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# Dietary exposure of juvenile common sole (*Solea solea* L.) to polybrominated diphenyl ethers (PBDEs): Part 2. Formation, bioaccumulation and elimination of hydroxylated metabolites

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**Abstract:**

The uptake, elimination and transformation of six PBDE congeners (BDE-28, -47, -99, -100, -153, -209) were studied in juvenile common sole (*Solea solea* L.) exposed to spiked contaminated food over a three-month period, and then depurated over a five-month period. Methoxylated (MeO-) and hydroxylated (OH-) PBDEs were determined in fish plasma exposed to PBDEs and compared to those obtained in control fish. While all MeO- and some OH- congeners identified in fish plasma were found to originate from non-metabolic sources, several OH- congeners, i.e., OH-tetraBDEs and OH-pentaBDEs, were found to originate from fish metabolism. Among these, 4′-OH-BDE-49 was identified as a BDE-47 metabolite. Congener 4′-OH-BDE-101, identified here for the first time, may be the result of BDE-99 metabolic transformation. Our results unequivocally showed that PBDEs are metabolised in juvenile sole via the formation of OH- metabolites. However, this was not a major biotransformation route compared to biotransformation through debromination.

Juvenile sole exposed to artificially-contaminated food showed the ability to biotransform PBDEs into hydroxylated metabolites; these meta-bolites accumulated in fish.

**Keywords:** Marine flatfish; PBDEs; Bioaccumulation; Biotransformation; Hydroxylated metabolites

## 1. Introduction

Polybrominated diphenyl ethers (PBDEs) are among the most widely-used brominated flame retardants and their presence has now been shown worldwide in all environmental compartments, including the deep ocean (Hites, 2004; Law et al., 2006; Tanabe et al., 2008; Shaw and Kannan, 2009). These contaminants have been a source of scientific concern for the last 10 years due to their persistence, bioaccumulation potential and toxicity to wildlife and humans (Darnerud et al., 2001; de Wit, 2002; Law et al., 2003; Legler, 2008; Ross et al., 2009).

Hydroxylated (OH-) and methoxylated (MeO-) PBDEs have also been the focus of several studies for approximately the last 15 years. However, data on their occurrence, fate and origins is scarce, especially in regard to marine biota. OH-PBDEs are now of particular interest, as their various toxic effects can be more potent than those of their non-hydroxylated parent congeners (Hamers et al., 2008). OH-PBDE effects on thyroid hormone homeostasis have already been reported (Brouwer et al., 1998; Meerts et al., 2000; 2001) resulting from their similarity to thyroxine (T4). These compounds can also act on oestradiol synthesis, elicit neurotoxic effects, and inhibit aromatase activity: most of these effects having been studied in mammal cells, including human cells (Meerts et al., 2001; Shaw and Kannan, 2009; Wan et al., 2009).

The industrial production or use of MeO- and OH-PBDEs has not been described to date (Haglund et al., 1997; Malmvärn et al., 2005; Valters et al., 2005). Methoxylated PBDEs have been previously identified in marine biota samples, such as algae, sponges, mussels, fish and mammals (Marsh et al., 2004; Malmvärn et al., 2005; Valters et al., 2005). MeO-PBDE concentrations in wild marine animals are generally higher than parent PBDE concentrations (Teuten et al., 2005; Valters et al., 2005; Malmvärn et al.,

2005; Malmvärn et al., 2008). In the marine environment, MeO-PBDEs are reported to originate mainly from natural products, i.e., bioformation in sponges or algae (Marsh et al., 2004; Valters et al., 2005; Teuten et al., 2005), although the possibility of formation via methylation of their hydroxylated homologues has also been reported (Haglund et al., 1997; Marsh et al., 2004; Teuten et al., 2005; Valters et al., 2005; Kelly et al., 2008; Malmvärn et al., 2008). MeO-PBDEs could also be formed directly in sediment or in organisms via hydroxylation followed by methylation by microorganisms (Haglund et al., 1997). However, they have never been reported as originating directly from PBDE metabolism in laboratory experiments, and this route, if it does occur, is considered as minor (Marsh et al., 2004).

OH-PBDEs may originate from natural sources or from in vivo metabolism. They have been identified in marine organisms such as algae, mussels and fish (Marsh et al., 2004; Malmvärn et al., 2005), as well as in abiotic compartments such as rain, snow and water (Ueno et al., 2008). OH-PBDEs have occasionally been found at higher concentrations than MeO-PBDEs in marine algae, mussels and fish (Malmvarn et al., 2005; de la Torre et al., 2009). Their production could originate directly from the algae itself, from associated microfauna or microflora, or from other organisms such as cyanobacteria (Unson et al., 1994; Malmvärn et al., 2005). More recently, it has been shown that MeO-PBDEs can also be demethoxylated to OH-PBDEs in vitro, at a faster rate than that of PBDE transformation to OH-PBDEs. This new finding could explain the high concentrations of OH-PBDEs sometimes reported in wildlife and increase the risk of biota exposure to OH-PBDEs (Wan et al., 2009). The occurrence of MeO- and OH-PBDEs at higher concentrations than those of PBDEs generally suggests that they are formed naturally (Wan et al., 2009).

However, data on the unequivocal identification of OH-PBDEs in fish as a result of metabolism remains scarce. The formation of OH-PBDEs via metabolism is cytochrome P450 enzyme-mediated (Hakk and Letcher, 2003). As recently reviewed by Kelly et al. (2008), very few studies report the formation of OH-PBDEs from specific congeners in fish under experimental conditions. Kierkegaard et al. (2001) reported the formation of 6 mono-hydroxylated metabolites of BDE-47 in pike (*Esox lucius*) after dietary exposure, with different profile distributions and levels in the various studied organs. Conversely, Burreau et al. (2000) reported no formation of hydrophilic metabolites in pike (*Esox lucius*) after dietary exposure to [$^{14}$C]-BDE-47 using whole-body autoradiography rather than GC-MS. Similarly, OH-PBDE metabolites were not detected in the blood serum of juvenile carp (*Cyprinus carpio*) exposed to spiked food (Stapleton et al., 2004), or in Chinook salmon microsomes (*Onchorhynchus tshawytscha)* after exposure to BDE-99 (Browne et al., 2009). However, OH-PBDEs are likely to accumulate in organisms due to their high log $K_{ow}$, in the 4.5 – 10.7 range (Kelly et al., 2008).

Other studies have reported the identification of OH-PBDEs in fish collected in the field, although their natural origin or formation due to metabolism could not be demonstrated unequivocally. It has been shown that identified OH-PBDEs may be formed, for example, by oxidative processes in effluent discharged from wastewater and sewage treatment plants, then further accumulated in fish (Valters et al., 2005). More recently, de la Torre et al. (2009) reported the identification of both MeO- and OH-PBDEs in the plasma of fish from Lake Ontario, with high concentrations of OH-PBDEs, some of which could possibly originate from fish metabolism.

Although it remains difficult to firmly identify the origin of OH-PBDEs in environmental samples, this hurdle can be overcome by studying their occurrence in fish exposed to artificially-contaminated food, in controlled conditions.

The results presented in this paper are part of a larger project on the fate and effects of selected organic contaminants (PCBs, PBDEs, PAHs) in juvenile sole *(Solea solea* L.*)* exposed to food artificially contaminated with PBDEs in experimental conditions. The study aimed to identify the in vivo transformation products of selected PBDEs in sole and determine their accumulation and elimination kinetics. Results relating to PBDEs and their debrominated metabolites in fish tissues are presented in a separate paper (Munschy et al., 2011). The present paper focuses on the study of MeO-PBDEs and OH- PBDEs in fish exposed to PBDEs, and results were compared with those obtained in control fish followed up simultaneously. As OH-PBDEs have a higher affinity for plasma proteins than for lipids (Gebbink et al., 2008), fish blood plasma was examined for the presence of OH- congeners. For analytical reasons, fish blood was also examined for MeO- congeners.

## 2. Materials and Methods

The experimental design and analytical procedures used for the experiment are described in detail in the first part of this study and references therein, including the treatment of wastes and water decontamination design (Munschy et al., 2011). The main aspects are briefly summarized below.

### *2.1. Experiment*

Juvenile sole (*Solea solea* L.) obtained from a commercial hatchery (Solea BV, Ijmuiden, Netherlands) were maintained in separate 4 $m^2$ (circa 400 L) tanks receiving a continuous flow of sea water from the roadstead of Brest (Brittany, France), maintained at 12 h light / 12 h dark photoperiods and at a constant temperature (19 ± 1°C). The fish were allowed to acclimatize to experimental conditions for 5 weeks before the experiment began.

Spiked food was prepared by slowly adding 160 ml of a solution of known-amounts of PBDE congeners (i.e., BDE-28, BDE-47, BDE-99, BDE-100, BDE-153, BDE-209) diluted in iso-octane to 4 kg of commercial food (DAN-EX 1562, pellet size 2mm, produced by Dana Feed, Denmark) using an automatic mixer. Spiked food was stored in amber containers in a dark and cool place during its use (circa 1.5 month). Contamination levels of the individual congeners were systematically checked on each of the three food batches used, and followed over the duration of the batch use. Concentrations of individual congeners were between 82 ± 5 ng $g^{-1}$ wet weight (ww) and 93 ± 6 ng $g^{-1}$ ww for BDE-28, BDE-47, BDE-99 and BDE-100, and 181 ± 16% ng $g^{-1}$ ww and 184 ± 23 ng $g^{-1}$ ww for BDE-153 and BDE-209, respectively (mean value ± standard deviation -SD, n= 20 batch replicates). Non-spiked food was also analysed for PBDE levels, and concentrations were between < 0.005 ng $g^{-1}$ ww and 0.39 ng $g^{-1}$ ww depending on the congener.

The fish (n = 470) were exposed to artificially-contaminated food for 84 days followed by non-spiked food for 149 days. In order to check if the addition of solvent to food had any effect on the study parameters, two types of control fish (mean initial weight = 17.4 g) were considered and separated in between two tanks: one tank corresponded to fish (n = 474) fed commercial food, and one tank to fish (n = 492) fed commercial food to

which solvent (iso-octane) was added. Individual daily food rations were adjusted according to fish size and biomass in each tank throughout the experiment, and were 0.8% of fish body weight / day, on average.

Fish were sampled from the tank receiving contaminated food on days 0, 8, 14, 28, 56, 84 (contamination period), and on days 91, 98, 112, 140, 168 and 233 (depuration period). Fish from the control tanks were sampled on days 0, 14, 84, 91, 140, 168 and 233. Fish were systematically sampled after a 24-hour period of fasting. Fish size and weight were recorded individually, immediately after anaesthesia with phenoxy-ethanol. Fish blood was collected from cardiac muscle, placed in tubes containing the anticoagulant heparin and immediately centrifugated at high speed (12 000 rpm) for 5 minutes. The resulting plasma was stored at -80°C pending further processing. Samples were made from pooled individuals of n = 8 to 20 fish, depending on fish size, and replicate pools (n = 2 to 3) were generally processed. Animal care guidelines were applied throughout the experiment by experienced staff.

*2.2. Standards and reagents*

The solvents (DCM, cyclohexane, acetone, ether, methanol, n-hexane, toluene) used for analyses were of trace analysis grade and supplied by SDS (France). Polystyrene gel beads Bio-Bead S-X3 (200-400 Mesh) were supplied by Bio-Rad Laboratories Inc. (USA). Silica gel (100-200 Mesh) and aluminium oxide (90 standardized) were supplied respectively by Sigma Aldrich (Germany) and Merck (Germany). PBDEs used to prepare spiked food were purchased from AccuStandard® Inc. (New Haven, CT, USA) and their purity was above 98%. Standard solutions used for calibration, recovery surrogates added before extraction and internal standard solution added before injection,

were obtained from Wellington Laboratories Inc. (Ontario, Canada), Cambridge Isotope Laboratories Inc. (Andover, MA, USA) or AccuStandard® Inc. (New Haven, CT, USA).

*2.3. Sample preparation*

Fish plasma samples were analysed for PBDEs, MeO-PBDEs and OH-PBDEs using a protocol adapted from Berger et al. (2004). Plasma samples, mixed with acidified sodium sulphate (1% concentrated sulphuric acid 96%), were spiked with recovery standards (BDE-139, and the labelled $^{13}$C-6-MeO-BDE-47, $^{13}$C-6'-MeO-BDE-100, $^{13}$C-6-OH-BDE-47 and $^{13}$C-6'-OH-BDE-100), extracted using cyclohexane:acetone 3:1 v/v, purified by Gel Permeation Chromatography and fractionated on Florisil columns (1.5 g de-activated at 0.5% w/w water, 60-100 Mesh particles) eluted with sequential mixes of 11 ml of *n*-hexane:DCM (3:1 v/v), 6 ml of *n*-hexane:acetone (85:15 v/v), and 10 ml of DCM:methanol (88:12, v/v). The neutral fraction containing PBDEs and MeO-PBDEs was eluted first. The second and the third fractions containing OH- congeners, including the two OH-BDE recovery standards, were combined and derivatised overnight in the fridge using diazomethane. Diazomethane was prepared using Diazald (Sigma Chemical Co) in ether for each sample batch and used on the day of preparation. PBDE, MeO- and OH- fractions were subsequently purified on acidified (minimum of 22% sulphuric acid) silica column (100-200 Mesh) eluted with 40ml of 15% DCM in hexane.

Lipid content (determined as solvent-extractable organic matter) was determined in plasma samples using a gravimetric method and ranged between 0.7% and 3% ww, i.e., very similar to the levels found in various species of pelagic fish collected in the Detroit river in Canada (Li et al., 2003). These levels were fairly low, as the fish analysed in our study were fed with food containing a higher lipid content (13%) than usually found in

208 the wild. As lipid percentages do not generally correlate with MeO- and OH-PBDE
209 concentrations, no lipid correction was applied and concentrations were expressed on a
210 wet weight basis (Valters et al., 2005).
211
212 *2.4. Instrumental analysis*
213 Fish plasma samples were analysed for PBDEs using a Gas Chromatograph (Agilent
214 6890) coupled to a Mass Spectrometer (5973N) operated in negative chemical
215 ionisation mode as described in the first part of this study (Munschy et al., 2011).
216 Both the MeO- and OH- fractions were analysed using High Resolution Gas
217 Chromatography - High Resolution Mass Spectrometry with an AutoSpec Ultima
218 (Micromass, Manchester, UK) operated in electronic impact ionisation mode at a
219 minimum resolution of 10 000 in the selected ion monitoring mode. The source was
220 maintained at 280°C and the filament was operated at a trap current of 650 µA and an
221 electron voltage of 35 eV, although the latter value was optimised for each filament.
222 The MS was calibrated using perfluorokerosene and resolution was checked after each
223 sample injection throughout the whole sequence of runs. The GC was equipped with a
224 DB-1 (J&W Scientific, USA) capillary column (30 m x 0.25 mm x 0.1 µm), which
225 allowed good separation of MeO- derivatives within the 20-minute runs. The column
226 was programmed as follows: the initial oven temperature was 100°C held for 1 minute,
227 ramped to 180°C at 40°C/minute with no hold time, ramped to 240°C at 15°C/minute
228 with no hold time, ramped to 280°C at 4°C/minute with no hold time, then ramped to
229 320°C at 20°C/minute and held for 1 minute. Gas flow of Helium was 0.8 ml/minute.
230 This column was used for quantification, while a longer column (Rtx - 5MS, 60 m x
231 0.25 mm x 0.1 µm, Restek Corp., USA) was also used to confirm the identification of

the compounds. The program used for the longer column was: 80°C (1 minute), to 250°C at 10°C/minute (held for 5 minutes), to 300°C at 3°C/minute (held for 15 minutes). Gas flow of He was 0.8 ml/minute. Injections of 1 µl of samples and standards were done in toluene in the splitless mode.

Identification of MeO- and OH-PBDEs was achieved by comparing their retention times to those of authentic MeO- or derivatised OH- standards used as reference and by comparing their isotopic ratio with their theoretical ratio. The calibration standards (Wellington Laboratories, Canada) used to identify and quantify the MeO-PBDEs contained four native MeO-tetraBDEs (5-MeO-BDE-47, 6-MeO-BDE-47, 4'-MeO-BDE-49, 2'-MeO-BDE-68), four native MeO-pentaBDEs (5'-MeO-BDE-99, 5'-MeO-BDE-100, 4'-MeO-BDE-101, 4'-MeO-BDE-103), plus $^{13}$C-labelled 6-MeO-BDE-47 and $^{13}$C-labelled 6'-MeO-BDE-100 used as internal recovery standards, added at the beginning of the sample treatment. The native OH- congeners used for OH-PBDE quantification were obtained from individual solutions (AccuStandard Inc., USA), i.e., the OH-triBDE 4'-OH-BDE-17, the OH-tetraBDEs 4-OH-BDE-42, 3-OH-BDE-47, 5-OH-BDE-47, 6-OH-BDE-47, 4'-OH-BDE-49 and 2'-OH-BDE-68, the OH-pentaBDEs 6'-OH-BDE-85, 4'-OH-BDE-90 and 6'-OH-BDE-99. $^{13}$C labelled 6-OH-BDE-47 and $^{13}$C labelled 6'-OH-BDE-100 (Wellington Laboratories, Canada) were used as internal recovery standards after derivatisation.

MeO-PBDEs were quantified using the most abundant ions $[M]^+$: m/z 357.9028 for MeO-Br2-BDEs, 435.8133 for MeO-Br3-BDEs, 515.7217 for MeO-Br4-BDEs, 593.6323 for MeO-Br5-BDEs, and 673.5408 for MeO-Br6-BDEs, and the corresponding $[M+2]^+$ identity confirmation ions reflecting the $^{81}$Br and $^{79}$Br isotopic contributions.

Quantification of both MeO- and OH-PBDEs was performed by isotopic dilution method. Injection standards used to calculate the internal recovery standards were $^{13}$C-BDE-79 (for tribrominated to tetrabrominated analogues) and $^{13}$C-BDE-139 (for pentabrominated to hexabrominated analogues). Whenever possible, individual MeO- and OH-PBDEs were quantified in relation to their corresponding authentic standard. MeO-PBDEs were quantified using the relative response factors (RRFs) obtained from a five-level calibration of the MeO-PBDEs mix. OH-PBDEs were quantified against derivatised standard solutions of OH-PBDEs. However, if authentic standards were unavailable, certain OH-PBDEs were directly quantified in relation to their MeO-analogues rather than to derivatised OH-PBDEs (i.e., 4'-OH-BDE-101 and 4'-OH-BDE-103).

*2.5. Quality assurance/quality control*

The entire analytical procedure was performed in clean laboratories maintained under positive atmospheric pressure and supplied with high-purity filtered air. Quality Assurance / Quality Control procedures were included for each batch of eight to ten samples. Blank samples were analysed using the same protocol as for samples and blanks were generally found to be free of any contamination by the targeted analytes. Method Detection Limits –MDLs-, calculated with the instrumental limit of detection, the mass of sample extracted and the final volume injection, ranged from 0.12 pg $g^{-1}$ ww to 0.52 pg $g^{-1}$ ww depending on the congeners. The two congeners 2'-MeO-BDE-68 and 6-MeO-BDE-47 were nevertheless identified in some blanks (MeO- fraction), at 0.26-0.53 pg $g^{-1}$ ww and 0.37-0.48 pg $g^{-1}$ ww, respectively, i.e., at levels 10 times to several orders of magnitude lower than the levels determined in samples.

Recovery rates for individual internal standards $^{13}$C- labelled 6-MeO-BDE-47 and 6'-MeO-BDE-100 were 84 ± 7% (SD) and 98 ± 16%, respectively (n = 33) for the MeO-fraction, and 84 ± 19% and 103 ± 31% (n = 26) for $^{13}$C labelled 6-OH-BDE-47 and 6'-OH-BDE-100, respectively. Recovery rates for individual MeO-PBDEs and OH-PBDEs were evaluated on replicate plasma samples (n = 5) obtained from a control fish pooled sample spiked with a mix of the standard solution congeners. Replicates of non-spiked samples were also analysed and results were used to correct the recoveries of added congeners, whenever necessary. Mean recovery rates (n = 5) were between 99 ± 8% (SD) and 102 ± 11% for MeO-BDEs, and between 95 ± 3% and 99 ± 16% for targeted OH-PBDEs.

## 3. Results and Discussion

### *3.1. Fish health and growth parameters*

Fish health and growth parameters were presented in the first part of this study (Munschy et al., 2011). Briefly, fish growth, liver somatic index and condition index showed no significant difference ($p < 0.05$) in fish exposed to PBDE contaminated food and control fish. Mortality was low (< 2%) and was similar between both categories. As experimental parameters for health were good and similar for all conditions throughout the experiment, the results of PBDE biotransformation in fish could be compared all together.

### *3.2. MeO-PBDEs*

Among the eight specifically-targeted MeO-PBDEs, two congeners were identified by comparison with authentic standards in the fish plasma samples, i.e., 6-MeO-BDE-47

304 and 2'-MeO-BDE-68. In addition, another pentabrominated MeO- congener was
305 detected, although at much lower concentrations, but could not be identified using any
306 of the authentic standards used in HRMS. The three congeners were quantified at levels
307 between 116-270 pg $g^{-1}$ ww, 58-158 pg $g^{-1}$ ww and 5-12 pg $g^{-1}$ ww, respectively
308 (average values calculated on pooled fish replicates). Levels did not show any trends
309 over time during the study period. The three congeners were also present in the plasma
310 of the control fish and at the same concentration ranges as in fish exposed to
311 contaminated food (Fig. 1), suggesting that the MeO-PBDEs detected in fish plasma did
312 not result from fish exposure to PBDEs. Similar observations were made by Lebeuf et
313 al. (2006) for 6-MeO-BDE-47 and 2'-MeO-BDE-68 in both control and PBDE-exposed
314 Atlantic Tomcod. In addition, 6-MeO-BDE-47 and 2'-MeO-BDE-68 possess a MeO-
315 group in the *ortho* position, supporting the hypothesis that these compounds may
316 originate from natural sources rather than PBDE metabolism (Malmvärn et al., 2005;
317 Valters et al., 2005). Both compounds have already been identified in marine biota such
318 as sponges, green algae, mussel and fish, including deep-sea species, (Kierkegaard et
319 al., 2004; Marsh et al., 2004; Malmvärn et al., 2005; Covaci et al., 2008; de la Torre et
320 al., 2009), as well as in marine mammals (Melcher et al., 2005; Weijs et al., 2009). Both
321 congeners have also been unequivocally identified as being naturally produced using
322 $^{14}C$ analysis of a True's beaked whale blubber sample (Teuten et al., 2005). Their
323 presence has also been detected in the commercial food used in this experiment. In
324 addition, fish were directly exposed to natural sea water throughout the experiment, as
325 the tanks were supplied with a continuous flow of sea water. Therefore, accumulation
326 from food and/or direct accumulation from water could have contributed to the
327 occurrence of 6-MeO-BDE-47 and 2'-MeO-BDE-68 in fish studied during our

328 experiment. These compounds have log $K_{ow}$ values in the range of 6.3 to 7.2 (Kelly et
329 al., 2008), enabling them to accumulate in tissues.
330
331 *3.3. OH-PBDEs*
332 Several OH-PBDEs were detected in fish plasma samples during this study. The
333 following congeners were identified in relation to their retention time, isotopic ratio and
334 derivatised authentic standards: 6-OH-BDE-47, 4'-OH-BDE-49, 4'-OH-BDE-101 and
335 4'-OH-BDE-103. Accumulation kinetics are shown in Fig. 2 for both control and
336 exposed fish. The two major OH- congeners, 6-OH-BDE-47 and 4'-OH-BDE-49, were
337 detected at concentrations ranging from 9 pg $g^{-1}$ ww to 95 pg $g^{-1}$ ww (in both control
338 and exposed fish) and from 6 pg $g^{-1}$ ww to 30 pg $g^{-1}$ ww (exposed fish), respectively.
339 The other two identified congeners, 4'-OH-BDE-101 and 4'-OH-BDE-103, were found
340 to be in the 1.8-5.5 pg $g^{-1}$ ww and 0.6-1.8 pg $g^{-1}$ ww ranges, respectively, in exposed
341 fish (Fig. 2). In addition, two OH-pentaBDEs were also detected, but could not be
342 identified in relation to any of the standards employed. The latter congeners were
343 detected at low levels, i.e., 0.5-1.8 pg $g^{-1}$ ww, and in exposed fish only (results not
344 shown).
345 While 6-OH-BDE-47 was detected in the plasma of control fish in a similar
346 concentration range to that of exposed fish, 4'-OH-BDE-49 and 4'-OH-BDE-101 were
347 found at much higher levels in exposed fish (Fig. 2). Congener 4'-OH-BDE-103
348 exhibited an intermediate behaviour.
349 Congeners 4'-OH-BDE-49 and 4'-OH-BDE-101 were below MDL (i.e., < 0.1-0.8 pg $g^{-1}$
350 ww) in most control fish samples, and their levels remained significantly lower than
351 those found in exposed fish (Fig. 2). This suggests that they probably originate from an

352 in vivo PBDE transformation. Moreover, the OH- groups of both congeners are in the
353 *para* position, which may indicate that they originate from PBDE metabolism (Marsh et
354 al., 2004; Valters et al., 2005). Congener 4'-OH-BDE-49 may be derived from BDE-47
355 via CYP enzyme-mediated metabolism (Valters et al., 2005) or from BDE-49
356 hydroxylation (Marsh et al. 2004). BDE-49 was identified in both the tissues and blood
357 of the same fish (Munschy et al., 2011, and below in paragraph 3.4). The formation of
358 4'-OH-BDE-49 (2, 2',4, 5') from BDE-47 (2, 2',4, 4') is due to a 1,2 shift in the *para*-
359 bromine atom during the formation of OH-metabolites (Qiu et al., 2007). This process
360 could also explain the formation of 4'-OH-BDE-101 (2, 2',4,5, 5') from BDE-99 (2,
361 2',4, 4',5). To our knowledge, 4'-OH-BDE-101 has never previously been identified as
362 a PBDE metabolite in fish. The two other unknown pentabrominated OH- congeners are
363 also thought to be of metabolic origin, as they were not identified in the control fish
364 (i.e., below LOD). However, we were unable to ascertain the position of their OH-
365 groups.
366 Both hydroxylated congeners accumulated in a linear manner during the 84-day fish
367 exposure to food (Fig. 2). Rates of formation assessed using the linear relationship were
368 0.202 pg $g^{-1}$ $day^{-1}$ and 0.046 pg $g^{-1}$ $day^{-1}$ for 4'-OH-BDE-49 and 4'-OH-BDE-101,
369 respectively. Interestingly, this increase in concentrations was followed by a rapid drop
370 once exposure to contaminated food ceased, before reaching higher levels after the first
371 month of depuration. These results would suggest a two-step formation of hydroxylated
372 metabolites in blood, with metabolites initially formed from "fresh" contaminants
373 brought through diet, and metabolites which may be formed subsequently from
374 contaminants stored in tissues and remobilised via blood during the depuration period.
375 Conversely, 6-OH-BDE-47 was detected at similar levels in both exposed fish and

376 control fish (Fig. 2), ruling out the possibility of its main origin being metabolic
377 transformation of PBDEs in sole exposed to PBDEs. Data from the literature reports
378 that the presence of this congener in aquatic biota has been attributed to both natural and
379 metabolic sources. 6-OH-BDE-47 has frequently been reported in aquatic biota at high
380 levels, i.e., in red algae and salmon blood (*Salmo salar*) from the Baltic, in various fish
381 species from the Detroit River and in marine sponges and ascidians (Marsh et al., 2004;
382 Malmvärn et al., 2005; Valters et al., 2005). In addition, this congener has been reported
383 as originating from various metabolic routes: it was one of the metabolites identified in
384 the plasma and faeces of rodents exposed to PBDEs (Malmberg et al., 2005; Marsh et
385 al., 2006), and the main congener detected in rain and snow from Ontario, Canada,
386 where its origin from wastewater and sewage treatment plants could be partly due to
387 human and animal metabolites (Ueno et al., 2008). Congener 6-OH-BDE-47 could also
388 theoretically be formed from direct hydroxylation of BDE-47 (Marsh et al., 2004),
389 although in our experiment, 6-OH-BDE-47 was found in similar levels in control fish
390 and exposed fish, ruling out this process as its main origin. As presented above in
391 paragraph 3.2, the MeO- analogue of 6-OH-BDE-47, i.e., 6-MeO-BDE-47, was also
392 identified in our samples; this methoxylated congener could possibly be formed by the
393 methylation of 6-OH-BDE-47 (Haglund et al., 1997; Marsh et al., 2004; Teuten et al.,
394 2005). However, 6-MeO-BDE-47 was present at higher levels than 6-OH-BDE-47,
395 hence arguing in favour of an origin other than 6-OH-BDE-47 methylation. The lack of
396 formation of MeO-PBDEs from OH-PBDEs has also recently been reported during fish
397 microsome incubations (Wan et al., 2009). Conversely, the same study reported the
398 formation of 6-OH-BDE-47 via demethoxylation of 6-MeO-BDE-47: this could partly
399 explain the formation of 6-OH-BDE-47 in our samples. The ratio between the MeO-

400 and the OH-PBDE congeners determined in our samples (about 4:1) is closed to what
401 was previously found in the wild in Baltic sea salmon blood (Marsh et al., 2004;
402 Asplund et al., 1999). In addition, OH- substitution in the *ortho* position with bromine
403 atoms in the 2,4- positions in the non-hydroxylated ring reinforces the hypothesis that
404 the 6-OH-BDE-47 identified in this study was of natural origin rather than a result of
405 PBDE metabolism (Malmberg et al., 2005; Malmvärn et al., 2005).
406 Congener 4'-OH-BDE-103 was identified at similar levels in both exposed and control
407 fish (Fig.2), although its levels were slightly higher in exposed fish. However,
408 concentrations were very low and are hence to be judged with caution. To our
409 knowledge, 4'-OH-BDE-103 has never previously been reported. Its molecular structure
410 suggests that it may originate from BDE-100 hydroxylation. However, the likelihood of
411 it originating from direct PBDE metabolism in our experiment is small, as this congener
412 was also identified in the control fish. On the other hand, its OH- substitution in the
413 *para* position is characteristic of metabolism rather than of natural origin. The presence
414 of this compound in control fish at levels close to those found in exposed fish is still not
415 fully understood and should be further examined.
416 *Para*- and *meta*- OH- metabolites, such as those found in fish plasma, are reported to be
417 the most potent in terms of thyroxine-like activity and/or oestrogen-like activities (Qiu
418 et al., 2007; Hamers et al., 2008). Their concentrations in the plasma of exposed fish
419 were very low, i.e., in the pg $g^{-1}$ ww range, which is far below the levels reported as
420 producing in vitro effects (Meerts et al., 2000; 2001; Hamers et al., 2008). However,
421 several studies in the wild have shown that OH-PBDEs may be present at high levels,
422 and that MeO- congeners, which are sometimes present at high levels too, may also
423 represent a source of OH-PBDEs (Wan et al., 2009). In addition to this, fish may be

424 exposed to other hydroxylated organohalogens in the wild, which would lead to
425 combined/additive effects, hence enhancing fish exposure to endocrine system function
426 disrupters (Brouwer et al., 1998; Li et al., 2003; Hamers et al., 2008).
427
428 *3.4. OH-PBDEs to PBDEs ratio*
429 In order to calculate the ratio of PBDE congeners versus their metabolites in blood
430 plasma, PBDEs were also quantified in blood plasma. All congeners to which fish were
431 exposed through diet (i.e., BDE-28, BDE-47, BDE-99, BDE-100, BDE-153, BDE-209)
432 were identified in blood, as well as some debrominated congeners such as BDE-49 and
433 an unknown pentabrominated congener. These debrominated congeners have been also
434 identified in fish tissues, and are more than likely the result of PBDE metabolism in fish
435 (Munschy et al., 2011). Maximum concentrations in blood were in the 1.3-13.3 ng $g^{-1}$
436 ww range depending on the congener; these levels were in the same range as those
437 estimated in the whole body, except for BDE-209, which showed higher maximum
438 levels in blood. Interestingly, higher-brominated congener concentrations were higher in
439 plasma than in the whole fish body during the first weeks of exposure; concentrations
440 were subsequently similar in fish tissues. An example of this is illustrated in Fig. 3 for
441 BDE-153. This observation indicates an initial faster rate of blood contamination after
442 exposure through diet, followed by a subsequent transport of contaminants throughout
443 the body via the blood. Conversely, as shown in Fig. 3, concentrations determined in
444 plasma exhibited higher variations between pooled samples than those determined in the
445 whole fish body. This could be explained by the dynamic properties of this circulating
446 media.
447 Considering that both BDE-47 and BDE-49 were metabolised into 4'-OH-BDE-49, we

calculated the ratio 4'-OH-BDE-49 / (BDE-47 + BDE-49), which was found to be between 0.11% and 0.51%. This ratio did not show any trends over time during the study period (Fig. 4). In the same manner, based on the fact that BDE-99 was metabolised into 4'-OH-BDE-101, we calculated the ratio 4'-OH-BDE-101 / BDE-99. This ratio was found to be between 0.07% and 4.2%, and showed a linear increase during the depuration period (Fig. 4). This would tend to suggest that the transformation of BDE-99 into 4'-OH-BDE-101 was a continuous process during the whole experiment.

The ratios calculated in this study are consistent with data reported previously in the literature for fish or other marine species in the wild and show that OH-PBDEs do not represent major metabolic residues in fish exposed to PBDEs (Valters et al., 2005; Kelly et al., 2008).

## Conclusions

This study highlighted the in vivo accumulation of OH-PBDEs in the plasma of sole exposed to food artificially contaminated with PBDEs. MeO-PBDEs were also found to accumulate in fish plasma, but were not found to originate from PBDE transformation. As expected from previously-published results, trends observed here for MeO-PBDEs suggest they are most likely of natural origin, i.e., originating from non-metabolic sources. On the other hand, we demonstrated that several OH-PBDEs were derived from PBDE metabolism in fish. The main identified hydroxylated metabolites were 4'-OH-BDE-49 and 4'-OH-BDE-101. In addition, two unknown OH-pentaBDEs were identified as a result of PBDE metabolism in fish. OH-PBDEs accumulated in fish plasma during the exposure period and were still present at similar levels five months after exposure to contaminated food has ceased, suggesting a longer depuration rate

than for the parent congeners. The detected hydroxylated congeners were present in fish plasma at low levels, hence demonstrating that they are not a major route of PBDE degradation in the study species in comparison to debrominated metabolites.

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## References


Asplund, L., Athaniasou, M., Sjodin, A., Bergman, A., Borjeson, H., 1999. Organohalogen substances in muscle, egg and blood from healthy Baltic salmon (*Salmo salar*) and Baltic salmon that produced offspring with the M74 syndrome. Ambio 28, 67-76.

Berger, U., Herzke, D., Sandanger, T.M., 2004. Two trace analytical methods for determination of hydroxylated PCBs and other halogenated phenolic compounds in eggs from Norwegian birds of prey. Analytical Chemistry 76, 441-452.

Brouwer, A., Morse, D.C., Lans, M.C., Schuur, A.G., Murk, A.J., Klasson Wehler, E., Bergman, Ä., Visser, T.J., 1998. Interactions of persistent environmental organohalogens with the thyroid hormone system: mechanisms and possible consequences for animal and human health. Toxicology and Industrial Health 14, 59-84.

Browne, E.P., Stapleton, H.M., Kelly, S.M., Tilton, S.C., Gallagher, E.P., 2009. In vitro hepatic metabolism of 2,2',4,4',5-pentabromodiphenyl ether (BDE-99) in Chinook salmon (*Onchorhynchus tshawytscha*). Aquatic Toxicology 92, 281-287.

Burreau, S., Broman, D., Orn, U., 2000. Tissue distribution of 2,2'24,4'-tetrabromo[$^{14}$C]diphenyl ether ([$^{14}$C]-PBDE-47) in pike (*Esox lucius*) after dietary exposure – a time series study using whole body autoradiography. Chemosphere 40, 977-985.

Covaci , A., Losada, S., Roosens, L., Vetter, W., Santos, F.J., Neels, H., Storelli, A., Storelli, M.M., 2008. Anthropogenic and naturally occurring organobrominated

511 compounds in two deep-sea fish species from the Mediterranean Sea. Environmental
512 Science and Technology, 42, 8654-8660.

513 Darnerud, P-O., Eriksen, G.S., Jóhannesson, T., Larsen, P.B., Viluksela, M., 2001.
514 Polybrominated Diphenyl Ethers: Occurrence, Dietary Exposure, and Toxicology.
515 Environmental Health Perspectives 109 (suppl. 1), 49-68.

516 de la Torre, A., Pacepavicius, G., Martinez, M.A., Darling, C., Muir, D., Alaee, M.,
517 2009. Methoxylated and hydroxylated polybrominated diphenyl ethers in brown
518 bullhead (*Ameiurus nebulosus*) plasma from Lake Ontario. Organohalogen
519 Compounds, 71, 2120-2123.

520 de Wit, C.A., 2002. An overview of brominated flame retardants in the environment.
521 Chemosphere 46, 583-624.

522 Gebbink, W.A., Sonne, C., Dietz, R., Kierkegaard, M., Born, E.W., Muir, D.C.G.,
523 Letcher, R.J., 2008. Target tissue selectivity and burdens of diverse classes of
524 brominated and chlorinated contaminants in polar bears (*Ursus maritimus*) from East
525 Greenland. Environmental Science and Technology 42, 752-759.

526 Haglund, P.S., Zook, D.R., Buser, H.-R., Hu, J., 1997. Identification and quantification
527 of polybrominated diphenyl ethers and methoxy-polybrominated diphenyl ethers in
528 Baltic biota. Environmental Science and Technology 31, 3281-3287.

529 Hakk, H., Letcher, R.J., 2003. Metabolism in the toxicokinetics and fate of brominated
530 flame retardants – a review. Environment International 29, 801-828.

531 Hamers, T., Kamstra, J.H., Sonneveld, E., Murk, A.J., Visser, T.J., Van Velzen, M.J.M.,
532 Brouwer, A., Bergman, Ä., 2008. Biotransformation of brominated flame retardants
533 into potentially endocrine-disrupting metabolites, with special attention to 2,2',4,4'-

534 tetrabromodiphenyl ether (BDE-47). Molecular Nutrition and Food Research 52,
535 284-298.

536 Hites, R.A., 2004. Polybrominated diphenyl ethers in the environment and in people: a
537 meta-analysis of concentrations. Environmental Science and Technology 38, 945-
538 956.

539 Kelly, B.C., Ikonomou, M.G., Blair, J.D., Gobas, F.A.P.C., 2008. Hydroxylated and
540 methoxylated polybrominated diphenyl ethers in a Canadian Arctic marine food web.
541 Environmental Science and Technology 42, 7069-7077.

542 Kierkegaard, A., Burreau, S., Marsh, G., Klasson-Wehler, E., de Wit, C., Asplund, L.,
543 2001. Matabolism and distribution of 2,2',4,4'-tetrabromo[$^{14}$C]diphenyl ether in pike
544 (*Esox lucius*) after dietary exposure. Organohalogen Compounds, 52, 58-61.

545 Kierkegaard, A., Bignert, A., Sellström, U., Olsson, M., Asplund, L., Jansson, B., de
546 Wit, C.A., 2004. Polybrominated diphenyl ethers (PBDEs) and their methoxylated
547 derivatives in pike from Swedish waters with emphasis on temporal trends, 1967-
548 2000. Environmental Pollution 130, 187-198.

549 Law, R.L.J., Alaee, M., Allchin, C.R., Boon, J.P., Lebeuf, M., Lepom, P., Stern, G.A.,
550 2003. Levels and trends of and other brominated flame retardants in wildlife.
551 Environment International 29, 757-770.

552 Law, R.LJ., Allchin, C.R., de Boer, J., Covaci, A., Herzke, D., Lepom, P., Morris, S.,
553 Tronczynski, J., de Wit, C., 2006. Levels and Trends of Brominated Flame
554 Retardants in the European Environment. Chemosphere 64, 187-208.

555 Lebeuf, M., Couillard, C.M., Légaré, B., Trottier, S., 2006. Effects of DeBDE and PCB-
556 126 on hepatic concentrations of PBDEs and methoxy-PBDEs in Atlantic Tomcod.
557 Environmental Science and Technology 40, 3211-3216.

558 Legler, J., 2008. New insights into the endocrine disrupting effects of brominated flame
559 retardants. Chemosphere 73, 216-222.

560 Li, H., Drouillard, K.G., Bennett, E., Haffner, G.D., Letcher, R.J., 2003. Plasma-
561 associated halogenated phenolic contaminants in benthic and pelagic fish species
562 from the Detroit River. Environmental Science and Technology 37, 832-839.

563 Malmberg, T., Athanasiadou, M., Marsh, G., Brandt, I., Bergman, Ä., 2005.
564 Identification of hydroxylated polybrominated diphenyl ether metabolites in blood
565 plasma from polybrominated diphenyl ether exposed rats. Environmental Science
566 and Technology 39, 5342-5348.

567 Malmvärn, A., Marsh, G., Kautsky, L., Athanasiadou, M., Bergman, A., Asplund, L.,
568 2005. Hydroxylated and methoxylated brominated diphenyl ethers in the red algae
569 *Ceramium tenuicorne* and blue mussels from the Baltic Sea. Environmental Science
570 and Technology 39, 2990-2997.

571 Malmvärn, A., Zebühr, Y., Kautsky, L., Bergman, A., Asplund, L., 2008. Hydroxylated
572 and methoxylated polybrominated diphenyl ethers and polybrominated dibenzo-p-
573 dioxins in red alga and cyanobacteria living in the Baltic Sea. Chemosphere 72, 910-
574 916.

575 Marsh, G., Athanasiadou, M., Bergman, A., Asplund, L., 2004. Identification of
576 hydroxylated and methoxylated polybrominated diphenyl ethers in Baltic Sea salmon
577 (Salmo salar) blood. Environmental Science and Technology 38, 1, 10-18.

578 Marsh, G., Athanasiadou, M., Athanassiadis, I., Sandholm, A, 2006. Identification of
579 hydroxylated metabolites in 2,2',4,4'-tetrabromodiphenyl ether exposed rats.
580 Chemosphere 63, 690-697.

581 Meerts, I.A.T.M., van Zanden, JJ., Luijks E.A.C., Van Leeuwen-Bol, I., Marsh, G.,
582 Jakobsson, E., Bergman, A, Brouwer, A. 2000. Potent competitive interactions of
583 some brominated flame retardants and related compounds with human transthyretin
584 in vitro. Toxicological Sciences 56, 95-104.

585 Meerts, I.A.T.M., Letcher, R.J., Hoving, S., Marsh, G., Bergman, Ä., Lemmen, J.G.,
586 van der Burg, B., Brouwer, A., 2001. In vitro estrogenicity of polybrominated
587 diphenyl ethers, hydroxylated PBDEs, and polybrominated bisphenol A compounds.
588 Environmental Health Perspectives 109, 399-407.

589 Melcher, J., Olbrich, D., Marsh, G., Nikiforov, V., Gaus, C., Gaul, S., Vetter, W., 2005.
590 Tetra- and tribromophenoxyanisoles in marine samples from Oceania. Environmental
591 Science and Technology 39, 7784-7789.

592 Munschy, C., Héas-Moisan, K., Tixier, C., Olivier, N., Gastineau, O., Le Bayon, N.,
593 Buchet, V., 2011. Dietary exposure of juvenile common sole (*Solea solea* L.) to
594 polybrominated diphenyl ethers (PBDEs): Part 1. Bioaccumulation and elimination
595 kinetics of individual congeners and their debrominated metabolites. Environmental
596 Pollution 159, 229-237.

597 Qiu, X., Mercado-Feliciano, M., Bigsby, R.M., Hites, R.A., 2007. Measurement of
598 polybrominated diphenyl ethers and metabolites in mouse plasma after exposure to a
599 commercial pentabromodiphenyl ether mixture. Environmental Health Perspectives
600 115, 1052-1058.

601 Ross, P.S., Couillard, C.M., Ikonomou, M.G., Johannessen, S.C., Lebeuf, M.,
602 Macdonald, R.W., Tomy, G.T., 2009. Large and growing environmental reservoirs of
603 Deca-BDE present an emerging health risk for fish and marine mammals. Marine
604 Pollution Bulletin 58, 7-10.

605 Shaw, S.D., Kannan, K., 2009. Polybrominated diphenyl ethers in marine ecosystems of
606 the American continents : foresight from current knowledge. Reviews on
607 Environmental Health 24, 157-229.

608 Stapleton, H.M., Letcher, R.J., Li, J., Baker, J.E., 2004. Dietary accumulation and
609 metabolism of polybrominated diphenyl ethers by juvenile carp (*Cyprinus carpio*).
610 Environmental Toxicology and Chemistry 23, 1939-1946.

611 Tanabe, S., Ramu, K., Isobe, T., Takahashi, S., 2008. Brominated flame retardants in
612 the environment of Asia-Pacific: an overview of spatial and temporal trends. Journal
613 of Environmental Monitoring 10, 188-197.

614 Teuten, E.L., Xu, L., Reddy, C.M., 2005. Two abundant bioaccumulated halogenated
615 compounds are natural products. Science 307, 917-920.

616 Ueno, D., Darling, C., Alaee, M., Pacepavicius, G., Teixeira, C., Campbell, L., Letcher,
617 R.J., Bergman, A., Marsh, G., Muir, D., 2008. Hydroxylated polybrominated
618 diphenyl ethers (OH-PBDEs) in the abiotic environment : surface water and
619 precipitation from Ontario, Canada. Environmental Science and Technology 42,
620 1657-1664.

621 Unson, M.D., Holland, N.D., Faulkner, D.J., 1994. A brominated secondary metabolite
622 synthesized by the cyanobacterial symbiont of a marine sponge and accumulation of
623 the crystalline metabolite in the sponge tissue. Ambio 119, 1-11.

624 Valters, K., Li, H., Alaee, M., D'Sa, I., Marsh, G., Bergman, A., Letcher, R.J., 2005.
625 Polybrominated diphenyl ethers and hydroxylated and methoxylated brominated and
626 chlorinated analogues in the plasma of fish from the Detroit river. Environmental
627 Science and Technology 39, 5612-5619.

628 Wan, Y., Wiseman, S., Chang, H., Zhang, X., Jones, P.D., Hecker, M., Kannan, K.,
629 Tanabe, S., Hu, J., Lam, M.H.W., Giesy, J.P., 2009. Origin of hydroxylated
630 brominated diphenyl ethers: natural compounds or man-made flame retardants ?
631 Environmental Science and Technology 43, 7536-7542.

632 Weijs, L., Losada, S., Das, K., Roosens, L., Reijnders, P.J.H., Santos, J.F., Neels, H.,
633 Blust, R., Covaci, A., 2009. Biomagnification of naturally-produced methoxylated
634 polybrominated diphenyl ethers (MeO-PBDEs) in harbour seals and harbour
635 porpoises from the Southern North Sea. Environment International 35, 893-899.
636

**Figure legends**

**Figure 1**

Concentrations (pg $g^{-1}$ ww ± SD) of MeO-PBDEs in blood plasma of fish (pooled samples) exposed to PBDEs through artificially-contaminated diet and in control fish. Standard deviations of the mean obtained on replicate pooled samples are presented.

**Figure 2**

Concentrations (pg $g^{-1}$ ww ± SD) of OH-PBDEs in blood plasma of fish (pooled samples) exposed to PBDEs through artificially-contaminated diet and in control fish.. Standard deviations of the mean obtained on replicate pooled samples are presented.

**Figure 3**

Concentrations (ng $g^{-1}$ ww ± SD) of BDE-153 in blood plasma and whole body of fish exposed to PBDEs through artificially-contaminated diet. Standard deviations of the mean obtained on replicate pooled samples are presented.

**Figure 4**

Variation of ratios (%) of OH-PBDEs to their PBDE precursors in blood plasma over the experiment of fish exposed to PBDEs through artificially-contaminated diet.

**Figure 1**

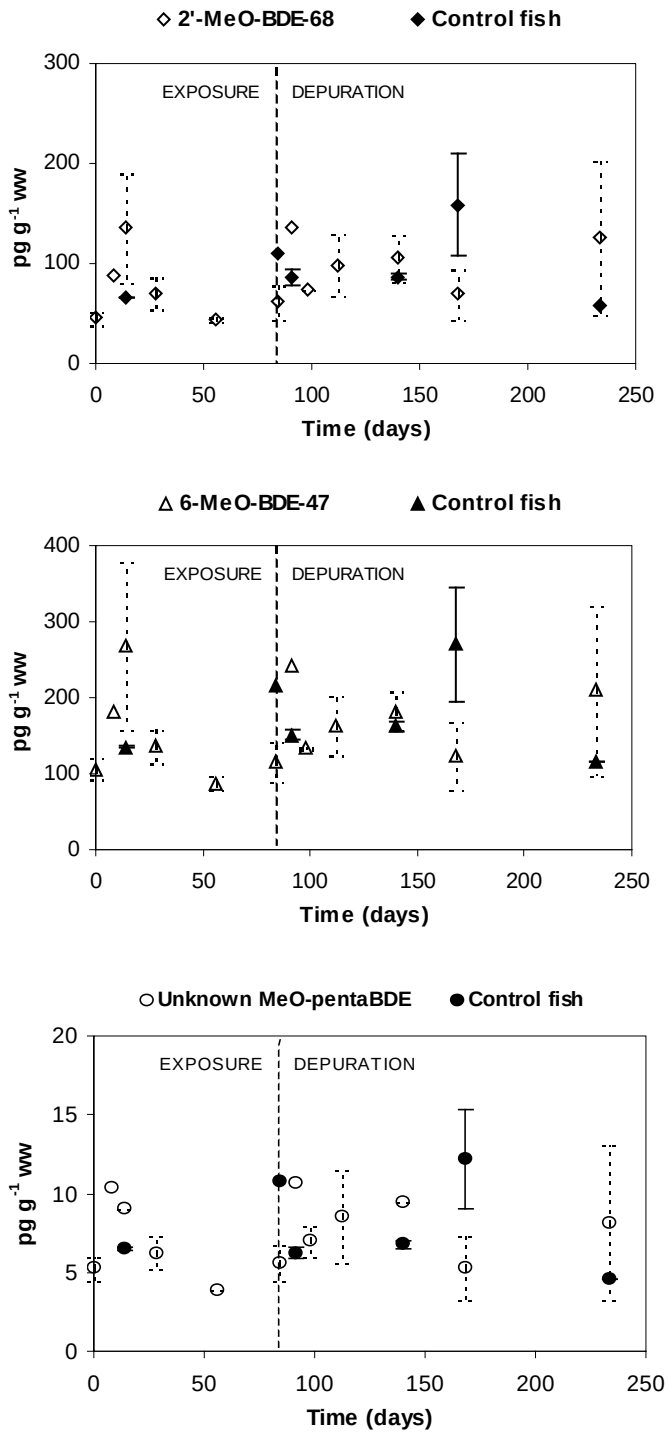

◇ 2'-MeO-BDE-68
◆ Control fish
EXPOSURE
DEPURATION
pg g-1 ww
Time (days)
△ 6-MeO-BDE-47
▲ Control fish
EXPOSURE
DEPURATION
pg g-1 ww
Time (days)
○ Unknown MeO-pentaBDE
● Control fish
EXPOSURE
DEPURATION
pg g-1 ww
Time (days)

**Figure 2**

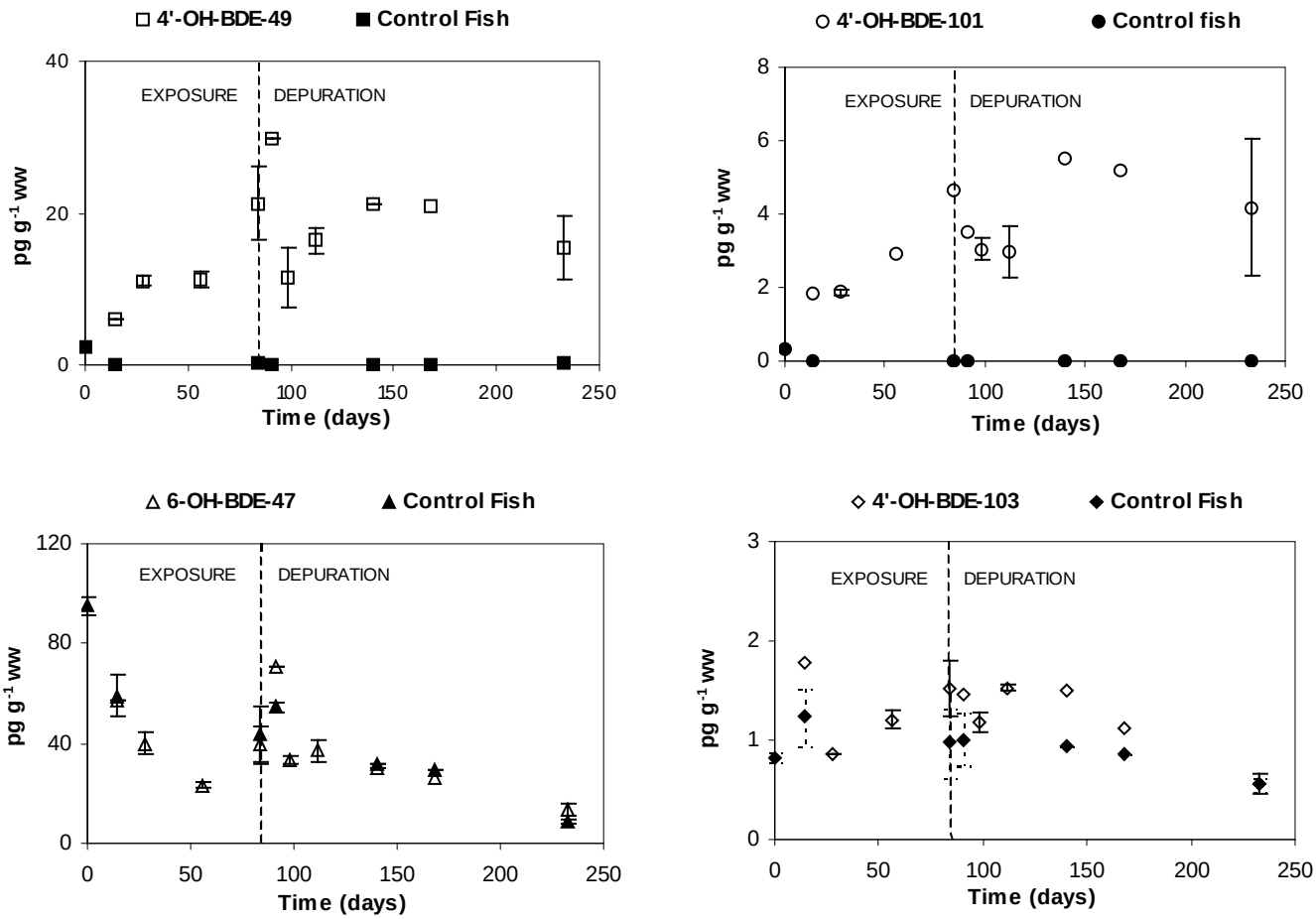

□ 4'-OH-BDE-49
■ Control Fish
EXPOSURE
DEPURATION
pg g-1 ww
Time (days)
○ 4'-OH-BDE-101
● Control fish
EXPOSURE
DEPURATION
pg g-1 ww
Time (days)
△ 6-OH-BDE-47
▲ Control Fish
EXPOSURE
DEPURATION
pg g-1 ww
Time (days)
◇ 4'-OH-BDE-103
◆ Control Fish
EXPOSURE
DEPURATION
pg g-1 ww
Time (days)

**Figure 3**

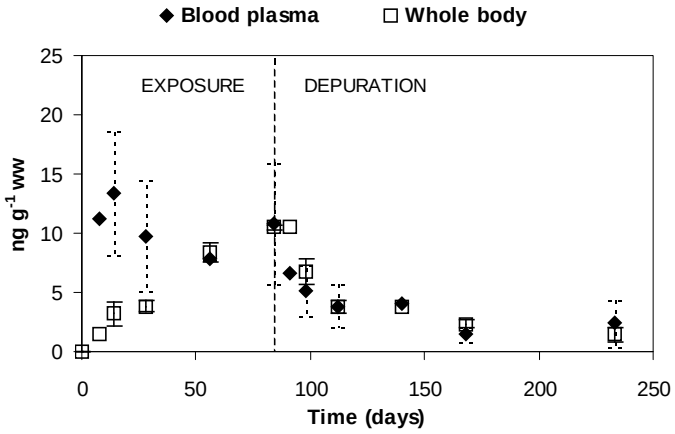
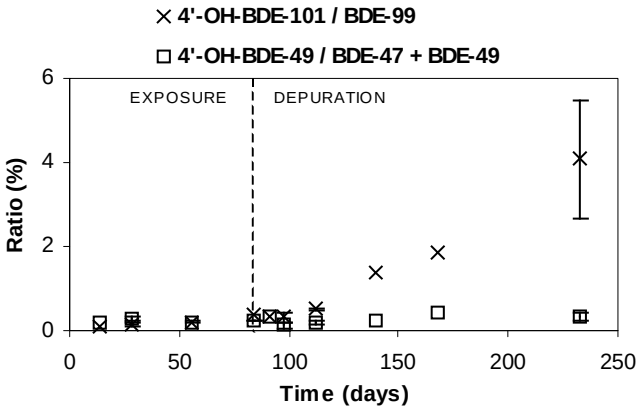

◆ Blood plasma
□ Whole body
EXPOSURE
DEPURATION
ng g-1 ww
Time (days)
0
5
10
15
20
25
0
50
100
150
200
250

**Figure 4**


× 4'-OH-BDE-101 / BDE-99
□ 4'-OH-BDE-49 / BDE-47 + BDE-49
EXPOSURE
DEPURATION
Ratio (%)
Time (days)
0
2
4
6
0
50
100
150
200
250