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Maternal transfer of emerging brominated and chlorinated flame retardants in European eels

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Abstract

The European eel (*Anguilla anguilla*) is regarded as a critically endangered species. Scientists are in agreement that the “quality of spawners” is a vital factor for the survival of the species. This quality can be impaired by parasites, disease and pollution. Especially endocrine disrupting organic chemicals pose a potential threat to reproduction and development of offspring.

To our knowledge, the findings in this publication for the first time describe maternal transfer of contaminants in eels. We analysed the concentrations of in total 53 polybrominated diphenyl ethers (PBDEs) and their halogenated substitutes in muscle, gonads and eggs of artificially matured European eels and in muscle and gonads of untreated European eels that were used for comparison. We found evidence that persistent organic pollutants such as PBDEs, as well as their brominated and chlorinated substitutes are redistributed from muscle tissue to gonads and eggs. Concentrations ranged from 0.001 ng g\(^{-1}\) ww for sum Dechlorane metabolites (DPMA, aCL\(_{10}\)DP, aCl\(_{11}\)DP) to 2.1 ng g\(^{-1}\) ww for TBA in eggs, 0.001 ng g\(^{-1}\) ww for Dechlorane metabolites to 9.4 ng g\(^{-1}\) ww for TBA in gonads and 0.002 ng g\(^{-1}\) ww for Dechlorane metabolites to 54 ng g\(^{-1}\) ww for TBA in muscle tissue. Average egg muscle ratios (EMRs) for compounds detectable in artificially matured eels from both Schlei Fjord and Ems River ranged from 0.01 for Dechlorane 602 (DDC-DBF) to 10.4 for PBEB. Strong correlations were found between flame retardant concentrations and lipid content in the analysed tissue types, as well as transfer rates and octanol-water partitioning coefficient, indicating that these parameters were the driving factors for the observed maternal transfer. Furthermore, indications were found, that TBP-DBPE, TBP-AE, BATE and TBA have a significant uptake from the surrounding water, rather than just food and might additionally be formed by metabolism or biotransformation processes. Dechloranes seem to be of increasing relevance as contaminants in eels and are transferred to eggs. A change of the isomer pattern in comparison to the technical product of Dechlorane Plus (DP) was observed indicating a redistribution of DP from muscle tissue to gonads during silvering with a preference of the syn-isomer. The highly bioaccumulative DDC-DBF was the most
abundant Dechlorane in all fish of the comparison group even though it is not produced or imported in the EU. The aldrin related “experimental flame retardant” dibromoaldrin (DBALD) was detected for the first time in the environment in similar or higher concentrations than DP.

Introduction

The survival of any species highly depends on its ability to produce healthy, fertile offspring. A failure to do so will substantially affect the overall population or even lead to its extinction. In case of the European eel (*Anguilla anguilla*) the strong decline of glass eel recruitment during the last 30 years (Quebec declaration 2003, ICES 2012) has led to the classification “critically endangered” by the International Union for Conservation of Nature (IUCN).

As of today, the reason for this drastic decline has not been ultimately determined. A variety of factors have been postulated, including overfishing, obstruction of migration, parasitism, predation, and pollution as well as climatic changes that might affect larval transport and survival (ICES 2006). However, scientists are in agreement, that the “quality of spawners” (the fitness of mature silver eels migrating back to their spawning ground in the Sargasso Sea) is vital for the survival of the species (ICES 2012). This quality seems to be seriously impaired by e.g. pollution, parasites and disease (Kirk 2003, Van Ginneken et al. 2005, Geeraerts and Belpaire 2010). The high body fat content of eels (up to 40% of total body weight (Svedäng and Wickström, 1997)) and their longevity favour the accumulation of lipophilic contaminants (Geeraerts and Belpaire 2010). Since fat reserves are the primary energy source during spawning migration and gonad development (Boëtius and Boëtius, 1985) it must be considered that the accumulated contaminants reach their toxic potential maximum during this crucial life-history phase and might affect egg and embryo development. Because eels only reproduce once in their life this could be especially problematic. The accumulated contaminants of the entire lifetime could therefore potentially transferred at once to their gonads and offspring. Contaminants might,
furthermore, weaken the eel during its migration back to the supposed spawning grounds in the Sargasso Sea, which means the spawner might not even be able to complete its journey. Among these, halogenated contaminants have been postulated to be of major concern (Palstra et al. 2006). They are suspected to affect the eel’s lipid metabolism, thereby lowering its chance to migrate back to the spawning grounds in the Sargasso Sea, to decrease its ability to reproduce or affect the viability of offspring (Palstra et al. 2006). To be able to test these hypotheses and assess the impact of halogenated contaminants on the quality of spawners it is vital to measure the transfer rates from mother into eggs, determine the decisive factors for these transfer rates and find contaminant patterns, as well as study the toxic effects on eels and their eggs. However, since the European eel spawning grounds have still not been located (Miler et al., 2014), the actual levels of contaminants in eel eggs could, so far, not be studied. The impact of halogenated contaminants could therefore only be estimated based on concentrations found in muscle or gonad tissue. With advances in the artificial reproduction of the European eel, it is now and for the first time possible to measure potentially hazardous compounds directly in eggs.

Eels are not only exposed to contaminants including legacy POPs, such as Polybrominated Diphenylethers (PBDEs), but also to substitutes for these banned compounds (Geeraerts and Belpaire 2010). Many of these substitutes have structures and properties similar to the replaced compounds and can therefore be expected to have similar adverse effects. In case of PBDEs, brominated (alternate BFRs) as well as chlorinated (Dechloranes) substitutes are in use. Many of them are now detected in similar or higher concentrations than PBDEs in the environment (Harju et al. 2009). There is little information available on production, usage, persistence, or toxicity of these substitutes, yet many are suspected to at least partially fulfil the criteria for POPs or have endocrine disrupting properties (Harju et al. 2009, Sverko et al. 2011).

In this study we analysed the contamination patterns of flame retardants (FR) in muscle and gonads of artificially matured silver eels (mature eels that would naturally be on the migration back to their spawning grounds and have stopped feeding) and their striped eggs.
as well as in muscle and gonads of untreated eels that were kept for comparison. The aim was to better understand mobilization and redistribution of contaminants during maturation and to investigate the impact of the maturation process on FR patterns (Table 1). An additional important factor determining potential negative impacts of contaminants on *A. anguilla* is the degree of interaction of the compound and the eel’s metabolism. It is therefore important to assess whether a compound was distributed throughout the body during uptake and “merely” stored, or whether it was redistributed specifically during maturation and lipid metabolism. In order to address this question, we compared the contamination patterns detected in artificially matured eels with patterns found in muscle and gonads of yellow and silver eels collected in a previous study (Sühring et al. 2013). Yellow eels are mostly sedentary in their habitats (mostly rivers), where they build up high lipid reserves in preparation of the maturation to silver eel and the migration back to the spawning grounds in the Sargasso Sea. As silver eels they stop feeding and use their stored lipid as energy reserve for the journey as well as the development of gonads and eggs. A detection of compounds in muscle as well as in gonads of yellow eels therefore indicates their distribution throughout the different tissue types during uptake rather than during maturation.

The aim of this investigation was, to determine if and to what extent PBDEs and their halogenated substitutes are transferred from parent eel through gonads to eggs, identify processes driving the transfer of these compounds and investigate their relevance. To the best of our knowledge, no data on maternal transfer of contaminants in eels and on levels of the here analysed compounds in eel eggs are available.

**Materials and methods**

**Experimental design**

Between October and November 2012, a total of 16 female European eels were caught in two German drainage systems (Ems River, Schlei Fjord) at the onset of spawning migration and held in freshwater tanks for up to seven days. 11 individuals were sacrificed and used as
a comparison group to determine the contaminant load before the onset of artificial maturation. Five specimens were transferred to a 1500 L saltwater recirculation system and kept in a moderate circular current. The system was equipped with a trickle filter for mechanical filtration and denitrification and with aquarium bubblers for the supply of oxygen. To simulate seasonal variability as well as temperature changes based on e.g. change in water depth water temperature was varied between 15.4°C and 22.0°C during weeks 1-11. From week 11 onwards temperature was controlled and kept between 18.1°C and 18.8°C until week 17. Afterwards temperature was increased and varied between 21.4°C and 22.2°C for the remaining time of the experiment. Salinity was kept between 34 and 37.

After an acclimatization phase of 11 days a weekly dose of 20 mg kg⁻¹ salmon pituitary extract (SPE) (Argent Aquaculture, Redmond, USA) was injected intramuscularly for up to 20 weeks to induce gonad maturation and ovulation. Prior to injections eels were anesthetised with 2-Phenoxyethanol (Carl Roth, Karlsruhe, Germany). Body weight, total length (L_T) and body girth (B_G) were recorded to calculate the Body Girth Index (BGI = B_G L_T⁻¹) (Palstra & Van den Thillart, 2009). From week 16 onwards, egg samples were taken by biopsy and staged according to Palstra & Van den Thillart (2009) to document oocyte maturation. 48 hours after the sudden increase of body weight and BGI, final oocyte maturation and ovulation was induced by an additional SPE injection (20 mg kg⁻¹) and a subsequent intraperitoneal injection of 2 mg kg⁻¹ 17, 20/3-dihydroxy-4-pregnen-3-one (DHP) (Sigma-Aldrich, St. Louis, USA) another 10 hours later.

In case of two eels the additional SPE injection was waived because egg development was already advanced. One eel did not respond to the SPE treatment after 22 weeks and neither the additional SPE nor the DHP injection was applied. Eels were killed by an overdose of 2-Phenoxyethanol and the remaining gonadal tissue was removed from the peritoneal cavity. Muscle samples were taken from the epaxial muscle. All samples were stored in aluminium foil at -20°C until further analysis.
Muscle and gonad samples from 10 yellow and 10 silver eels from a sampling station near the city of Cuxhaven in the Elbe River in 2012 originated from a previous study (Sühring et al. 2013). Since then samples were stored at -20°C in aluminium containers.

**Extraction and clean-up**

The frozen egg, muscle and gonad samples were homogenised with anhydrous Sodium sulfate (Na₂SO₄) (Merck) using a stainless steel/glass 1 L laboratory blender (neoLab Rotorblender). Prior to extraction all samples were spiked with mass labelled surrogate standards ¹³C-BDE-28, ¹³C-BDE-47, ¹³C-BDE-99, ¹³C-BDE-153, ¹³C-BDE-183, ¹³C-MeOBDE-47, ¹³C-MeOBDE-100, ¹³C-HBB, ¹³C-synDP and ¹³C-PBBz (Wellington Laboratories, Cambridge Isotopes).

Extraction and clean-up were performed in accordance with the method described in Sühring et al. (2013), using accelerated solvent extraction with subsequent gel permeation chromatography and silica gel clean-up. 500 pg (absolute) ¹³C-PCB-141 and ¹³C-PCB-208 was added as an injection standard to each sample. The lipid content of samples was determined gravimetrically from separate aliquots following a method described in Sühring et al. (2013). 2 x 200 L tank water of the recirculation tank for the hormone treated eels was enriched on PAD3 sorbent filled glass cartridges at 1 mL per minute. Surrogate standards were added (see above) prior to extraction. Extraction and clean-up was performed using a method by Möller et al. (2010). The water samples were analysed for all studied compounds.

The aqueous salmon pituitary extract (SPE) was ultrasonic extracted with 2:1 hexane:SPE (v/v) for 2 x 15 minutes and analysed.

**Instrumental Analysis**

In order to obtain maximum sensitivity as well as selectivity all extracts were analysed by gas chromatography/mass spectrometry (Agilent QQQ 7000) in electron capture negative ionisation mode (ECNI) with single MS (GC-MS) as well as in electron ionisation mode (EI) with tandem-mass spectrometry GC-MS/MS. Results for target analytes in both methods
were statistically indistinguishable (MEMO Test). Concentrations were therefore calculated as the average of the four measurements per sample (both analytical methods of each two aliquots).

For analysis in EI the instrument was fitted with a Restek 1614 column (15m x 0.25mm i.d. x 0.10 µm film thickness, Restek) with Helium (purity 99.999%) as carrier gas and Nitrogen as collision gas. The instrument was operated in multiple reactions monitoring mode (MRM) at 70 eV. Samples were analysed for nine PBDE congeners (BDE-28, -47, -66, -85, -99, -100, -153, -154, -183), eight methoxylated PBDEs (5MeOBDE-47, 6MeOBDE-47, MeOBDE-49, -68, -99, -100, -101, -103), twenty four alternate BFRs (2,4,6-tribromophenol (2,4,6-TBP), 2,4,6-tribromophenyl allylether (TBP-AE), 2-bromoallyl 2,4,6-tribromophenyl ether (BATE), 1,2-bis(2,4,6-tribromophenoxy)ethane (BTBPE), Decabromodiphenylethane (DBDPE), 2,3-dibromopropyl-2,4,6-tribromophenyl ether (TBP-DBPE), 2-ethyl-1-hexyl 2,3,4,5-tetrabromobenzoate (EH-TBB), Hexabromobenzene (HBB), Hexachlorocyclopentadiene (HCCPD), Hexachlorocyclopentadienyl-dibromocyclooctane (DBHCTD), Pentabromobenzyl acrylate (PBBA), Pentabromobenzylbromide, 1-bromoethy-2,3,4,5,6-pentabromobenzene (PBBB), Pentabromobenzene (PBBz), Pentabromoethylbenzene (PBEB), Pentabromotoluene (PBT), Tetrabromo-p-xylene (TBX), 2,4,6-tribromoanisole (TBA), Tris-(2,3-dibromopropyl) isocyanurate (TBC), Tetrabromo-o-chlortoluene (TBCT), Tetrabromophtalic anhydride (TEBP-Anh), Bis(2-ethyl-1-hexyl)tetra bromophthalate (TBPBH), α/β-tetrabromoethylcyclohexane (α/β-DBE-DBCH), α/β-1,2,5,6-tetrabromocyclooctane (α/β-TBCO)), an 12 Dechloranes (Dechlorane Plus (DP), the one- and two-fold dechlorinated DP species (aCl11DP [-1Cl+1H], aCl10DP [-2Cl+2H]), 1,5-Dechlorane Plus monoadduct (DPMA), Dechlorane 601, 602 (DDC-
DBF), 603 (DDC-Ant) and 604 (HCTBPH), Chlordene Plus (Cplus), Dibromochlordene (DBCD), 
Dibromoaldrin (DBALD), Hexachlorocyclopentadiene (HCCPD) and 
Hexachloro(phenyl)norbornene (HCPN).

ECNI analysis was based on a method developed by Möller et al. (2011). The method was 
extended to include further analytes and a backflush system. The instrument operated in 
selected ion monitoring mode (SIM) with methane as reactant gas. It was fitted with a HP-
5MS column (30m x 0.25mm i.d. x 0.25µm film thickness, J&W Scientific). In both EI and 
ECNI a restriction capillary (0.8m x 0.1mm i.d., deactivated) with a backflush system was 
used. In ECNI eels were analysed for fourteen alternate BFRs, eight Dechloranes and three 
PBDE congeners.

A detailed list of standards, MRM transitions as well as commonly used acronyms and 
acronyms used in this paper are presented in supplement information Tables S3 and S4.

Peak areas of the obtained chromatograms were integrated using Agilent Technologies 
MassHunter Workstation Software Quantitative Analysis B.06.00. Further data analysis was 
performed with Microsoft Office Excel 2010. Statistical analysis, including normality test, 
outlier test, and t-test were performed using Origin Lab 9.1 Pro software. T-test was only 
applied for normally distributed data.

**QA/QC**

Extraction and clean-up were conducted in a clean lab (class 10000). Materials containing 
FRs were avoided during sample preparation and analysis.

Surrogate recoveries were determined for every eel sample. Mean recoveries were 116 ± 
27% for $^{13}$C-BDE-28, 134 ± 25% for $^{13}$C-BDE-47, 90 ± 20% for $^{13}$C-BDE-99, 136 ± 47% for $^{13}$C-
BDE-153, 125 ± 58% for $^{13}$C-BDE-183, 73 ± 38% for $^{13}$C-MeOBDE-47, 139 ± 24% for $^{13}$C-
MeOBDE-100, 87 ± 35% for $^{13}$C-HBB, 112 ± 38% for $^{13}$C-synDP and 98 ± 36% for $^{13}$C-PBBz. All 
concentrations were recovery corrected.
A blank test, using \( \text{Na}_2\text{SO}_4 \) treated similar to real samples, was conducted with every extraction batch (five samples). Concentrations of FR in blanks were between 1 pg absolute for HBB and 136 pg absolute for TBP. Average blank values were subtracted from concentration found in the samples.

The limit of detection (LOD) was calculated from a signal to noise ratio of three or by using the average blank + three times the standard deviation (if the analyte was present in the blanks). The limit of quantification (LOQ) was calculated from a signal-to-noise ratio of ten or using the average blank + ten times the standard deviation (if the analyte was present in the blanks). LODs in ECNI ranged from 0.17 pg absolute for DDC-DBF to 190 pg absolute for HCTBPH. In EI LODs ranged from 0.34 pg absolute for DPMA to 25 ng absolute for BDE-183.

Recoveries of target analytes and 13C- standards were tested with and without matrix during method validation of both used analytical methods. The reproducibility was good, with an average of < 10 % deviation for five measurements.

The salmon pituitary extract (SPE) as well as the water of the recirculation tanks were analysed as described above. In SPE none of the target analytes could be detected. In water of the recirculation tanks TBA, TBP-DBPE as well as trace amounts (< 1pg L\(^{-1}\) after blank subtraction) of TBP-AE and BATE were detected.

A detailed list of blank values, LOD and LOQ is presented in supplement information Table S5.

**Results and discussion**

The maternal transfer of 53 FRs of the three compound groups PBDEs, alternate BFRs and Dechloranes was investigated. 32 of these compounds were detectable in muscle tissue of hormone treated silver eels. 29 compounds could additionally be detected in eggs, indicating a further maternal transfer. Within the maternally transferred contaminants three types of maternal transfer were observed.

1) DDC-DBF, PBT, PBEB, BDE-28, BDE-47, BDE-66, BDE-99, BDE-100, BDE-153 and BDE154 were detected in all tissue types of hormone treated silver eels as well as the comparison
group from all sampling sites and yellow and silver eels from the Elbe. The detection in all tissue types of eels from various life stages indicated that these compounds were distributed into various tissue types during uptake and also redistributed into eggs during artificial maturation.

2) EH-TBB, HBB, synDP, antiDP, aCl10DP and aCl11DP were not detected in yellow eel gonads and showed increasing concentrations in gonads and eggs of hormone treated eels compared to the comparison group. This indicates that these compounds were not distributed throughout the body during uptake, but transferred or redistributed into gonads and eggs specifically during the artificial maturation process.

3) TBA and TBP-DBPE were present in various tissue types of yellow eels, untreated silver eels and hormone treated eels, but additionally displayed a high continued uptake from the water phase during the artificial maturation process. This resulted in a strong increase of these substances in hormone treated silver eels compared to the comparison group, indicating a high bioaccumulation as well as transfer rate for these compounds.

BATE, BTBPE, CPlus, DDC-Ant, HCCPD, TBP-AE, 5MeOBDE47, 6MeOBDE47, MeOBDE49 and MeOBDE68 were detected in various tissue types of hormone treated eels and the comparison group. In eels from Elbe River these compounds were not detectable. It could therefore not be determined whether these compounds were distributed throughout the body during uptake or exclusively during the maturation process. However, as will be discussed later, a change in the MeOBDE congener pattern between comparison group and hormone treated eels indicated metabolism or transformation processes for this compound group specifically during artificial maturation.

For BDE-85, DBALD, DBCD, DBE-DBCH, DBHCTD, DPMA, TBP, TBX, MeOBDE99, MeOBDE100, MeOBDE101 and MeOBDE103 no maternal transfer into eggs could be observed, even though many of the compounds were detected in comparably high concentrations in muscle and gonad tissue.

Results on detected concentrations, patterns, maternal transfer and observed decisive processes are described in detail in the following sections.
### Concentrations of BFRs and Dechloranes in different tissue types

<table>
<thead>
<tr>
<th>Name</th>
<th>hormone treated [yes/no]</th>
<th>life cycle stage</th>
<th>Habitat</th>
<th>tissue type</th>
<th>lipid [%]</th>
<th>weight [g]</th>
<th>stage</th>
</tr>
</thead>
<tbody>
<tr>
<td>yellow Elbe</td>
<td>no</td>
<td>yellow</td>
<td>Elbe</td>
<td>Muscle</td>
<td>27 ± 8</td>
<td>412 ± 160</td>
<td>2 and 3</td>
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<tr>
<td>n = 10</td>
<td></td>
<td></td>
<td></td>
<td>Gonads</td>
<td>n.a.</td>
<td>n.a.</td>
<td></td>
</tr>
<tr>
<td>silver Elbe</td>
<td>no</td>
<td>silver</td>
<td>Elbe</td>
<td>Muscle</td>
<td>25 ± 4</td>
<td>655 ± 125</td>
<td>5</td>
</tr>
<tr>
<td>n = 10</td>
<td></td>
<td></td>
<td></td>
<td>Gonads</td>
<td>n.a.</td>
<td>n.a.</td>
<td></td>
</tr>
<tr>
<td>comp Ems</td>
<td>no</td>
<td>silver</td>
<td>Ems</td>
<td>Muscle</td>
<td>26 ± 5</td>
<td>684 ± 129</td>
<td>5</td>
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<tr>
<td>n = 7</td>
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<td></td>
<td></td>
<td>Gonads</td>
<td>22 ± 5</td>
<td>10 ± 3</td>
<td></td>
</tr>
<tr>
<td>comp Schlei</td>
<td>no</td>
<td>silver</td>
<td>Schlei</td>
<td>Muscle</td>
<td>22 ± 4</td>
<td>611 ± 121</td>
<td>5</td>
</tr>
<tr>
<td>n = 4</td>
<td></td>
<td></td>
<td></td>
<td>Gonads</td>
<td>24 ± 4</td>
<td>10 ± 2</td>
<td></td>
</tr>
<tr>
<td>ht Ems</td>
<td>yes</td>
<td>silver</td>
<td>Ems</td>
<td>Muscle</td>
<td>28 ± 6</td>
<td>1014 ± 403</td>
<td>5</td>
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<td>n = 3</td>
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<td></td>
<td></td>
<td>Gonads</td>
<td>24 ± 13</td>
<td>256 ± 262</td>
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<td></td>
<td></td>
<td>Eggs</td>
<td>18 ± 11</td>
<td>238 ± 81</td>
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<tr>
<td>ht Schlei</td>
<td>yes</td>
<td>silver</td>
<td>Schlei</td>
<td>Muscle</td>
<td>15 - 35</td>
<td>567 - 1177</td>
<td>5</td>
</tr>
<tr>
<td>n = 2</td>
<td></td>
<td></td>
<td></td>
<td>Gonads</td>
<td>33</td>
<td>131 - 161</td>
<td></td>
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<td></td>
<td>Eggs</td>
<td>4 - 8</td>
<td>194 - 498</td>
<td></td>
</tr>
</tbody>
</table>

Table 1: Analysed eel samples including information on treatment, life cycle phase, habitat, tissue types and sample number (n).
General contamination pattern

Figure 1: Average concentrations of total PBDEs in grey, MeOBDEs in dark green, total alternate BFRs in blue, TBP-DBPE transformation products (TBP-AE, BATE) in light green and total Dechloranes in red in ng g\(^{-1}\) wet weight in eggs, gonads and muscle tissue of hormone treated silver eels and muscle tissue and gonads of comparison group silver eels from Ems River (left) and Schlei Fjord (right).

PBDEs were the predominant contaminants in muscle tissue as well as gonads of all non-hormone-treated eels, regardless of developmental stage (yellow or silver) and origin with average concentrations for total PBDEs between 0.23 ± 0.11 ng g\(^{-1}\) wet weight (ww) in gonads of silver eels from Schlei Fjord to 8.9 ± 3.4 ng g\(^{-1}\) ww in muscle tissue of yellow eels from Elbe River (Table 2). BDE-47 was the predominant congener in all analysed samples,
with an average contribution of 64% to total PBDEs. Sum concentrations of alternate BFRs, Dechloranes and methoxylated BDEs (MeOBDEs) in these eels were below 1 ng g$^{-1}$ ww in all analysed tissue types. In hormone treated silver eels, on the other hand, alternate BFRs were found in significantly higher concentrations than PBDEs (t-test at level 0.05). Average total alternate BFR concentrations were between 0.70 ± 0.10 ng g$^{-1}$ ww in eel eggs from Ems River and 7.4 ng g$^{-1}$ ww in muscle tissue from Ems River, whereas total PBDE concentrations ranged between 0.16 ± 0.05 ng g$^{-1}$ ww and 1.07 ng g$^{-1}$ ww in the same samples (Table 2, Figure 1). This significant increase could be an indication, that alternate BFRs either have a higher uptake through skin and gills than PBDEs or are remobilised from other tissue types during artificial maturation. Another significant (t-test at level 0.05) difference between hormone treated eels and the comparison group were the increase of potential TBP-DBPE metabolites/transformation products (TBP-AE and BATE) with median contribution to sum contamination of 1% in silver eels of the comparison group from Ems River and 12% in hormone treated eels from the same habitat; correspondent to the high observed concentrations of alternate BFRs. This trend was less distinct, but still existent in eels from Schlei Fjord, with 3% in the comparison group and 7% in hormone treated eels (Table 2, Figure 2). Dechloranes had the lowest average concentrations in all analysed specimens. Interestingly, Dechlorane contribution to sum FR concentration was higher in eels of the comparison group than hormone treated eels from both Ems River and Schlei Fjord (Table 2). This decrease could be caused by a removal via e.g. excretion, metabolism or redistribution into other lipid rich tissues such as the liver. Overall concentrations of MeOBDEs differed largely between individual samples of hormone treated eels as well as the comparison group. However, only low brominated MeOBDEs (up to MeOBDE-68) were detected in hormone treated eels whereas low and high brominated MeOBDEs (up to MeOBDE-103) were detectable in the comparison group. All analysed contaminant groups, observed trends and patterns will be discussed individually in the next sections.
The detection of PBDEs in yellow as well as silver eel gonads indicated a distribution of contaminants into various tissues during uptake but could also be an indication of a redistribution of contaminants during maturation. The latter assumption was supported by the observed pattern of PBDE congeners in the analysed eels. Yellow eels were contaminated with congeners of both the technical Penta- (BDE-47 (24- 38 %), BDE-82, BDE-85, BDE-99, BDE-100 (50- 62 %), BDE-153 and BDE-154 (4-8 %)) and technical Octa-BDE (BDE-153, BDE-154 (10-12 %), BDE-183 (43-44 %))mixtures while all analysed silver eels showed an increase of congeners attributed to the technical PentaBDE mixture to up to 98% of the total PBDE contamination. Additionally an increase of lower brominated BDE congeners could be observed in gonads and eggs of hormone treated eels compared to the pattern in muscle tissue. In muscle tissue of hormone treated eels BDE congener distribution was similar to profiles previously reported by Belpaire et al. (2008) with BDE-47 > BDE-100 > BDE-153 >
BDE-99. In gonads and eggs however, BDE-99 had a similar contribution to total PBDEs as BDE-100, followed by low brominated congeners such as BDE-28 and BDE-66. PBDEs are known to undergo enzymatic debromination (Eljarrat et al. 2011), which could explain the change in the contamination pattern. However, in the comparison group, PBDE congener profile in gonads remained similar to the profiles in muscle tissue, indicating, that the changes were caused by the artificial maturation process.

Interestingly, MeOBDEs concentrations were about tenfold higher in comparison group silver eels from Ems River than hormone treated eels from the same habitat and differed significantly (t-test at level 0.05) concerning the congener pattern. Only low brominated MeOBDE congeners up to MeOBDE-68 could be found in hormone treated eels, whereas muscle as well as gonad tissue of silver eels of the comparison group from the same habitat were contaminated with MeOBDE congeners up to MeOBDE-103 (Figure 1, Table 2). This change in the contamination pattern could be an indication that MeOBDEs undergo similar debromination process observed for PBDEs, leading to an increase of lower brominated congeners over time. This would again imply that contaminants are not merely redistributed, but subjected to metabolism. However, metabolism studies have to be conducted to confirm this hypothesis. In eels from Schlei Fjord no MeOBDEs could be detected.

**Alternate brominated flame retardants**

A higher number of alternate BFRs were detected in the comparison group, compared to hormone treated eels. However, detection frequencies and overall concentrations were up to fifty times higher in all tissue types of hormone treated eels (Figure 1, Table 2). 1,3,5-tribromo- 2-(2,3-dibromopropoxy)- benzene (TBP-DBPE) was the most abundant with the highest concentration of alternate BFR in all analysed eels (Figure 1,2). It was detected in all analysed tissue types of yellow and silver eels indicating distribution of this compound into various tissues during uptake, rather than just remobilisation during artificial maturation. Interestingly, concentrations in hormone treated eels were significantly (t-test
at level 0.05) higher than concentrations found in the comparison group; with 0.85 ng g⁻¹ ww, 2.0 ng g⁻¹ ww and 8.6 ng g⁻¹ ww in eggs, gonads and muscle tissue of hormone treated eels and 0.21 ng g⁻¹ ww and 0.18 ng g⁻¹ ww in gonads and muscle tissue of the comparison group. The increase cannot have been caused by ingestion, because both groups had stopped feeding.

To assess potential sources for this increase in TBP-DBPE concentration, the water from the tanks of the hormone treated group was analysed to confirm if changes in contamination patterns between hormone treated eels and comparison groups were caused by uptake of contaminants through the surrounding water or the artificial maturation process. TBP-DBPE concentrations higher than 1 ng L⁻¹ were detected in the tank water, suggesting that the increased concentrations in hormone treated eels might have indeed been caused by a continued uptake from TBP-DBPE leaching out of the recirculation system into the tank water during the maturation process. The repeated detection of TBP-DBPE in eels that had not been kept in tanks prior to sampling tissue remained inexplicable, because there is no report on current production or use and the only known producer, Chemische Fabrik Kalk, Germany, ceased its production in the 1980s (von der Recke & Vetter, 2007). Another potential source of TBP-DBPE could be remobilisation from other tissues during the artificial maturation process. Corresponding TBP-DBPE transformation products 1,3,5-tribromo- 2-(2-propen-1-yloxy) benzene (TBP-AE) and 2-bromoallyl-2,4,6- tribromophenyl ether (BATE) (von der Recke & Vetter, 2007) had the second and third highest concentration and detection frequency (>90%) of alternate BFRs in all tissue types of hormone treated eels, while detection frequencies in the comparison group were below 50% and TBP-AE could not be detected in gonads (Table 2). The transformation products were also found in water samples, however in much lower concentrations (< 1pg L⁻¹). The detection of transformation products in eggs and gonads of hormone treated eels rather than the comparison group could be an indication that TBP-DBPE might not just be redistributed into gonads and eggs. TBP-DBPE seems to be subjected to metabolism or biotransformation during maturation resulting in the increase of its transformation products in gonads and eggs of hormone
treated eels (Figure 2). A continuous uptake as well as a potential metabolism of TBP-DBPE during maturation is reason for concern, because TBP-DBPE as well as its transformation products TBP-AE and BATE are known endocrine disrupters and able to penetrate the brain-blood-barrier (von der Recke & Vetter, 2007), making them a potential danger to the healthy development of offspring.

EH-TBB was only detected in two muscle samples but in all gonad samples and all but one of the egg samples of the hormone treated eels, making it the most abundant alternate BFR after TBP-DBPE and its metabolites in hormone treated silver eels (Table 2). EH-TBB is the principal component in the additive flame retardant Firemaster 550 (FM 550) produced since 2003 by Chemtura as a replacement for PentaBDE in polyurethane foam (PUF) applications (Covaci et al. 2011). The high contribution of EH-TBB in gonads and eggs of hormone treated eels rather than muscle tissue could be the result of redistribution from tissue types other than muscle during the artificial maturation. The high lipid content of the liver in eels (Lewander et al., 1974, Dave et al. 1975) could make the liver a storage medium for BFRs, including EH-TBB. During maturation the eel uses its stored lipid to develop gonads and eggs, resulting in high lipid contents in these tissue types (as discussed above). This lipid metabolism pathway is driven by processes in the liver (Boëtius and Boëtius, 1991). Contaminants stored in the liver could be remobilised during the process. This is very likely in case of EH-TBB, which is known to be biologically metabolised (Baerr et al. 2010).

Further alternate BFRs detected in hormone treated eels were concentration wise TBP > BTBPE > PBEB > HBB > PBT. In eels of the comparison group additional alternate BFRs were TBP > DBE-DBCH > HBB > PBT > PBEB > BTBPE > TBX > HCCPD > DBHCTD. The noticeable fewer compounds in hormone treated eels might be an indication of a removal of the contaminants from muscle as well as gonad tissue during the artificial maturation process. The removal cannot be explained by redistribution into eggs, yet substances could be either excreted, distributed into other tissue types, or transformed.

**Dechloranes**
The Aldrin-related experimental flame retardant Dibromoaldrin (DBALD) was found to be the highest concentrated Dechlorane in muscle tissue of hormone treated eels (up to 0.98 ng g\(^{-1}\) ww) and the third highest in the comparison group (up to 0.18 ng g\(^{-1}\) ww). This is, to our best knowledge, the first time that DBALD has been reported in the environment. DBALD was first mentioned in US patent 3941758 as a fire retardant additive for polymers (Maul et al. 1976). Recently, Riddell et al. (2012) conducted a research project on the “structural confirmation of legacy halogenated flame retardants derived from hexachlorocyclopentadiene”, naming DBALD as one of the relevant monoadducts. However, there is no information available on current use or production. DBALD is structurally similar to the banned insecticide Aldrin, with two chlorine atoms substituted by bromine. The presence of an Aldrin-related contaminant could be problematic due to the potentially very high toxicity for fish (the LC50 of Aldrin is 0.006 – 0.01 mg/kg for trout and bluegill (Metcalf 2000)). However, DBALD could not be detected in gonads or eggs of hormone treated eels and was detected in only two gonad samples of the comparison group. It therefore seems that it is not readily distributed into these tissue types during uptake or maturation (Table 2).

The most frequently detected and highest concentrated Dechlorane in muscle as well as gonads of the comparison group was DDC-DBF. Whereas the anti-stereoisomer of Dechlorane Plus (DP) was more abundant in tissue of hormone treated eels. \(\Sigma\text{DP}\) as well as DDC-DBF could be found in all samples of the comparison group from Ems River and Schlei Fjord with \(\Sigma\text{DP}\) concentrations up to 0.079 ± 0.064 ng g\(^{-1}\) ww in muscle tissue and 0.12 ± 0.064 ng g\(^{-1}\) ww in gonads. DDC-DBF concentrations reached up to 0.11 ± 0.039 ng g\(^{-1}\) ww in muscle tissue and 0.068 ± 0.022 ng g\(^{-1}\) ww in gonads. In hormone treated eels \(\Sigma\text{DP}\) was in the 10 – 100 pg g\(^{-1}\) ww range as well, with highest concentrations in muscle tissue and < 30 pg g\(^{-1}\) ww in eggs. DDC-DBF in hormone treated eels was low, compared to the comparison group with maximum concentrations of 30 pg g\(^{-1}\) ww in muscle tissue from Schlei Fjord (Table 2) and concentrations below the limit of quantification in gonads or eggs. The similar or even higher concentrations of DDC-DBF in eels of the comparison group compared to DP
remained inexplicable, because DDC-DBF is, other than DP, not produced or imported to the EU. However, DDC-DBF is known to be highly bioaccumulative (Shen et al. 2011). Small amounts, below the registration limit of the REACH legislation, leaching out of imported products could therefore potentially be the origin of the observed contamination in eels. The decrease of DDC-DBF in hormone treated eels indicates excretion, redistribution or metabolism/biotransformation of DDC-DBF during the artificial maturation process. None of the observed DDC-DBF concentration levels induced effects in mutagenic and genotoxicity tests (see supplement information 2.6).

In contrast to results of all earlier life stages, e.g. yellow eels (Sühring et al. 2013) the anti-isomer of DP was predominant in muscle tissue and gonads of silver eels of the comparison group from both Ems River and Schlei Fjord with synDP/∑DP ratio (fsyn) of as low as 0.09 ± 0.18 in muscle and 0.44 ± 0.23 in gonads from Ems River. SynDP could not be detected in gonads of comparison group eels from Schlei Fjord. This low fsyn ratio in silver eels had already been reported in silver eels from Elbe River (Sühring et al. 2013), however it remained surprising, because in all other analysed eels from both this study and Sühring et al (2013), synDP was clearly predominant with fsyn of up to 0.9. In Sühring et al. (2013) it was concluded, that this observed change in the isomer contributions had probably been caused by either excretion or redistribution into other tissue types of synDP. A selective uptake of antiDP was unlikely, because the overall concentrations of antiDP were similar in yellow and silver eels. Gonads were postulated as one of the possible tissue types into which redistribution might occur, which would indicate a selective redistribution of the DP isomers with a preference of the syn-isomer. This hypothesis was supported by the higher contribution of synDP in silver eel gonads (as shown above), but especially by the results of the hormone treated eels. In hormone treated eels syn- and antiDP were detected in 93% of the samples. The overall concentrations were similar in hormone treated eels and comparison group (not significantly different, t-test at level 0.05). The fsyn ratio however, differed strongly from the comparison group as well as between the different tissue types (Table 2). In muscle tissue of hormone treated eels fsyn was as low as 0.3 ± 0.04.
Contributions in gonads on the other hand were similar to previously observed levels in yellow eel muscle tissue with up to 0.9 ± 0.02. SynDP was also predominant in eggs with up to 0.7 ± 0.1. In yellow eel gonads from Elbe River no DP could be detected, indicating a preferred distribution of synDP into gonads and eggs specifically during maturation (Table 2).

Apart from DBALD, DDC-DBF and DP a variety of other Dechloranes and metabolites could be detected in individual fish. Interestingly, the DP metabolites aCl10DP, aCl11DP and DPMA were primarily detected in muscle samples of the comparison group, rather than gonads or tissue of hormone treated eels (Table 2), indicating, that DP is not subjected to additional metabolism during maturation. Further Dechloranes that could be detected in individual samples were DDC-Ant and CPlus in one sample of the hormone treated eels and 29%, 18% of the samples from the comparison group, respectively.
Table 2: overview results of all discussed samples in ng g⁻¹ wet weight as well as fsyn [g/g], lipid content [%] and number of samples (n). Data marked with * was published in Sühring et al. 2013, data marked with ** was published in Sühring et al. 2014
Maternal transfer to eggs

The ratio of FR concentrations between maternal muscle tissue and eggs (EMR) as well as between muscle tissue and gonads (GMR) were calculated to assess maternal transfer efficiencies.

The transfer rates were calculated using following equations:

\[
EMR = \frac{c_{\text{egg}}}{c_{\text{muscle}}} \tag{1}
\]

\[
GMR = \frac{c_{\text{gonad}}}{c_{\text{muscle}}} \tag{2}
\]

Where \( c \) is the concentration [ng g\(^{-1}\) lw] in paired egg and muscle or gonad and muscle tissue.

Average EMRs for compounds detectable in artificially matured eels ranged from 0.01 for DDC-DBF to 10.4 for PBEB. The higher EMRs matched the general higher FR concentrations in fish from this habitat, indicating that transfer efficiencies increase with tissue concentration.

EMRs and GMRs could provide further indications for potential metabolism or transformation processes in e.g. the case of the observed increase of the relative contribution of BDE congeners attributed to technical PentaBDE (mostly BDE-47) in silver eels. The redistribution of Penta and OctaBDE congeners in silver eel gonads of the comparison group was similar, with GMRs of 0.5 and 0.4, respectively. In hormone treated eels on the other hand, PentaBDEs had significantly higher maternal transfer efficiencies than OctaBDEs, with GMRs for PentaBDE of 0.7 and 0.4 for OctaBDEs and EMRs of 0.08 and 0.05 for Penta- and OctaBDEs, respectively. The increase of the relative contribution of PentaBDEs could be caused by either selective redistribution or metabolism processes such as the enzymatic debromination. As with every metabolism process this would imply an interaction of contaminant and organism, potentially inducing adverse effects, especially in case of known reproduction toxicants such as the technical OctaBDE mixture (de Wit, 2002).
Driving factors for maternal transfer

A major observed difference between the analysed eel groups was the lipid content in different tissue types. Yellow eels had very high lipid contents of up to 35% in muscle tissue, while the lipid content of the scarcely developed gonads was only around 1% (Table 2). Silver eels from the comparison group had slightly lower lipid contents in muscle (around 25%) and larger gonads with lipid contents similar to the muscles. During the maturation process eels use the lipid stored in their muscle to develop gonads and eggs (Boëtius and Boëtius, 1991). An increase of lipid content in gonads and eggs along with a decrease in muscle is therefore an indication for the progress of maturation. This change was observed in artificially matured eels with a significant negative correlation between lipid content in muscle tissue, eggs and gonads of \( r = -0.73 \). The lipid content in muscles was in some cases as low as 15%, while lipid content in gonads reached up to 35% and up to 29% in eggs.

This change in pattern of lipid distribution throughout the body can be expected to highly impact the distribution of BFRs and Dechloranes, because both groups are lipophilic. As expected significant positive correlation \( (r = 0.82) \) was found between lipid content and absolute FR load for all tissue types (Figure 3). The correlation between lipid and total FRs for eggs was above average with \( r = 0.86 \). The decreasing lipid content in muscle and the increase of lipid in eggs and gonads represent the use of lipids for development of gonads and eggs during the maturation process. These observed changes in lipid and contaminant distribution give a strong indication that the lipid content in muscle as well as the therein-stored contaminants are transferred into eggs specifically during the maturation process. As eels are undergoing a similar starvation and lipid metabolism process during the natural maturation process, a similar transfer of contaminants is likely to occur in naturally maturing eels.
Figure 3: Correlation of lipid mass in gram per fish and sum contaminants of halogenated flame retardants in ng per fish.

The lipid driven transfer will be impacted by the physical-chemical properties of different compounds and especially their ability to bind to lipids. The octanol-water partition coefficient (Log\textsubscript{kOW}) can be used as a proxy to describe and quantify this ability. Positive correlations between log\textsubscript{kOW} and logEMR were observed for all analysed eels (r up to 0.47). Recent studies found similar correlations for PCB EMRs in drum (\textit{Aplodinotus grunniens}) (Russel et al. 1999) with r = 0.41 and zebrafish (\textit{Danio rerio}) exposed to BFRs, with r = 0.89 (Nyholm et al. 2009) (Figure 4). Peng et al. (2012), on the other hand, observed strong negative correlation between log\textsubscript{kOW} and EMRs for Dechloranes in Chinese sturgeon (\textit{Acipenser sinensis}), indicating potentially high inter-species differences in the maternal transfer mechanism. The difference could be caused by differences in lipid metabolism during gonad development in different fish species.
Figure 4: Correlation of log EMR (egg muscle ratio) and log $k_{OW}$ (octanol-water partitioning coefficient) of halogenated flame retardants and polychlorinated biphenyls (PCBs) in different fish species.

The correlation between log$k_{OW}$ and transfer rates explains the higher transfer rates into eggs of e.g. DP (average EMR in analysed eels: 3.5, log$k_{OW}$: 11.27) compared to DDC-DBF (average EMR in analysed eels: 0.01, log$k_{OW}$: 8.05), which was rarely detectable in any eggs, even though the concentrations in muscle were higher than DP and it is known to be a highly bioaccumulative and bioavailable substance (Shen et al. 2011).

Combining $EMR = \frac{C_{egg}}{C_{muscle}}$ with the observed relation for log$k_{OW}$ of a compound x and its EMR in artificially matured silver eels $EMR_x = 0.9801 \times logk_{OW} - 4.3303$ provides a first estimate for the maternal transfer of a compound x based on its log$k_{OW}$ and the concentration found in muscle tissue; with:

$C_{egg} = 0.981 \times log k_{OW} \times C_{muscle} - 4.3303 \times C_{muscle}$. 
Plotting the concentration in eggs against the lipid content showed the following relationship for the artificially matured eels: \( C_{\text{egg}} = 1.5 \times \%\text{lipid}_{\text{egg}} + 1.6 \). As discussed above the lipid content in gonads and eggs increases during the maturation process, as lipid stored in muscles is used to develop gonads and eggs. The lipid content in muscle of the analysed eels was in some cases as high as 35%. To assess the potential concentrations in eel eggs, assuming a complete transfer of lipids, a lipid content of 35% was used for calculation. The average weight of a single egg was 0.07 g ww or 0.025 g lw. The average total FR load per egg after a complete transfer of lipids from muscle to eggs would therefore theoretically be

\[
Total_{\text{egg}} = C_{\text{egg}} \left[ \frac{ng}{g} \right] \times m_{\text{egg}} [g lw] = 1.5 \times 35 + 1.6 \left[ \frac{ng}{g} \right] \times 0.025 \ [g lw] = 1.3 \ ng,
\]

with varying of the contributions of individual compounds based on their \( \log k_{\text{OW}} \).

The observed relations are just a first and rough estimate, describing overall trends rather than exact transfer rates for individual compounds. They do, for example, not explain the observed difference in EMR of the stereoisomers of DP, with average EMR of 3 for the syn-isomer and 0.5 for the anti-isomer. Despite the overall different trend Peng et al. (2012) reported similar observations for the maternal transfer of the DP isomers in Chinese sturgeon, providing further indications that additional factors to lipid content and \( k_{\text{OW}} \) might affect the maternal transfer of BFRs and Dechloranes.

**Metabolism and continued uptake**

Lipid metabolism and subsequent metabolism of stored contaminants during maturation could potentially have a high impact on contamination patterns throughout the body. Especially in eels, different uptake pathways of contaminants could also lead to major changes in contamination patterns during maturation. Eels stop feeding during that period, which could increase the relative contribution of contaminants with continued uptake through gills, skin or the ingestion of water compared to contaminants with primary uptake through food.
As shown above indications were found that several compounds might not only be redistributed into gonads and eggs, but could be continuously absorbed from the water as well as subjected to metabolism or biotransformation during the maturation process. This leads to a significant change in the contamination pattern between hormone treated eels and comparison group from the same habitat as well as hormone treated eels from different habitats. Especially in case of TBP-DBPE and its transformation products the significant increase in hormone treated eels (Figure 2), along with high concentrations in the water phase indicated a high continued uptake from the water phase.

Further investigations are necessary to determine which compounds are accumulated through the water phase and whether observed changes were caused by metabolism processes or other physical or habitat based changes during the maturation process. Especially because the increase of potential metabolites such as PentaBDE, low brominated MeOBDEs as well as TBP-AE and BATE in gonads of hormone treated eels indicate metabolism processes.

Another potential BFR and PBDE metabolite is tribromoanisole (TBA) (Nyholm et al. 2009). It was detected in all analysed eels and tissue types with the highest average concentrations of several ng g\(^{-1}\) ww (Table 2). Determining the potential origin of this contamination proved difficult, because TBA is also a naturally occurring substance and was found in the water samples from the tanks. However, a significant increase (t-test at level 0.05) of the TBA concentration was observed in hormone treated eels from Ems River, compared to the comparison group from the same habitat, indicating either continued uptake during the artificial maturation (i.e. tank water) or formation through metabolism of other brominated compounds. TBA did not induce effects at any detected concentrations in a standardised fish embryo toxicity test (see supplement information 2.5. for details.)

The difference between the artificial and natural maturation was too high to draw conclusions regarding effects on eels in general. The repeated observation of increased levels of potential BFR metabolites as well as contamination of the tank water in hormone
treated eels compared to the comparison groups from the same habitat call for further investigation of uptake pathways during the silver eel life stage as well as BFR metabolism or transformation in eels. This is especially important regarding the essential process from maturation to reproduction, where the quality of spawners might be particularly at risk due to endocrine disrupting or in general toxic substances.

**Conclusion**

This study provided evidence that PBDEs as well as their brominated and chlorinated substitutes are redistributed to gonads and eggs during maturation. The driving factors for this maternal transfer seem to be primarily the transfer through lipids dependent on the log\textsubscript{OW} of the individual compound. Based on these observed correlations a contaminant load of > 1 ng per egg was estimated for sum brominated and chlorinated flame retardants. Correlations were also found for the maternal transfer and the concentration in muscle tissue, which provides a potential possibility to assess the maternal transfer of halogenated flame retardants in eels without actually having to sample eggs. Further studies should be conducted to verify these correlations for other compounds potentially affecting the quality of spawners, especially information on PCBs and Dioxins in eel eggs are needed to assess whether the critical concentrations for impairment of embryo development reported by Palstra et al. (2006) are reached in the environment.

Additionally, indications were found that the brominated flame retardant TBP-DBPE and potentially other BFRs are not merely redistributed to gonads and eggs, but continuously absorbed from the surrounding water and potentially subjected to metabolism or transformation processes, resulting in the increase of transformation products such as low brominated MeOBDEs, PentaBDE, TBP-AE and BATE. Studies regarding the potential impact of this continued exposure, metabolism or transformation processes on the maturation and reproduction success of eels or fish in general are needed. Especially considering that the release of stored chemicals in eels occurs during their maturation phase and that not only a
fraction, but a lifetime worth of accumulated contaminants are potentially released, affecting the quality of spawners.

The results of this study also emphasize the necessity to further increase the research on emerging brominated and chlorinated flame retardants. A variety of potentially hazardous non-PBDE flame retardants were detected, such as the aldrin related DBALD and the highly bioaccumulative DDC-DBF. Even though neither are officially produced or imported into the EU. The observed maternal transfer of potentially hazardous and endocrine disrupting contaminants could impair “quality of spawners”, reproduction success and development of offspring.

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