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Biota monitoring under the Water Framework Directive: On tissue choice and fish species selection☆

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ABSTRACT

The study addresses the topic of suitable matrices for chemical analysis in fish monitoring and discusses the effects of data normalization in the context of the European Water Framework Directive (WFD). Differences between species are considered by comparing three frequently monitored species of different trophic levels, i.e., chub (*Squalius cephalus*, n = 28), (bream, *Abramis brama*, n = 11), and perch (*Perca fluviatilis*, n = 19) sampled in the German Danube. The WFD priority substances dioxins, furans and dioxin-like polychlorinated biphenyls (PCDD/F + dl-PCB), polybrominated diphenyl ethers (PBDE), α-hexabromocyclododecane (α-HBCDD), hexachlorobenzene (HCB), mercury (Hg), and perfluorooctane sulfonic acid (PFOS) as well as non-dioxin-like (ndl)-PCB were analyzed separately in fillet and carcass and whole body concentrations were calculated. Hg was analyzed in individual fish fillets and carcasses, all other substances were determined in pool samples, which were compiled on the basis of fish size (3 chub pools, 1 bream pool, 2 perch pools). The data were normalized to 5% lipid weight (or 26% dry mass in the case of Hg and PFOS) for comparison between matrices and species.

Hg concentrations were generally higher in fillet than in whole fish (mean whole fish-to-fillet ratio: 0.7) whereas all other substances were mostly higher in whole fish. In the case of lipophilic substances these differences leveled after lipid normalization.

Significant correlations ($p \leq .05$) were detected between Hg and fish weight and age. Hg concentrations varied least among younger fish. PCDD/F, dl-PCB, ndl-PCB, PBDE, α-HBCDD and HCB correlated significantly ($p \leq .05$) with lipid concentrations. Fillet-to-whole fish conversion equations and/or conversion factors were derived for all substances except α-HCBDD. Although more data also for individual fish would be desirable the results are nevertheless a step on the way to translate fillet concentrations of priority substances to whole fish concentrations.

1. Introduction

Biota monitoring has become a valuable instrument in environmental assessment complementing the analysis of water, suspended particulate matter and sediment especially in the case of those substances that tend to accumulate in organisms and are difficult to determine in other matrices. In the European Water Framework Directive (WFD) eleven substances and substance groups have been identified for which the assessment of compliance with environmental quality standards (EQSs) is required in biota. For nine of these the EQS refers to fish, i.e., dicofol, dioxins, furans and dioxin-like polychlorinated biphenyls (PCDD/F+dl-PCB), heptachlor and heptachlor epoxide, hexabromocyclododecane (HBCDD), hexachlorobenzene (HCB), hexachlorobutadiene (HBCDD), mercury (Hg), perfluorooctane sulfonic acid (PFOS), and polybrominated diphenyl ethers (PBDE) (EC, 2000, EC, 2013). The EQSs were derived for the protection goals 'human health' and 'secondary poisoning of wildlife' with the more sensitive protection

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goal being decisive for determining the EQS.

The biota monitoring community typically faces the questions of what fish species to choose, what size of fish to target, what matrix to analyze (e.g., fillet or whole fish), whether to pool samples or analyze individual fish, how to convert data from one matrix or species to another, and how to assess compliance with target values. Fish species and size play a crucial role in contamination especially when it comes to substances that bioaccumulate and biomagnify in the food web. Normally the contamination increases with trophic position and age of the fish (Driscoll et al., 2013; EC, 2014).

Decisions are mostly governed by the underlying question regarding the protection goals – does the program address primarily the human health aspect – which would favor the analysis of fillet of large (predatory) fish - or is its major focus on the protection of piscivorous wildlife and relatively small whole fish would be the appropriate matrix?

This in concert with the wide range of monitored fish species and the analysis of pool samples as well as individual fish has resulted in a wide variety of data sets that are difficult to compare.

Examples are, e.g., Germany, where some federal states have generated long time series of monitoring data analyzing fillets and/or livers of individual fish belonging to more than 30 species (e.g., FGG Elbe, 2016; Fliedner et al., 2016a; Guhl et al., 2014; ICPR, 2011). Additionally, the German Environmental Specimen Bank (ESB) has generated a broad data base for pool samples of bream fillets and livers (www.umweltprobenbank.de). Likewise, multiple data exist from fish monitoring in other countries, e.g., in Europe (compilations see EC, 2014; Fliedner et al., 2016b), the U.S., and Canada (Batt et al., 2017; Environment and Climate Change Canada, 2017; Lazorchak et al., 2003; Stahl et al., 2009, 2013, 2014; U.S. EPA, 2017; Wathen et al., 2015).

For ecological and economic reasons it would be desirable to address both protection goals, human health and the protection of piscivorous predators, in just one program by converting fillet data to whole fish or *vice versa* and translating data from one fish species to another and from young fish to old (or *vice versa*). Moreover, from the economic point of view pooling of samples would be preferable.

The EU Guidance document No 32 (EC, 2014) addresses these aspects in the context of EQS compliance monitoring and gives general recommendations. It states, for instance, that when monitoring fish fillets "… Conversion factors for fillet-to-whole fish contaminant levels should be used, when available, to give more accurate risk estimates for secondary poisoning. …. Thus, MS (Member States) that wish to consider this option should derive conversion factors for HCBD, dicofol, HBCDD, HCB, PFOS, and preferably mercury, before implementing such an approach". Alternatively, lipid-normalized concentrations in any matrix/tissue can be used, provided the contaminant concentrations correlate with the lipid content.

The present study addresses these issues by presenting data of a tailored monitoring study conducted in the Danube in 2015. The focus is on the aspect fillet *vs.* whole fish, younger *vs.* older fish, differences between fish species and effects of normalization.

The data are analyzed and discussed with respect to the following questions relevant for risk assessment and EQS compliance check:

- How do contaminant concentrations in fillet and whole fish relate to one another?
- What are the effects of data normalization to lipid (respectively dry mass in the case of Hg and PFOS)?
- Can data normalization overcome tissue and species specific differences in contamination thus superseding the need for monitoring different matrices (e.g., whole fish and fillet) and supporting the comparison between different monitoring programs?

2. Material & methods

2.1. Sampling

Chub (*Squalius cephalus*, n = 28), bream (*Abramis brama*, n = 11), and perch (*Perca fluviatilis*, n = 19) were sampled at Kelheim in the middle section of the German Danube. All three are frequent species in German freshwaters and are already included in national monitoring programs. The sampling site Kelheim (Danube km 2404) is located downstream of the confluence of Danube and Rhine-Main-Danube Canal and upstream of the barrage Bad Abbach (Fig. 1). It reflects the state of the shipped Middle Danube. Fish migration in this area is hampered by many barrages.

The sampling took place in September 2015 after the spawning season. It was performed on two consecutive days using gillnets. Until processing the fish were interim-stored in freezers up to 48 h. For every fish biometric data (length, weight, age, and sex) were recorded. Then one fillet was removed completely and separated from its skin while the second fillet remained on carcass. Fillet and carcass (including the second fillet and the skin of the removed fillet) were weight separately before being individually shock-frozen in liquid nitrogen. Next, the tissue was pre-crushed, cryo-milled and stored as homogenized powder at temperatures below −150 °C in an inert atmosphere to minimize chemical alterations (Rüdel et al., 2009, 2015; Rüdel and Weingärtner, 2008).

In the following, the term 'carcass' refers to the carcass plus the one remaining fillet.

2.2. Pool preparation

Hg was analyzed in fillet and carcass of individual fish while all other substances and substance groups were determined in pool samples of fillets, respectively carcasses. The pools were composed of fish of comparable size (Table S1, Supplementary material).

2.3. Chemical analysis

Fillet and carcass were analyzed for the WFD priority substances mercury (Hg), dioxins and furans (PCDD/Fs) and dioxin-like polychlorinated biphenyls (dl-PCB), polybrominated diphenyl ethers (PBDE, sum of BDE-congeners −28, −47, −99, −100, −153, −154), hexabromocyclododecane (HBCDD), hexachlorobenzene (HCB), and perfluorooctane sulfonic acid (PFOS). Additionally, non-dioxin-like (ndl-) PCB (sum of congeners CB-28, -52, −101, −138, 153, −180) were analyzed.

The analytical methods applied are widely used methods that are confirmed by regular analysis of certified reference materials and validated regularly in inter-laboratory proficiency test.

Analysis of Hg was performed at Fraunhofer IME by a dedicated atomic absorption spectrometry (AAS) method applying Direct Mercury Analyzer (DMA) instruments (Rüdel et al., 2010). All other substances were analyzed by Eurofins GfA Lab Service GmbH, Hamburg. The methods have been described in more detail in Fliedner et al. (2016b). Briefly, PCDD/F, PCB, and HCB were determined by high resolution gas chromatography and high resolution mass spectrometry (HRGC/HRMS). Liquid chromatography and tandem mass spectrometry (LC-MS/MS) was used for the analysis of HBCDD and PFOS. PBDE were determined by means of gas chromatography and mass spectrometry (GC/MS). Identification of target compounds was based on the comparison of retention time