Selection and application of trophic magnification factors for priority substances to normalize freshwater fish monitoring data under the European Water Framework Directive: a case study

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Abstract

Background: The European Water Framework Directive (WFD) requires the monitoring of biota—preferably fish—to check the compliance of tissue concentrations of priority substances (PS) against substance-specific environmental quality standards (EQSs). In monitoring programs, different fish species are covered, which often are secondary consumers with a trophic level (TL) of about 3. For harmonization, a normalization of monitoring data to a common trophic level is proposed, i.e., TL 4 (predatory fish) in freshwaters, so that data would be sufficiently protective. For normalization, the biomagnification properties of the chemicals can be considered by applying substance-specific trophic magnification factors (TMFs). Alternatively, TL-corrected biomagnification factors (BMFTLs) may be applied.

Since it is impractical to derive site-specific TMFs or BMFTLs, often data from literature will be used for normalization. However, available literature values for TMFs and BMFTLs are quite varying. In the present study, the use of literature-derived TMFs and BMFTLs in data normalization is studied more closely.

Results: An extensive literature evaluation was conducted to identify appropriate TMFs for the WFD PS polybrominated diphenyl ethers (PBDE), hexachlorobenzene, perfluorooctane sulfonate (PFOS), dioxins and dioxin-like compounds (PCDD/F + dl-PCB), hexabromocyclododecane, and mercury. The TMFs eventually derived were applied to PS monitoring data sets of fish from different trophic levels (chub, bream, roach, and perch) from two German rivers. For comparison, PFOS and PBDE data were also normalized using literature-retrieved BMFTLs.

Conclusions: The evaluation illustrates that published TMFs and BMFTLs for WFD PS are quite variable and the selection of appropriate values for TL 4 normalization can be challenging. The normalized concentrations partly included large uncertainties when considering the range of selected TMFs, but indicated whether an EQS exceedance at TL 4 can be expected. Normalization of the fish monitoring data revealed that levels of substances accumulating in the food web (TMF or BMF > 1) can be underestimated when relying on fish with TL < 4 for EQS compliance assessment. The evaluation also revealed that TMF specifically derived for freshwater ecosystems in Europe would be
Introduction

The European Water Framework Directive 2000/60/EC (WFD; [1]) aims to achieve a “good status” for all water bodies in Europe. One requirement is the compliance with a “good chemical status” of surface waters for which environmental quality standards (EQSs) for contaminants were derived [2]. While most of the EQSs refer to concentrations in water, the EQSs for a number of priority substances (PS) relate to biota. The monitoring of fish (or, in the case of polycyclic aromatic hydrocarbons, crustaceans, or mollusks) is recommended for compliance assessment [3]. However, European Union (EU) Member States may derive and implement EQSs for alternative biota taxa provided that they offer the same level of protection as the EQSs laid down in Directive 2013/39/EU [2].

Biota EQSs were derived to protect the health of human consumers and to prevent secondary poisoning of predatory wildlife by residues of PS [4]. The respective EQSs addressed by the WFD are directed to the receptor the most at risk (e.g., human health for hexachlorobenzene or secondary poisoning for mercury). Table 1 summarizes the EQSs for fish [2].

Fish monitoring has to rely on species abundant in the water body under consideration. This inevitably leads to the use of different fish species in Europe and even within one water body to assess compliance with the EQSs. Often, fish of a trophic level (TL) of about 3 (secondary consumers) are selected, because they are abundant in most waterbodies and reflect the prey of many top predators. To allow for spatial and temporal comparison of fish data, the EU WFD Guidance Document on Biota Monitoring [3] proposes normalizing the monitoring data of freshwater fish to a common trophic level (i.e., predatory fish with TL = 4, as these are preferably consumed by humans). It is assumed that TL 4-normalized monitoring data would be sufficiently protective (for both, top predators and human health), more comparable between sites and sampling campaigns, and less dependent on the locally occurring fish species. In case of PCDD/F + dl-PCB, a normalization may only be required in specific cases (e.g., for temporal or spatial comparisons), since the derived EQS can be applied to the organisms of different trophic levels [2].

In the EU guidance [3], also a normalization of the PS concentrations in fish to a lipid content of 5% (or, for PFOS and mercury to a dry weight content of 26%) is proposed. This additional lipid/dry weight normalization was not applied in this investigation, since the EQS compliance checking needs ww data (for comparison

Table 1 List of priority substances and EQSs for fish as µg kg⁻¹ wet weight (ww) according to Directive 2013/39/EU [2]. The last column lists the protection goals on which the EQS is based [3]

| Substance                                                                 | EQS for fish [µg kg⁻¹ ww] | Protection goal     |
|---------------------------------------------------------------------------|---------------------------|---------------------|
| Polybrominated diphenyl ethers (PBDE; sum of congeners BDE 28, 47, 99, 100, 153, 154)§ | 0.0085                    | Human health        |
| Hexachlorobenzene (HCB)                                                   | 10                        | Human health        |
| Perfluorooctane sulfonate (PFOS) and derivatives                          | 9.1                       | Human health        |
| Dioxins and dioxin-like compounds (PCDD/F + dl-PCB)§                      | 0.0065§                   | Human health        |
| (as WHO2005-TEQ)#                                                        |                            |                     |
| Heptachlor and heptachlor epoxide (sum)                                   | 0.0067                    | Human health        |
| Hexachlorobutadiene (HCBD)                                               | 55                        | Secondary poisoning |
| Hexabromocyclododecane (HBCDD; sum of α-, β- and γ-HBCDD)§               | 167                       | Secondary poisoning |
| Dicofol                                                                  | 33                        | Secondary poisoning |
| Mercury (Hg)                                                             | 20                        | Secondary poisoning |

§ Expressed as WHO toxicity equivalents (TEQ) according to Van den Berg et al. [5]

Groups of chemicals consisting of several congeners or diastereomers (details are provided as Additional file 1: section “List of compounds covered in the substance groups”)

EQS also applicable for mollusks, crustaceans
with the ww-based EQSs). It is also doubtful whether the 5% lipid content of fish is a realistic base for normalization of PS fillet concentrations. In a previous study [6], the lipid content of fish from six German water bodies was 1.5±0.5% (mean ± standard deviation; n =36) in fillets and 5.2±1.6% (n =36) in whole fish. The fish lipid contents were not correlated to the trophic positions (for example, predatory perch had often lowest fillet lipid contents in comparison to lower trophic level fish).

Normalizing substance concentrations in fish tissues to levels of fish of a higher TL requires the knowledge of the substance-specific biomagnification properties expressed as biomagnification factor (BMF) or trophic magnification factor (TMF). BMFs are defined as the ratio of fish to diet concentrations (which may be corrected for trophic level), whereas TMFs represent the diet-weighted averaged BMFs of chemical residues across food webs [7, 8] calculated from the slope of the logarithmic substance concentrations plotted against the trophic level of the investigated organisms [9]. While the BMF characterizes the increase of substance concentrations from prey to predator, the TMF integrates enrichment processes in the whole food web [10, 11]. An example of a pelagic food chain, in which chemicals are enriched at different levels from primary producers to consumers and from which TMFs can be derived, may be simplified as phytoplankton => zooplankton => plankton-feeding fish => predatory fish; if a BMF is calculated, especially the biomagnification in fish seems relevant in the present context (e.g., plankton-feeding fish => predatory fish).

The application of the TMF concept and its potential use for regulatory purposes has been described in previous publications [8, 9, 11]. When used for regulatory assessment of the chemicals’ bioaccumulation properties, e.g., under the European Regulation EC/1907/2006 (REACH—Registration, Evaluation, Authorisation and Restriction of Chemicals [12]), it is sufficient to know whether the TMF of a chemical is significantly >1 or not. By contrast, a more reliable and precise value is needed when the TMF is used in predictive models [10, 11].

TMFs often vary strongly depending on the characteristics of the studied food web (e.g., biodiversity, food web dynamics, energy, and nutrient cycling), season and temperature of the studied ecosystem, the sampling strategies and coverage of taxa and species, and the system dynamics [9, 13–20]. For TL normalization, it would, therefore, be ideal to derive the TMFs directly in the ecosystem under consideration. This, however, is impractical given the large number of WFD monitoring sites in Europe (about 30,000 for fish [21]).

To support and standardize the selection of appropriate TMFs from the literature for WFD purposes, Kidd et al. [22] recently proposed a step-by-step procedure. In the present study, a literature search was carried out to apply this approach to TMF values of a number of WFD PS and to identify possible pitfalls. The selected TMFs were then used for TL 4 normalization of monitoring data of fish (fillet and whole fish) from two German rivers representing three trophic levels. Special emphasis was put on the question of what TMF to choose if the substance, for which EQS compliance is to be assessed, includes several congeners, diastereomers, or compounds as is the case for PBDE, HBCDD and PCDD/F + dl-PCB. Furthermore, this contribution discusses the use of fish-derived BMFs adjusted to the difference of one trophic level between prey and predator [11] as possible alternative to TMFs in normalizing substance concentrations in fish from monitoring studies to fish of a higher TL.

Materials and methods

Chemicals considered in the study

The PS covered in the literature search are summarized in Table 1. Additional file 1: Table S1 lists the CAS registry numbers; for PS consisting of different congeners or diastereomers, the selected compounds are listed for which TMF data could be retrieved.

Literature search

For the literature search, different online databases were used (e.g., PubMed, Science direct, Scopus, and Google Scholar). Search terms applied were: trophic magnification, TMF, food web magnification, and biomagnification. The time period covered was 1995–2020. Original papers on TMF studies as well as reviews and meta-analyses (e.g., [14, 19]) were gathered and TMF values compiled in a data base.

For TL 4 normalization with TMFs from literature, Kidd et al. [22] recommend the selection of TMFs that were derived in the same or a similar ecosystem characterized for example by latitude, mean annual temperature, and ecosystem type (rivers or lakes). In the present study, the fish monitoring data to which the TMFs were to be applied refer to German rivers and the results of the study are intended to be used for TL normalization of fish monitoring data in the context of the European WFD. Thus, the focus of the literature review was on studies related to freshwater ecosystems (preferably riverine ecosystems) in Europe, North America, and North China, which are assumed to be comparable regarding climate and water conditions. This first selection included studies with common European but also with other fish species.
Selection of TMF for data normalization

The selection of appropriate TMFs followed the recommendations given in Kidd et al. [22]. As far as possible, TMFs relating to species similar to those occurring in European water bodies were selected. Where available, TMFs referring to pelagic food webs and whole fish concentrations were preferred. In all but two studies [38, 39] TMFs selected for normalization based on TL calculations with a trophic enrichment increment $\Delta^{15}N$ of 3.4‰.

Fish sampling, preparation, and analysis

Fish monitoring data used in the present study originate from two German sites, the Moselle River and a lake-like widening of the slow-flowing Havel River (Großer Wannsee). Details of fish sampling, preparation, and analysis are described in detail by Radermacher et al. [23] and Rüdel et al. [6].

Briefly, fish were caught with nets either by a federal state authority (Fischereiamt Berlin, sampling at Havel River; September 2016) or by an authorized commercial fisher (Moselle River, near Trier; October 2017). At each site, about 60 fish were caught (three species; about 20 fish per species grouped in two size classes). Sacrificed fish were either processed immediately or kept on ice until dissection (maximum storage on ice: 48 h). For further processing, this sample was allowed to thaw at room temperature for about 3 h.

Before processing, fish sizes were measured and scales were taken for age determinations of each individual (age determination by Institut für Angewandte Ökologie Dr. Schwevers, Kirtorf-Wahlen, Germany). Fish were dissected, and liver, brain (only sampled for some fish), one fillet, and the remaining carcass were weighed and frozen separately with liquid nitrogen, and stored until further treatment at $-150 \, ^\circ \mathrm{C}$ in the gas phase over liquid nitrogen. Sample treatment followed the procedures of the German environmental specimen bank (ESB) program [24].

Frozen fillet and carcass samples were manually crushed and cryo-milled [25]. Composite samples of fillets and carcasses were prepared from fish grouped according to their previously determined age. Proportional amounts of tissues of about 10 fish were applied for preparing six composite samples per sampling site (three species, two different size classes each).

Stable isotope determinations of fish composite samples were performed by agrosolab GmbH, Jülich, Germany, and evaluated for fish trophic level as described in Rüdel et al. [6]. Mussel samples collected in parallel to the fish samples from each site were used as baseline organisms (TL = 2). For the trophic level calculations according to Eq. (1), a trophic enrichment increment $\Delta^{15}N$ of 3.4‰ was applied [3, 26]:

$$TL_{fish} = (\delta^{15}N_{fish} - \delta^{15}N_{mussel}) / \Delta^{15}N + 2.$$  \tag{1}

Chemical analysis of PS in fish and mussel tissues followed WFD requirements [2] and technical specifications [27]. Fillet and carcass were analyzed for PCDD/F + dl-PCB (reported as WHO2005-TEQ excluding concentrations below the limits of quantification, LOQs), PBDE (EQS-conform as sum of six congeners; see Table 1), HBCDD (sum of $\alpha$-, $\beta$-, $\gamma$-diastereomers), PFOS, and HCB by Eurofins GfA Lab Service GmbH, Hamburg, Germany. The methods applied are outlined in Rüdel et al. [6]. Mercury was quantified by Fraunhofer IME following the procedure described by Ngquetseng et al. [28]. Fat content of all composite samples was determined according to Smedes [29]. Tissue dry mass contents were determined after freeze-drying.

Data are reported on a wet weight (ww) basis for fillet and whole fish. Whole fish concentrations of all analytical parameters were calculated from tissue weights and measured concentrations in fillet and carcass composite samples according to Bevelhimer et al. [30] (see also [31]).

Normalization approach

TL 4 normalization using TMFs: The EU guidance [3] proposes a normalization to a TL of 4 according to the following equation:

$$C_{TL-adj(y)} = C_{meas(y)} \cdot TMF^{4 - TL(x)},$$  \tag{2}

with $C_{TL-adj(y)} = $ TL-adjusted concentration of substance $y$, $C_{meas(y)} = $ measured concentration of substance $y$ in the sample, TMF = TMF of substance $y$, and $TL(x) = $ measured trophic level of fish sample $x$.

Important note: In cases where the applied TMF was derived from lipid-normalized biota concentrations, lipid-based concentrations have to be applied in Eq. (2) (concentration units: $\mu$g $kg^{-1}$ lipid weight). Accordingly, wet weight concentrations have to be used for wet weight-derived TMFs ($\mu$g $kg^{-1}$ wet weight). Since EQS compliance is checked on the basis of wet weight concentrations, all lipid-normalized, TL-adjusted data resulting from Eq. (2) have to be back-calculated to wet weight data. For this purpose, it is assumed that the lipid content of TL 4 fish is similar to the lipid content of the originally analyzed fish sample.

TL 4 normalization using BMFs: Normalization by applying a BMF$_{TL}$ (i.e., the BMF adjusted to a prey–predator difference of 1 TL of substance $y$) was performed analogously [11]:
\[ C_{\text{TL}, \text{adj}(y)} = C_{\text{meas}(y)} \times \text{BMF}^{(4 - \text{TL}(x))}. \quad (3) \]

For this equation, as mentioned above for Eq. (2), the basis of the BMF (lipid/wet weight) has to correspond to the measured concentration of the substance in the fish (same units). For the back-calculation of lipid-normalized data, it is also assumed that the lipid contents of the TL 4 fish and the measured fish is similar.

**Results and discussion**

**Literature search**

The results of the literature search are summarized in Table 2. Shown are only TMFs for which the correlation between TL and log-transformed concentrations was significant (\( p < 0.1 \)).

In total, 31 studies were identified that refer to freshwater ecosystems in temperate zones and report TMFs for at least one PS (Table 2). The vast majority of the studies (about 80%) refer to lake ecosystems; only five studies report TMFs for PS in riverine ecosystems. Overall, about 88 TMF values for 27 compounds including isomers, congeners, and diastereomers were retrieved.

Regarding the analyzed tissue of fish, about 50% of the studies and roughly 55% of the TMF values listed in Table 2 refer to substance concentrations in whole fish, while most others are based on fillet concentrations. One study used whole fish concentrations for small fish and fillet concentrations of larger ones to calculate the TMF [32], while Simonnet-Laprade et al. [33] also included whole fish concentrations calculated with conversion factors. The only TMF available for heptachlor epoxide is calculated from data including a marine food web with marine mammals [19, 34]. For dicofol, the estimated TMF is based on a number of bioconcentration factors (BCFs), some of which are based on studies including early life stages of fish, while others are based on model calculations [22].

In the stable isotope analysis, which is essential for the calculation of the trophic position of the analyzed organisms (and thus a pre-requisite for the TMF calculation), mostly an trophic enrichment increment from one trophic level to the next of \( \Delta^{15}N = 3.4\%o \) was applied [26]. Only a few studies used other factors, i.e., 2.79\%o [35], 3\%o [13], and 3.8\%o [36–40]. Jardine et al. [41] provide mercury TMFs for stream ecosystems based on \( \Delta^{15}N = 2 \%o \) and also recalculated TMFs using \( \Delta^{15}N = 3.4\%o \), which resulted in higher TMFs (i.e., TMFs of 1.6–4.8 for \( \Delta^{15}N = 3.4\%o \) vs. 1.3–2.5 for \( \Delta^{15}N = 2\%o \)).

In most studies, zooplankton served as baseline organisms for trophic position calculations (i.e., in 16 of 30 studies that reported 72% of all TMFs listed in Table 2). Others used long-living benthic invertebrates like bivalves and snails or herbivore fish. In one study, the TL calculation is based on biofilms [41]. No respective data were available for five studies.

The TMF data in Table 2 show a high variability. TMFs varied between different kinds of ecosystems and food webs (e.g., geographical location, pelagic or benthopelagic food webs, riverine or lake ecosystems, including invertebrates vs. only fish) and the type of analyzed tissue in fish (whole fish or fillet). Further factors that influenced the TMF calculation were the kind of data on which the TMF was based (i.e., wet weight data, dry weight data, or lipid weight data) and the applied enrichment increment for the trophic position calculations (e.g., in [41]).

**Selection of TMF for data normalization**

Data normalization in the present study focused on PBDE, PCDD/F + dl-PCB, HCB, HBCDD, PFOS, and mercury, whereas dicofol, HCBD, and heptachlor + heptachlor epoxide were not considered. In the case of heptachlor + heptachlor epoxide, all concentrations in fish analyzed here were below the LOQ (the available analytical method was also not sensitive enough to detect concentrations in the range of the EQS). Dicofol and HCBD were not analyzed, because concentrations in fish from German freshwaters are typically near or below the LOQ [62, 63].

In the following, the TMF selection process and problems encountered therein are discussed. According to Kidd et al. [22], if several appropriate TMF for one PS are available, a geometric mean value shall be calculated. The selected TMFs or geometric means used for normalization of the fish monitoring data in the present study are summarized in Table 3.

**TMF for groups of substances**

The TMF selection procedure proposed in [22] is suitable for single substances like HCB, or for compounds that usually are determined as single substances (PFOS isomers, measured, e.g., as linear PFOS, or mercury compounds, analyzed as total mercury). For those PS, however, that cover more than one chemical group, congeners or diastereomer, the selection of an appropriate TMF was more difficult. This applied to PCDD/F + dl-PCB for which the EQS refers to 7 polychlorinated dibenzo-p-dioxins, 10 polychlorinated dibenzofurans, and 12 dioxin-like polychlorinated biphenyls, to PBDE, where the EQS is based on the sum of six congeners, and to HBCDD for which the EQS refers to the sum of three diastereomers.

In principle, different approaches are conceivable here (the note stated for Eq. (2) regarding the base of the TMF
Table 2 Trophic magnification factors (TMFs) and biomagnification factors (BMFs) for WFD priority substances that refer to freshwater ecosystems in the temperate zones (Europe, North America, and North China)

| Substance, congener, diastereomer | TMF | BMF | Water body | Type of aquatic food web | Analyzed fish tissue | Source |
|-----------------------------------|-----|-----|------------|--------------------------|---------------------|--------|
| BDE 28                            | 0.53|     | Lake Erie, Can, USA | Pelagic fish | Whole fish | [42] |
| BDE 28                            | 1.6 |     | Lake Ontario, Can | Pelagic fish | Whole fish | [42] |
| BDE 28                            | 2.7 | 0.9–2.0 (TL-corrected, fish–fish) | Lake Erie, Can, USA | Pelagic fish | Whole fish | [43] |
| BDE 28                            | 0.6 | 0.78–0.60 (TL-corrected, zooplankton–fish) | Lago Maggiore, Italy | Pelagic | Fish fillet | [15]; [44] |
| BDE 47                            | 5.72|     | Lake Mjøsa, Norway | Pelagic | Fish fillet | [45] |
| BDE 47                            | 5.82|     | Lake Mjøsa, Norway | Pelagic | Fish fillet | [9] |
| BDE 47                            | 6.95|     | Lake Mjøsa, Norway | Pelagic | Fish fillet | [46] |
| BDE 47                            | 5.2 | 0.1–8.9 (fish–fish) | Lake Winnipeg, Can | Pelagic | Fish fillet | [47] |
| BDE 47                            | 1.31|     | Lake Baiyangdian, China | Benthopelagic | Fish fillet | [48] |
| BDE 47                            | 1.9 | 0.4–3.3 (TL-corrected, fish–zooplankton) | Lake Erie, Can, USA | Benthopelagic | Whole fish | [43] |
| BDE 47                            | 4.2 | 0.3–3.6 (TL-corrected, fish–fish) | Lake Erie, Can, USA | Pelagic fish | Whole fish | [43] |
| BDE 47                            | 6.34±1.19| 4.1–7.6 (mean TL-corrected fish–fish) | Lake Ontario, Can | Pelagic | Whole fish | [20] |
| BDE 47                            | 2.11|     | Lake Ontario, Can | Pelagic fish | Whole fish | [42] |
| BDE 99                            | 0.46|     | Lake Erie, Can, USA | Pelagic | Whole fish | [42] |
| BDE 99                            | 2.95|     | Lake Mjøsa, Norway | Pelagic | Fish fillet | [45] |
| BDE 99                            | 1.39|     | Lake Baiyangdian, China | Benthopelagic | Fish fillet | [48] |
| BDE 99                            | 2.43|     | Lake Mjøsa, Norway | Pelagic | Fish fillet | [9] |
| BDE 99                            | 6.36±1.19| 8.0–22.1 (mean TL-corrected, fish–fish) | Lake Ontario, Can | Pelagic | Whole fish | [20] |
| BDE 99                            | 0.23|     | Lake Erie, Can, USA | Pelagic fish | Whole fish | [42] |
| BDE 100                           | 1.82|     | Lake Baiyangdian, China | Benthopelagic | Fish fillet | [48] |
| BDE 100                           | 0.44|     | Lake Erie, Can, USA | Pelagic fish | Whole fish | [42] |
| BDE 100                           | 3.7 | 0.4–4.9 (TL-corrected, fish–fish) | Lake Erie, Can, USA | Pelagic fish | Whole fish | [43] |
| BDE 100                           | 6.18±1.19| 4.5–11.0 (mean TL-corrected fish–fish) | Lake Ontario, Can | Pelagic | Whole fish | [20] |
| BDE 153                           | 0.50|     | Lakes Maggiore and Lugano, Italy | Pelagic | Fish fillet | [15]; [44] |
| BDE 153                           | 0.28|     | Lake Baiyangdian, China | Benthopelagic | Fish fillet | [48] |
| BDE 153                           | 0.08|     | Lake Erie, Can, USA | Pelagic fish | Whole fish | [42] |
Table 2 (continued)

| Substance, congener, diastereomer | TMF       | BMF                  | Water body          | Type of aquatic food web | Analyzed fish tissue | Source       |
|-----------------------------------|-----------|----------------------|---------------------|--------------------------|----------------------|-------------|
| BDE 153                           | 6.34 ± 1.23 | 3.4–13.2 (mean TL-corrected, fish–fish) | Lake Ontario, Can   | Pelagic                  | Whole fish           | [20]        |
| BDE 154                           | 1.65      |                      | Lake Baiyangdian, China | Benthopelagic           | Fish fillet          | [48]        |
| BDE 154                           | 9.46 ± 2.17 | 2.9–7.6 (mean TL-corrected fish–fish) | Lake Ontario, Can   | Pelagic                  | Whole fish           | [20]        |
| BDE 154                           | 0.47      |                      | Lake Erie, Can, USA  | Pelagic fish             | Whole fish           | [42]        |
| Sum PBDE^2                        | 1.51 (sum 9 congeners) | 0.4–40 (TL-corrected, fish–fish) | Lake Ontario, Can   | Pelagic                  | Whole fish           | [43]        |
| HCB                               | 2.4       |                      | Lake Como, Italy     | Pelagic                  | Fish fillet          | [49]        |
| HCB                               | 2.9 ± 1.7 |                      | Lake Como, Italy     | Pelagic                  | Fish fillet          | [50]        |
| HCB                               | 2.1 ± 1.8 |                      | 5 lakes in Can and North USA^A | Pelagic                  | Fish fillet          | [50]        |
| PCB 77                            | 1.7–7.1 (fish–fish) |                      | Lake Ontario, Can   | Benthopelagic           | Whole fish           | [51]        |
| PCB 81                            | 2.67      |                      | Lake Hartwell*, SC, USA | Benthopelagic           | Whole fish           | [52]        |
| PCB 126                           | 2.96      |                      | Lake Ontario, Can   | Benthopelagic           | Whole fish           | [51]        |
| PCB 169                           | 3.20      |                      | Lake Ontario, Can   | Benthopelagic           | Whole fish           | [51]        |
| PCB 105                           | 4.01      |                      | Lake Hartwell*, SC, USA | Benthopelagic           | Whole fish           | [52]        |
| PCB 105                           | 3.52      |                      | Lake Ontario, Can   | Benthopelagic           | Whole fish           | [51]        |
| PCB 105                           | 1.46      |                      | Lake Kernaalanjärvi, Finland | Benthopelagic           | Fish fillet          | [35]        |
| PCB 114                           | 4.66      | 2.4–28 (fish–fish)   | Lake Ontario, Can   | Benthopelagic           | Whole fish           | [51]        |
| PCB 114                           | 1.27      |                      | Lake Kernaalanjärvi, Finland | Benthopelagic           | Fish fillet          | [35]        |
| PCB 118                           | 1.57      |                      | Lake Kernaalanjärvi, Finland | Benthoplegic            | Whole fish           | [35]        |
| PCB 118                           | 4.10      |                      | Lake Hartwell*, SC, USA | Benthopelagic           | Whole fish           | [52]        |
| PCB 118                           | 2.9       |                      | Lake Como, Italy     | Pelagic                  | Fish fillet          | [49]        |
| PCB 118                           | 3.40      | 1.3–8.8 (fish–fish)  | Lake Ontario, Can   | Benthopelagic           | Whole fish           | [51]        |
| PCB 118                           | 0.73      | 0.17–0.57 (TL-corrected; zooplankton-fish) | Lago Maggiore, Italy | Pelagic                  | Fish fillet          | [15]        |
| PCB 156                           | 6.24      |                      | Lake Hartwell*, SC, USA | Benthopelagic           | Whole fish           | [52]        |
| PCB 156                           | 1.63      |                      | Lake Kernaalanjärvi, Finland | Benthopelagic           | Fish fillet          | [35]        |
Table 2 (continued)

| Substance, congener, diastereomer | TMF | BMF | Water body | Type of aquatic food web | Analyzed fish tissue | Source |
|-----------------------------------|-----|-----|------------|--------------------------|----------------------|--------|
| PCB 156                           | 2.2 |     | Lake Como, Italy | Pelagic | Fish fillet | [49] |
| PCB 156                           | 4.06 | 2.1–15.6 (fish–fish) | Lake Ontario, Can | Benthopelagic | Whole fish | [51] |
| PCB 180                           | 4.58 |     | Lake Mjøsa, Norway | Pelagic | Fish fillet | [45] |
| PCB 180                           | 6.01 |     | Lake Mjøsa, Norway | Pelagic | Fish fillet | [9] |
| PCB 180                           | 1.2 |     | Lake Erie, Can | Benthopelagic | Whole fish | [53] |
| PCB 180                           | 1.79 |     | Lake Kernaalanjärvi, Finland | Benthopelagic | Fish fillet | [35] |
| PCB 180                           | 4.3±19 |     | 17 lakes in Can and North USA | Benthopelagic | Fish fillet | [50] |
| PCB 180                           | 3.8±19 |     | 5 lakes in Can and North USA | Pelagic | Fish fillet | [50] |
| PCB 180                           | 4.3 |     | Lake Como, Italy | Pelagic | Fish fillet | [49] |
| Sum PCBs$   | 2.40 |     | Lake Superior | Pelagic | Whole fish | [54] |
| Sum PCBs$   | 1.6 (FWMF; sum of 20 congeners) |     | Twelvemile Creek, SC, USA | Pelagic | Whole fish | [55] |
| Sum PCBs$   | 3.09 (sum of 127 congeners) |     | Lake Hartwell*, SC, USA | Benthopelagic | Whole fish | [52] |
| Sum PCBs$   | 1.6 (FWMF; sum 7 chiral congeners) | 2.5–5.5 (fish–fish) | Lake Superior | Pelagic | Whole fish | [37] |
| Sum PCBs$   | 1.44 (sum 8 congeners?) |     | Lake Ontario, Can | Benthopelagic | Whole fish | [51] |
| Sum PCBs$   | 2.8 (sum 31 congeners) |     | Lake Como, Italy | Pelagic | Fish fillet | [49] |
| α-HBCDD          | 2.58 |     | Ponds, Dahuangpu Wetland Natural Conservation, Tianjin, China | Benthopelagic incl. herring gull | Fish fillet | [56] |
| γ-HBCDD          | 4.8 | 0.1–6.3 | Lake Winnipeg, Can | Pelagic | Fish fillet | [36] |
| Sum HBCDDs       | 3.1 | 1.9 (arithmetic mean BMF fish–fish) | Lake Winnipeg, Can | Pelagic | Fish fillet | [36] |
| Sum HBCDDs       | 2.44 |     | Ponds, Dahuangpu Wetland Natural Conservation, Tianjin, China | Benthopelagic incl. herring gull | Fish fillet | [56] |
| PFOS               | 2.23 |     | Lake Ontario, Can | Pelagic fish | Whole fish | [42] |
| PFOS               | 6.3 (ww) |     | Lake Ontario, Can | Pelagic | Whole fish | [38] |
| PFOS               | 5.9 (ww) |     | Lake Ontario, Can | Pelagic | Whole fish | [39]; Δ15N = 3.8‰ |
| PFOS               | 6.9 (ww) |     | Lake Mjøsa, Norway | Pelagic | Fish fillet | [46] |
Table 2 (continued)

| Substance, congener, diastereomer | TMF | BMF | Water body | Type of aquatic food web | Analyzed fish tissue | Source |
|-----------------------------------|-----|-----|------------|-------------------------|----------------------|--------|
| PFOS                             | 3.0 (ww, sum linear + branched) | Lake Mergozzo, Italy | Pelagic | Fish fillet | [57] |
| PFOS                             | 4.2±0.87 (ww, sum PFOS) | Sum PFOS: 0.13–0.9 (ww, TL-corrected, fish–fish) | Lake Ontario, Can | Pelagic | Whole fish | [40]; Δ¹⁵N = 3.8‰ |
| PFOS                             | 3.8±0.98 (ww, sum PFOS) | 0.5–16 (ww, TL-corrected, invertebrate–fish) | Lake Ontario, Can | Benthopelagic | Whole fish | [40]; Δ¹⁵N = 3.8‰ |
| PFOS                             | 1.5 (ww, sum linear + branched) | 2.0–169 (ww, TL-corrected, macrophytes, invertebrates–fish) | River Orge*, France | Benthopelagic | Whole fish | [33] |
| PFOS                             | 2.4–4.1 (ww, sum linear + branched; geometric mean: 2.9) | | Rivers Rhône, Bourde, Furan, Luynes, France | Benthopelagic | Whole fish (partly calculated with CF) | [58] |
| Hg                               | 4.9–13 (ww, mean 6 years: 7.06 ww) | Lake Mjøsa, Norway | Pelagic | Fish fillet | [46] |
| Hg                               | 2.3 (dw); 2.4 (ww) | | Lake Mergozzo, Italy | Pelagic | Fish fillet | [57] |
| Hg                               | 1.3–2.5 (dw; mean: 1.9); 2.79 (ww) | 21 rivers, New Brunswick, Can | Benthopelagic incl. biofilm | Fish fillet | [41]; Δ¹⁵N = 2.0‰; italics: recalculated with ww data given by [14] |
| Hg                               | 1.6–4.8 (dw); 4.73 (ww) | 21 rivers, New Brunswick, Can | Benthopelagic incl. biofilm | Fish fillet | [41]; Δ¹⁵N = 3.4‰; italics: recalculated with ww data given by [14] |
| Hg                               | 5.4 | | River Laagen, Norway | Benthopelagic | Fish fillet | [13] |
| Hg                               | 3.98 | 3.21–668 (TL-corrected; zooplankton–fish) | Lago Maggiore, Italy | Pelagic | Fish fillet | [15] |
| MeHg                             | 2.8–6.0 (dw; mean: 3.5) | 1.8–94 (dw, TL-corrected, 21 rivers, New Brunswick, Can mean 4.5 ± 2.3 for predatory invertebrates – fish) | Benthopelagic incl. biofilm | Fish fillet | [41]; Δ¹⁵N = 2.0‰ |
| MeHg                             | 5.6–21.2 (dw) | 21 rivers, New Brunswick, Can | Benthopelagic incl. biofilm | Fish fillet | [41]; Δ¹⁵N = 3.4‰ |
| Heptachlor                        | 0.33 | | Lake Superior | Benthopelagic | Lake Superior: fish fillet (lake trout) and whole fish | [19] with data from [32] |
| Substance, congener, diastereomer | TMF | BMF | Water body | Type of aquatic food web | Analyzed fish tissue | Source |
|----------------------------------|-----|-----|------------|-------------------------|----------------------|--------|
| Heptachlor epoxide               | 4.93|     | Lake Superior, arctic marine food web | Benthopelagic or pelagic + marine mammals | Lake Superior: fish fillet (lake trout) and whole fish; Arctica: whole fish, blubber + muscle of mammals | [19] with data from [32] (Lake Superior) and [34] (Arctica) |
| HCBD                            | (No TMF available; no evidence on biomagnification; TMF set as 1.0) | | | | | |
| Dicofol                         | 0.75 (Estimated with an aquatic food web model) | | | | | [22] and references cited therein |

Data basis for TMFs and BMFs: lipid-normalized concentrations unless otherwise indicated (e.g., PFOS, Hg); significant correlation between TL and log-transformed concentrations (p ≤ 0.1)

CF: conversion factor F-WF; Can: Canada; SC: South Carolina; FWFMF: food web magnification factor (previously used term synonym for TMF); MeHg: methyl mercury. Trophic enrichment increment Δ15N used in TL calculation: 3.4‰ unless otherwise indicated; ww: wet weight; dw: dry weight

5 Sum of congeners that partly include - but are not restricted to—the WFD relevant congeners

*Includes benthic organisms

a Subgroup of the 17 lakes shown in the line above
and the concentrations units to be applied is also valid for the following Eqs. (4)-(7):

(1) Assess each congener or diastereomer of the respective group separately by normalizing the concentration of each congener or diastereomer using its specific TMF and use the sum of the normalized congener or diastereomer concentrations for EQS assessment, that is:

\[
C_{\text{TL}-\text{adj}} = \sum (C_{\text{meas}(a)} \times \text{TMF}_a^{(4-TL(x))} + C_{\text{meas}(b)} \times \text{TMF}_b^{(4-TL(x))} + \ldots).
\]

(2) Apply a mean TMF calculated from the individual TMFs of the congeners or diastereomers to the sum of all congeners, that is:

\[
C_{\text{TL}-\text{adj}} = \left( \sum C_{\text{meas}(a)} + C_{\text{meas}(b)} + \ldots \right) \times \text{TMF}_{\text{mean}}^{(4-TL(x))}. \tag{5}
\]

(3) Use a TMF, which is representative for a whole group (e.g., the TMF for one dl-PCB) or a worst case TMF WC (e.g., for the PBDE congener with the highest TMF), i.e.,

\[
C_{\text{TL}-\text{adj}} = \left( \sum C_{\text{meas}(a)} + C_{\text{meas}(b)} + \ldots \right) \times \text{TMF}_{\text{R or WC}}^{(4-TL(x))}. \tag{6}
\]
(4) Use a TMF derived for the whole group (TMF<sub>G</sub>), e.g., for total HBCDDs as quantified by gas chromatography), i.e.,

\[
C_{TL-adj} = \left( \sum C_{\text{meas}(a)} + C_{\text{meas}(b)} + \ldots \right) \times \text{TMF}_{G}^{(4-TL(x))}.
\]

(7)

Which approach to choose largely depends on the available TMFs and the structure of the monitoring data. Approaches (1) and (2) require complete data sets for all components of the respective group. If TMFs for certain chemicals are either not available or only for individual congeners or diastereomers, only approach (3) can be applied. Approach (4) is reasonable if TMFs are reported for the sum of congeners, diastereomers, or chemical groups, whereby the sum must include only the relevant congeners or diastereomers (e.g., those required for WFD EQS compliance assessment (Table 2).

In the present study with monitoring data of fish from German rivers (Additional file 1: Table S5), approaches (1) and (2) were applicable only to PBDE, because only for this substance group, TMFs were available for all relevant congeners (Table 2). In the case of PCDD/F + dl-PCB, no TMFs could be retrieved for dioxins, furans and some dl-PCB, so only approach (3) could be applied. Approach (4) was adopted for HBCDD for which TMFs for the sum of HBCDD diastereomers were available. Since mainly the three diastereomers α-, β-, and γ-HBCDD are found in organisms, one can assume that reported sum-TMFs also relate mainly to these three diastereomers. By contrast, reported sum-concentrations and sum-TMFs of PBDE and PCB mostly include many more congeners than relevant for EQS compliance assessment (Table 2).

To get an impression of the influence of the different TMF calculation approaches on normalization, approaches (1), (2), and (3) were compared using the PBDE monitoring data of fish from German rivers (Additional file 1: Table S5).

The geometric mean TMFs of single congeners used in approach (1) are shown in Additional file 1: Table S3 (i.e., pelagic food webs, derived for whole fish). Approach (2) applied a TMF of 1.73 [arithmetic mean of the congener-specific TMFs used in approach (1)]. In approach (3), the worst case TMF (i.e., the TMF for BDE 47) of 2.25 (geometric mean TMF for BDE 47, pelagic food web, derived for whole fish). Figure 1 shows the \( \sum_6 \) PBDE concentrations in fish fillet normalized to TL 4 using the different approaches (fillet data are shown, since this is the relevant tissue for compounds with human health-related EQS; Table 1).

Normalization to TL 4 resulted in higher PBDE concentrations for all fish except those of TL 4 (perch 2 in Fig. 1. Comparison of TL 4-normalization approaches using \( \sum_6 \) PBDE concentrations in composite samples of fillet (F) of fish from the rivers Moselle (M) and Havel (H). Samples in Moselle and Havel are arranged according to increasing TL. Approach (1): sum of TL 4-normalized PBDE congener concentrations; congener-specific TMFs (Additional file 1: Table S3) were used for normalizing single congener concentration, which were then summed up. Approach (2): sum of congener concentrations normalized to TL 4 by applying the arithmetic mean TMF of the individual congener TMFs (= 1.73) used in approach (1). Approach (3): sum of PBDE concentrations normalized to TL 4 using the geometric mean TMF for BDE 47 (= 2.25)
the Havel). A comparison of the different approaches shows that approach 2 leads to the lowest concentrations. Approaches 1 and 3 lead to comparable results with slightly higher concentrations when the “worst case” scenario was assumed with the TMF for BDE 47 (approach 3). Based on these findings, the TMF for the most accumulating congener (BDE 47) was used to normalize the ∑6 PBDE concentrations in fish (Table 3).

No TMFs were available for dioxins and furans. From the available data for dl-PCBs, the TMF of the most toxic congener (PCB-126) was selected for data normalization (Additional file 1: Table S2). Choosing TMFs for PBDE and PCDD/F + dl-PCBs that represent “worst case” scenarios is in line with the precautionary principle and should, therefore, be the preferred approach.

**TMFs based on different ecosystem types or reference values**

For non-polar PS, most freshwater TMFs available in the literature refer to lake ecosystems and are based on lipid-normalized concentrations (Table 2). Except for HCB, TMFs based on whole fish concentrations could be retrieved.

For PFOS and mercury, the data were more variable as suitable TMFs for stream ecosystems were also available [14, 33, 41, 58]. In the case of PFOS, TMFs related to wet weight concentrations, and whole fish TMFs were available. By contrast, for mercury only fillet-based TMFs were available and the basis of the concentrations from which the TMF were derived differed: Jartun et al. [46] derived a TMF for mercury based on wet weight concentrations, whereas the TMFs of Govaerts et al. [13] and Guzzella et al. [15] refer to lipid-normalized concentrations, and Jardine et al. [41] report dry weight-based TMFs. Mazzoni et al. [57] report wet weight and dry weight-based TMF for the same food web and found no significant differences between both. In the comprehensive meta-analysis by Lavoie et al. [14], TMFs for mercury are based on wet weight concentrations that have in part been recalculated from dry weight data using either the organisms’ water content given in the respective publication or—in the case no water content had been reported—on a default value of 75 ± 8% water content. The latter decision may cause some uncertainty since mussels and invertebrates usually have higher water contents than 75% (partly more than 90%) [24, 64, 65]. Using too low water contents potentially yields lower target substance concentrations in low TL biota and therefore higher TMFs.

The variability of the TMFs makes the selection of a suitable TMF for TL 4 normalization difficult. In the present study, we considered TMFs based on wet weight concentrations only for TL 4 normalization of mercury (whole fish) and PFOS (fillet) concentrations in fish from German rivers. Figures 2 and 3 illustrate the effects of different ww-based TMFs for these PS on TL 4-normalized data. Shown are the tissue concentrations relevant for the respective EQS protection goal [i.e., whole fish for Hg (secondary poisoning) and fillet for PFOS (human health); Table 1]. The respective data are summarized in Additional file 1: Table S4.

Compared to measured concentrations, normalization to TL 4 resulted in higher mercury concentrations (Fig. 2). The extent of this effect depended on the TMF and the TL of the fish.

For fish from the Moselle, mercury concentrations in composite samples of chub (TL 2.9 and 3.0) and roach (TL 3.0 and 3.1) increased by factors 3.6–5.5 after normalization with highest increases associated with TMF 4.73. By contrast, mercury levels in perch (TL 3.8 and 3.9) were barely affected by normalization (factors 1.2–1.5 between measured and TL 4-normalized concentration) due to their already high TL. With respect to EQS compliance, all samples exceed the EQS of 20 µg kg⁻¹ ww after TL 4 normalization, whereas both roach samples comply with the EQS when measured concentrations are used for assessment.

The picture was similar for fish from the Havel: Mercury concentrations in bream (TL 2.9 and 3.1) and roach (TL 3.2 and 3.3) were strongly affected by TL 4 normalization (factors 2.7–5.5 between TL 4 normalized and measured concentrations), while concentrations in perch (TL 3.8 and 4.0) changed only marginally or not at all (factors 1.0–1.4). The strongest effect was observed for the sample “bream 1” which was the only sample to comply with the EQS when measured concentrations are considered.

A similar picture emerges for PFOS. While concentrations in perch fillet hardly changed with TL 4 normalization, they increased strongly in fish of low trophic levels depending on the TMF (Fig. 3). This substantially altered the compliance with the EQS of 9.1 µg kg⁻¹: In both rivers, only perch exceeded the EQS when the assessment was based on measured concentrations. After TL 4 normalization, EQS compliance was only detected for roach samples from the Havel. All other samples clearly exceed the EQS by factors of 1.4–3.4.

The findings highlight the flaws associated with a TL 4 normalization of data: A correction for the trophic level may lead to a more consistent EQS compliance assessment (e.g., for mercury: after TL 4 normalization, all concentrations are exceeding the EQS). However, the TL 4 normalized data may be much higher than those actually observed in TL 4 fish (Fig. 2).

Kidd et al. [22] recommend to use TMFs of similar ecosystems. However, appropriate TMFs for riverine ecosystems—as required in the present study with fish
monitoring data from German rivers—were available only for PFOS and Hg. Accordingly, only for these two substances, it was possible to study whether fundamental differences are to be expected when using TMFs for lake ecosystems instead of river TMFs for data normalization. For PFOS, TMFs for benthopelagic river food webs in southeastern France and near Paris [33, 58] range between 1.5 and 4.1, while TMFs for lakes (i.e., Lake Ontario) are in the range of 4.2–5.9 for pelagic food webs and around 3.9 when benthic organism is included [39, 40] (Table 2).

For mercury, river TMFs are available for rivers in New Brunswick (Canada) [41] and Norway [13], and are based on dry weight and lipid-normalized mercury concentrations, respectively. For lake ecosystems, TMFs are available from Italy (wet weight-based [57] and lipid-based [15]) and Norway (wet weight-based [46]) (Table 2). Lavoie et al. [14] calculated different wet weight-based TMFs for mercury, e.g., TMFs of 3.7 ± 5.4 for lake freshwater food webs and 3.7 ± 3.6 for riverine food webs, as well as TMFs of 4.5 ± 5.2 for freshwater ecosystems in temperate zones, 3.8 ± 2.9 for freshwater food webs that include fish and other species, and 3.6 ± 2.1 for mesotrophic freshwater food webs. The overall TMF for mercury including all studies was 4.3 ± 4.8.

These data show that the variability of TMFs within one ecosystem type (i.e., lake or river) can be large and may overrule the differences between rivers and lakes. Based on a global analysis, Walters et al. [19] suggest that TMFs are primarily influenced by food web composition, whereas latitude and associated temperature-dependent factors are not as relevant. For mercury, by contrast, latitude, next to the trophic state of the ecosystem, seems to be an important factor in biomagnification [13, 14]. Moreover, TMFs may vary by season due to changes in exposure and food web dynamics (e.g., [9, 15–17]).

**Application of selected TMF for TL normalization of fish monitoring data**

Based on the data compiled in Table 2 and the considerations on the handling of substances consisting of more than one congener or diastereomer or for which TMFs from different ecosystem types are available, the following TMFs for the WFD PS covered in the present

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**Fig. 2** Effect of TMF choice on TL 4-normalized mercury concentrations (µg kg$^{-1}$ ww) in composite samples of whole fish (W) from the rivers Moselle (M) and Havel (H). TMF 4.73: geometric mean for stream ecosystems and Δ^15N of 3.4 ‰ [41] recalculated from dw- to ww basis by [14]; TMF 4.31: geometric mean of lake [46, 57] and stream ecosystems [14, 41]; TMF 4.12: geometric mean of lake ecosystems [46, 57]; samples in Moselle and Havel are arranged according to increasing TL; red line: EQS (20 µg kg$^{-1}$ ww)
evaluation were selected for normalization of the fish monitoring data from German rivers (Table 3).

Measured and TL 4-normalized concentrations of PS in fish from the rivers Moselle and Havel are shown in Additional file 1: Table S5 and S6. Considering the relevant matrices for EQS exceedance (Table 1), TL 4 normalization had little effect on EQS compliance or exceedance in the case of PCDD/F + dl-PCB, PBDE, HCB, and HBCDD, whereas for mercury and PFOS, the assessment partially changed.

In the case of PCDD/F + dl-PCB, when measured concentrations in fillet are used for compliance check, no sample exceeded the EQS and it was only one sample (Havel bream 2) when the assessment was based on TL 4-normalized concentrations (Additional file 1: Table S6). For PBDE, 100% EQS exceedance was observed for both measured and TL 4-normalized fillet concentrations, whereas concentrations of HCB in fillet and HBCDD in whole fish were always well below the respective EQS.

Concentrations of mercury exceeded the EQS in 9 of 12 whole fish samples when measured concentrations were used for assessment, while all samples exceeded the EQS after TL 4 normalization. In the case of PFOS, only 4 of 12 fillet samples (perch from Moselle and Havel) had concentrations above the EQS when measured data were considered, whereas 10 of 12 samples exceeded the EQS when normalized concentrations were used for compliance assessment.

These results highlight the need for caution in interpreting measurement data in terms of compliance with the EQS. As shown by the results for Hg and PFOS, an EQS compliance assessment based solely on measured concentrations in fish of lower trophic levels can be misleading: the status of the waterbody would be considered good, although it is not.

**TL 4 normalization based on trophic level-corrected BMF (BMF_{TL})**

Kidd et al. [22] suggest that in case no appropriate TMFs are available, TL 4 normalization may resort to BMFs if these reflect a TL step of 1. When dealing with simple food webs, a fish-to-fish BMF may be a good alternative to the TMF, because TL differences between two fish species are small (1–2 TL) and variations in BMFs are expected to be less pronounced than in TMFs, which average biomagnification across the whole food web [15]. However, apart from fish–fish pairs, BMFs are also calculated for other prey–predator relationships.
At best, published BMFs are available that are corrected for TL. The derivation of TL-corrected BMFs (and their relation to TMFs) is discussed, e.g., by Conder et al. [11], Poma et al. [44], and Guzzella et al. [15]. A report of the European Centre for Ecotoxicology and Toxicology of Chemicals [66] discusses the comparability of field-derived TMFs and BMFs, and concludes that BMF_{TL}s can serve as substitutes for TMFs when assessing biomagnification of substances in case no TMFs are available.

Among the studies compiled here, six report TL-corrected BMFs derived in addition to TMFs from parallel measurements of fish of different TL from one ecosystem (i.e., [15, 20, 33, 40, 41, 43]; Table 2).

Most data are available for PBDE. Guzzella et al. [15] calculated TL-corrected zooplankton-fish BMFs for a number of PBDE and PCB congeners for a food web in the Lago Maggiore, and compared these to the respective TMF values. Pérez-Fuentetaja et al. [43] report TL-corrected BMFs and TMF for individual PBDE congeners and the sum of 10 PBDE congeners for two kinds of food webs in Lake Erie, i.e., one including zooplankton, benthic invertebrates, and fish, and the other including only fish. Kurt-Karakus et al. [20] analyzed a pelagic food web in Lake Ontario and derived TL-corrected BMFs and TMF values for some PBDE congeners. Furthermore, they studied the effects of the structure of the food web by calculating TMFs for “simplified” food webs by excluding species.

In all three studies, the calculation of TL-corrected BMFs [BMF_{TL}, calculated according to Eq. (8)] was based on lipid-normalized PBDE congener concentrations using the equation:

\[
BMF_{TL} = \frac{C_{\text{predator}}/C_{\text{prey}}}{(TL_{\text{predator}}/TL_{\text{prey}})}.
\] (8)

Houde et al. [40] analyzed PFOS accumulation in a pelagic and a benthopelagic food web in Lake Ontario and derived wet weight-based BMF_{TL}s also using Eq. (8).

By contrast, Jardine et al. [41] and Simonnet-Laprade et al. [33] determined TL-corrected BMFs (designated as BMF_{TL}s) for benthopelagic food webs in streams with an equation proposed by Conder et al. [11]:

\[
BMF_{TL} = 10^{\left(\log(C_{\text{predator}}/C_{\text{prey}})/(TL_{\text{predator}} - TL_{\text{prey}})\right)}.
\] (9)

Jardine et al. [41] studied food webs including biofilms in Canadian streams and calculated BMF_{TL} and TMF values for methyl mercury (MeHg) based on dry weight concentrations, while Simonnet-Laprade et al. [33] report the BMF_{TL} and TMF for PFOS obtained in the Orge River in France based on wet weight concentrations.

The suitability of field-derived BMF_{TL}s for TL 4 normalization of fish monitoring data was studied more closely for PBDE and PFOS. For both substances, BMF_{TL}s for pelagic food webs and predator–prey pairs of fish–fish were available, i.e., from Pérez-Fuentetaja et al. [43] and Houde et al. [40], respectively. Since the BMF_{TL}s given in the publications were calculated according to Eq. (8), we recalculated the values as BMF_{TL} using Eq. (9), because, according to Conder et al. [11], TL-corrected BMFs calculated in this manner compare best to TMFs.

For PBDE, the calculation was based on lipid-normalized concentrations of the WFD relevant congeners in smallmouth bass (TL about 4.4), round goby (TL about 3.5), steelhead trout (TL about 4.6), and emerald shiner (TL about 3.75) [43]. For each congener, BMF_{TL}s were calculated for the predator–prey pairs smallmouth bass–round goby and steelhead trout-emerald shiner. The arithmetic mean BMF_{TL} of 4.54 was used for TL 4 normalization of fillet concentrations of fish from Moselle and Havel.

Regarding PFOS, the wet weight-based concentrations of total PFOS in lake trout (TL 4.0), alewife (TL 2.3), rainbow smelt (TL 3.1), and slimy sculpin (TL 3.2) [40] were used to calculate BMF_{TL}s for the predator–prey pairs trout-alewife, trout-rainbow smelt, and trout-sculpin. For TL 4 normalization of fish fillet data from Moselle and Havel, the arithmetic mean BMF_{TL} of 0.82 was used. The results of the data normalization using BMF_{TL}s are shown in Table 4. For comparison, the TMFs used in the present study are included in Table 4.

For PFOS, the differences between TL-normalized concentrations based on the BMF_{TL} and those calculated with the TMF were quite high in fish from lower trophic levels (chub, roach, and bream). In contrast, the corresponding PBDE data differed only slightly. Due to the uncertainty introduced by the range of TMFs included in the geometric mean calculation, the concentration ranges of the BMF_{TL}- and TMF normalized data overlap (refer to “Assessment of the uncertainty of the TL 4 normalization” section). For perch, the BMF_{TL} and TMF normalizations show only small differences, because the TL difference to TL 4 is only low.

In the case of PFOS, it is doubtful whether the trophic level normalization of fish concentrations using a TMF is appropriate. Noticeable is the fish–fish BMF_{TL}<1, in comparison to the selected TMFs in the range 1.5–4.1 derived from whole food webs (Table 3). Apparently, PFOS biomagnification in fish is lower than at lower trophic levels (see also discussion in [57]). Thus, the PFOS tissue levels calculated on base of the TMF may be too high (which, however, would be a conservative approach since it results in higher concentrations applied for the EQS compliance check).
The BMF TL s differed from the TMFs, and accordingly, the resulting TL 4 normalized concentrations also differed. In the case of PBDE, the BMF TL was twice as high as the TMF and the normalized concentrations using the BMF TL were also mostly higher. For PFOS, it was the other way around. The BMF TL used here was very low, because in the study of Houde et al. [40], the top predator (lake trout) had accumulated comparably low PFOS concentrations. Thus, when applying this BMF TL to the monitoring data from Moselle and Havel, the resulting concentrations were mostly lower compared to those normalized with the TMF.

These data show that the selection of appropriate BMF TL s can be quite a challenge. On one hand, only very few suitable field-derived BMF TL s are available from the literature. On the other hand, field-derived BMF TL s exhibited larger variations than TMFs depending, e.g., on the predator–prey pairs and on uncertainties in feeding ecology of [9–11]. The application of an unsuitable BMF TL may lead to erroneous results when used in TL 4 normalization. Possibly, high quality laboratory-derived BMFs would be a better alternative to field-derived values [10, 22]. This, however, needs to be studied more closely in future evaluations.

### Assessment of the uncertainty of the TL 4 normalization

A number of uncertainties should be considered when using the normalization approach according to the EU guidance document [3]: First of all, the uncertainty of the fish analysis has to be mentioned (analysis of the PS concentrations). Depending on compound and concentration level, the extended measurement uncertainty is in the range of 10–30% of the PS concentration [6]. Moreover, the measurement of δ15N for the determination of the trophic level of the fish is subject to some uncertainty (estimated: ±0.1–0.2 TL). Another uncertainty comes from the assumption that the TL 4 fish has the same lipid content as the analyzed fish. For the two sites investigated here, factors of 4 and 2 were found between the lowest and highest lipid content (both, in fillet and whole fish; Table S5, Additional file). Last but not least, the choice of the TMF adds uncertainty to the TL adjustment of PS concentrations. As shown in Table 3, the range of the TMFs that were selected for the geometric mean calculation can be quite high (e.g., for PBDE, a factor of about 14 between lowest and highest selected TMFs).

When looking at Eq. 2, the highest uncertainty seems to be introduced by the TMF. To illustrate this, TL-normalized concentrations based on different TMFs were calculated for the three PS for which several TMFs had been selected and corresponding geometric TMFs calculated (i.e., PBDE, HCB, and PFOS; Additional file 1: Tables S7–S9). The effect of TL normalization is the highest for fish with the lowest measured TL (here: chub, roach) and lowest for perch (measured TL only slightly different from TL 4). For the TMF range found for PBDE (0.46–6.41; Table 3), the TL-normalized concentrations for chub 1, Havel river (TL 2.9) range from 0.5 to 8.2 µg kg⁻¹ (factor 17; Additional file 1: Table S7). For perch 1 from the Havel river (TL 3.9), the concentration span is lower (factor 1.3). The uncertainty of the TL 4 normalization of the HCB fish concentrations (Additional file 1: Table S8) was only small, since the TMF range was small, too. For PFOS, again, a larger uncertainty is observed due to the range of the selected TMFs (1.5–4.1; Table 3). This results in a factor of 3 between the TL-normalized concentrations calculated with the lowest TMF and those

### Table 4 Comparison of TL 4 normalized PBDE and PFOS concentrations (µg kg⁻¹ wet weight) in fillet (F) of fish from the rivers Moselle (M) and Havel (H) using field-derived TMFs and BMF TL s (fish–fish) from literature

| Sample | PBDE (sum WFD relevant congeners) [µg kg⁻¹ ww] | PFOS [µg kg⁻¹ ww] |
|--------|-----------------------------------------------|------------------|
|        | BMF TL 4: 4.54ᵃ | TMF: 2.25ᵇ | BMF TL 4: 0.82ᶜ | TMF: 2.60ᵈ |
| Moselle, fillet | | | |
| Chub 1 M F | 5.65 | 2.61 | 4.28 | 15.2 |
| Chub 2 M F | 5.58 | 2.77 | 3.99 | 12.6 |
| Roach 1 M F | 1.36 | 0.68 | 3.61 | 11.4 |
| Roach 2 M F | 2.54 | 1.35 | 3.53 | 10.0 |
| Perch 1 M F | 1.58 | 1.47 | 10.9 | 12.2 |
| Perch 2 M F | 1.76 | 1.53 | 14.6 | 18.4 |
| Factor max/min | 4.13 | 4.10 | 4.14 | 1.85 |
| Havel, fillet | | | |
| Roach 1 H F | 0.72 | 0.44 | 1.36 | 3.05 |
| Roach 2 H F | 2.35 | 1.34 | 1.54 | 3.87 |
| Bream 1 H F | 2.01 | 0.93 | 3.83 | 13.6 |
| Bream 2 H F | 1.63 | 0.87 | 3.88 | 11.0 |
| Perch 1 H F | 0.22 | 0.19 | 12.9 | 16.2 |
| Perch 2 H F | 0.18 | 0.18 | 16.0 | 16.0 |
| Factor max/min | 13.4 | 7.62 | 11.8 | 5.33 |

In Italics: Factors between minimum and maximum concentrations for the respective sample set

-ᵃ Arithmetic mean fish–fish BMF TL based on lipid-normalized data of Pérez-Fuentetaja et al. [43], recalculated according to [11]
-ᵇ Geometric mean TMF for BDE 47 [20, 42, 43]
-ᶜ Arithmetic mean fish–fish BMF TL based on wet weight data of Houde et al. [40], recalculated according to [11]
-ᵈ Geometric mean TMF for stream ecosystems [33, 58]
calculated with the highest TMF value (Additional file 1: Table S9).

As discussed above, TL 4 normalization for mercury reduced the uncertainty of the EQS compliance assessment: normalization resulted in a more consistent assessment (the lower TL fish also exceeded the EQS after TL4 normalization). However, calculated TL 4 concentrations were higher than the measured data for perch. In the case of PFOS, the assessment outcome was also more consistent (although not all lower TL fish at the Havel site exceeded the EQS), but the concentrations of TL 4 normalized lower TL fish were mostly in the same range as those measured for perch. The normalization of PS fish concentrations to fish of TL 4 did not change the assessment of EQS compliance or exceedance in the case of HCB (all concentrations before and after normalization compliant) and PBDE (all fish concentrations above the EQS).

Conclusions
Published TMFs and BMFs for the WFD PS are quite variable and the choice of appropriate values for TL 4 normalization can be challenging. Here, the approach suggested by Kidd et al. [22] was helpful to guide the selection of relevant TMFs. Despite the uncertainties associated with the published values, they could, nevertheless, be used for TL 4 normalization of fish monitoring data. Only in the case of PBDE, the high range of the TMF found in the previous studies introduces a high uncertainty (Table 3). Normalization of the data ultimately draws attention to the fact that, when relying on fish with TL < 4 for EQS compliance assessment, water pollution associated with substances that accumulate in the food web (TMF, BMF > 1) can be underestimated. The normalized concentrations are of course subject to uncertainties, but they do indicate whether an exceedance of EQS at trophic level 4 is likely.

The present study highlights that in the context of the WFD [1] additional TMF data for freshwater ecosystems in Europe would be of benefit. This would make more suitable TMFs from comparable ecosystems available. Future TMF studies should follow the recommendations outlined by Borga et al. [9] and Conder et al. [11] and consider aspects discussed in Kidd et al. [22]. Ideally, future TMF studies should try to cover the analysis of a large number of chemicals with a broader range of properties. This would allow a better comparability of data (benchmarking) and may even offer the possibility, given the database is large enough, to apply quantitative structure–activity relationships (QSARs) to estimate TMFs for additional compounds of interest.

Field-derived BMF_{TLs} may not be an appropriate alternative to TMFs, because they can vary even stronger than TMFs depending—among others—on the respective predator–prey pairs (e.g., BMF_{TL5} for PFOS in the range of 2–169 for fish-invertebrate pairs in a French river [33]). Uncertainties related to field-derived BMF_{TLs} have already been discussed elsewhere (e.g., [10, 66]). Whether laboratory-derived BMF_{TLs} are suitable substitutes for TMFs needs further investigation.

Supplementary information
Supplementary information accompanies this paper at https://doi.org/10.1186/s12302-020-00404-8.

Additional file 1. Supporting information on evaluations and detailed data tables: List of compounds covered in the substance groups to which the WFD EQS refers; Additional information to Table 3; Additional Tables S1—S9; References for the Additional file.

Abbreviations
BCF: Bioccentration factor; BDE: Brominated diphenyl ether; BMF: Biomagnification factor; BMF_{TL}: Trophic level-corrected BMF; C: Concentration; Can: Canada; dw: Dry weight; ESB: Environmental specimen bank; EQS: Environmental quality standard; EU: European Union; FWMF: Food web magnification factor; H: Havel River; HBCDD: Hexabromocyclododecane; HCB: Hexachlorobenzene; HCBD: Hexachloroburadiene; Hg: Mercury; LOQ: Limit of quantification; M: Moselle river; MeHg: Methyl mercury; PBDE: Polybrominated diphenyl ethers; PCB: Polychlorinated biphenyls; PCDD/F + dioxins and dioxin-like compounds; PFOS: Perfluorooctane sulfonate; PS: Priority substances; TEQ: Toxicity equivalents; TL: Trophic level; TMF: Trophic magnification factor; W: Whole fish; WFD: Water Framework Directive; WHO: World Health Organization; ww: Wet weight; \( \delta^{15}N \): Stable nitrogen isotope ratio; \( \Delta^{15}N \): Trophic enrichment increment for \( ^{15}N \).

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Authors’ contributions
HR, VK, CS, CR, and JK conceived the project; VK, HR, and AF compiled and evaluated the TMF study database; GR, AF, HR, and AD evaluated the fish monitoring data; AF wrote a first version of the manuscript; all authors contributed to the optimization of the manuscript and the discussion of the data. All authors read and approved the final manuscript.

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The study relied on publicly available fish monitoring data.

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Not applicable.

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The authors declare that they have no competing interests.

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