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Dietary exposure of juvenile common sole (*Solea solea* L.) to polybrominated diphenyl ethers (PBDEs): Part 1. Bioaccumulation and elimination kinetics of individual congeners and their debrominated metabolites

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Abstract:

The uptake and elimination 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, then depurated over a five-month period. The results show that all of the studied PBDEs accumulate in fish tissues, including the higher brominated congener BDE-209. Several additional PBDE congeners were identified in the tissues of exposed fish, revealing PBDE transformation, mainly via debromination. The identified congeners originating from PBDE debromination include BDE-49 and BDE-202 and a series of unidentified tetra-, penta-, and hepta- BDEs. Contaminant assimilation efficiencies (AEs) were related to their hydrophobicity (log K_{ow}) and influenced by PBDE biotransformation. Metabolism via debromination appears to be a major degradation route of PBDEs in juvenile sole in comparison to biotransformation into hydroxylated metabolites.

Juvenile sole exposed to artificially-contaminated food accumulate PBDEs, including the higher brominated congeners, and have a debromination capacity.

Keywords: Marine flatfish; PBDEs; Bioaccumulation; Biotransformation; Debromination

1. Introduction

Polybrominated Diphenyl Ethers (PBDEs) have been extensively studied for around a decade, due to the exponential increase of their environmental levels. These compounds have been identified worldwide and in all environmental compartments (de Wit, 2002; Law et al., 2003; Hites, 2004; Law et al., 2006; Tanabe et al., 2008; Shaw and Kannan, 2009). They are also cause for concern due to their potential adverse effects on wildlife and humans (Darnerud et al., 2001; de Wit, 2002; Legler, 2008; Ross et al., 2009). These contaminants may exert toxic effects such as endocrine disruption, alteration of thyroid hormone homeostasis and neurotoxicity (Legler, 2008). Their physicochemical properties and fate in the environment (persistence, bioaccumulation, long-range transport and toxicity) qualify them as persistent organic pollutants (POPs); therefore, commercial Penta-bromodiphenyl ether and Octa-bromodiphenyl ether have been added to the list of POPs established by the United Nations Stockholm Convention since May 2009 (BSEF, 2010). PBDEs also feature on the list of priority substances drawn up by the European Water Framework Directive (Decision 2455/2001/EC).

Over 67,000 metric tons of PBDEs were produced worldwide in 2001, mostly comprising Deca-bromodiphenyl ether (DecaBDE) (over 80%) (Law et al., 2006). The commercial mixtures Penta-bromodiphenyl ether and Octa-bromodiphenyl ether have been banned from the European market since 2003 (Directive 2003/11/EC) and effectively eliminated in North America since 2005 (Ward et al., 2008). DecaBDE remains on the list of authorized chemicals. However, it has been banned for use in all electrical and electronic applications in Europe since July 2008, and its production and use will be voluntarily phased out in the U.S. market by the end of 2012 (BSEF, 2010). BDE-209, - the main component of DecaBDE – was initially thought to represent a low

threat to biota due to its high hydrophobicity and high molecular size. However, several studies have demonstrated that this compound is bioavailable and can be transformed into more bioaccumulable and toxic PBDEs (Kierkegaard et al., 1999; Stapleton et al., 2006). Moreover, BDE-209 accumulation in sediment has recently become a matter of concern, as this compartment represents large environmental reservoirs and could therefore be a potential threat to biota in the long-term (Ross et al., 2009).

PBDE behaviour in fish has been the focus of several studies. Studies dealing with fish artificially exposed to PBDEs via food have shown that the fate of individual congeners is highly dependent upon the species as well as the congener itself. Fish have widely varying capacities to assimilate and metabolise PBDEs via debromination processes, both in terms of efficiency and metabolite profiles. For example, juvenile carp (*Cyprinus carpio*) showed no accumulation of BDE-99 following dietary exposure (Stapleton et al., 2004a), whereas juvenile trout (*Salvelinus namaycush*) (Tomy et al., 2004) and pike (*Esox lucius*) (Burreau et al., 1997) were shown to accumulate this congener. Different fish species also varied in their ability to transform BDE-99 into BDE-49 or BDE-47 (Stapleton et al., 2004b; Benedict et al., 2007; Browne et al., 2009). Congeners with 3 to 10 bromine atoms were all found to accumulate in juvenile lake trout with varying assimilation efficiencies, widely influenced by debromination (Tomy et al., 2004). BDE-47 generally showed the highest uptake (Burreau et al., 1997; Tomy et al., 2004; Stapleton et al., 2004a), while BDE-209 assimilation by fish was generally low and gave rise to lower brominated congeners. No BDE-209 was found in juvenile carp tissues after a 60-day food exposure experiment, but several penta- to octa-BDEs accumulated in the exposed fish (Stapleton et al. 2004c). BDE-209 was shown to accumulate in the tissues of rainbow trout (*Oncorhynchus mykiss*) - mainly in the liver -

and uptake of this congener was around 3% when all debrominated congeners, mainly octa- and nona-BDEs, were taken into account (Stapleton et al., 2006). This study also revealed that 22% of BDE-209 was biotransformed in liver microsomes of rainbow trout. In another study, BDE-209 uptake by rainbow trout after 120 days of exposure was reported to be even lower, i.e., in the 0.02-0.13% range (Kierkegaard et al., 1999). The purpose of our study was to determine accumulation and elimination kinetics, and to identify the metabolites of selected PBDEs in common sole (*Solea solea* L.) exposed to spiked food under experimental conditions. Common sole was chosen as the target species for this project in view of its benthic habitat and potential exposure to sediment-associated persistent organic contaminants such as PBDEs, plus its high commercial value on European coasts (Riou et al., 2001; Nicolas et al., 2007).

The results presented in this paper are part of a larger project on the fate and effects of selected organic contaminants (PCBs, PBDEs, and PAHs) in juvenile sole. In this paper, we have focused on the accumulation and depuration kinetics of PBDEs and the formation of debrominated congeners. The occurrence of other biotransformation products such as hydroxylated PBDEs has also been studied and the results are presented in a separate paper (Munschy et al., part 2, submitted for publication).

2. Materials and Methods

2.1. Experiment

The experimental design used for the fish exposure is described in detail in the Supplementary Information (SI). Only the main aspects are summarized below.

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 commercial food, obtained from Dan Feed (Denmark), consisted of formulated feed containing 58% crude protein and 15% crude fat (DAN-EX 1562, pellet-size 2 mm). Spiked food was prepared by slowly adding a solution in iso-octane of known amounts of six PBDE congeners (i.e., BDE-28, BDE-47, BDE-99, BDE-100, BDE-153, BDE-209, AccuStandard® Inc., New Haven, USA, purity above 98%) to commercial food. Spiked food was stored in amber containers in a cool, dark place throughout its use (circa 1.5 month). Individual PBDE congener concentrations were determined in each batch of spiked food, and mean values were considered for the calculation of Assimilation Efficiencies (AEs). 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, 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, Table 1). 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 (mean value calculated on n = 4 replicates) depending on the congener. The congeners detected in non-spiked food included the congeners used to spike the contaminated food, plus BDE-49 (0.17 ng g^{-1} ww), a non-identified penta-BDE (Penta-1 at 0.02 ng g^{-1} ww) and BDE-154 (0.04 ng g^{-1} ww) (Table 1).

The fish (n = 470, one tank) were exposed to food spiked with PBDEs 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 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 feeds were adjusted according to fish size and biomass in each tank throughout the experiment, and were equivalent to 0.8% of fish body weight / day, on average. In order to minimize uneaten food, this daily feeding rate was slightly reduced compared to those found in the literature for sole grown in captivity (Coutteau et al., 2001; Schram et al., 2006).

Individual fish size and weight were recorded immediately after anaesthesia with phenoxy-ethanol. Fish were sampled from the tank receiving spiked food on days 0, 8, 14, 28, 56, and 84 (contamination period) and on days 91, 98, 112, 140, 168 and 233 (depuration period). Fish from both control tanks were sampled on days 0, 14, 84, 91, 140, 168 and 233. Fish were then brought to the laboratory for further dissection in clean conditions. Muscle (without skin) and liver were dissected to determine PBDE concentrations. Samples were made from pooled individuals of n = 8 to 20, depending on fish size, and replicate pools (n = 2 to 3) were generally processed.

This study was conducted under the approval of the Animal Care Committee of France under the official licence (972-1) of V. Buchet. Special attention was given to the treatment of wastes, including water decontamination design (see detailed information in the SI).

2.2. Sample preparation

The detailed standards, reagents and analytical protocols for extraction and clean-up have been described previously (Johansson et al., 2006; Munschy et al., 2008). Briefly, PBDE analyses were conducted using approximately five grams of freeze-dried fish tissue. The freeze-dried fish tissue samples were spiked with recovery standards (BDE

139 and ^{13}C labelled BDE-209 – Wellington Laboratories Inc., Ontario, Canada-) prior to extraction, and then extracted with dichloromethane (DCM) using Accelerated Solvent Extraction (ASE, Dionex Corp., USA). The extracts were cleaned on a gel permeation chromatography glass column (460 mm x 26 mm) filled with styrene-divinylbenzene (Bio-beads S-X3), eluted with DCM, fractionated on a silica and alumina column and treated with concentrated sulphuric acid followed by several rinses with iso-octane.

Total lipid content was systematically determined in duplicate in muscle (1 g ww) and liver (150 mg ww) according to the Folch et al. (1957) method, using dichloromethane instead of chloroform. The samples were homogenized and extracted in an excess of dichloromethane/methanol (2/1, v/v) solution and lipid content was determined gravimetrically.

2.3. Instrumental analysis

All fish tissue and spiked food samples were analysed for PBDEs using a Gas Chromatograph (Agilent 6890) coupled to a Mass Spectrometer (5973N) operated in electron capture negative ionisation (ECNI) mode. Detailed chromatographic conditions are given in Hong et al. (2009) and in the SI.

In order to confirm identification of PBDE congeners and to determine the number of bromine atoms of unidentified PBDEs, selected samples were also analysed by High Resolution Gas Chromatography - High Resolution Mass Spectrometry (HRGC-HRMS). Instrumental details can be found in the SI.

2.4. Quality assurance/quality control

186 Samples were processed in the laboratory in a clean, low-dust atmosphere at positive
187 pressure. Quality Assurance / Quality Control procedures were implemented for each
188 batch of eight to ten samples, including procedural blanks, analysis of replicate samples,
189 use of recovery surrogates in all samples, analysis of certified reference material and
190 participation to intercomparison exercises.
191 Blank samples were analysed using glass powder, extracted and processed in the same
192 manner as the fish samples. Blanks were contamination-free, with the exception of
193 BDE-209, which was found at 2 pg.μl^{-1} injected (median value calculated on 17 blanks).
194 BDE-209 limit of detection (LOD) was set at 8 pg.μl^{-1} injected, i.e., blank mean value
195 plus 3 SDs. The LODs for other congeners were defined as 3 times the signal to noise
196 ratio, i.e., 0.5 pg μl^{-1} injected. Method Detection Limits (MDLs) were calculated for
197 each sample taking into account the injection volume, the volume of the concentrated
198 extract before injection and the extracted sample mass. They ranged from 0.001 ng g^{-1}
199 ww to 0.06 ng g^{-1} ww in fish tissues, and from 0.005 ng g^{-1} ww to 0.5 ng g^{-1} ww in
200 food. High variations in BDE-209 levels in blanks were found between batches, with no
201 obvious relation to the BDE-209 concentrations found in samples, hence ruling out
202 cross-contamination problems. Therefore, BDE-209 concentrations were corrected from
203 blank values in both exposed and control fish. The values obtained in each blank were
204 used to correct the values in samples analysed in the same batch.
205 Replicate fish muscle (n = 7) and liver (n = 7) samples were analysed to evaluate our
206 method accuracy in terms of repeatability, resulting in a relative standard deviation
207 (RSD) in the 12-15% range in muscle samples and in the 9-20% range in liver samples
208 for all congeners except BDE-209, for which a higher RSD was observed (36% and
209 32% in muscle and liver, respectively).

Surrogate recoveries were 101 ± 14% for BDE 139 and 91 ± 16% for ^{13}C BDE 209 (mean ± SD calculated on n = 75 analyses) and no surrogate correction was done for the reported concentrations.

Certified reference material (WMF-01, supplied by Wellington laboratories Inc., Ontario, Canada) was routinely included in each sequence of samples and the results were within certified values. WMF-01 consists of a freeze-dried fish sample for which certified or indicative values are given for 7 congeners (namely, BDE-28, BDE-47, BDE-99, BDE-100, BDE-153, BDE-154, BDE-183) at concentrations of between 0.53 ± 0.40 ng g^{-1} dry weight (dw) and 123.2 ± 24.8 ng g^{-1} dw. Recoveries were between 67 ± 23% and 117 ± 9% (mean value ± SD calculated on n = 13 replicate samples) depending on the congener.

The laboratory regularly takes part in Quality Assurance of Information for Marine Environmental Monitoring in Europe (QUASIMEME) intercomparison exercises for PBDEs in biota and our Z-scores are satisfactory, i.e., between -2 and +2.

2.5. Data analysis

Growth rates were calculated by fitting all fish weights to an exponential model over the exposure time:

$$\text{Fish weight at } t = a \times \exp(b \times t)$$

whereby a is a constant, b is the growth rate in g day^{-1}, and t the time in days (Martin et al., 2003).

In order to take into account the dilution of contaminant concentrations due to growth during the depuration phase, concentrations were corrected for growth rate by multiplying the concentrations by [1 + (b × t)] (Fisk et al., 1998).

234 Depuration rate coefficients (k_d) were calculated by fitting the data to a first-order decay
235 curve:

236 $$\ln(\text{concentration}) = a + (k_d \times t)$$

237 whereby a is a constant, k_d is the depuration rate coefficient and t is the exposure time
238 (days) (Fisk et al., 1998).
239 Depuration half-lives were calculated on the exponential part of the depuration curve
240 using:

241 $$t_{1/2} = \frac{\ln(2)}{k_d}$$

242 Apparent (i.e., net) AEs were calculated throughout the exposure period using the
243 following equation (from Tomy et al., 2004):

244 $$\text{AE}\ (\%) = \frac{(C_{fish}) \times (\text{fish body weight})}{(C_{food}) \times (\text{cumulative mass of ingested feed})}$$

245 where C_{fish} (ng g^{-1} ww) is the concentration in fish and C_{food} (ng g^{-1} ww) the
246 concentration in food. PBDE concentrations in fish were calculated using the
247 concentrations determined separately in liver and muscle, the fish liver weight and the
248 fish muscle weight, which was estimated to be the whole body weight minus the liver
249 weight. Remaining fish parts were not kept for contaminant analysis, and were therefore
250 not taken into account for concentration calculations.
251 PBDE concentrations determined in fish tissues were not normalized to total lipid
252 content, due to higher variations of these concentrations when normalized to lipid
253 content.
254

255 **3. Results and Discussion**

256 *3.1. Fish health and growth parameters*

Fish health and growth parameters are presented in Table 2 up to day 168. Because of a smaller number of fish available for PBDE analysis at day 233 (n= 16 and n = 6 for PBDE and solvent-control tanks, respectively), parameters are presented for fish maintained up to day 168. Mortality was low throughout the experiment (< 2% of initial fish numbers) for all conditions (PBDE exposure, solvent-control and control tanks). No significant differences (Paired Student's t-test; $p < 0.05$) were observed in fish growth, liver somatic index and condition index between fish exposed to PBDE-spiked food and both control fish. Total lipid content in muscle and liver tissue was determined on each pooled sample and did not show any significant trends over the time period or in relation to exposure conditions. As experimental parameters for health were good and similar for all conditions throughout the experiment, the results of PBDE accumulation and depuration in fish obtained in all conditions could be compared all together.

Fish growth obeyed an exponential kinetic law for PBDE-exposed fish and both control fish. However, fish growth rate calculated at day 233 was lower (0.0058 g day^{-1}) than fish growth rate calculated up to day 168 (0.0076 g day^{-1}). Contaminant concentrations were therefore corrected for growth using the two different growth rates.

3.2. Bioaccumulation and depuration kinetic parameters

Concentrations of PBDE congeners used to spike food were determined in control fish: they ranged from 0.003 ng g^{-1} ww for BDE-153 to 0.093 ng g^{-1} ww for BDE-47 (estimated in whole body). Since concentrations in control fish and solvent-control fish showed no significant difference (Paired Student's t-test; $p < 0.05$), they were considered all together. All congeners administered in spiked food were detected in both the muscle and liver of exposed fish, at higher concentrations than in control fish (Fig.

281 1). The highest concentrations were in the 2.8-10.6 ng g^{-1} ww range for tri- to hexa-
282 BDEs, depending on the congeners, and 0.06 ng g^{-1} ww for BDE-209. These maximum
283 concentrations were generally observed at the end of the exposure period (84 days).
284 Increased concentrations of all congeners were detected in the samples after 7 days of
285 exposure, revealing a fast food uptake and assimilation. As shown in Fig. 1, linear
286 PBDE accumulation kinetics were observed in fish during the exposure phase (3
287 months) followed by an exponential decrease during the depuration phase (5 months).
288 Similar kinetics, contamination levels and standard deviations were observed for BDE-
289 47 and BDE-28 (data not shown). No steady-state was achieved for any congener
290 during the 3-month exposure time. The observed depuration curves showed two-stage
291 depuration kinetics, with a fast initial depuration up to day 112 (i.e., 28 days of
292 depuration), followed by a slower depuration rate until the end of the experiment and
293 reaching an apparent steady-state. At the later stage of the depuration phase, most
294 congeners showed an increase of their concentrations. This may have been enhanced by
295 the lower growth rate used at day 233 for correction of the concentrations. The uptake
296 and depuration curves observed in this experiment were typical of those observed by
297 other authors during bioaccumulation studies of hydrophobic contaminants in fish (Fisk
298 et al., 1998; Tomy et al., 2004).
299 Assimilation, depuration rate constants and half-lives are presented in Table 3. Half-
300 lives ranged from 37 days for BDE-99 to 86 days for BDE-28. These values were
301 slightly higher than those previously reported for juvenile carp (*Cyprinus carpio*) of
302 initial body masses of 17-18 g exposed to food spiked at 100 ng g^{-1} ww per PBDE
303 congener (Stapleton et al., 2004a). In juvenile lake trout exposed to low-dose (1-3 ng g^{-1}
304 dw) contaminated food, Tomy et al. (2004) reported half-lives of between 39 and 115

305 days for the same congeners as in our experiment, while half-lives were higher for the
306 same fish exposed at higher doses (6-27 ng g^{-1} dw), i.e., in the 26-346 days range. In our
307 experiment, fish were exposed to higher doses, i.e., in the 100-200 ng g^{-1} ww range, but
308 smaller fish (mean weights 17 ± 5 g) than in Tomy et al. (2004) experiment (55 ± 5 g)
309 were studied, which may lead to higher depuration rates and hence lower half-lives
310 (Sijm and van der Linde, 1995; Fisk et al., 1998).
311 Apparent assimilation rates, estimated in whole fish body from concentrations
312 determined in muscle and liver separately, were calculated using a linear regression
313 relationship: they were in the (3.1-12.4) × 10^{-2} ng g^{-1} day^{-1} range for tetra- to hexa-BDEs
314 and much lower for BDE-209 (i.e., 0.08 × 10^{-2} ng g^{-1} day^{-1}). Apparent depuration rates
315 in whole body were in the (0.81-1.85) × 10^{-2} ng g^{-1} day^{-1} range, hence of a similar order
316 to those reported by Stapleton et al. (2004a) in juvenile carp and by Tomy et al. (2004)
317 in juvenile lake trout. Previously published studies have shown that depuration rates and
318 half-lives varied inversely with hydrophobicity, estimated with log K_{ow}, with a
319 maximum half-life for log K_{ow} of around 7 and slower depuration kinetics for highly
320 hydrophobic compounds (Fisk et al., 1998, Stapleton et al., 2004a). In this study, no
321 significant relationship was observed between calculated PBDE half-lives or depuration
322 rates and log K_{ow}, molecular weight or number of bromine atoms. This coincides with
323 the results presented by Tomy et al. (2004). The absence of relationship observed in our
324 study is probably due to the biotransformation of certain congeners. Generally speaking,
325 debromination would enhance the depuration rates of metabolised congeners and lower
326 the depuration rates of the subsequently-produced debrominated metabolites.
327 Accumulation and depuration kinetics were also determined separately in muscle and
328 liver. Interestingly, although the accumulation of all congeners followed a linear

329 increase in muscle, some differences between congeners were noted in liver. BDE-28,
330 BDE-47 and BDE-100 showed linear increases, whereas BDE-99 and BDE-153 showed
331 a two-stage increase, with a rapid linear increase during the first two weeks of exposure,
332 followed by a decrease in the accumulation rates (as illustrated for BDE-99 and BDE-
333 153 in Fig.2). After the first two weeks, BDE-99 accumulation rate nearly reached a
334 steady-state. This would suggest an initial rapid assimilation of these two congeners in
335 the liver, followed by a slow-down due to metabolism. BDE-99 and BDE-153
336 assimilation rates in liver were therefore estimated for both sections of the assimilation
337 curve and showed much lower values after day 14 (Table 3). Assimilation rates in liver
338 for all congeners were higher than those estimated in the whole body, especially for
339 BDE-99 and BDE-153 during the first two weeks of exposure (Table 3). Antunes et al.
340 (2008) reported higher assimilation rates for contaminants with high log K_{ow} in liver
341 than in muscle. Similar observations could be made in our experiment, as illustrated in
342 Fig. 3 by the ratio of BDE-100 concentrations in liver versus those in muscle: this ratio
343 increased sharply between day 0 and day 8, then decreased until the end of the exposure
344 time. Interestingly, this phenomenon was not observed for BDE-28 during the exposure
345 time (Fig. 3), indicating that higher brominated congeners accumulate more easily in
346 liver than lower brominated congeners. Depuration kinetics also showed some
347 differences between the two tissues (see SI for illustration): while depuration started in
348 muscle after day 91 (i.e., 7 days after the termination of the exposure), depuration in
349 liver started immediately after exposure to PBDEs had ceased (i.e., after day 84). While
350 in muscle, all congeners showed the same depuration curve, differences could be
351 noticed in liver, in which the concentrations of the lower brominated congeners BDE-28
352 and BDE-47 showed no decrease during the depuration period, while other congeners

353 exhibited a quick decrease between day 84 and day 91, followed by a stabilisation of the
354 concentrations.
355
356 *3.3. Biotransformation of PBDE congeners in fish*
357 BDE-28, BDE-47 and BDE-100, administered at similar levels in food (circa 100 ng g^{-1}
358 ww), were measured at similar levels in fish, whereas BDE-99, which was also present
359 at circa 100 ng g^{-1} ww in food, showed lower concentrations in fish (Fig. 1). This may
360 be explained by a lower AE or by rapid transformation in fish. Some authors have
361 shown that BDE-99 is either not assimilated, as in juvenile carp (Stapleton et al.,
362 2004a), or degraded to lower brominated congeners, as in Chinook salmon
363 (*Onchorhynchus tshawytscha)* and common carp (Stapleton et al., 2004c; Browne et al.,
364 2009). Assimilation is partly controlled by passive diffusion and also by mediated
365 uptake (Gobas et al., 1993; Burreau et al., 1997). Since passive diffusion is related to
366 molecule physicochemical properties, BDE-99 and BDE-100 assimilation should be
367 relatively similar. The lower assimilation observed for BDE-99 was hence probably due
368 to its biotransformation in sole. BDE-153 was given at ca 200 ng g^{-1} ww in food, but
369 was present in fish tissues at similar levels to BDE-28, BDE-47 and BDE-100. BDE-
370 209 (200 ng g^{-1} ww in food) was detected at the lowest concentrations in exposed fish,
371 and these concentrations were still higher than those determined in control fish (Fig.1).
372 The lower concentrations observed for BDE-153 and BDE-209 in comparison to
373 exposure levels may be due to a combined effect of physicochemical properties (higher
374 molecular weight, log K_{ow}, number of bromine atoms) and biotransformation. This issue
375 will be further discussed below.

376 Moreover, certain PBDE congeners not added to the spiked food were detected
377 throughout the whole study period in fish exposed to spiked food. They include two
378 tetra-brominated congeners (BDE-49 and one unidentified -Tetra-1), one penta-
379 brominated congener (Penta-1), the hexa-brominated BDE-154, two hepta-brominated
380 congeners (BDE-183, Hepta-1), and the octa-brominated BDE-202. These congeners
381 were present in exposed fish at higher concentrations than in control fish (Fig. 1); the
382 concentrations of BDE-183, Hepta-1 and BDE-202 were below the MDL (i.e., < 0.001
383 ng g^{-1} ww), while BDE-49, Tetra-1, Penta-1, and BDE-154 concentrations ranged from
384 0.003 ng g^{-1} ww for Tetra-1 and Penta-1 to 0.03 ng g^{-1} ww for BDE-49. Identification of
385 these congeners was confirmed by High Resolution Mass Spectrometry (HRMS). Since
386 congeners Tetra-1, Penta-1, Hepta-1 could not be identified against authentic standards,
387 they were quantified relative to BDE-49, BDE-100 and BDE-183 calibration curves,
388 respectively. BDE-202, which was not present in the calibration solution used for
389 quantification by GC/ECNI-MS, was quantified relative to BDE-183.
390 In order to check if these additional congeners were the result of accumulation from
391 food or from metabolism in fish, theoretical concentrations in fish after 84 days of
392 exposure were calculated. In order to do so, we considered the concentration determined
393 in spiked food, or the MDL (i.e., 0.5 ng g^{-1} ww, see Table 1) for congeners < MDL, the
394 total biomass of fish at day 84, and the total mass of food given to the fish up to day 84.
395 Theoretical concentrations were corrected for the variation of AEs with log K_{ow}, using
396 the relationship estimated in this study (see paragraph 3.5).
397 BDE-154 was initially present in non-spiked food, at 0.043 ng g^{-1} ww, and was
398 determined in spiked food at a higher level (1 ng g^{-1} ww, Table 1). This was most
399 probably explained by the presence of BDE-154 identified in BDE-99 solution used to

spike food, at a level of circa 1% of BDE-99, which would give a theoretical concentration in spiked food of around 1.5 ng g^{-1} ww. BDE-154 theoretical concentration calculated in fish after 84 days of exposure to spiked food would be circa 0.08 ng g^{-1} ww, which is close to the concentration determined at that time (0.13 ng g^{-1} ww). BDE-183 was detected in spiked food at 1.5 ng g^{-1} ww, while it was not detected (i.e., < 0.005 ng g^{-1} ww) in non-spiked food. Its presence in spiked food is likely due to impurity in BDE-153 spiking solution (at circa 1% of BDE-153). BDE-183 theoretical concentration in fish after 84 days would be circa 0.08 ng g^{-1} ww, while the measured concentration at day 84 was 0.06 ng g^{-1} ww. Hence, BDE-154 and BDE-183 identified in fish fed spiked food resulted more probably from direct accumulation from food than from metabolism in fish. The same calculations were done for the other congeners (BDE-49, Tetra-1, Penta-1, Hepta-1 et BDE-202), showing that they were originating from metabolism in fish, as discussed below.

The congeners originating from parent PBDE debromination showed similar increasing and decreasing profiles over time to the congeners given in food (Fig. 1). Their accumulation rates -or “bioformation” rates-, depuration rates and half-lives are presented in Table 4. Most exhibited lower accumulation rates than the parent congeners, while their depuration rates, estimated in the whole fish body, were of the same order of magnitude as those of the parent congeners. The increase in concentrations at day 233, which was observed for most parent PBDEs, was also observed for the debrominated congeners, especially for Tetra-1, Penta-1 and Hepta-1.

3.4. Debromination pathways

423 During our experiment, although fish were exposed to a mix of PBDEs and not to single
424 congeners, an attempt was made to explain the presence of the congeners thought to
425 originate from the debromination of parent PBDEs.
426 Debromination of BDE-99 has already been shown in different fish species, although
427 with different debromination pathways. For example, Chinook salmon debrominated
428 BDE-99 into BDE-49, while common carp preferentially debrominated BDE-99 into
429 BDE-47 (Stapleton et al., 2004a; Benedict et al., 2007; Browne et al., 2009). In our
430 experiment, it is not unreasonable to assume that BDE-49 may originate from BDE-99.
431 However, debromination of BDE-153 into BDE-49 cannot be absolutely dismissed.
432 Further experiments during which fish would be exposed to only BDE-153 should be
433 conducted in order to verify this assumption. BDE-49 shows much higher levels in
434 exposed fish versus control fish (Fig.1). BDE-49 was present initially in non-spiked
435 food at 0.18 ng g^{-1} ww, and, due to a higher MDL, was not detected in spiked food
436 (Table 1). Using the calculation explained above, BDE-49 concentration in fish after 3
437 months of exposure to food would be 0.05 ng g^{-1} ww, hence far lower than the
438 concentration determined at day 84 (3.7 ng g^{-1} ww) in exposed fish. BDE-49 was
439 present at similar levels to BDE-99 in fish during the contamination phase and was even
440 higher during the depuration phase, which would indicate a high debromination rate of
441 BDE-99 to BDE-49.
442 Tetra-1 was determined in exposed fish at 0.5 ng g^{-1} ww at day 84 (Fig. 1). This level
443 could not result from the accumulation from food, as Tetra-1 was below the MDL in
444 both non-spiked and spiked food (theoretical concentration in fish after 84 days of
445 exposure would be 0.05 ng g^{-1} ww). The origin of Tetra-1 remains uncertain: it could
446 originate from the direct loss of one bromine atom of one of the congeners given in

447 food, or from successive and staggered debrominations from higher-brominated
448 congeners.
449 Previously-published studies have reported BDE-209 debromination into lower
450 brominated congeners – mainly hexa- to nona-BDEs - in juvenile carp and rainbow
451 trout (*Oncorhynchus mykiss*) (Kierkegaard et al., 1999; Stapleton et al., 2004c; Tomy et
452 al., 2004; Stapleton et al., 2006). In our experiment, several PBDEs could originate
453 from BDE-209.
454 The Penta-1 congener was detected in all samples at concentrations of up to 2.5 ng g^{-1}
455 ww at the end of the exposure time. Similarly to BDE-49, Penta-1 was identified in non-
456 spiked food, at 0.022 ng g^{-1} ww, and was below the MDL in spiked food (Table 1). The
457 theoretical concentration in fish at the end of the exposure period would be 0.04 ng g^{-1}
458 ww, while Penta-1 was determined at 2.5 ng g^{-1} ww in the exposed fish at day 84. We
459 can thus hypothesize that Penta-1 results from debromination in fish. BDE-209 could be
460 a potential parent congener, although BDE-153 debromination could not be completely
461 ruled out. Exposure of juvenile carp (*Cyprinus carpio*) to BDE-209 lead to the
462 formation of one penta-brominated metabolite, and to similar congener profiles as those
463 observed during our experiment (Stapleton et al., 2004c). Thus, in sole, Penta-1 could
464 also originate from BDE-209. However, in our experiment, Penta-1 was determined at
465 fairly high concentrations (up to 2.5 ng g^{-1} ww at day 84), which would therefore imply
466 a high assimilation of BDE-209. Exposure to BDE-153 or BDE-209, individually,
467 would be necessary to confirm the origin of Penta-1 in sole.
468 Hepta-1 and BDE-202 theoretical concentrations were both estimated in exposed fish at
469 day 84 as 0.03 ng g^{-1} ww and 0.02 ng g^{-1} ww, respectively. Their concentrations
470 determined in exposed fish at day 84 were 0.17 ng g^{-1} ww and 0.07 ng g^{-1} ww,

471 respectively. We can then reasonably assume that both congeners originated mostly
472 from BDE-209 debromination in fish. (Hepta-1 + BDE-202) / BDE-209 ratio showed an
473 increase during the exposure period, followed by a stabilization during the depuration
474 phase (Fig. 4). This ratio exhibited a higher value at day 233, in relation to the increase
475 of Hepta-1 concentration observed at that time, and a higher variability, mostly due to
476 low-level concentrations of BDE-209.
477 Information on the pathways and mechanisms of in vivo debromination in fish is still
478 scarce, although, as previously suggested by Stapleton et al. (2004c), one could assume
479 that PBDE debromination occurs via the removal of one bromine atom at a time.
480 Deiodinase enzymes - which regulate thyroid hormones- and the cytochrome P450
481 system may play a role in PBDE debromination in fish (Burreau et al., 2000, Tomy et
482 al., 2004; Benedict et al., 2007). Preferential removal of *meta*-substituted bromines
483 suggests that deiodinase enzyme systems may play a role in debromination (Stapleton et
484 al., 2004c). In our study, the hypothesized debromination pathways presented above
485 would suggest that debromination occurred via the removal of a Br atom in the *para*
486 position, i.e., during BDE-99 transformation into BDE-49 and during BDE-209
487 transformation into BDE-202.
488
489 *3.5. Assimilation efficiencies (AEs)*
490 The apparent AEs of BDE-28, BDE-47, BDE-99, BDE-100 and BDE-153 were, on
491 average throughout the exposure time, in the 10-16% range, whereas BDE-209 showed
492 a lower value, i.e., 1.4% (Table 3). To take into account the biotransformation of BDE-
493 99 and BDE-209, and in accordance with the above discussion on hypothesized
494 debromination routes, these AEs were calculated in relation to the summed

concentrations of BDE-99 + BDE-49, and BDE-209 + Hepta-1 + BDE-202, respectively. They may very well be underestimated, as not all the debrominated metabolites were taken into account for the calculation. Hydroxylated metabolites were shown to be negligible (Munschy et al., 2010) and hence have not been considered for the estimation of AEs. On the other hand, AEs may be overestimated if some of the congeners to which fish were initially exposed through diet were also produced via debromination processes. However, the AEs calculated in this study did not show any increase throughout the exposure period for most congeners; if this had been the case, debromination of congeners into the ones to which the fish were exposed via spiked food could have occurred. Only the AEs calculated for BDE-209 showed an increase during the exposure time, varying from 0.4% at day 8 to 2.6% at day 84. Since BDE-209 have no precursor, and showed no increase in spiked food over time, this could be explained by the increase of Hepta-1 concentration relative to BDE-209 concentration with time (Fig. 1).

Unlike previously observed by various authors (Burreau et al., 1997; Stapleton et al., 2004a; Tomy et al., 2004), the AEs calculated in this study showed an inverse linear relationship with log K_{ow} (Fig. 5), molecular weight and number of bromine atoms (not shown). Their values differed from those previously reported in the literature. Burreau et al. (1997) reported AEs of 90%, 60% and 40% for BDE-47, BDE-99 and BDE-153, respectively, in pike (*Esox lucius*). Stapleton et al. (2004a) found AEs of 20%, 93% and 4% for BDE-28, BDE-47 and BDE-153, respectively, in common carp, whereas BDE-99 was not assimilated at all. Tomy et al. (2004) reported AEs ranging from 31% for BDE-99 to 53% for BDE-28 in juvenile lake trout. BDE-209 was systematically found

518 to have lower AEs, ranging from less than 1% (Kierkegaard et al., 1999) to 5.2% (Tomy
519 et al., 2004).
520 Estimation of AEs depends on numerous factors related to exposure parameters, such as
521 contaminant levels in food and food quality. AEs calculated by Tomy et al. (2004) were
522 generally lower when fish were exposed to low doses rather than high doses. Food lipid
523 content is another parameter which may influence AEs, although the way in which this
524 parameter influences efficiency is a matter of controversy (Burreau et al., 1997; Fisk et
525 al., 1998; Gobas et al., 1993).
526 Additionally, AEs are influenced by the calculation method used to estimate whole
527 body concentrations. While our calculations were based on concentrations determined
528 separately in muscle and liver (as per Kierkegaard et al., 1999), other authors used
529 either whole body (with emptied stomach cavity) and liver separately (Stapleton et al.,
530 2004a), whole body minus the gastrointestinal tract (Burreau et al., 1997), or whole
531 body minus liver and gastrointestinal tract (Tomy et al., 2004). Calculating whole fish
532 body concentrations on the basis of concentrations determined separately in liver and
533 muscle may lead to an underestimation of whole body contamination, especially if
534 contaminants are stored in significant amounts in tissues not taken into account for the
535 calculation, and if these tissues represent a significant mass versus fish body weight.
536 Among the fish parts which had not been analysed in our study, and which may
537 represent a significant accumulation compartment, is the tissue surrounding the fins.
538 Observations conducted on dorsal and ventral fins under a microscope revealed lipid
539 accumulation at the base of the fins in juvenile sole (personal communication). Boon et
540 al. (1984) have shown that, in addition to the liver, gut, skin, carcass and interparietal
541 fat may accumulate significant amounts of organic contaminants in sole. Our AEs may

542 therefore have been underestimated, mainly because they were calculated on the basis
543 of contaminant concentrations in liver and muscle only. This underestimation may
544 nevertheless be limited in view of the short exposure time in our experiment. The extent
545 of contaminant accumulation in tissues other than muscle and liver is related to
546 accumulation kinetics, which differ according to tissue type (Gobas et al., 1999). The
547 relative contribution of various tissues to whole body PBDE concentrations over time in
548 sole will be examined in more detail in the future.
549

550 **Conclusions**

551 The results obtained in our study demonstrated that juvenile sole accumulate PBDEs
552 from diet in a linear manner, and that this accumulation is followed by an exponential
553 decrease after exposure to PBDEs through diet has ceased. Apparent assimilation rates
554 in fish were influenced by PBDE debromination, and showed different values in liver,
555 compared to those estimated in whole body. The results also showed that juvenile sole
556 have the ability to bio-transform certain PBDEs into debrominated congeners. The main
557 debrominated metabolities found during this study were BDE-49 and an unknown
558 pentabrominated congener; other tetra-brominated to octa-brominated congeners were
559 also detected, at lower concentrations. In common sole, BDE-99 debromination may
560 preferentially yield BDE-49, although BDE-49 could also originate from BDE-153
561 debromination. Sole was shown to assimilate BDE-209 and noticeably metabolise it
562 into a series of lower brominated congeners. The hypothesized debromination pathways
563 mainly involved the removal of one Br atom in the *para* position. AEs were influenced
564 by PBDE transformation in fish and were inversely correlated with log K_{ow}.
565

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Figure captions

Figure 1

Accumulation and depuration of PBDEs in common sole exposed to PBDEs through diet. Concentrations are expressed in ng g^{-1} ww in whole body. Mean concentrations and standard deviations were calculated and are shown when replicate pooled samples were analysed. In most control fish samples, standard deviations are low and hence do not appear on the figures. Concentrations of Hepta-1 and BDE-202 in control fish are below the MDL.

Figure 2

BDE-99 and BDE-153 concentrations (ng g^{-1} ww) in the liver of PBDE-exposed sole during the exposure period.

Figure 3

Ratio of PBDE concentrations in liver (ng g^{-1} ww) versus PBDE concentrations in muscle (ng g^{-1} ww) for BDE-28 and BDE-100.

Figure 4

(Hepta-1 + BDE-202) / BDE-209 ratios during the 3-month exposure period and the 5-month depuration period.

Figure 5

Assimilation efficiencies (%) of the six PBDEs given in food at t=84 days in common sole. Log K_{ow} values are those of Kelly et al. (2008).

Tables

Table 1

PBDE concentrations (ng g^{-1} ww) in non-spiked and spiked food

Congener	Concentration in non-spiked food[a]	Concentrations in spiked food[b]
BDE-28	0.028 ± 0.004	84.8 ± 4.4
BDE-47	0.390 ± 0.046	82.2 ± 4.5
BDE-49	0.165 ± 0.012	< 0.5
Tetra-1	< 0.005	< 0.5
BDE-99	0.093 ± 0.008	85.7 ± 5.2
Penta-1	0.022 ± 0.0002	< 0.5
BDE-100	0.116 ± 0.011	93.1 ± 6.4
BDE-153	0.015 ± 0.001	181.1 ± 15.7
BDE-154	0.043 ± 0.003	1.0 ± 0.5
BDE-183	< 0.005	1.5 ± 0.3
Hepta-1	< 0.005	< 0.5
BDE 202	< 0.005	< 0.5
BDE-209	0.083 ± 0.008	184.2 ± 23.0

[a] mean concentration ± standard deviation determined on 4 replicates of non-spiked commercial food

[b] mean concentration ± standard deviation determined on a total of 20 replicates from the various batches of spiked food

738 **Table 2**
739 Health and growth parameters monitored in exposed and control fish during an 8-month fish
740 exposure experiment
741

		PBDE exposed fish	Solvent-control fish	Control fish
Number of fish	t 0	470	492	474
	t 168	37	14	64
Mortality (total during experiment)		9	4	7
Fish mass (g) [a]	t 0	17.4 ± 5.4	17.4 ± 5.4	17.4 ± 5.4
	t 168	61.3 ± 20.6	63.3 ± 17.5	57.5 ± 23.1
Growth rate (10^{-3}/d)[a,b]	Whole fish	7.6 ± 0.3	7.7 ± 0.4	7.4 ± 0.4
Lipids (%) ww[c]	Muscle	2.0 ± 0.5	2.0 0.5	1.8 ± 0.3
	Liver	8.8 ± 1. 5	7.5 ± 1.5	7.1 ± 1.0
LSI (%)[c,d]		1.01 ± 0.28	1.02 ± 0.31	1.00 ± 0.30
Condition index (%)[c,e]		0.97 ± 0.15	0.97 ± 0.14	0.96 ± 0.12

742 [a] Fish mass and growth rates were calculated from fish sampled for PBDE analysis
743 [b] Growth rates were calculated from: weight = a × exp (b × time), whereby b is the growth
744 rate
745 [c] mean value ± standard deviation calculated throughout the experiment
746 [d] LSI: (liver somatic index) = (mass liver ww / mass whole body ww) × 100
747 [e] Condition index = (weight (g) / length^3 (cm)) × 100
748
749
750

Table 3

Bioaccumulation and elimination parameters of selected PBDE congeners using artificially-contaminated food during an 8-month fish exposure experiment

Congener	Assimilation rate (whole body) 10^{-2} ng/g/day	Assimilation rate (liver) 10^{-2} ng/g/day	Depuration rate (whole body) [c] 10^{-2} ng/g/day	Half-life days	Assimilation Efficiency [d] %
BDE-28	11.3 ± 0.7	41.2 ± 1.4	0.81 ± 0.25	86 ± 27	16.1 ± 5.3
BDE-47	10.3 ± 0.6	42.1 ± 2.4	1.18 ± 0.41	59 ± 21	15.4 ± 5.0
BDE-99	3.1 ± 0.4	50.9 ± 4.0 [a]	1.85 ± 0.35	37 ± 7	13.2 ± 4.1
		2.2 ± 1.5 [a]			
BDE-100	10.8 ± 0.6	58.3 ± 6.7	1.11 ± 0.44	62 ± 24	13.8 ± 4.7
BDE-153	12.4 ± 1.0	300.7 ± 46.9 [a]	0.83 ± 0.33	83 ± 33	10.1 ± 4.1
		41.6 ± 12.3 [a]			
BDE-209	0.08 ± 0.03 [b]	3.1 ± 0.8 [b]	1.62 ± 0.30	43 ± 8	1.4 ± 0.9

[a] BDE-99 and BDE-153 assimilation rates in liver were calculated for both parts of the assimilation curve (see discussion in 3.2 and Fig. 2).

[b] BDE-209 assimilation rates (whole-body and liver) were calculated from concentrations determined up to day 56 (linear section of the assimilation curve).

[c] Depuration rates were calculated using the exponential part of the curve

[d] Assimilation efficiencies are mean values (± standard deviation) calculated throughout the exposure time

762 **Table 4**
763 Bioaccumulation and elimination parameters of debrominated metabolites of PBDE congeners
764 during an 8-month fish exposure experiment using artificially-contaminated food. Calculations
765 are based on concentrations estimated in whole fish body
766

Congener	Accumulation rate 10^{-2} ng/g/day	Depuration rate [a] 10^{-2} ng/g/day	Half-life days
Tetra-1	0.60 ± 0.07	0.63 ± 0.33	110 ± 57
BDE-49	4.38 ± 0.17	0.60 ± 0.23	115 ± 44
Penta-1	3.05 ± 0.08	1.17 ± 0.21	59 ± 11
Hepta-1	0.22 ± 0.01	1.44 ± 0.44	48 ± 15
BDE-202	0.09 ± 0.01	0.80 ± 0.23	86 ± 25

767 [a] Depuration rates were calculated using the exponential part of the curve

Figure 1

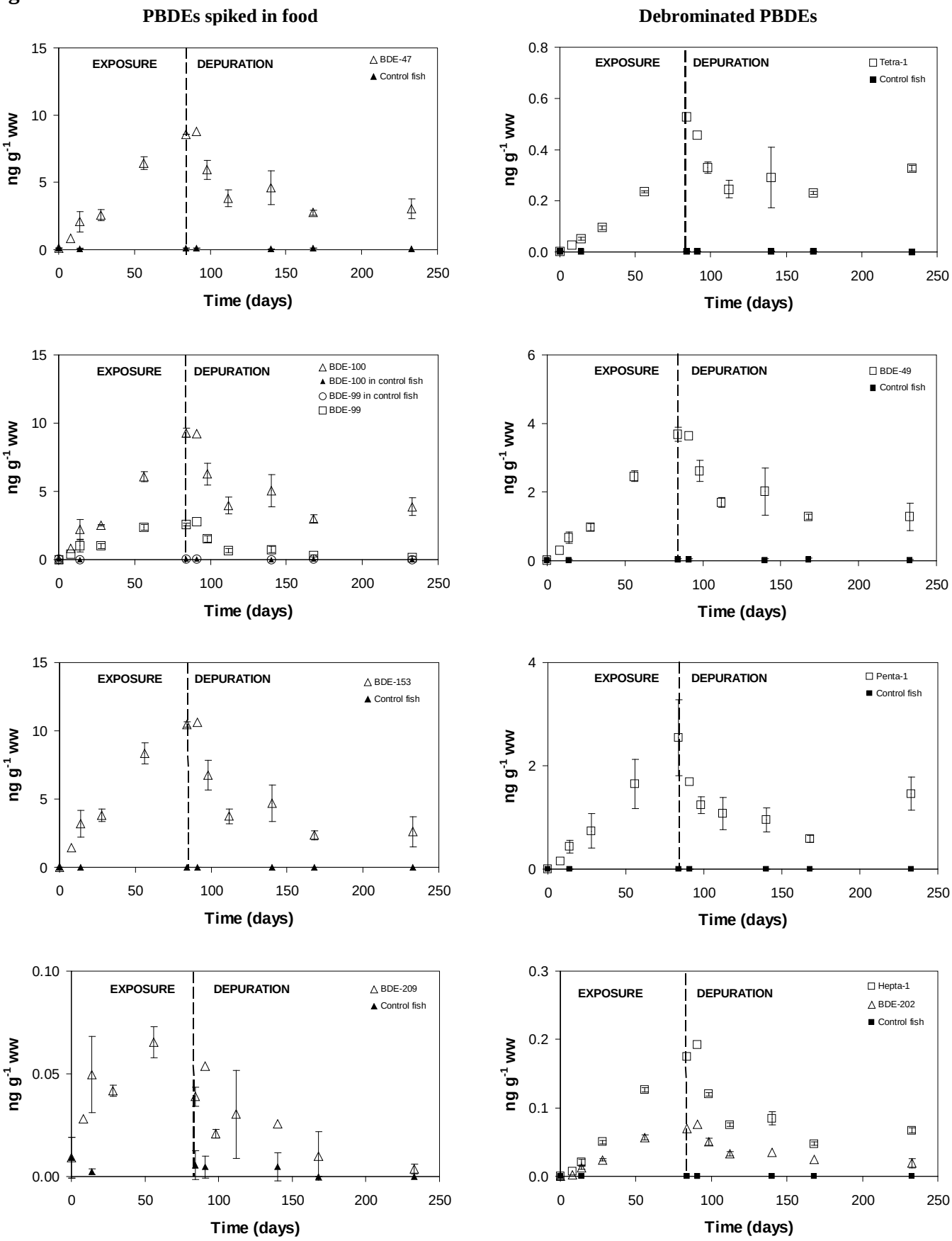

PBDEs spiked in food
Debrominated PBDEs
EXPOSURE
DEPURATION
BDE-47
Control fish
Tetra-1
BDE-100
BDE-100 in control fish
BDE-99 in control fish
BDE-99
BDE-49
BDE-153
Penta-1
BDE-209
Hepta-1
BDE-202
ng g-1 ww
Time (days)

Figure 2

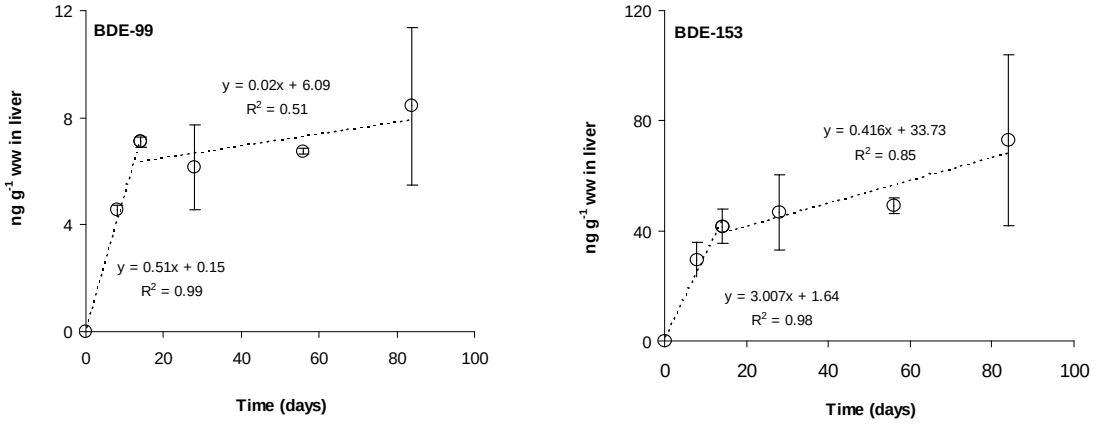

BDE-99
y = 0.02x + 6.09
R2 = 0.51
y = 0.51x + 0.15
R2 = 0.99
ng g-1 ww in liver
Time (days)
0
4
8
12
0
20
40
60
80
100
BDE-153
y = 0.416x + 33.73
R2 = 0.85
y = 3.007x + 1.64
R2 = 0.98
ng g-1 ww in liver
Time (days)
0
40
80
120
0
20
40
60
80
100

Figure 3

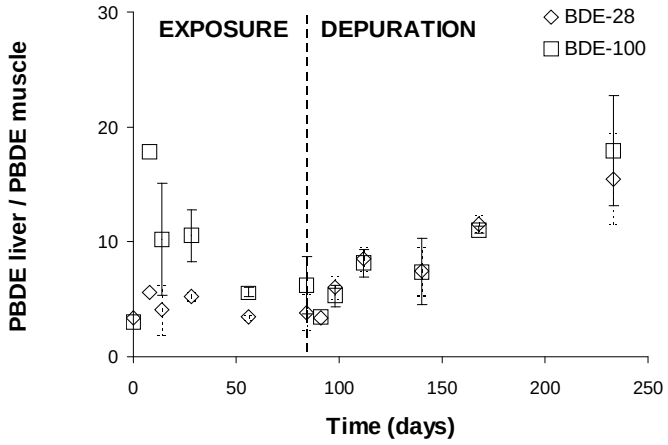

EXPOSURE
DEPURATION
BDE-28
BDE-100
PBDE liver / PBDE muscle
Time (days)
0
10
20
30
0
50
100
150
200
250

Figure 4

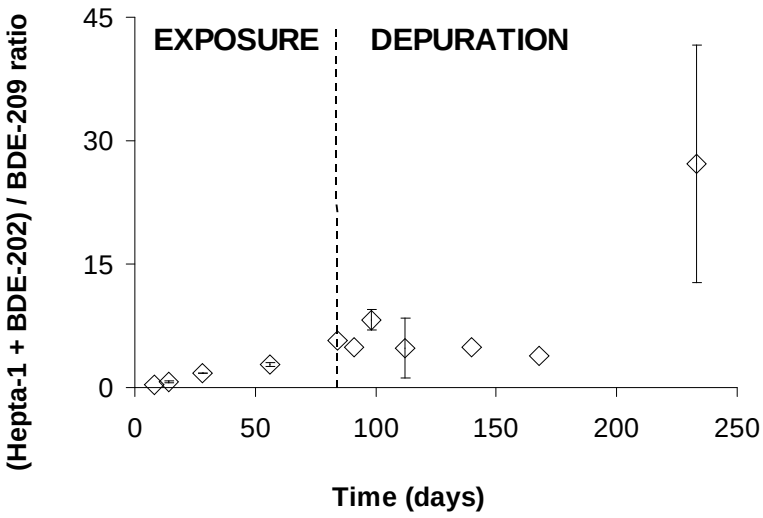
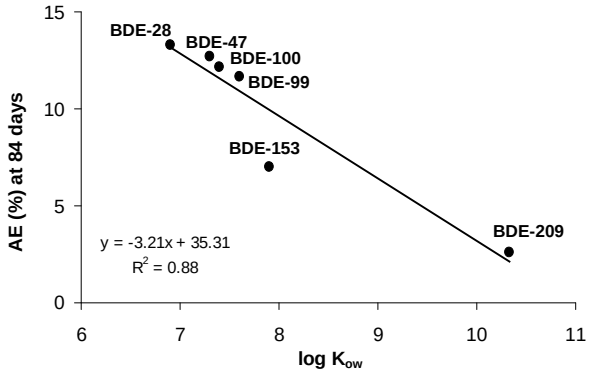

EXPOSURE
DEPURATION
(Hepta-1 + BDE-202) / BDE-209 ratio
Time (days)
0
15
30
45
0
50
100
150
200
250

Figure 5


15
10
5
0
6
7
8
9
10
11
AE (%) at 84 days
log K$_{ow}$
BDE-28
BDE-47
BDE-100
BDE-99
BDE-153
BDE-209
y = -3.21x + 35.31
R^2 = 0.88