



Current levels and trends of selected EU Water Framework Directive priority substances in freshwater fish from the German environmental specimen bank[☆]



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ABSTRACT

Under the German environmental specimen bank programme bream (*Abramis brama*) were sampled in six German rivers and analysed for the priority hazardous substances dicofol, hexabromocyclododecane (HBCDD), hexachlorobenzene (HCB), hexachlorobutadiene (HCBD), heptachlor + heptachlor epoxide (HC + HCE), polybrominated diphenylethers (PBDEs), polychlorinated dibenzo-p-dioxins and -furans and dioxin-like polychlorinated biphenyls (PCDD/Fs + dl-PCBs), and perfluorooctane sulfonic acid (PFOS). The aim was to assess compliance with the EU Water Framework Directive environmental quality standards for biota (EQS_{Biota}) for the year 2013, and to analyse temporal trends for those substances that are of special concern. General compliance was observed for dicofol, HBCDD and HCBD whereas PBDEs exceeded the EQS_{Biota} at all sites. For all other substances compliance in 2013 varied between locations. No assessment was possible for HC + HCE at some sites where the analytical sensitivity was not sufficient to cover the EQS_{Biota}. Trend analysis showed decreasing linear trends for HCB and PFOS at most sampling sites between 1995 and 2014 indicating that the emission reduction measures are effective. Mostly decreasing trends or constant levels were also observed for PCDD/Fs and dl-PCBs. In contrast, increasing trends were detected for PBDEs and HBCDD which were especially pronounced at one Saar site located downstream of the industries and conurbation of Saarbrücken and Völklingen. This finding points to new sources of emissions which should be followed in the coming years.

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1. Introduction

The EU Water Framework Directive (WFD) (Directive, 2000/60/EC) aims at the protection and improvement of the aquatic environment (EC, 2000). This includes the reduction of emissions of hazardous substances and the enhancement of the ecological status of the aquatic environment. Achieving this goal Europe-wide requires common definitions of the chemical status of the water quality. In this context, priority substances were identified that require action at the European Union (EU) level. For these

substances environmental quality standards (EQSs) were derived which serve as benchmark concentrations for harmful effects to wildlife and humans. Among the priority substances a group of hazardous substances was identified that represent a significant risk to or via the aquatic environment (EC, 2008, EC, 2013).

Most priority hazardous substances are persistent organic pollutants (POPs) characterized by chemical properties that may significantly affect human health and the environment, i.e. chemical stability, toxicity and a high potential for bioaccumulation. The latter leads to higher concentrations in biota compared to the water phase. Biota monitoring is therefore an alternative to the measurement of these substances in water. In the EU Directive 2013/39/EU for eight of these substances, respectively substance groups, namely dicofol, hexabromocyclododecane (HBCDD), hexachlorobenzene (HCB), hexachlorobutadiene (HCBD), mercury and

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its compounds (Hg), heptachlor + heptachlor epoxide (HC + HCE), polybrominated diphenylethers (PBDEs), and perfluorooctane sulfonic acid (PFOS) a monitoring in fish is advised. For another group of chemicals, i.e. polycyclic aromatic hydrocarbons (PAHs), the monitoring of benzo(a)pyrene and fluoranthene in mussels is requested since these compounds are metabolized by fish. For polychlorinated dibenzo-p-dioxins and -furans and dioxin-like polychlorinated biphenyls (PCDD/Fs + dl-PCB) the EQS_{Biota} relates to fish, crustaceans and molluscs. Table S1 (Supplementary material 1) gives an overview on compounds, protections goals and EQSs. For some compounds the EQS_{Biota} is intended to protect the human health (i.e. safe consumption of fish) while for others the main focus is on the prevention of secondary poisoning of fish-eating top predators.

Meanwhile, all of these substances are regulated to reduce their emissions to the environment. Some bans and restrictions go back to the 1970s and 1980s (e.g. for dl-PCBs and HCB) while others have only been banned recently (e.g. PFOS, HBCDD and HCBd). It is therefore of interest to analyse the temporal trends and current environmental concentrations of these POPs with respect to compliance with the EQS_{Biota}.

In this context, the German Environmental Specimen Bank (ESB) is a valuable tool because highly standardized sampling and archiving of environmental samples allows the (retrospective) assessment of temporal and spatial trends of chemicals (www.umweltprobenbank.de/en/). The present study focuses on bream (*Abramis brama*) sampled in three major German stream systems and their tributaries. The aim was to determine the EQS_{Biota} compliance for the eight organic priority hazardous substances and chemicals' groups for which compliance monitoring is requested in fish. Furthermore, temporal trends were analysed for HCB, HBCDD,

PBDEs, PCDD/Fs + dl-PCBs, and PFOS to evaluate the success of emission reduction measures.

2. Materials and methods

2.1. Sampling

Sampling and processing under the German ESB programme is highly standardized and follows standard operating procedures (German Environment Agency, 2016; Paulus et al., 1996; Rüdél et al., 2015). In brief, bream are collected every year after spawning between mid-July and early October. Routinely, 20 fish aged 8–12 years are taken at each sampling site (Klein et al., 2012). The muscle tissue (filet) is dissected and immediately shock-frozen in liquid nitrogen as a pooled sample of all bream from one site. In the laboratory, the tissue is pre-crushed, cryo-milled and finally stored in an archive as homogenized powder at temperatures below –150 °C in an inert atmosphere to minimize chemical alterations (Rüdél and Weingärtner, 2008; Rüdél et al., 2009).

Fig. 1 shows the locations of the sampling sites and Table 1 gives a brief description of the sites and a rationale why they were chosen by the ESB. The sites are located in the three main streams in Germany, i.e. the Rhine, Elbe and Danube, as well as in the Elbe tributaries Saale and Mulde and the Rhine tributary Saar. Lake Belau is located in an agricultural region in Schleswig-Holstein and is representative of remote sites with relatively low anthropogenic influence.

2.2. Chemical analysis

Tissue samples of bream muscle were retrospectively analysed



Fig. 1. Freshwater sampling sites of the German environmental specimen bank.

Table 1
Freshwater sampling sites of the German environmental specimen bank.

	Sampling site	Code	River km	Short characterization of the sites
Elbe	Prossen	E1	13	Site in the upper reach of the Elbe shortly downstream of the Czech-German border; designed to study the contaminant load of the Elbe at its entry to Germany
	Zehren	E2	96	Site in the transition area between upper and middle section of the Elbe about 30 km downstream of the city of Dresden and 5 km downstream of Meißen; reflects the water pollution of the urban area of Dresden
	Barby	E3	296	Site in the middle section of the Elbe about 1 km downstream of the confluence of the river Saale and about 30 km downstream of the Mulde mouth; captures the contaminant loads of the two tributaries
	Cumlosen	E4	470	Site in the relatively natural section of the Middle Elbe in the North German Plain; reflects the pollution at the former cold war German-German border and the industries at Magdeburg
	Blankenese	E5	634	Site in the tidal section of the Lower Elbe located downstream of the city of Hamburg and the Hamburg harbour; documents the contaminant load discharged into the North Sea
Mulde/tributary of the Elbe	Mulde	Mu	near mouth	Site covering the Jonitzer Mulde and Mulde between the branch off of the Jonitzer Mulde and the confluence with the Elbe at Dessau-Rosslau; about 20 km downstream of Bitterfeld; captures the contamination of the industrial area around Bitterfeld discharged into the Elbe.
Saale/tributary of the Elbe	Saale	Sa	near mouth	Site between the harbour of Halle and the barrage at Wettin; reflects the contaminant load of the Halle conurbation discharged into the Elbe
	Weil	R1	174	Site in the Upper Rhine at the entry into the Upper Rhine Valley and the beginning of the Rhine canal, located at the Swiss-German border just downstream of the city of Basel; captures the contamination load of the industries in the Basel area
	Iffezheim	R2	334	Site in the upper Rhine at the end of the Rhine canal at the German-French border, located in the head- and tailwater of the barrage of Iffezheim; reflects the contamination of the Rhine canal
	Koblenz	R3	590	Site in the Middle Rhine after the river has crossed the Rhenish Slate Mountains and directly upstream of the Moselle confluence, about 4 km downstream of the Lahn confluence and 100 km downstream of the Rhine-Main conurbation; reflects the pollution of the Middle Rhine
Saar/tributary of the Rhine	Bimmen	R4	865	Site in the Lower Rhine along the German-Dutch border about 85 km and 50 km downstream of the Ruhr and Lippe confluences; captures the contaminant load of the Rhine downstream of the industrial region of the Ruhr conurbation
	Güdingen	S1	barrage Güdingen	Site in the area of the barrage of Güdingen at the entry of the Saar into the Saar conurbation directly downstream of the German-French border, influenced by the 2nd largest sewage treatment plant of the Saarland; reflects the contaminant load of the Saar upstream of the industrial Saar conurbation
	Rehlingen	S2	barrage Rehlingen	Site located in the area of the barrage of Rehlingen, where the Saar leaves the Saarland conurbation, directly downstream of the industrial area of Dillingen/Völklingen and 20 km downstream of the Rossel mouth; influenced by a sewage treatment plant; captures the contamination of the Saar downstream of the industrial sites of the Saar conurbation
Danube	Ulm	D1	2593	Site in the reservoir basins at a barrage in the headwaters of the Danube below the Swabian Alp, downstream of the villages of Ehingen and Erbach; reflects the water quality of the near natural part of the river
	Kelheim	D2	2404	Site in the Middle Danube and the central section of the German Danube downstream of the mouth of the Altmühl, which connects the Danube with the Rhine-Main-Danube Canal; reflects the state of the shipped Middle Danube
	Jochenstein	D3	2210	Site in the Middle Danube at the German-Austrian border about 20 km downstream of the Inn confluence; reflects the contaminant load of the Danube when leaving Germany
Lake Belau	Lake Belau	LB	–	Site in a lake in northwestern Germany in the Bornhöved Lake District fed by the small river Schwentine; reflects the water quality in an area with relatively low anthropogenic impact

for dicofol, HBCDD, HCB, HCBd, HC + HCE, PBDEs, PCDD/Fs + dl-PCBs, and PFOS. Brief descriptions of the analytical methods and references are summarized in Table S2 (Supplementary material 1).

Identification of target compounds was based on the comparison of retention time and relative isotope ratios between native and isotopic labelled internal standards. Quantification of target compounds was carried out by means of isotope dilution analysis with the use of internal and external standards. Method blanks including extraction, clean-up and measuring were monitored in parallel to each batch of samples consisting of not more than 12 single samples. Furthermore, precision and accuracy were checked by analysing in-house quality assurance-pool samples, sample material of previous interlaboratory proficiency studies or certified reference material along with each batch of samples.

Lipid determination was performed gravimetrically using an aliquot of the crude extracts obtained during the extraction with a mixture of appropriate polar and non-polar solvents performed for the HCB analysis.

2.3. Data evaluation

Unless otherwise indicated, all concentrations (including those from literature) are reported on a wet weight (ww) basis as µg/kg ww.

In accordance with the EU Directive 2013/39/EU (EC, 2013), the

data for PCDD/Fs and dl-PCB are expressed in terms of toxicity equivalents (TEQ) as defined by the WHO in 2005 (Van den Berg et al., 2006), neglecting non-quantifiable compounds (lower bound procedure). TEQs are used as descriptors for the combined toxicity of dioxins, furans and dioxin-like PCBs.

Temporal trends were analysed for HCB, HBCDD, PCDD/Fs + dl-PCBs, PBDEs, and PFOS at selected sites in the main streams Rhine, Elbe and Danube as well as in the Saar. For trend analysis a software tool from the German Environment Agency was applied (LOESS-Trend, Version 1.1, based on Microsoft Excel). This tool fits a locally weighted scatterplot smoother (LOESS; fixed window width of seven years) through the yearly contaminant levels and then tests for significance of linear and non-linear trend components by means of an Analysis of Variance (ANOVA) following the approach of Fryer and Nicholson (1999) (for details refer to the Supplementary material 1). The time window of seven years for the smoother function has proven to be well suited, particularly for the evaluation of marine and limnetic monitoring data.

Trend analysis was based on substance concentrations normalized to 5% lipid, except for PFOS for which measured wet weight concentrations were used as input data. The normalization to 5% lipid follows the recommendations of the EU WFD Guidance Document 32 on biota monitoring (EC, 2014).

Comparison with published data from European waters focused on monitoring data of fish other than eel monitored after the year

2000. Published lipid-based data were converted to wet weight data, based on the lipid concentrations given in the respective publication (Table S7, Supplementary material 2).

3. Results

3.1. Spatial distribution and EQS_{Biota} compliance in 2013

Dicofol and HCBd were not detected in any of the bream samples in 2013. General compliance was also observed for HBCDD, whereas PBDEs exceeded the EQS_{Biota} at all sites. For all other compounds compliance differed between sites (Fig. 2, Table S3, Supplementary material 1).

Fig. 2 reveals a similar spatial distribution for all substances except HCB in so far, as highest contaminations are detected in fish from the Rhine and (with the exception of PFOS) in the Saar. Furthermore, clear spatial trends are discernable for PBDEs and PFOS in the Rhine, i.e. a downstream increase for PBDEs and a downstream decrease for PFOS.

HCB concentrations in bream ranged between 0.17 and 22.2 µg/kg in 2013 (Table S3, Supplementary material 1) and exceeded the EQS_{Biota} of 10 µg/kg in the upper and middle Rhine (R2, R3), at all Elbe sites and in the Mulde.

HC and its metabolite HCE were detected only in bream from the Saar and the Rhine at concentrations of 0.08–0.14 µg/kg ww, thus exceeding the EQS_{Biota} of 0.0067 µg/kg by 1200–2100%. No

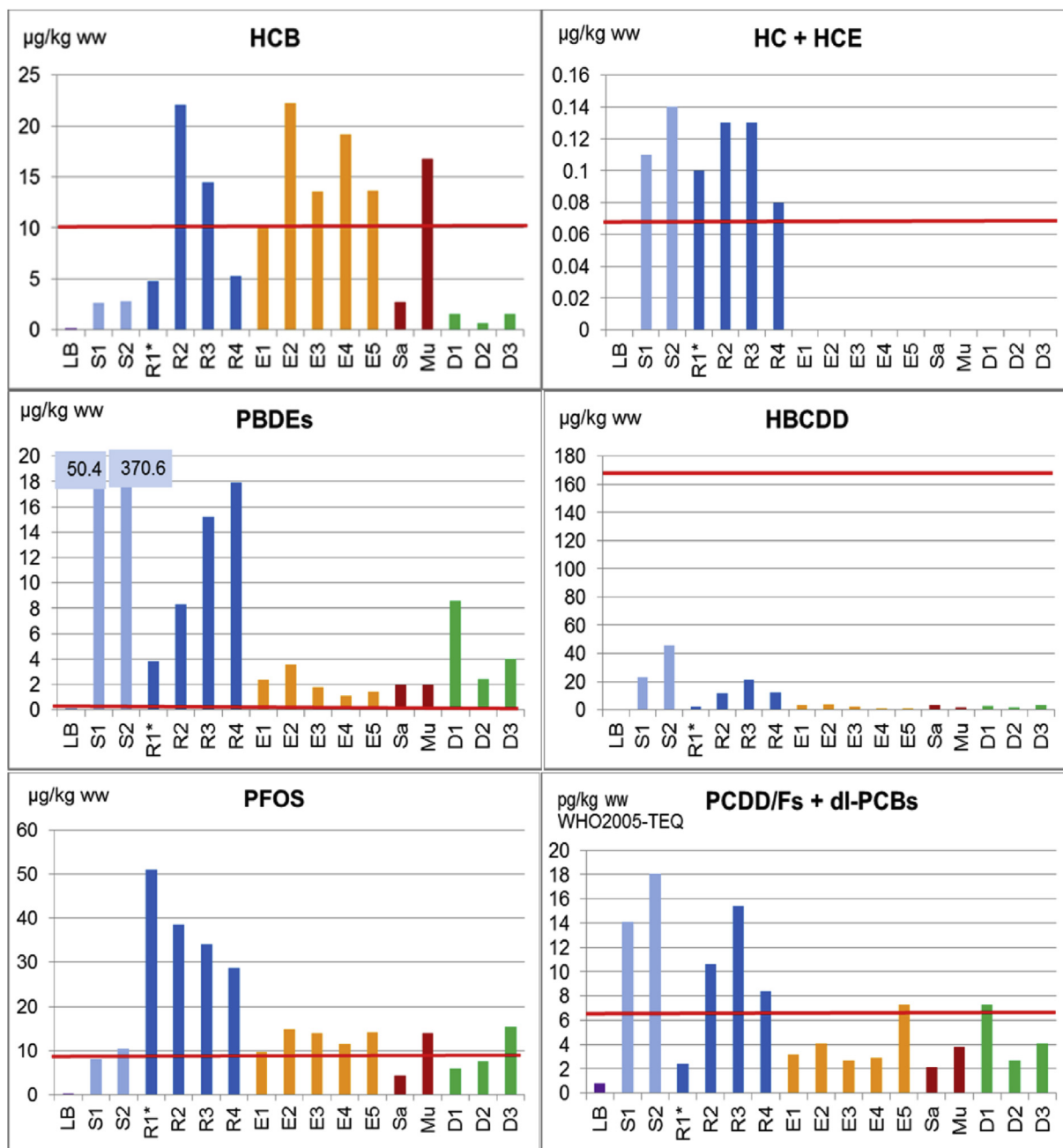


Fig. 2. EQS_{Biota} compliance check for priority hazardous substances in bream (*Abramis brama*, muscle pool samples of approximately 20 bream each) sampled in German freshwaters under the German environmental specimen bank programme in 2013 (* in 2011 at R1). LB: Lake Belau; S: Saar; R: Rhine, E: Elbe, Sa: Saale, Mu: Mulde, D: Danube. Red line: EQS_{Biota} (in µg/kg wet weight): HCB: 10; HC + HCE: 0.0067; PBDEs: 0.0085; HBCDD: 167; PFOS: 9.1; PCDD/Fs + dl-PCB: 0.0065 WHO2005-TEQ. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

assessment of HC + HCE was possible for fish from the other sites because the sensitivity of the analytical method was too low.

PBDEs exceeded the EQS_{Biota} at all ESB sampling sites in 2013, with concentrations in the range of 0.14 and 371 µg/kg. Contamination was lowest at Lake Belau while extremely high levels of 50.4 and 371 µg/kg were detected in bream from the Saar sites S1 and S2, respectively. Concentrations were also relatively high in bream from the middle and lower Rhine (R3, R4), whereas considerably lower levels were observed in the Elbe, its tributaries Mulde and Saale and the Danube. BDE-47 was the dominant congener at all sites (Table S4, Fig. S1, Supplementary material 1). Interestingly, BDE-209, the major congener of the technical mixture DecaBDE, which is not considered in the EQS_{Biota} (and thus not included in the sum of PBDEs presented here) was relatively high at the Rhine site R1 in 2011 and at the Danube sites D1 and D3 in 2013 (Fig. S1, Supplementary material 1).

HBCDD (as sum of the α -, β - and γ -diastereomers) was detected in all bream samples at concentrations in the range of 0.17 and 45.6 µg/kg and thus well below the EQS_{Biota} of 167 µg/kg. Highest contaminations were observed in the Saar and at the Rhine sites R3 and R4. At many sites (e.g. in the Elbe, Mulde, the Danube and the lower and middle Rhine) HBCDD concentrations were roughly in the same range as PBDEs (Table S3, Supplementary material 1). α -HBCDD was the dominant diastereomer in all samples accounting for 71–97% of the total measured HBCDD concentrations. The dominance of the α -HBCDD diastereomer in biota has been reported previously (e.g. Gerecke et al., 2003; Rüdell et al., 2012).

PFOS levels in bream ranged between 0.3 and 38.5 µg/kg in 2013. An even higher concentration of 51 µg/kg was observed in bream sampled at the upper Rhine site R1 in 2011 (Table S3, Supplementary material 1, no sample in 2013). The EQS_{Biota} of 9.1 µg/kg was exceeded at 12 of 17 sampling sites.

PCDD/Fs and dl-PCBs were detected in bream at concentrations in the range of 0.0008–0.0181 µg WHO₂₀₀₅-TEQ/kg. Levels were generally higher in bream from the western part of Germany and, accordingly, it was only sites in western Germany where the EQS_{Biota} was exceeded in 2013 (Table S3, Supplementary material 1).

Mostly dl-PCBs dominated in the samples (Fig. S2, Table S5, Supplementary material 1) with dl-PCB:PCDD/F ratios in the range of 1.1–7. An extremely high ratio of dl-PCB:PCDD/F concentrations of 21 was detected at the sampling sites LB (Lake Belau). In contrast, at the Elbe sites E4 and E5, PCDD/Fs were higher than dl-PCBs (ratios of 0.74 and 0.76, respectively). Relatively low ratios were also observed in the Mulde and at the confluence of Mulde and Elbe (E3).

3.2. Temporal trends

For selected sampling sites in the Rhine, Elbe, Danube and Saar temporal trends were analysed retrospectively for those substances that exceeded the EQS_{Biota} in 2013, i.e. HCB, PBDEs, PCDD/Fs + dl-PCBs and PFOS. Additionally, trends for HBCDD were determined to evaluate in how far this substance, which has been introduced as substitute for PBDEs in some applications, might be a problem in the future. HC + HCE were not included in trend analysis because the analytical method was not sufficiently sensitive to cover concentrations in the range of the respective EQS_{Biota}.

Fig. 3 gives an overview of the strength and direction of the linear trends. The accompanying data of the trend analysis are summarized in Table S6 (Supplementary material 1).

HCB concentrations have decreased at all sampling sites since 1995. At the Danube sampling site D3, however, the trend was not significant. Decreases were most pronounced in the Elbe where initial concentrations were very high and are still relatively high in 2014 (Fig. 4A + B, Table S6, Supplementary material 1). HCB levels

in bream from the Rhine were almost 10-times lower and the decreases were less pronounced. Trend curves in the Rhine were not steady but show a maximum around 1999/2001 (Fig. S3, Supplementary material 1). Much lower HCB levels and weaker decreases were detected in the Saar and Danube.

In 1998, PCDD/Fs and dl-PCBs contamination was highest in bream from the Saar and the lower Rhine (R4). Since then, concentrations have clearly decreased at the Saar site S1 and R4 (Fig. S4, Supplementary material 1) but not at the Saar site S2. Decreasing trends are also detected in the Elbe and at the Rhine site R2, whereas increasing contaminations are noticeable for bream from the Danube (Table S6, Supplementary material 1, significant linear trend ($p = 0.01$) only at D1).

PBDE contamination of fish was high at all sampling sites (Table S6, Supplementary material 1). Decreasing trends, however, were detected only in the Rhine and at the Saar site S1, while no significant trends or even increases were observed at the other sites. In 1995, extremely high levels were detected in bream from the Saar and, by a factor of about 5 lower, in the lower Rhine (R4). Since then, concentrations at the Saar site S1 have decreased steadily, whereas a strong increase was noticed in bream from S2 (Fig. 4C + D). The respective trend curve shows a decline between 1995 and 2001 followed by a sharp increase thereafter.

In the Rhine, PBDE contamination of bream decreased at all sites (Table S6, Fig. S5, Supplementary material 1). Trends, however, were not steady. This was especially pronounced at R4 where concentrations in bream decrease until 2005 then increased again until 2011 followed by a new decrease (Fig. 4E).

Relatively low but increasing PBDE concentrations were observed in bream from the Elbe (significant linear trend with $p = 0.05$ only at E1). Concentrations varied between years indicating that diffuse emissions may contribute to the increase. Comparably low but more or less constant PBDE levels are found in bream from the Danube.

HBCDD concentrations seem to be increasing at many sites (Table S6, Supplementary material 1). In 1995, lowest levels were found in the Elbe while contamination of bream from the Saar and Rhine and also from the Danube site D1 (in 2003) was relatively high. In the lower Rhine (R2) and the upper Danube (D1), HBCDD decreased with time whereas increasing concentrations were observed in bream from the Saar, the Elbe and the Rhine site R3 (Fig. S5, Supplementary material 1). At both Saar sites, concentrations were more or less constant until 2009 and increased thereafter indicating the rise of new emissions in this area (Fig. S6, Supplementary material 1). At the Saar site S2 levels seem to drop again in 2014. HBCDD concentrations in bream from the Rhine site R3 and the Elbe sites E1 and E3 showed very little variations between years and increased slowly but steadily. Highest initial HBCDD concentrations and strong variations in trend directions were noticeable in bream from the lower Rhine site R4 (Fig. 4F). The trend curve resembles that of PBDEs with decreasing contaminations until 2005, steep increases thereafter and a new decrease in recent years.

PFOS contamination of bream was highest in the Rhine and Elbe in 1995 (Table S6, Supplementary material 1). Since then contaminations have decreased steadily in the Elbe and the Danube whereas trends in the Rhine and Saar were less even with either constant or increasing levels in the first years followed by a decrease. The turning point was around 2007 at both Saar sites and the Rhine sites R2 and R3, and in 1999–2001 at R4 (Fig. 4G + H).

4. Discussion

The presented German Environmental Specimen Bank (ESB) data show that the contamination of bream varies considerably

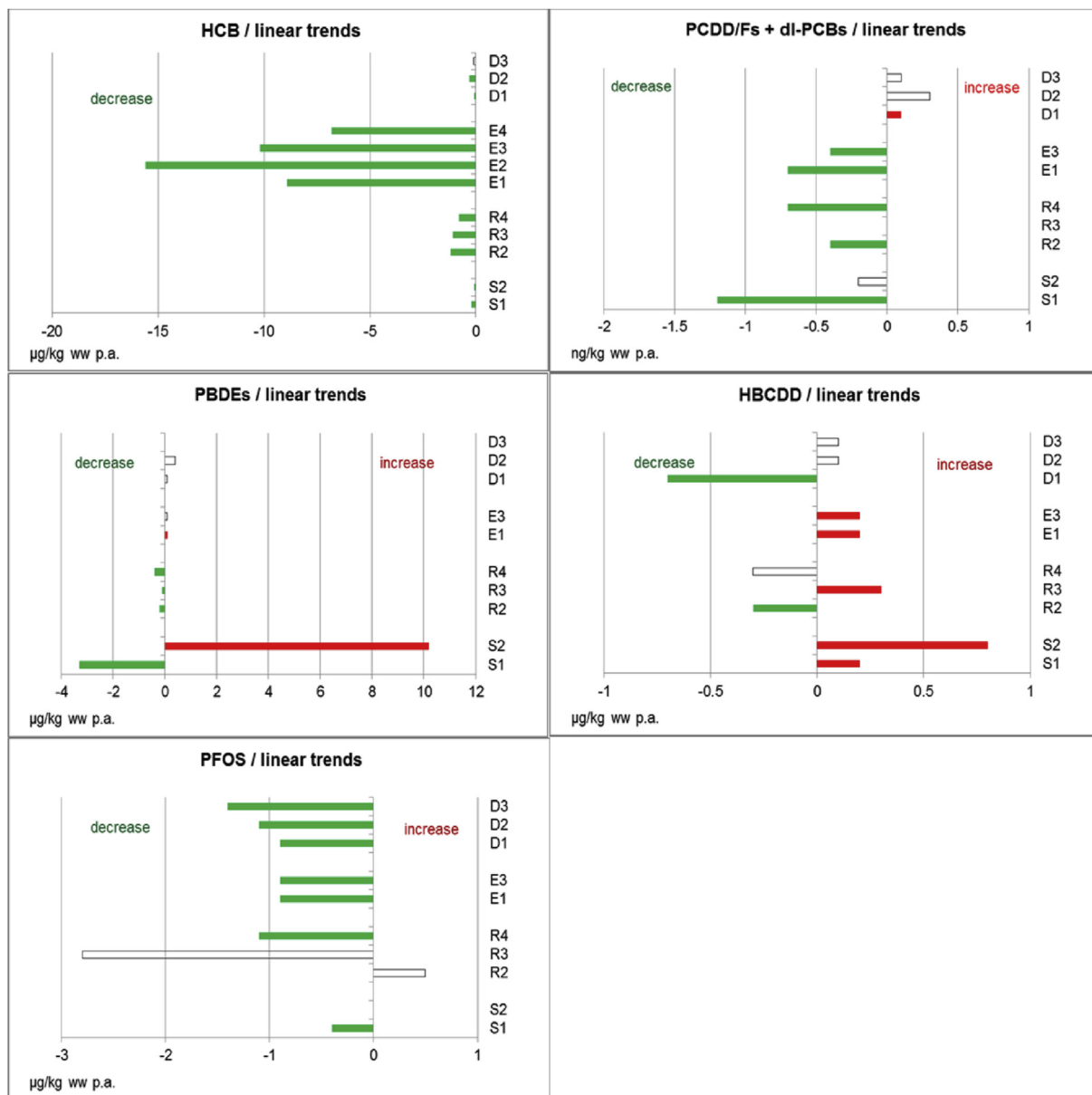


Fig. 3. Direction and strength of linear temporal trends in muscle tissue of bream (*Abramis brama*, pool of 20 fish) sampled under the German environmental specimen bank programme in 1995* – 2014. Data refer to concentrations ($\mu\text{g}/\text{kg}$ wet weight) normalized to 5% lipid except for PFOS where measured concentrations were used in trend analysis. Concentrations of PCDD/Fs and dl-PCBs are reported as ng/kg WHO₂₀₀₅-TEQ. Green bars: significant decrease; red bars: significant increase; blank bars: non-significant increases and decreases. * deviating sampling periods: PCDD/Fs and dl-PCBs: 1998–2014, Danube: 2004–2014; HCBDD (Danube): 2003–2014. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

between rivers and sampling sites. Nevertheless, certain general patterns can be observed that are related to former production and usage of the respective compounds.

Highest contaminations were mostly detected in the highly industrialized and densely populated areas, i.e. the conurbations at the Saar and the Ruhr and the region around Bitterfeld at the Mulde and Elbe.

The Saar region is characterized by metal working and steel industry and carries the legacies of former mining activities. Coal and steel industry have strongly influenced also the Ruhr region, next to numerous chemical industries. The industrial region in the middle Elbe was dominated by chemical industries, as are the upper Rhine region around Basel and the areas around Ludwigshafen, Mannheim and Frankfurt. The river sediments in these areas still carry the legacies of former emissions while new emissions are

introduced mostly by waste water treatment plants (WWTPs), which reflect the ongoing usage of products containing the respective substances. Floods may have shifted contaminated sediments and fish downstream and blurred the picture but typical contamination patterns related to former production and usages are still present.

HCB was highest in bream from the ESB sampling sites in the Rhine, Elbe and Mulde.

In the Rhine, HCB pollution is related to the former chlorine producing industries along the river, e.g. in the upper Rhine at Rheinfelden (around Rhine km 150), the middle Rhine downstream of Mannheim and Ludwigshafen (around Rhine km 440) and the lower Rhine around km 650–760. Further HCB contaminations originate from chemical industries in the Frankfurt area (Heinisch et al., 2006, 2007). Production of HCB in Germany ended in 1993

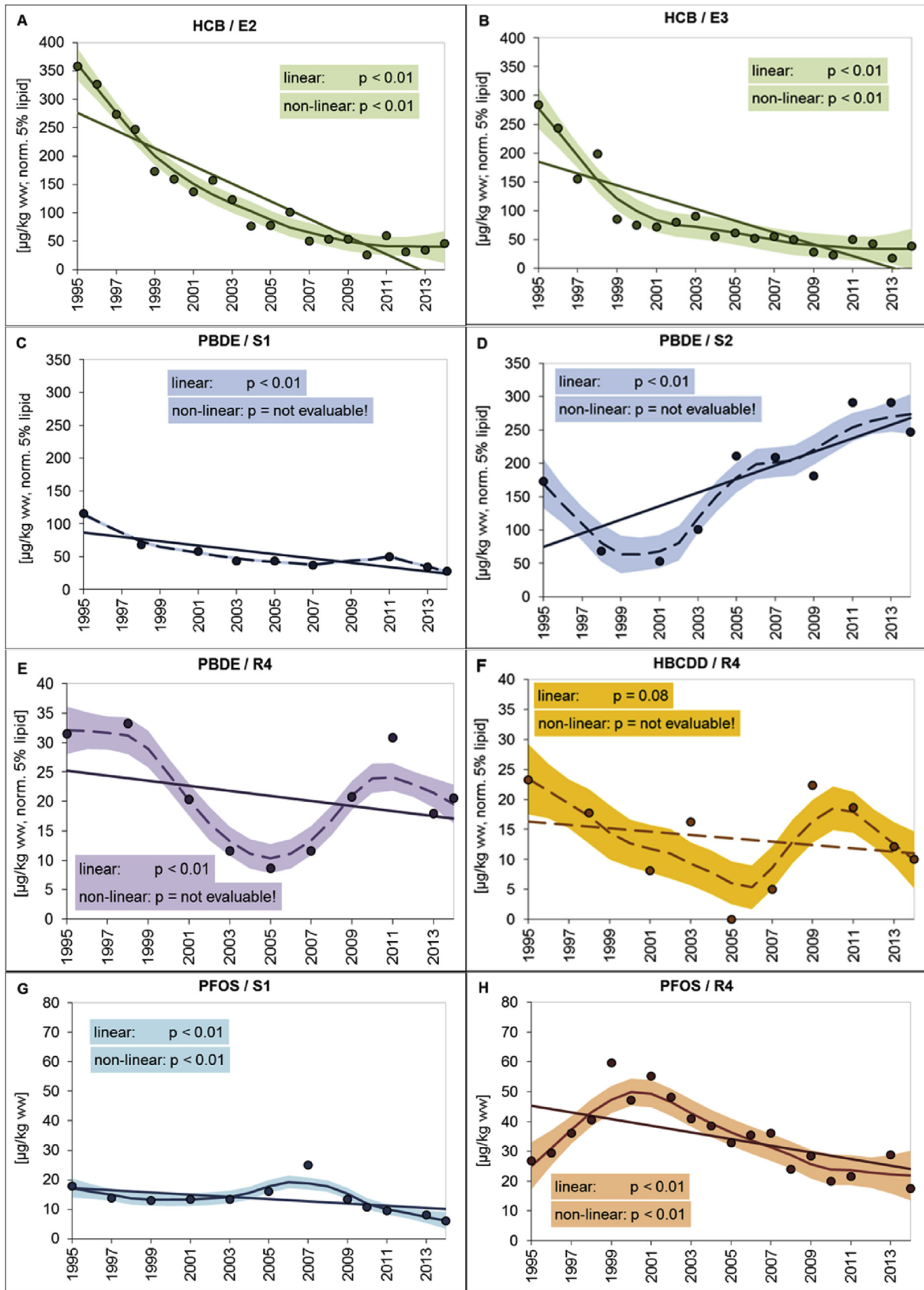


Fig. 4. Temporal trends of HCB, PBDE, HBCDD and PFOS in muscle tissue of bream (*Abramis bama*) sampled under the German environmental specimen bank programme. Concentrations are given as $\mu\text{g}/\text{kg}$ wet weight (ww) normalized to 5% lipid, except for PFOS (no lipid normalization). Data refer to annual pool samples of 20 fish each. Shaded area: 95% confidence interval of LOESS function; linear and non-linear regression lines: solid lines are significant, broken lines are not. A + B: HCB at the Elbe sites E2/Elbe km 96 and E3/Elbe km 296; C + D + E: PBDE at the Saar sites S1/Güdingen and S2/Rehlingen and the Rhine site R4/km 865; F: HBCDD at the Rhine site R4/km 865; G + H: PFOS at the Saar site S1/Güdingen and the Rhine site R4/km 865.

which is reflected in decreasing bream contaminations at all Rhine sites.

The ESB site R2 (Rhine km 334/Iffezheim) may be influenced by the former point source near Rhine km 150 from where sediments have travelled downstream and settled around the barrage at Iffezheim. Moreover, sediment studies by Schönberger et al. (2006) point towards additional unidentified sources of HCB between Rhine km 180 and 240. The contamination of bream from the ESB site R3 might be related to the chemical industries in the areas around Mannheim/Ludwigshafen and Frankfurt. In 1999, a flood occurred in the upper Rhine area (BfG/Undine information BfG – Undine information platform, 2016) that probably carried contaminated sediment and/or contaminated bream downstream to R2 and R3. This would explain the peak in the trend curves at these sites between 1999 and 2001 (Fig. S3, Supplementary material 1). HCB levels in bream from the sampling site R4 downstream of the former production sites in the Ruhr region were always lower compared to R2 and R3 and variations between the years were relatively small indicating that production or usage of HCB were less relevant in this area and/or emission were effectively reduced.

In the Elbe, major HCB contaminations originate from production sites and landfills in the Czech Republic. HCB processing industries were also located around Magdeburg and in the industrial region around Bitterfeld (Heinisch et al., 2006). However, in our study HCB levels were always highest in bream from the Elbe site E2 downstream of the cities of Dresden and Meißen. No HCB point sources are known for this area and no effects of the Elbe floods in 1999 and 2002 are noticeable, which might have caused a downstream travelling of contaminated sediment from the Czech Elbe. Presumably the contamination at this site is related to the former use of HCB in wood preservatives or in seed treatment that was allowed in the former German Democratic Republic until 1984. In the Mulde and middle Elbe (E3, E4) legacies in the sediments originating from the industries around Bitterfeld and Magdeburg (Heinisch et al., 2006, 2007) are probably responsible for the relatively high HCB levels in bream. The observed decreases in HCB contamination of bream from the upper and middle Elbe and its tributaries indicate that emissions have ceased and emission control measures are effective.

Our data from the Elbe compare quite well to published data e.g. from the FGG Elbe (2016; FIS databank) and Hrádková et al. (2012; Table S7, Supplementary material 2).

No chlorine producing industries were located at the Saar, Danube and Saale, which explains the low HCB levels in bream from these rivers.

HCB concentrations in fish from other European freshwaters were mostly quite low and comparable to the less contaminated ESB sites without known HCB sources, e.g. Lake Belau (LB) and the sites in the Danube (Jürgens et al., 2013; Miège et al., 2012; Schmid et al., 2007; Waszak and Dąbrowska, 2009; Table S7, Supplementary material 2).

PCDD/Fs and dl-PCBs were mostly higher in bream from the ESB sites in western Germany. Highest levels were detected in the Saar and the middle and lower Rhine. In the Saar, the intensive mining activities and associated industries are probable sources. In 1998, especially bream from the site S1 were highly contaminated indicating that also French industries contributed to the emissions (Neugebauer et al., 2011). Levels dropped between 2002 and 2004 which points towards the shut-down of a point source or effective emission reduction measures. At S2, however, no significant decline was noticeable. This site is located downstream of the industrial region around Völklingen and Saarbrücken where legacies in the sediments are probably still high. Krauß-Kalweit (2005) reports similar PCDD/F + dl-PCB concentrations in cyprinids sampled in the Saar near the ESB site S2 in 2004, while fish sampled near the ESB

site S1 were clearly less contaminated than our bream in the same year.

Potential former PCDD/F + dl-PCB sources in the Rhine were again the chemical industries located in the upper Rhine region and in the area around Ludwigshafen, Mannheim and Frankfurt as well as the coal and steel industries and chemical manufactures in the lower Rhine region. Since 1998, contamination of bream has decreased significantly at R4 where initial concentrations were highest, indicating that emissions have declined considerably in this area. Decreasing contaminations are also apparent in the lower Rhine at R2 whereas no changes are detected in the middle Rhine at R3. Comparative data from the ICPR (2011) for the middle Rhine near R3 were lower in 2010 (Table S7, Supplementary material 2).

Bream from the ESB sites in the Elbe and its tributaries had comparably lower PCDD/F and dl-PCB levels in 2013 (Tables S3 and S5, Supplementary material 1). The data are in accordance with concentrations reported by Stachel et al (2007; Table S7, Supplementary material 2) for the middle Elbe. Decreasing trends point towards effective emission reductions. Low levels were also detected in bream from the Danube. At the ESB site D2 our data are in the same range as those of the 3rd JDS from the same site (Liška et al., 2015; Table S7, Supplementary material 2).

The ratios of dl-PCB to PCDD/Fs in the samples indicate that the sources of contamination differed between the sampling sites and also between the western and the eastern part of Germany. In the Saar, Danube and Rhine, dl-PCBs clearly dominated in the samples (Fig. S2, Table S5, Supplementary material 1). Such a dominance of dl-PCBs has also been reported by others (e.g. Blanchet-Letrouvé et al., 2014; Liška et al., 2015; Neugebauer et al., 2011; Pacini et al., 2013; Stachel et al., 2007) and is attributed to the wide use of technical PCB mixtures in industrial applications in the past. An extremely high dl-PCB:PCDD/F ratio was detected at the sampling sites LB (Lake Belau). Since Lake Belau is a site with low anthropogenic influence, aerial deposition of dl-PCBs is the most likely source.

In the middle and lower Elbe and the Mulde the higher or equally high shares of PCDD/Fs in the samples point to additional emissions from other sources (e.g. the chloralkali production and nonferrous metal industries). Severe PCDD/F contamination of sediments and floodplains in the Mulde and Elbe downstream of the Mulde mouth have already been described by Umlauf et al. (2005, 2010) after the Elbe flood of 2002 and were attributed to the former metallurgical industry (especially the production of magnesium) in the Bitterfeld region. In the course of the years, PCDD/F-contaminated sediments may have travelled downstream leading to the observed dominance of PCDD/Fs in bream at E4 and E5.

Potential emissions of the brominated flame retardants (BFRs) PBDE and HBCDD originate from industries (production and usage) and WWTPs, which reflect the usage of products containing flame retardants (Hillenbrand et al., 2007; Jürgens, 2015). Accordingly, bream contaminations were again highest in the industrialized areas and urban congregations around Saarbrücken, Völklingen and Saarlouis and the Ruhr region.

Mostly both BFRs were in the same concentration range, except for the Saar where PBDEs were clearly higher than HBCDD throughout the study (at S1 by a factor around 4, at S2 by a factor around 14). High levels of PBDEs and HBCDD in sediments and bream from the Saar have already been reported by Stiehl et al. (2008) and Sawal et al. (2011). The dominant BDE-congeners in the sediments were BDE-47, and –99, while BDE-183 and –209 (the major components of technical Octa- and Deca-BDE, respectively) were relatively low. The authors therefore concluded that technical Penta-BDE mixtures were responsible for the contamination (Stiehl et al., 2008). However, BDE-47, –99 and –100 can

also be formed during degradation of technical Octa-BDE and Deca-BDE (Gerecke et al., 2005; He et al., 2006) making it difficult to clearly identify the origin of contamination.

The strong increase of PBDE contamination at S2 indicates that the emissions are related to an industrial point source reflecting e.g. a switch in production. Since no increase was detected at S1 the source must be located between both sampling sites in the area around Saarbrücken, Völklingen, and Dillingen. Compared to PBDEs, HBCDD increased only slightly at both Saar sites. Possibly these emissions come from WWTPs and reflect an increased use of HBCDD in the investigated period.

High BFR levels were also detected in bream from the Rhine sites R2, R3 and R4. At the upper Rhine site R2 the industries and WWTPs around Basel are possible sources. Since levels of both BFRs have decreased since 1995, emission control measures are obviously effective here. At R3, a close-by abandoned industrial site might be responsible for HBCDD emissions (Stiehl et al., 2008). Further emissions may originate from the industries around Frankfurt, Mannheim and Ludwigshafen. The trend curves for both groups of BFRs suggest that there are still emissions in this area (slight increase in HBCDD levels at R3 and constant levels of PBDEs since 2007). The ESB site R4 in the lower Rhine is influenced by the Ruhr region and the confluence with the river Lippe. The Lippe has been identified as potential PBDE source for the lower Rhine because of emissions from an industrial complex with mainly chemical factories (Guhl et al., 2014). The decrease of both BFRs since 2010/2011 indicates that emission reduction measures were successfully implemented here.

Bream from the Elbe and its tributaries had comparably low BFR levels. HBCDD, however, increased slowly but steadily since 1995 reflecting the increased use of HBCDD whereas no consistent trends were detected for PBDE.

PBDE concentrations in bream from the Czech section of the Elbe were higher compared to our data, while HBCDD was mostly within the same range or even lower (Hajšlová et al., 2007; Hloušková et al., 2013; Hrádková et al., 2012; Pulkrabová et al., 2007, Table S7, Supplementary material 2). Significantly higher levels are reported from sites downstream of chemical factories and a paper mill (Hajšlová et al., 2007; Hloušková et al., 2013).

Published fish monitoring data on BFRs from other European freshwaters confirm the ubiquitous presence of these compounds in the environment with highest contaminations at sites near former industrial point sources and urban congregations (Allchin and Morris, 2003; Cheaib et al., 2009; Eljarrat et al., 2004, 2005; Gerecke et al., 2003; Harrad et al., 2009; Isoaari et al., 2006; Jürgens et al., 2013; Miège et al., 2012; Poma et al., 2014; Remberger et al., 2004; Rüdél et al., 2012; Schlabach et al., 2004; Zennegg et al., 2003; Table S7, Supplementary material 2). The HBCDD concentrations observed in bream at the ESB sites are comparable to published data from sites without point sources.

PFOS was used in Germany mainly in metal plating, firefighting foams, hydraulic fluids, photographic and semiconductors industries and textile and paper industries (Carloni, 2009). Therefore highest contaminations are expected in the vicinity of respective industries. Furthermore, WWTPs are relevant sources which mirror the usage of PFOS containing products. Both, industries and WWTPs may play a role in the contamination of bream from the Rhine. At the lower Rhine the plating industry seems to be the major source (Guhl et al., 2014). The decreasing PFOS levels which are noticeable since 2007 in the upper and middle Rhine area (R2 and R3), and since 2001 in the lower Rhine region (R4) indicate that the industrial applications of PFOS have either stopped and/or that effective emission reduction measures were introduced.

In the Elbe, PFOS was relatively high in 1995 but decreased steadily since then suggesting that former sources have been

eliminated. Relatively low PFOS concentrations and mostly decreasing trends were detected in bream from the Saar and the Danube. Industrial usage of PFOS was obviously not relevant in these areas. In 1995, PFOS contamination was still rather high at the Saar site S1 where a close-by WWTP is a likely source of contamination.

Published data on PFOS in freshwater fish from European freshwaters cover a relative wide concentration range (0.1–752 µg/kg ww) depending on the proximity of point sources (Berger et al., 2009; Hloušková et al., 2013; Hrádková et al., 2012; Järnberg and Holmström, 2003; Labadie and Chevreuil, 2011; Miège et al., 2012; Schuetze et al., 2010; Squadrone et al., 2015; Table S7, Supplementary material 2).

The determination of HC + HCE in bream was limited by the insufficient sensitivity of the analytical method. Contaminations were only detected in bream from the Saar and Rhine and are attributed to legacies in the sediments that originate from former production and usage. Comparable concentrations are also reported from Switzerland and Poland (Schmid et al., 2007; Waszak and Dąbrowska, 2009; Table S7, Supplementary material 2). No HC + HCE was detected in bream from the ESB sites in the Elbe and Danube which is in accordance with data from the FGG Elbe (2016) and Liška et al. (2015).

Dicofol and HCBd are obviously of no concern in German freshwaters. This is in line with reported data, e.g. from the Danube (Liška et al., 2015), the Elbe (FGG Elbe 2016), the Rhône (Miège et al., 2012), and the Thames and the Nene (Jürgens et al., 2013) (Table S7, Supplementary material 2).

5. Synopsis

No threshold exceedances were detected for those compounds for which the EQS_{Biota} was derived to protect secondary poisoning of wildlife (dicofol, HBCDD, HCBd) (Table S3, Supplementary material 1). The data of the ESB fish monitoring as well as published data from other European countries suggest that dicofol and HCBd are probably of no concern in European freshwaters to date. The comparatively low HBCDD levels in bream from the ESB sites indicate that there are no point sources in the vicinity of the covered sampling sites. All concentrations were well below the WFD EQS_{Biota}. However, mercury - a further priority compound not covered here which also has a secondary poisoning protection goal (Table S1, Supplementary material 1) - revealed a broad exceedance of the respective EQS_{Biota} in previous studies (e.g. Lepom et al., 2012; Fliedner et al., 2014; Nguetseng et al., 2015).

In the present study, EQS_{Biota} exceedances were detected only for those priority substances where the EQS is based on human health considerations (prevention of consuming contaminated fish; PBDEs, HCB, PFOS, HC + HCE, PCDD/Fs + dl-PCBs). In 2013, the respective EQS_{Biota} for PBDEs was exceeded at all sampling sites.

For PCDD/Fs and dl-PCBs, EQS_{Biota} exceedances were detected at ESB sites near former point sources. Concentrations of HCB are, like PFOS, still high in bream from many sites. Fish contamination with HC + HCE, for which the EQS_{Biota} was exceeded at the ESB sites in Rhine and Saar, seems to be related mainly to legacies in the sediments. However, the applied analytical method for HC + HCE in fish needs some improvement to allow an EQS_{Biota} compliance check at all sites.

The EQS Directive (EC, 2013) does not specify whether whole fish or certain fish parts (e.g. filet) should be analysed. While for the protection of wildlife whole fish seems to be an appropriate matrix (top predators like cormorants or otters feed on whole small or medium sized fish), the analysis of fish filet seems more suitable for the protection goal human health (humans prefer the filets of larger fish). Considering this, the filets of relatively large bream as selected

for the ESB monitoring seem to be a good choice for monitoring of the relevant human health-related priority substances.

The trend monitoring revealed that concentrations of PCDD/Fs and dl-PCBs in bream are decreasing at most sites. It can be assumed that legacies in the sediments are responsible for the observed contaminations. For HCB and PFOS decreasing trends point towards effective emission reduction management. In contrast, further efforts are required in reducing diffuse and point source emissions of PBDEs. The detected congener patterns suggest that technical PentaBDE contributes to the observed contaminations. PentaBDE is banned since 2004. Decreasing trends, however, were detected only in the Rhine and at the Saar site S1, while a strong increase at the Saar site S2 points towards new emissions in this area. It is not clear whether these emissions are caused by technical PentaBDE itself or are the result of degradation of technical Octa- or DecaBDE. Regarding HBCDD, diffuse emissions seem to increase as indicated by the increasing trends of HBCDD in bream from many sites. This should be followed critically in future.

Altogether, the prevalence of declining trends indicates that pollution in German freshwaters is declining. This is also reflected in improved fish health as demonstrated by Teubner et al. (2015) for the ESB sites in German rivers.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.envpol.2016.06.060>.

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