PREDICTING THE QUALITY STATUS OF GERMAN RIVER WATERS BY ENVIRONMENTAL SPECIMEN BANKING: FIRST EXPERIENCES CONCERNING PCDD/Fs, DL-PCBs AND HEXACHLOROBENZENE IN BREAM

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Introduction

Aquatic organisms can bioaccumulate environmental contaminants via the surrounding water or the aquatic food chain. Fish or mussels are therefore suitable matrices to detect accumulating pollutants in the water to represent long-term trends, to track down causes of contamination and to document success of redevelopment measures.
The European "Directive on environmental quality standards in the field of water policy" lays down environmental quality standards (EQS) for 45 priority substances and certain other pollutants with the aim of achieving good surface water chemical status [1]. For the purpose of trend monitoring, sediment and biota are regarded as the most suitable matrices for many substances [2]. By the implementation of the provisions of this directive and its update in 2013, the compliance biotamonitoring of hazardous priority substances has gained national and international importance [1, 3]. The guidance document standardizing trends and compliance biota-monitoring for all water bodies in Europe is still work in progress.
The German environmental specimen bank (ESB), one of the largest specimen collection programmes world-wide, is sampling and storing targeted matrices across the whole biosphere and ecosphere [4]. This collection is able to deliver the basis for retrospective evaluations of baseline contaminations and time trends for toxic pollutants as shown in this study for polychlorinated dibenzo-p-dioxins and -furans (PCDD/Fs), dioxin-like PCB (dl-PCBs) and hexachlorobenzene (HCB). Here, data for PCDD/Fs, dl-PCBs and HCB in archived muscle of bream samples from 16 sampling sites in Germany are compared with the respective European environmental quality standards (EQS) in fish [3]. An EQS resembles the concentration of a particular pollutant or group of pollutants in water, sediment or biota which is considered safe for human health and environment [5].

Materials and methods

Samples. Breams (*Abramis brama*) are caught annually after spawning between mid-July and early October by the ESB Project Team, Trier University, Germany. At least 20 fish with an age between 8 to 12 years are taken at each sampling site. The muscles are pooled, grinded and stored as homogenised powder in sub-samples of about 10 g each at temperatures below -150 °C in an inert atmosphere resulting from evaporating liquid nitrogen by the Fraunhofer Institute for Molecular Biology and Applied Ecology (Fraunhofer IME), Department Environmental Specimen Bank, Schmallenberg, Germany. Collection and processing is performed under well-defined and reproducible conditions according to standard operating procedures [6]. Sampling areas (fig. 1) are the rivers Rhine, Saar, Danube and Elbe with the tributaries Mulde and Saale as well the lake Belauer See as a non-polluted reference area [7].

Analysis of PCDD/Fs and DL-PCBs. Bream samples were analysed within the frame of a retrospective study for 17 2,3,7,8-substituted PCDD/Fs and 12 dl-PCBs using high resolution gas chromatography and high resolution

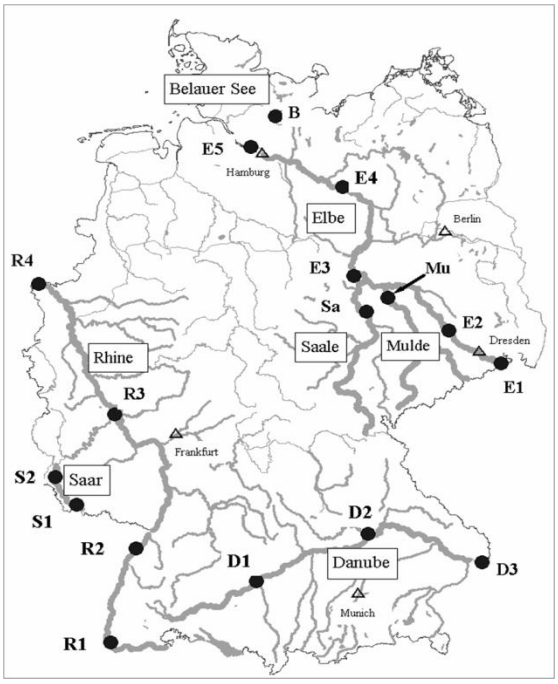

Figure 1: German river system and sampling sites

mass spectrometry (HRGC/HRMS) analogue to a method described before [8, 9]. TEQ values are calculated by using WHO-$TEFs_{2005}$ according to the lowerbound procedure. Results are related to the fresh weight of the samples.

Analysis of hexachlorobenzene (HCB). Bream samples were analysed on a yearly basis immediately after sample taking for continuous monitoring for a fixed set of organochlorine pesticides (including POPs like DDT, HCHs and HCB) since the late 1990s. Sample aliquots of about 5 g fresh weight material were homogenised with sodium sulphate followed by column extraction by means of hexane/acetone (v/v, 2:1). Before extraction, a mixture of ^{13}C-labelled internal standards (17 organochlorine pesticide-compounds) was added to the sample. An aliquot of the extract was used for gravimetric lipid determination after solvent evaporation. A multicolumn clean-up including alumina and florisil followed. $^{13}C_{12}$-PCB 105 was added to the final extract as syringe standard. The measurement was performed by HRGC/HRMS on Waters Autospec or DFS mass spectrometers. Quantification was done by means of isotope dilution method using multipoint calibration. QA/QC-measures for such a continuous monitoring were described before [10].

Analysis of time trends. Time trends are statistically analysed using a software tool from the German Federal Environment Agency (Umweltbundesamt (UBA); LOESS-Trend, Version 1.1, based on Microsoft Excel). This tool fits a locally weighted scatterplot smoother (LOESS, with a fixed window width of 7 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 [11].

Table 1: Description of sampling sites (amending figure 1)

Sampling site		**Years of sampling taking (HCB resp. PCDD/Fs + dl-PCBs)**
E1	River Elbe – Prossen (km 13)	1991, 1993 – 2013 resp. 2001 – 2007
E2	River Elbe – Zehren (km 93)	1993 – 2013 resp. 2003
E3	River Elbe – Barby (km 296)	1991, 1993 – 2013 resp. 2001 – 2007
E4	River Elbe – Cumlosen (km 470)	1991, 1993 – 2013 resp. 2003
E5	River Elbe – Blankenese (km 632)	1993 – 2013 resp. 2001 – 2007
Mu	River Mulde – Dessau (mouth)	1995 – 2013 resp. 2001 – 2007
Sa	River Saale – Wettin	1995 – 2013 resp. 2001 – 2007
R1	River Rhine – Weil (km 174)	1999 – 2013 resp. 2001 – 2007
R2	River Rhine – Iffezheim (km 334)	1995 – 2013 resp. 2001 – 2007
R3	River Rhine – Koblenz (km 590)	1999 – 2013 resp. 2001 – 2007
R4	River Rhine – Bimmen (km 865)	1999 – 2013 resp. 2001 – 2007
S1	River Saar – Güdingen	1999 – 2013 resp. 2001 – 2007
S2	River Saar – Rehlingen	1999 – 2013 resp. 2001 – 2007
D1	River Danube – Ulm (km 2593)	2001 – 2013 resp. 2002, 2004 – 2007
D2	River Danube – Kelheim (km 2404)	2001 – 2013 resp. 2002, 2004 – 2007
D3	River Danube – Jochenstein (km 2210)	2001 – 2013 resp. 2002, 2004 – 2007
B	Lake Belauer See	10 years irregularly between 1992 and 2013 resp. 2007

Results and discussion

According to the European Directive 2013/39/EU the following environmental quality standards (EQS) are applied for biota, i.e. fish: 0,0065 µg WHO-TEQ_{2005}/kg (being 6,5 ng WHO-TEQ_{2005}/kg) for the sum of PCDD/Fs and dl-PCBs resp. 10 µg/kg for HCB [3].

After 2001, levels of PCDD/Fs and dl-PCBs in bream were below the EQS at the sampling sites E3, E4, Mu, Sa, D1 and D2 in any year of sampling. Only the sampling sites S2 and R2 showed concentrations of PCDD/Fs and dl-PCBs above the EQS in each year of sampling. All other sampling sites showed lower levels of PCDD/Fs and DL-PCBs at least in the last year of sampling investigated here with the exception of D3. At this sampling site almost yearly fluctuating levels, ranging from 5,7 to 11 ng/kg, were observed. For Lake Belau, which is regarded as a non-polluted reference lake, about 0,43 ng WHO-PCDD/F-PCB-TEQ_{2005}/kg were found in bream muscle.

Levels of HCB in bream were below the EQS of 10 µg/kg at the following sampling sites in each year of sampling investigated here: S1, S2, D1, D2 and D3. The sites E1, E2, E4, E5, Mu and R2 exceeded the EQS in all samples investigated. Sampling sites showing levels of HCB below the EQS at least in the last years of

sampling were Sa, R1 and R4. For R3 HCB-levels were below the EQS in the past but increased to levels above the EQS over the very last years. For Lake Belau, which is regarded as a non-polluted reference lake, about 0,23 µg HCB/kg were detected in bream muscle.
Figures 2a and 2b show the time trends for HCB at the sampling site "E3 River Elbe – Barby" as an example. Red lines mark the EQS of 10 µg/kg for HCB. The blue lines show the linear trend which is significant if the stated value for p (linear) is below 0,05. The green lines show the non-linear trend, being also significant if p ("nicht-linear" meaning non-linear) is below 0,05. The grey shaded areas are the confidence intervals of the smoothing functions. These figures indicate that HCB-levels in bream at sampling site E3 were clearly above the corresponding EQS until 2004/2005. HCB-levels in bream at site E3, River Elbe – Barby, decline at a lower rate over the last years of sampling than in earlier times. Therefore, only the results since 2004 were considered to predict a future concentration of HCB to avoid an underestimation of the situation. On basis of this trend the expected values for HCB could be calculated as being 11 µg/kg in 2015 and 9 µg/kg in 2018 corresponding to a yearly decline of about 1,9 µg/kg. These results show that – provided that the environmental situation will not change – HCB-levels could be lowered below the EQS in the next years. But it should also be taken into account that this prediction contains a certain uncertainty depending on the length of the forecast period as shown in figure 2b.

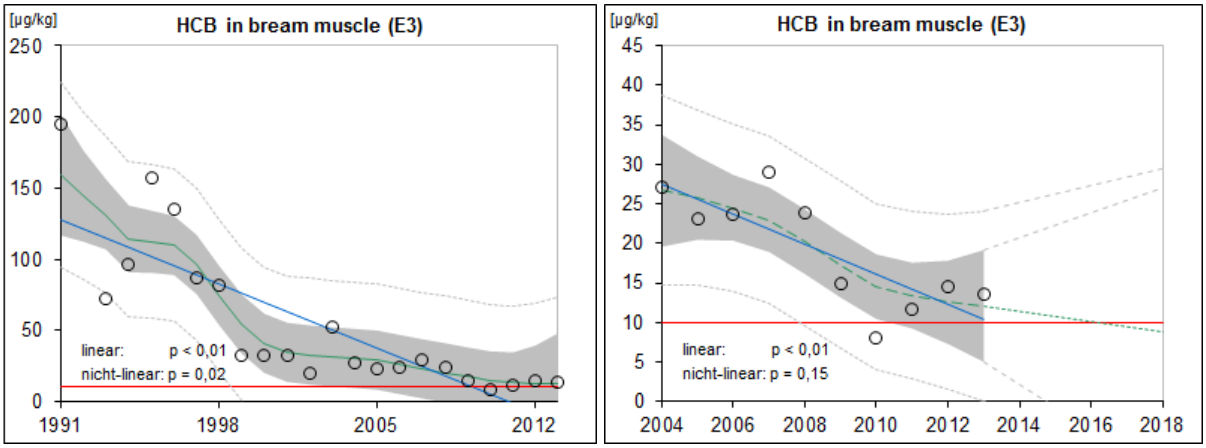

Figure 2: HCB at sampling site E3 (River Elbe –Barby): (a) sampling in 1991 to 2013 (left side), (b) segment for 2004 to 2013 inclusive of forecast until 2018 (right side)

An investigation of the HCB-data was performed for all other sampling sites according to the method described here for E3. For nearly all sampling sites a fair chance could be noted that environmental levels of HCB in bream may decline below the European EQS in the near future resp. these concentrations are already below this limit and might not rise above this limit – despite for the sampling sites E2, E4, E5, R2 and R3. Here it cannot be foreseen on basis of the available data that HCB-concentrations in bream will decline below the EQS.

Protection goals of the established EQS are either top predators (secondary poisoning via prey fish) or human health (consumption of fishery products). The mentioned EQS for hexachlorobenzene as well as PCDD/Fs and DL-PCBs were based on EQS values which were derived from human toxicity data [12, 13]. European Member States are likely to choose between different fish species for their biota monitoring programme. Based on the recommendations of a non-legally binding guidance document published by the European Commission the species to be selected should be representative in relation to the contaminant load and exposure at the studied monitoring site [2]. EU guidance for biota monitoring is currently prepared by an expert group at the European Commission. Depending on the protection goal either whole fish samples or filet can be considered appropriate for monitoring EQS. But various tissues within the same species may vary considerably with respect to concentration factors, bioaccumulation rates, metabolic capacity and / or excretion rates. In addition large differences among individual specimens could be noted for HCB in Wels catfish (*Silurus glanis*) caught from the Ebro River (Spain) with HCB-levels being in the range of 1,8 to 1017 µg/kg (n=27-29, median 20,4 µg/kg, mean 108 µg/kg) [14]. With regard to this background further information is needed to prove the fitness for purpose of

the sampling design and to show that bream muscle is an appropriate matrix for monitoring of HCB-, PCDD/F- and DL-PCB-levels in German river waters. Such information should include variations between bream and other fish species, between breams of different ages as well as variations between different tissues. A study, focussing on these points, is actually under work and results will be presented in due time.

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