

Monitoring of hexabromocyclododecane diastereomers in fish from European freshwaters and estuaries

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Abstract

Background and aims Hexabromocyclododecane (HBCD) is a brominated flame retardant used mainly in polystyrene foam as well as in textile applications. In recent years, measures were taken to reduce HBCD emissions during its production and use. To evaluate the efficacy of these measures, a monitoring project was initiated with fish as bioaccumulation indicators.

Methods Since 2007 bream was sampled annually at several European freshwater sites applying standardized methods. Muscle samples were analyzed by a validated liquid chromatography–tandem mass spectrometry (LC-MS/MS) method which allows the quantification of α -, β -, and γ -diastereomers of HBCD.

Discussion In most samples, α -HBCD was predominant. The lowest HBCD levels (11 $\mu\text{g}/\text{kg}$ lipid weight (lw), sum of three HBCD diastereomers) were detected in bream sampled in 2009 from Lake Belau which is situated in a rural area of Northern Germany. During the 4-year study period, statistical significant decreases of HBCD concentrations were detected in bream from the rivers Rhone (France; -85% , level 2010, 205 $\mu\text{g}/\text{kg}$ lw) and Western Scheldt (the Netherlands; -60% , level 2010, 36 $\mu\text{g}/\text{kg}$ lw). High HBCD concentrations (9,480–14,500 $\mu\text{g}/\text{kg}$ lw) without a clear time trend were observed in bream from the river Tees (UK). These high levels are in line with published data for fish from contaminated regions.

Conclusions Overall, the results indicate that environmental burdens of HBCD are decreasing at those locations where point sources and more diffuse emissions are expected.

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1 Introduction

1,2,5,6,9,10-Hexabromocyclododecane (HBCD; CAS nos. 25637-99-4 and 3194-55-6) is added as a flame retardant to extruded or expanded polystyrene foams used in building insulation as well as to flame-retarded textiles such as upholstery furniture. Commercial HBCD mainly consists of three diastereomers (the so-called α -, β -, and γ -isomers), although two other diastereomers (δ - and ϵ -HBCD) are also detectable (Heeb et al. 2005). The α -, β -, and γ -diastereomers are chiral and occur as pairs of enantiomers, while δ - and ϵ -HBCD exist as non-chiral meso-forms. In total, 16 HBCD diastereomers are known, but only these five seem relevant. The predominant component in technical HBCD is γ -HBCD with a fraction of 70–95%, whereas α - and β -HBCD account for 5–30% (European Commission 2008).

HBCD is only sparingly soluble in water (66 $\mu\text{g/L}$ for the technical product, sum of all diastereomers), only slightly volatile (vapor pressure, 6.3×10^{-5} Pa at 21°C), and has an octanol/water partition coefficient (as $\log K_{ow}$) of 5.625 (European Commission 2008). In the European Union Risk Assessment Report (EU RAR) on HBCD, the compound has been classified as persistent, bioaccumulating, and toxic (PBT), although it does not unequivocally fulfill the specific P criterion (European Commission 2008). HBCD has been identified as a “substance of very high concern,” meeting the criteria of a PBT substance pursuant to Article 57(d) in the REACH regulation, and is currently under assessment for the addition to the list of compounds covered by the Stockholm Convention on Persistent Organic Pollutants. The main reasons for the consideration of HBCD are its persistence in the environment, its susceptibility to long-range environmental transport, its bioaccumulation and biomagnification potential, and the fact that the measured levels in biota, including higher trophic levels such as birds and mammals, are of significant concern for human health and the environment (UNEP 2010).

EU-wide consumption of HBCD in 2007 was estimated to be 11,000 tonnes (ECHA 2008). During production, use, and disposal, HBCD may be released into the environment. Emissions of HBCD from polystyrene products are assumed to be low; it may, however, be released from HBCD-containing textiles during washing and wear. The European risk assessment of HBCD identified a former UK production site and emissions from the textile industry as important point sources (European Commission 2008).

It is assumed that the major fraction of HBCD which reaches wastewater treatment plants (WWTP) is eliminated by adsorption to sludge, and only about 20% is released into the environment via WWTP effluents (European Commission 2008). HBCD concentrations detected in effluents ranged from about 10 to 30 $\mu\text{g/L}$ (European Commission 2008). It is therefore likely that aquatic organisms downstream of WWTPs are exposed to HBCD.

In recent years, emissions control programs like VECAP (the Voluntary Emissions Control Action Programme) and SECURE (Self Enforced Control of Use to Reduce Emissions) have been implemented by HBCD producers and users in order to reduce environmental emissions of HBCD (EBFRIP 2010). To assess the impact and relevance of these implemented emission reduction measures, a monitoring project for HBCD in the aquatic environment in Europe was initiated by the “Industry working group for HBCD,” a sector group of the European Chemical Industry Council, in 2007. The project is designed to assess temporal trends and spatial differences in burdens over a period of up to 10 years

at different representative sites across Europe. The focus is on environmental compartments which are expected to be sinks for HBCD, as estimated from its physicochemical properties and based on knowledge about HBCD production and use. It was therefore decided to perform an environmental monitoring by sampling representative fish species as well as suspended particulate matter close to the fish collection sites. The present contribution summarizes the first results of the HBCD monitoring in fish using a diastereomer-specific analytical method (LC-MS/MS).

2 Materials and methods

2.1 Sampling and sample preparation

All steps of the sample treatment were performed according to standard operating procedures developed for the German Environmental Specimen Bank (ESB; Klein 1993; Paulus et al. 1996; Umweltbundesamt 1996). The sampling of bream (*Abramis brama*) is described in a specific guideline (Klein et al. 2010a). For the sampling of sole (*Solea solea*), the respective guideline for sampling of marine fish for the ESB was applied with slight adaptations (sampling of eelpout, *Zoarces viviparus*; Klein et al. 2010b). Routinely, 15 fish per site were collected and pooled for each annual sample. However, in two cases, only a lower number of fish could be caught. Sampling of fish was performed after the spawning season since alterations in the fat content of the muscle tissue are low in this phase. Directly after sampling and dissecting on a clean bench, the fish muscle tissue was cooled to temperatures below -150°C . In the laboratory, pooled samples were prepared by cryomilling according to a protocol described elsewhere (Rüdel et al. 2008). In two cases, samples from 15 individual fish were prepared by cryo-grinding each fillet separately in a Pulverisette 5 planetary ball mill (Fritsch, Idar-Oberstein, Germany). Before grinding, the zirconium oxide milling jars and balls were pre-cooled to a temperature below -150°C (Rüdel et al. 2008).

2.2 Analysis

2.2.1 Materials

^{13}C -labeled and non-labeled standards of α -, β -, and γ -HBCD with purities $>98\%$ were obtained from Wellington Laboratories via Campro Scientific (Berlin, Germany). Chemicals applied were of trace analysis grade or HPLC grade. Glassware (with the exception of volume measuring devices like measuring flasks) was heated at 250°C overnight before use. The volume measuring devices were cleaned by rinsing with *n*-pentane only.

2.2.2 Standards

Stock solutions of all standards had concentrations of 50.0 µg/mL. These were diluted to 100 ng/mL (¹³C-labeled HBCD diastereomers in acetonitrile, to be used as internal standard, IS) or 500 ng/mL (non-labeled HBCD diastereomers). From the latter calibration, solutions were prepared by using amounts of 8–1,000 µL and filling up with solvent to appropriate volumes of 1.50–10.0 mL. Fortifications for recovery experiments at different levels were performed with solutions containing 10 or 500 ng/mL non-labeled HBCD diastereomers.

2.2.3 Sample preparation

All fish samples were analyzed within 6 months after sampling. Up to 1.5 g tissue was weighted into 80-mL centrifuge vials with screw caps. Depending on the expected concentration, 20–100 µL of the IS solution was added.

2.2.4 Sample extraction

After the addition of 15 mL acetone, the mixture was homogenized for about 30 s, applying an Ultra-Turrax (IKA-Werke, Staufen, Germany), and shaken for 30 min. Subsequently, 25 mL of *n*-pentane was added, and, after further 30 min of shaking, 20 mL of deionized water. The mixture was shaken for another 15 min before centrifuging at about 3,000×*g* for 5 min. The pentane phase was removed using a glass pipette, transferred into a glass vial, and evaporated to almost complete dryness under a stream of nitrogen.

2.2.5 Lipid removal

For cleanup by gel permeation chromatography (GPC), 2.3 mL of the GPC solvent (dichloromethane/cyclohexane, 1:1) was added. GPC was performed on a Bio-Beads S-X3 200–400 mesh column (Bio-Rad, Munich, Germany; 10-mm diameter, 45-cm bed height) with an automated system (e.g., CleanUp XL, ABIMED, Langenfeld, Germany). The HBCD-containing fraction (15–28 min) was isolated and the solvent completely evaporated under a stream of nitrogen. After dissolution with ultrasonic treatment in 100 µL of acetonitrile, samples were analyzed by LC-MS/MS.

2.2.6 HPLC separation

HBCD diastereomers were separated by liquid chromatography and analyzed using a Waters Micromass Quattro Micro-triple quadrupole mass spectrometer with a Waters 2695

HPLC system (Waters, Eschborn, Germany) and a Phenomenex Gemini column (5 µm, C18, 150×3 mm; Phenomenex, Aschaffenburg, Germany). Chromatography was performed at 30°C with a gradient from three mobile phases (A: methanol; B: water with 2 mmol ammonium acetate/methanol, 9+1; C: acetonitrile; gradient details are given in “HPLC parameters” in the [Electronic supplementary material \(ESM\)](#)).

2.2.7 HPLC–tandem mass spectrometry coupling

The MS/MS system was operated with negative electrospray ionization in the multiple reaction monitoring mode with argon as collision gas. Details on monitored ion transitions and instrumental settings are given in ESM Tables S1 and S2 and Fig. S1 (which shows example chromatograms).

2.2.8 Calibrations

Daily before sample measurements, calibrations were performed with at least six concentration levels (range was chosen depending on the HBCD levels in samples; the solutions applied were 0.5, 1.0, 2.0, 4.0, 8.0, 15.0, 30.0, 50.0, 100, 200, 350, and 500 ng/mL plus blank). Procedural blanks were prepared from 100 µL acetonitrile and 20 µL IS solution and treated analogously to samples, but contained no sample matrix. The specificity of the HPLC-MS/MS procedure was checked by an analysis of unfortified biota samples which showed no disturbing peaks at the retention times of the HBCD diastereomers.

2.2.9 Fat determination

The total lipid contents of each sample were determined gravimetrically following a procedure according to Smedes (1999). Water, propan-2-ol, and cyclohexane were added to a tissue sample, resulting in a volume ratio of 8:10:11. After homogenizing with an Ultra-Turrax, the organic phase was separated by centrifugation, dried, and the amount of extracted lipid determined by weighing.

2.2.10 Reporting

Concentrations are given as micrograms per kilogram wet weight (ww) or lipid weight (lw) where appropriate.

2.2.11 Calculations

For arithmetic mean value calculations, the measured concentration was used even in cases where the latter was below the limit of determination (LOQ). In the tables, values below the LOQ are shown in parentheses.

2.3 Statistical evaluations

Time series with at least four data points from different years (arithmetic mean values for replicates) were analyzed for possible trends with the two-sided non-parametric Mann–Kendall test using a Microsoft Excel application developed by Salmi et al. (2002). As output, the significance level of the respective trend is received. All time series were also analyzed with a statistical program retrieved from the Arctic Monitoring and Assessment Programme homepage (Bignert 2007). The basic statistical methodology applied is a robust regression-based analysis to detect trends in time series datasets (Nicholson et al. 1998). The log-linear regression analyses yield a slope of the line which describes the annual concentration change as percent value. The type I error rate (α) was set as 5%. If a trend was detected, the actual probability (p value) was given out.

2.4 Validation and quality assurance

The validation of the method was performed according to the SANCO guidelines 3029 and 825 (European Commission 2000, 2004) by fortification experiments at three concentration levels (i.e., LOQ, $10 \times$ LOQ, $100 \times$ LOQ). For this purpose, a sample matrix with minimal contamination was used (bream sample from a pristine site, blank levels below $0.1 \mu\text{g/kg}$ ww for each of the three HBCD diastereomers). The concentrations for each HBCD diastereomer were derived from daily recorded calibration functions. Linear regressions yielded correlation coefficients >0.997 for α -, β -, and γ -HBCD. The method was validated for a LOQ of $0.1 \mu\text{g/kg}$ ww (Table 1). All fortification experiments showed recoveries in the range of 70–110% and relative standard deviations (RSD) below 20%. The precision of the method was determined by the RSD of the recoveries at each fortification level. They ranged from about 6% to 19% for the LOQ level and from about 2% to 9% for the higher concentrations levels. The reported data are not corrected for recovery.

The laboratory is accredited for this method according to ISO/IEC 17025 (2005). Internal (e.g., measurement of laboratory reference materials, daily calibrations, frequent control of balances and pipettes, maintenance of instruments) and external quality assurance measures (e.g., approval of the method protocol by the competent authority DAkS GmbH, regular laboratory audits) were applied accordingly.

From one bream sample, a larger amount was prepared and used as the laboratory reference material which was analyzed along with each series of samples. The reproducibility of the measured concentrations was sufficient with mean concentration \pm standard deviation of $9.6 \pm 1.2 \mu\text{g/kg}$

α -HBCD, $0.1 \pm <0.1 \mu\text{g/kg}$ β -HBCD, and $0.8 \pm 0.2 \mu\text{g/kg}$ γ -HBCD (wet weight data, $n=14$).

The precision of the method was verified regularly by replicate measurements of representative samples in each series. Typical relative standard deviations were about 5–15% at concentration levels above $1 \mu\text{g/kg}$ ww of the respective diastereomer. Individual fish samples were usually analyzed once, whereas pooled annual samples were analyzed mostly two to four times.

3 Results and discussion

3.1 Selection of fish as monitoring organisms

Fish is often used as a bioaccumulation indicator for environmental monitoring of the aquatic compartment. Bioaccumulation depends strongly on the lipophilic properties of the target compound, but also on the metabolism, habitat, and diet of the organism (van der Oost et al. 2003). In the present study, bream (*A. brama*) was chosen as monitoring species because of its widespread occurrence in European rivers and its limited migration behavior. As a bottom feeder, it is closely associated with the sediment compartment which is considered to be a sink for HBCD. Bream are also routinely collected within the German Environmental Specimen Bank Program (www.umweltprobenbank.de; Umweltbundesamt 2008). From investigations with archived bream muscle tissue samples, temporal concentration trends for compounds such as alkylphenols, triclosan/methyltriclosan, and polycyclic musk fragrances could be detected and partly correlated to the consumption and use patterns of these chemicals (Boehmer et al. 2004; Wenzel et al. 2004; Rüdél et al. 2006).

The uptake of HBCD by fish in laboratory tests and the respective bioconcentration factors (BCF) have been reported previously. The EU RAR (European Commission 2008) considers a BCF of 18,100 L/kg for HBCD, which was retrieved from a study with fathead minnow (*Pimephales promelas*) reported by Veith et al. (1979). Wildlife studies confirmed the presence of HBCD in fish tissue. Covaci et al. (2006) reviewed data on the environmental occurrence of HBCD. In freshwater fish from urban/suburban regions in Europe, HBCD concentrations were mostly between 10 and $1,000 \mu\text{g/kg}$ lw. Hajslová et al. (2007) reported HBCD levels of about 30– $6,000 \mu\text{g/kg}$ lw (calculated from reported wet weight and lipid percent data) in bream from the Czech part of the rivers Elbe and Vltava in 2005.

For the present HBCD monitoring project, bream were available at all selected sites. In the brackish water of the Western Scheldt estuary, sole (*S. solea*) was collected in addition. Because of its benthic lifestyle, sole is perma-

Table 1 Mean recoveries of α -, β -, and γ -HBCD from fortified fish muscle samples as percent of applied HBCD concentration

| Test item | Fortification level ($\mu\text{g}/\text{kg}$ ww) | <i>N</i> | Mean recovery (%) (\pm RSD, in %) | Overall recovery (%) (\pm RSD, in %) |
|----------------|---|----------|--------------------------------------|---|
| α -HBCD | 0.1 ^a | 5 | 76.2 (\pm 19.1) | 87.6 (\pm 14.2) |
| | 1.0 | 5 | 95.9 (\pm 8.1) | |
| | 10 | 5 | 90.6 (\pm 2.8) | |
| β -HBCD | 0.1 ^a | 5 | 91.2 (\pm 6.9) | 94.2 (\pm 5.1) |
| | 1.0 | 5 | 96.4 (\pm 1.7) | |
| | 10 | 5 | 95.1 (\pm 4.6) | |
| γ -HBCD | 0.1 ^a | 5 | 82.1 (\pm 19.1) | 91.2 (\pm 13.3) |
| | 1.0 | 5 | 92.6 (\pm 9.0) | |
| | 10 | 5 | 99.0 (\pm 4.6) | |

RSD relative standard deviation

^a LOQ level

nently in contact with the sediment. The suitability of sole as a bioaccumulation indicator for flame retardants was demonstrated by Janák et al. (2005).

3.2 Site selection

Originally, six sampling sites were evaluated covering different climate regions in Europe; the respective geographical coordinates are shown in Table 2. River sampling sites are located near their mouths or in the estuary area and are considered to be representative of both industrial HBCD emissions from point sources (e.g., textile industry in the Scheldt area) and more diffuse emissions from sources via municipal WWTPs. Based on analytical data from two sampling years (2007/2008), four of the six sites were finally selected for long-term monitoring (Tees, Western Scheldt, Rhone, Lake Belau). Decisions were based on regional distribution and on HBCD concentrations in fish. Lake Belau was selected because of its location in a rural area where only little anthropogenic influence is expected. It also serves as the reference site for the German Environmental Specimen Bank Program.

3.3 Biometric data

Age, weight, and length were determined for every fish individually, whereas the fat content was measured in annual pooled muscle tissue samples (Table 2). For Western Scheldt bream, all biometric parameters were relatively stable during the study period. For sole from the same site and bream from the other sites, fish age, weight, and length varied between years. In 2007, bream from the rivers Rhone and Lake Belau were relatively young, light, and small in size, and the fat contents were accordingly low (<1%). It is assumed, however, that these variations do not impair the comparison between years because all sampled fish were sexually mature and had comparable condition

factors (see below). Furthermore, all HBCD concentration data were normalized to fat content.

From weight and length data, the condition factor (CF) was calculated (formula: $100 \times \text{body weight [g]} / (\text{length [cm]})^3$). The mean CF values for all sampling years and sites were in the range 1.1–1.3 g/cm^3 , with low standard deviations ($\leq 0.1 \text{ g}/\text{cm}^3$). Low CFs generally indicate insufficient living conditions, possibly caused by, e.g., non-optimal water temperatures, chronic oxygen deficiency, or the presence of toxic compounds. The CFs found for bream are within the normal range (i.e., comparable to those determined for bream sampled since the 1990s for the German Environmental Specimen Bank Program; data are available at www.umweltprobenbank.de). A good nutritional state of bream corresponds to a CF of about 1.1, whereas 0.9 and below is considered insufficient. Thus, all sampled bream had a good nutritional status and encountered appropriate living conditions. These results suggest that the fish sampled in different years are of comparable status.

The sole sampled from the Western Scheldt were much younger compared with the bream, but were, nevertheless, mature. According to Bromley (2003), sole mature at an age of 3 years, so the collected sole were considered appropriate for this study. The fat content of the sole muscle tissue was lowest in 2009 (0.85%), while values ranged between 1.2% and 1.5% fat in the other years. The low fat content in 2009 is not mirrored by the mean weight and length data in this year. The mean CFs for sole ranged between 0.96 and 0.98 g/cm^3 . Unfortunately, no literature data on sole CFs are available and a comparison between bream and sole CFs is not possible due to, e.g., the different shapes of the two species.

3.4 HBCD diastereomer patterns and concentrations

In monitoring studies for HBCD, different methods are applied: (a) analysis of total HBCD diastereomers by GC/

Table 2 Site information (geographical coordinates) and biometric data (age, weight, total length, fat content) of fish sampled 2007–2010 (mean values ± SD)

| River/lake and geo-coordinates | Year | No. of fish | Mean age (years) | Mean weight (g) | Mean length (cm) | Fat content (%) |
|---|------|-------------|------------------|-----------------|------------------|-----------------------------|
| Bream | | | | | | |
| Tees, UK 54°33' N, 1°18' W | 2007 | 15 | 8.4±1.9 | 1,320±460 | 47±6 | 3.3 |
| | 2008 | 15 | 9.1±1.4 | 1,330±300 | 48±3 | 3.4 |
| | 2009 | 15 | 9.2±1.6 | 1,300±320 | 48±4 | 2.7 |
| | 2010 | 15 | 8.1±3.4 | 1,103±582 | 45±7 | 1.9 |
| Mersey, UK 53°23' N, 2°34' W | 2007 | 11 | 3.4±0.7 | 329±62 | 31±2 | 2.5 |
| | 2008 | 15 | 4.5±0.6 | 509±92 | 35±2 | 2.9 |
| Rhone, France 43°38' N, 4°36' E | 2007 | 15 | 4.1±0.9 | 405±108 | 32±3 | 0.75 |
| | 2008 | 15 | 6.5±0.9 | 760±200 | 36±3 | 2.9 |
| | 2009 | 15 | 6.2±1.0 | 646±131 | 37±3 | 3.6 |
| | 2010 | 15 | 5.2±1.3 | 609±230 | 37±4 | 2.4 |
| Western Scheldt, the Netherlands 51°23' N, 4°14' E | 2007 | 7 | 8.6±2.8 | 1,470±480 | 49±6 | 3.4 |
| | 2008 | 15 | 8.8±4.1 | 1,480±410 | 50±7 | 2.7 |
| | 2009 | 15 | 8.3±1.8 | 1,420±220 | 50±3 | 3.0 |
| | 2010 | 15 | 8.8±2.0 | 1,485±237 | 51±4 | 2.4 |
| Götaälv, Sweden 57°43' N, 11°59' E | 2007 | 15 | 8.5±3.0 | 1,210±360 | 47±5 | 2.5 |
| | 2008 | 15 | 9.6±4.2 | 1,280±380 | 46±4 | 3.6 |
| Lake Belau, Germany 54°06' N, 10°15' E | 2007 | 15 | 8.9±4.4 | 1,010±380 | 44±6 | 0.92 |
| | 2008 | 15 | 10.1±3.7 | 1,480±380 | 50±4 | 2.2±0.7 (n=15) ^a |
| | 2009 | 15 | 12.6±2.4 | 1,950±570 | 54±5 | 3.1 |
| | 2010 | 15 | 10.3±3.8 | 1,328±522 | 48±7 | 1.6 |
| Sole | | | | | | |
| Western Scheldt, the Netherlands 51°23' N, 4°14' E | 2007 | 15 | Approx. 3 | 239±24 | 29±1 | 1.4±0.6 (n=15) ^a |
| | 2008 | 15 | Approx. 3–4 | 396±82 | 34±2 | 1.2 |
| | 2009 | 15 | Approx. 3–4 | 438±169 | 35±4 | 0.85 |
| | 2010 | 15 | Approx. 3–4 | 300±116 | 31±4 | 1.5 |

Total length is the length from the tip of the mouth to the end of the compressed tips of the caudal fins. The fat content was determined for the final pooled sample (i.e., n=1, exceptions are marked)

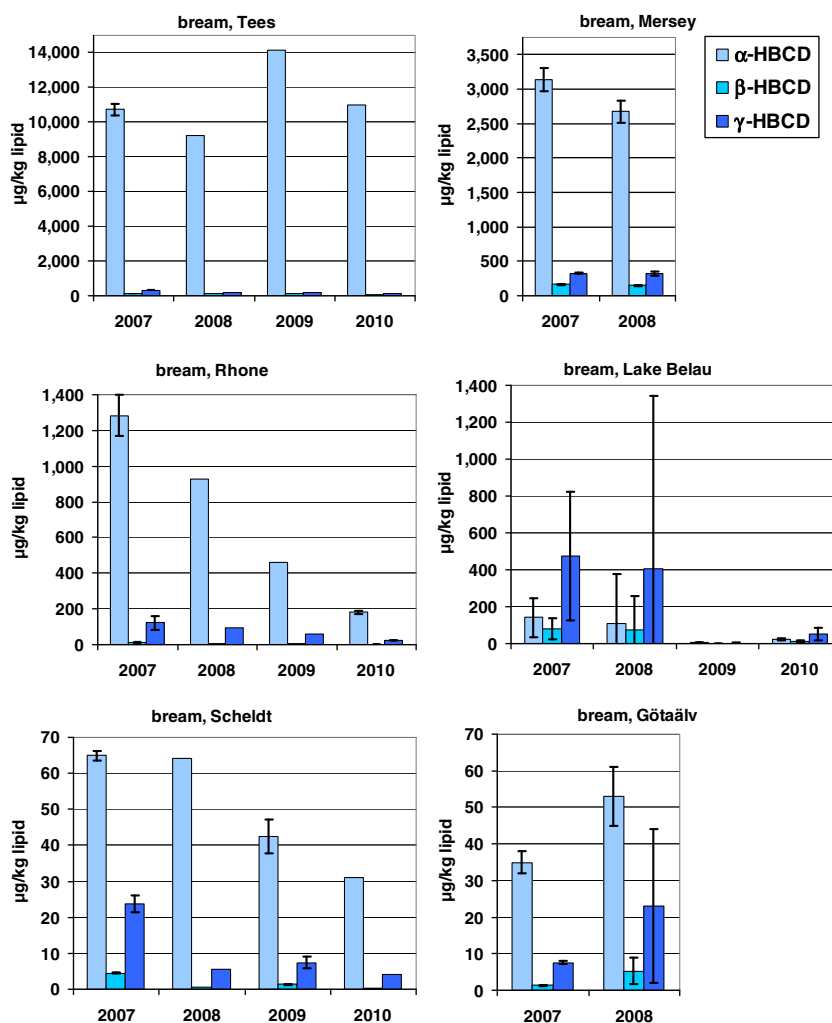
^aData from fillets of 15 individual fish

MS (e.g., Hajslová et al. 2007); (b) diastereomer-specific analysis by HPLC/MS (mostly for α-, β-, and γ-HBCD; e.g., Bogdal et al. 2009); and (c) enantiomer-specific analyses (e.g., Harrad et al. 2009). In the present study, diastereomer-specific analysis by HPLC/MS was chosen since the main purpose of this study was to follow the temporal trends of HBCD diastereomers in fish. Possible changes of enantiomer ratios in fish may occur by enantiomer-specific uptake, metabolism, or excretion (Harrad et al. 2009). However, this aspect is currently considered only to be of minor relevance for environmental monitoring since no differences in effects for pairs of HBCD enantiomers have been reported up to now.

Results from the diastereomer-specific analysis of HBCD are shown in Fig. 1 for bream and in Fig. 2 for sole. Detailed wet- and lipid weight-related numerical concentration data for bream are provided in ESM Tables S3 and S4.

The HBCD levels (sum of the three diastereomers) in bream from the different sampling sites can be ranked as follows: Götaälv = Western Scheldt < Lake Belau < Rhone < Mersey < Tees (2008 data). In 2010, only four sites were monitored; the respective ranking is Western Scheldt < Lake Belau < Rhone < Tees. α-HBCD was the dominant diastereomer in almost all fish samples, which is in accordance with previous studies (e.g., as summarized by Covaci et al. 2006 or Law et al. 2008b). The mean α-HBCD fractions in bream were 70–80% at Götaälv, 96–99% at Tees, 85–86% at Mersey, 87–91% at Rhone, and 70–91% at Western Scheldt (Fig. 1; percent distributions for bream are given in ESM Table S5). An exception was Lake Belau where γ-HBCD dominated, with about 60–70% in the years 2007, 2008, and 2010. Only in 2009, when HBCD levels were lowest, was an α-HBCD predominance observed (70% α-HBCD).

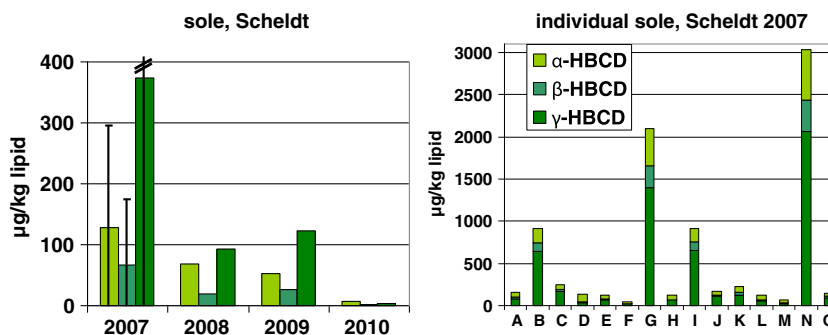
Fig. 1 Concentrations of α -, β -, and γ -HBCD in bream muscle tissue from six sites (lipid weight data). Standard deviations refer to replicate analysis of the same annual pool sample or to analysis of 15 individual fish (Lake Belau, 2008)



A predominance of γ -HBCD (i.e., 50–60%) was also detected in sole from the Western Scheldt in the years 2007–2009. In 2010, however, a shift from γ -HBCD to α -HBCD was observed, resulting in 56% α -HBCD. In most years, HBCD levels in sole were higher than those of bream from the same region (probably because of the differences in habitat exposure, feeding behaviors, and species differences in bioaccumulation). The sole data also confirm the decrease of HBCD levels detected for the bream from the Western Scheldt, although the decline is more pronounced for the sole.

The reason for the differing diastereomer distribution in bream from Lake Belau and in Western Scheldt sole as compared with the relative occurrence of the individual diastereomers in bream collected from the Western Scheldt and other locations is currently unknown. However, the data are consistent for samples/analyses from different years (also for individually analyzed samples; see below). For sole, it may be hypothesized that the high γ -HBCD concentration at the Western Scheldt stem from more recent emissions, e.g., emissions from textile industry upstream

Fig. 2 *Left*, Concentrations of α -, β -, and γ -HBCD in sole muscle tissue from the Western Scheldt (lipid weight data). Standard deviations refer to replicate analysis of the same annual pool sample or to analysis of 15 individual fish (2007). *Right*, Concentrations of α -, β -, and γ -HBCD in muscle tissue of individual sole sampled in 2007 (lipid weight data; $n=15$, A–O)



(Roosens et al. 2008). Another possible reason may be that these fish are in close contact with the sediment as they cover themselves with mud or sand during the day and sediment usually has a high γ -HBCD fraction (Covaci et al. 2006). For bream from Lake Belau, no relevant local HBCD sources are known. A recent study revealed HBCD levels of 2 $\mu\text{g}/\text{kg}$ dry weight in the sediment of Lake Belau, which was at the lower end of the range found in sediments of several German rivers in that study (range=1–20 $\mu\text{g}/\text{kg}$; Stiehl et al. 2008).

With regard to time trends, a decrease of HBCD levels (as the sum of α -, β -, and γ -HBCD) was noticeable in bream from the Rhone and the Western Scheldt between 2007 and 2010. In Rhone, bream levels decreased by about 85% (from 1,420 \pm 110 $\mu\text{g}/\text{kg}$ lw in 2007, $n=4$, to 205 \pm 9 $\mu\text{g}/\text{kg}$ lw HBCD in 2010, $n=2$; Fig. 1), and in bream from the Western Scheldt, decreases of about 60% were observed (from 93 \pm 2 $\mu\text{g}/\text{kg}$ lw in 2007, $n=2$, to 36 $\mu\text{g}/\text{kg}$ lw in 2010; Fig. 1). A decrease in HBCD was also observed in sole from the Western Scheldt, i.e., from about 600 $\mu\text{g}/\text{kg}$ lw in 2007 to about 12 $\mu\text{g}/\text{kg}$ lw for 2010, corresponding to a decrease of about 98% (for the sum of the three HBCD diastereomers, see Fig. 2). High HBCD levels with pronounced variations within the same year were detected in bream from the Tees River sampled downstream of the former production site in Newton Aycliffe (range=9,480–14,500 $\mu\text{g}/\text{kg}$ lw). Such high levels are in line with published data for fish from regions close to point sources (e.g., Eljarrat et al. 2004).

In Lake Belau bream, another interesting finding was made. Besides the different diastereomer pattern, the HBCD concentration in 2009 was extremely low (sum of three HBCD diastereomers, 11 \pm 2 $\mu\text{g}/\text{kg}$ lw, $n=3$), which corresponded to a decrease of about 98% from 2008 to 2009. In 2010, the levels increased again to 84 \pm 44 $\mu\text{g}/\text{kg}$ lw ($n=2$), but were still clearly lower than the values measured in 2007/2008. These findings may be explained partly by a higher fat content of the bream in 2010 as compared with 2009 (3.1% vs. 1.6%). However, the high variability of the analytical data of the homogenates from 2007 and of the body burdens of the individual fish analyzed in 2008 render the interpretation of the data with respect to a time trend difficult.

To assess the variability between different specimens, muscle tissue samples from individual fish and selected sites were analyzed, i.e., bream from Lake Belau sampled in 2008 and sole from the Western Scheldt sampled in 2007. The data for the individual sole investigated are given in Fig. 2. Mean and median concentrations for the sum of the three HBCD diastereomers are 568 and 163 $\mu\text{g}/\text{kg}$ lw, respectively. Four individual fish had very high HBCD levels (B, G, I, N). The ratio between the fish with the highest and the lowest HBCD levels is 66. Fat contents

varied less (range=0.8–2.9%, mean=1.4%, median=1.2%). The high differences in individual HBCD burdens probably reflect different HBCD levels in the respective habitats.

High variations of HBCD levels of individual fish sampled from one site are also reported by Pulkrabová et al. (2007) (coefficients of variation for total HBCD concentrations of bream of up to 95%) and Harrad et al. (2009) (high variability of individual HBCD tissue concentrations reported for several fish species at different sites). Despite the high variability between individual fish, it is assumed that with a pool of >12 fish, a reliable estimation of the average concentration of a lipophilic substance in a fish population is possible (UNEP 2004).

3.5 Comparison of HBCD levels with data from other studies

Comprehensive reviews of environmental levels of HBCD were published, e.g., by Covaci et al. (2006) or Law et al. (2008b). In the present study, the HBCD levels in bream are compared only with studies which also investigated muscle tissue from European freshwater fish (mainly sedentary species). Studies with eel, however, were not considered because eel populations are often altered by restocking programs, rendering the interpretation of concentration data in comparison to other fish difficult because of variable exposure times.

The lowest HBCD values (i.e., the sum of three HBCD diastereomers) were detected in bream from Lake Belau sampled in 2009 (mean value \pm SD=11 \pm 2 $\mu\text{g}/\text{kg}$ lw, $n=3$) and in bream from the Western Scheldt (mean value \pm SD=51 \pm 4 and 36 $\mu\text{g}/\text{kg}$ lw for 2009 and 2010, respectively). These values are comparable to the lower levels detected by Gerecke et al. (2003) in fish sampled from Swiss lakes in 2002 (range=25–210 $\mu\text{g}/\text{kg}$ lw; muscle of whitefish, *Coregonus* sp.), Cheaib et al. (2009) in Lake Geneva in 2004 (49–324 $\mu\text{g}/\text{kg}$ lw; lake trout), and Harrad et al. (2009) in fish samples from English lakes (14–290 $\mu\text{g}/\text{kg}$ lw; mainly perch, crucian carp, common roach, and northern pike).

The high HBCD levels found in bream from river Tees are in line with published data from contaminated regions. The highest values (Tees bream 2009, 386 $\mu\text{g}/\text{kg}$ ww, corresponding to 14,500 $\mu\text{g}/\text{kg}$ lw) are comparable to the total HBCD concentrations reported for barbel (*Barbus graellsii*) from a contaminated river in Spain (river Cinca, directly downstream of Monzon, a heavily industrialized town; only wet weight-based data available—up to 1,170 $\mu\text{g}/\text{kg}$ ww for total HBCD in fish muscle; Eljarrat et al. 2004).

For the Tees/Skerne Rivers system (UK), data for other fish species are available from the “Committee on Toxicity of Chemicals in Food Consumer Products and The

Environment” (COT 2004): HBCD concentrations in the edible portion of trout sampled at upstream control sites were 21–119 $\mu\text{g}/\text{kg}$ ww. At sites downstream of the mentioned former production plant, HBCD (sum of three diastereomers) in trout ranged from 159 to about 6,760 $\mu\text{g}/\text{kg}$ ww. The wet weight-related concentrations detected in the current study in Tees bream in the years 2007–2010, i.e., 320–386 $\mu\text{g}/\text{kg}$ ww (ESM Table S3) were at the lower end of the trout data range reported by COT (2004). However, a direct comparison seems not feasible since bioaccumulation of HBCD may differ between species.

In general, the HBCD levels in bream reported in the current study are comparable to those found by Hajslová et al. (2007) for muscle tissue of bream sampled in the Czech Republic in the rivers Elbe and Vlatava in 2005 (range reported for totals of HBCD diastereomers as analyzed by GC/MS, about 30–6,000 $\mu\text{g}/\text{kg}$ lw, mean=964 \pm 1,610 $\mu\text{g}/\text{kg}$ lw, calculated from reported wet weight and lipid percent data). For bream from other river sites in the Czech Republic, Pulkrabová et al. (2007) reported lower HBCD levels (<7–328 $\mu\text{g}/\text{kg}$ lw in 2001–2003; converted from the reported HBCD wet weight data and lipid contents, only total HBCD analyzed by GC/MS). The HBCD levels of other fish (trout, chub, perch, barbel) investigated in that study were in the same range.

The occurrence of HBCD and other organohalogen compounds was also monitored in different fish species collected from 11 locations in Czech rivers, situated in highly industrialized areas, in 2008/2009 (Hrádková et al. 2011). The concentrations of HBCD ranged from <0.02 to 1.8 $\mu\text{g}/\text{kg}$ ww (converted with reported lipid contents, about <0.2–40 $\mu\text{g}/\text{kg}$ lw). The highest levels were found in fish muscle tissue from Elbe River sites. However, these HBCD levels are lower than those reported earlier for fish from Czech rivers (Hajslová et al. 2007). In a further study from the Czech Republic, levels of HBCD diastereomers and other halogenated compounds were investigated in brown trout samples upstream and downstream of three WWTP (Li et al. 2011). While the concentrations of HBCD diastereomers upstream of the treatment plants were mostly below the LOQ (sum HBCD <2.5–3.7 $\mu\text{g}/\text{kg}$ lw), the levels downstream were clearly higher (4.0–29 $\mu\text{g}/\text{kg}$ lw).

Roosens et al. (2008) reported HBCD levels in several fish species sampled from the upper Scheldt (near Oudenaarde, Belgium) in 2006. The total HBCD levels in whole fish were 4,500 \pm 3,000 $\mu\text{g}/\text{kg}$ lw (range=390–12,100 $\mu\text{g}/\text{kg}$ lw, GC/MS and LC/MS data). The Scheldt area investigated by Roosens and co-workers is upstream of the Western Scheldt region covered in the present study and probably more influenced by point sources, which may explain the high HBCD levels.

For sole, only one study reporting HBCD levels was retrieved (Janák et al. 2005). Samples were also from the Scheldt area. For two upstream sites toward Antwerp, which are near to those investigated in the current study, Janák and co-workers reported α - and γ -HBCD levels in muscle tissue of 360 and 17 $\mu\text{g}/\text{kg}$ lw, and 110 and 11 $\mu\text{g}/\text{kg}$ lw, respectively. The range of concentrations is comparable to the present study. However, the high α -HBCD fraction is quite different from the findings reported here.

3.6 Assessment of time trends for HBCD

In a first approach, time series were tested for trends with the non-parametric Mann–Kendall test by applying the MAKESENS tool developed by Salmi et al. (2002). Slightly significant decreases ($p<0.1$) were observed for Rhone bream (all diastereomers and total HBCD) and bream from the Western Scheldt (α -HBCD and total HBCD). For the Scheldt sole, only the decrease of α -HBCD was slightly significant ($p<0.1$).

These trends were also evaluated for statistical significance by log-linear regression analysis where the slope of the line describes the annual percentage change in concentration (Bignert 2007). For the whole study period, a significant decreasing trend of 48% per year was detected for total HBCD in Rhone bream (with a 95% confidence interval of –76% to –20%, $p<0.032$). The decrease of α -HBCD levels alone was also significant ($p<0.030$), but not the trends for the other two diastereomers. In bream from the Western Scheldt, the concentrations of total HBCD (sum of three diastereomers) decreased significantly by 27% per year (confidence interval, –30% to –24%, $p<0.008$). The decrease in α -HBCD levels in the sole from the Western Scheldt was not significant at the $p<0.05$ level in this test.

In two cases (Lake Belau bream, Western Scheldt sole), the observed decreases of the HBCD levels were not significant, although declines were quite large (Figs. 1 and 2). This is probably caused by the partly high variations of HBCD levels in some years or, as for Lake Belau, by an increase of HBCD levels in 2010 from the very low concentration detected in 2009.

The findings of the present study indicate that the environmental burdens of HBCD are decreasing at those sites which are characterized by a combination of industrial point sources and diffuse contaminations, e.g., from municipal WWTPs (Western Scheldt, Rhone), although only 4 years have been evaluated so far.

Data from some other studies also point toward decreasing HBCD levels in biota, at least in Central Europe. Roosens et al. (2008) report decreasing HBCD burdens for eel from the upper Scheldt area in Belgium between 2000 and 2006. The authors suspect the presence of point sources

in the area and interpret the declining contamination as a result of lower emissions. Esslinger et al. (2011) performed a retrospective monitoring study for HBCD diastereomers with archived eggs of herring gulls (*Larus argentatus*) from three islands in the North and Baltic Seas covering the years 1988–2008. Since 2000, the HBCD levels in eggs from all sites decreased significantly. In the blubber of harbor porpoises (*Phocoena phocoena*) stranded or dying due to physical trauma in the UK between 1994 and 2006, the HBCD levels decreased since 2003 (Law et al. 2006, 2008a). The authors assume that the closure of the HBCD manufacturing plant in northeast England (Newton Aycliffe, UK) in 2003 and the industry voluntary HBCD reduction measures contributed to the decline of environmental HBCD levels.

4 Conclusions

The present study confirms that bream are appropriate bioindicators for the purpose of environmental monitoring as well as for the detection of time trends of lipophilic chemicals such as HBCD. Standardized methods for sampling and sample treatment, as well as reproducible, quality-assured analytical methods, are a solid basis for the detection of concentration changes over a relatively short period of time.

The HBCD concentrations detected in fish in the present monitoring project are in line with published data for similar species and/or locations with similar characteristics. At most sites, α -HBCD was the predominant diastereomer in fish, which is also in accordance with the majority of other studies. However, in the samples of bream from Lake Belau (all years but 2009) and of sole from Western Scheldt (all years but 2010), γ -HBCD was the main HBCD diastereomer. This finding could be confirmed by analyses of individual fish from these sites (Lake Belau bream 2008, sole Western Scheldt 2007).

The observed concentration changes of HBCD in fish tissue are only based on data from a relatively short time period of 4 years. Nevertheless, at two sites (Rhône, Western Scheldt), statistically significant decreases of HBCD concentrations could be detected. Further data need to be collected to confirm the currently available observations. Also, the partly high variability of the data (e.g., for Lake Belau) has to be considered when interpreting the data.

The observed decreases of HBCD levels in fish seem to be consistent with the implemented emission control measures, which were mentioned the “Introduction” of this contribution. Similar time trends were also found in a number of recent publications where HBCD concentrations in biota were compared over time. At the site which is

impacted by a former point source (i.e., Tees) and where higher HBCD concentrations were detected, however, no clear time trend is discernable so far.

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