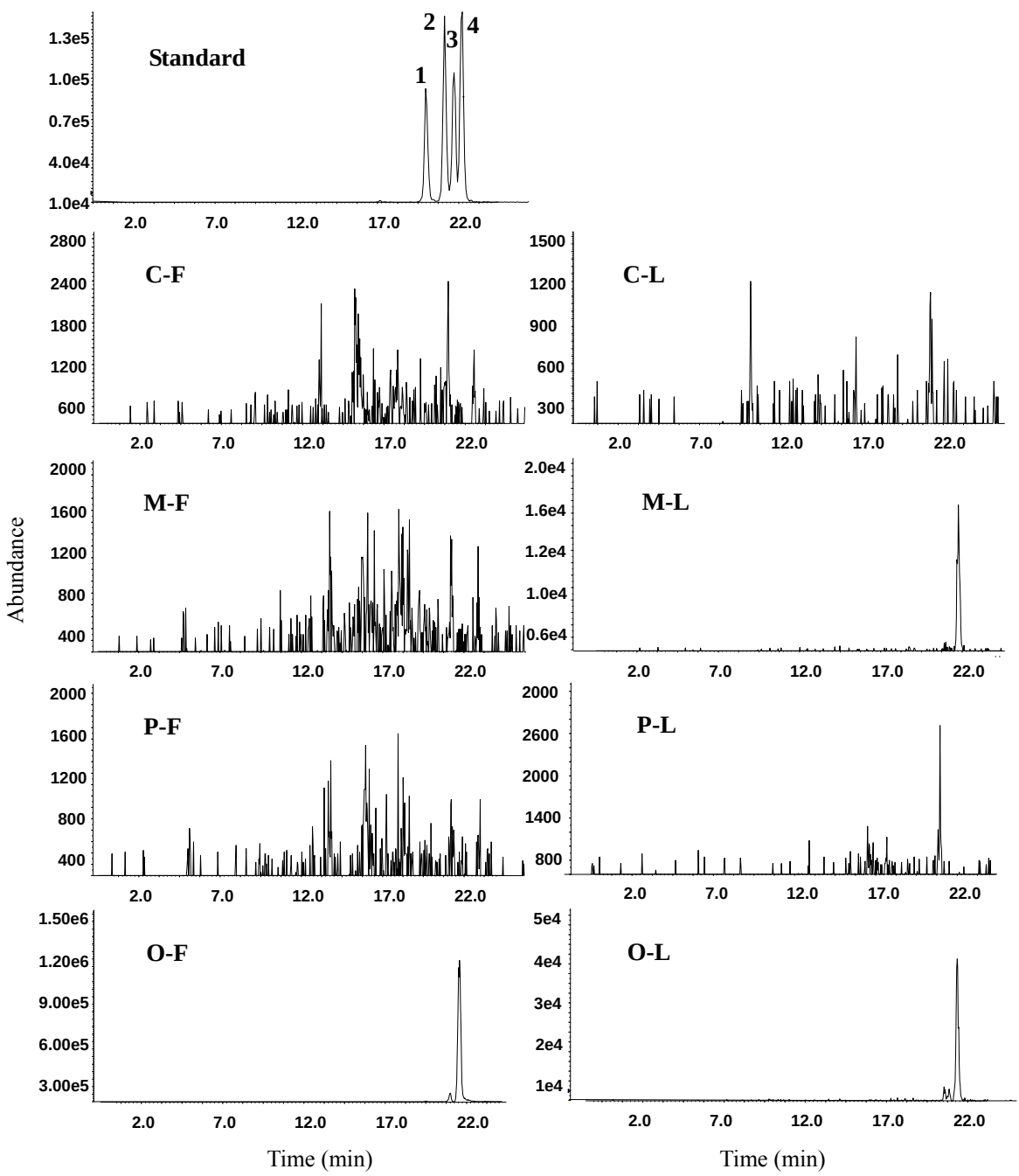


Figure S1. LC-MS/MS MRM chromatographic profiles of OH-Tetra-BDEs detected in prepared food and medaka livers after 14-day exposure. Standard: OH-Tetra-BDEs standard solution (5 ng/ml), 3-OH-BDE-47 (1), 5-OH-BDE-47 (2), 6-OH-BDE-47 (3), 4'-OH-BDE-49 (4); C-F: control food; M-F: 6-MeO-BDE-47 spiked food; P-F: BDE-47 spiked food; O-F: 6-OH-BDE-47 spiked food; C-L: liver of medaka in control group; M-L: liver of medaka exposed to 6-MeO-BDE-47; P-L: liver of medaka exposed to BDE-47; O-L: liver of medaka exposed to 6-OH-BDE-47.