Unraveling the relationship between concentrations of hydrophobic organic contaminants in freshwater fish of different trophic levels and water using passive sampling

Foppe Smedes[1,*], *Jaromír Sobotka*[1], *Tatsiana P Rusina*[1] *Pavla Fialová*[1], *Pernilla Carlsson*[1,2], *Radovan Kopp*[3] *and Branislav Vrana*[1]

[1] Masaryk University, Faculty of Science, Centre RECETOX, Kamenice 753/5, 625 00 Brno, Czech Republic

[2] Norwegian Institute for Water Research (NIVA), Fram Centre, Hjalmar Johansen gate 14, 9007 Tromsø, Norway

[3] Mendel University in Brno, Faculty of AgriSciences, Department of Zoology, Fisheries, Hydrobiology and Apiculture (FA), Zemědělská 1, 61300 Brno, Czech Republic

** Corresponding author:*
Foppe Smedes
foppe.smedes@recetox.muni.cz
Masaryk University, Faculty of Science, Centre RECETOX
Kamenice 753/5, pavilon A29
625 00 Brno
Czech Republic

Abstract

Concentrations of hydrophobic organic compounds (HOC) in aquatic biota are used for compliance, as well as time and spatial trend monitoring in the aqueous environment (EU WFD, OSPAR). Due to trophic magnification in the food chain, thermodynamic levels of HOC, e.g. PCB, DDT and BDE, in higher trophic level (TL) organisms are expected to be strongly elevated above those in water. This work compares lipid-based concentrations at equilibrium with the water phase derived from aqueous passive sampling ($C_{L\rightleftharpoons water}$), with lipid-based concentrations in fillet and liver of fish (C_L) at different TL for three water bodies in the Czech Republic and Slovakia. HOC's C_L in fish were near $C_{L\rightleftharpoons water}$, only after trophic magnification up to TL=4. For fish at lower TL, C_L progressively decreased relative to $C_{L\rightleftharpoons water}$ as HOC's K_{OW} increased above 10^6. The C_L decreasing towards the bottom of the food chain suggests non-equilibrium for primary producers (algae), which is in agreement with modeling passive HOC uptake by algae. Because tropic magnification and resulting C_L in fish exhibit large natural variability, $C_{L\rightleftharpoons water}$ is a viable alternative for monitoring of HOC using fish, showing a twofold lower confidence range while requiring less samples.

Introduction

Many chemical substances can leach into the environment as a result of their use in products, direct application or waste discharge. Their presence may harm ecosystems, depending on amount, distribution, degradation rate and toxicity. Hence, production and usage of several of these compounds are restricted or banned. To assess the effectiveness of measures to reduce pollution, trend and compliance monitoring is performed in water, sediment or biota.[1-3] Apart from assessing potential impact on organisms, concentrations of hydrophobic organic compounds (HOC, see Supporting information S1 for acronym explanation)) in biota are often used for monitoring the aquatic environment because bioaccumulation processes result in HOC concentrations in biota being much higher than in water and thus are analytically more feasible to measure. However, HOC levels in biota depend on various biological confounding factors, such as the lipid content of organisms, their sex, age, feeding habits, food availability, migration behavior and seasonality. Furthermore, biomagnification assessment of HOC requires consideration of organisms' trophic level (TL)[4,5], in combination with correction for other non-controllable factors such as lipid content. In monitoring programs, the influence of biological confounding factors is reduced by sampling pre-selected species of a certain age, size and sex.[5,6] However, despite the attempts to minimize the sampling variability, the inherent biological variability complicates the monitoring of HOC for compliance as well as for spatial and temporal trend assessment.[7,8]

Aqueous passive sampling (PS) has a great potential as an alternative to biota sampling[9,10] and the estimated freely dissolved concentration is proportional to the chemical activity, representing bio-availability of the HOC.[11] In contrast to aquatic organisms or whole water, partitioning passive samplers (e.g. based on silicone or low-density polyethylene polymer) provide "samples" from the environment with HOC concentrated in a matrix of well-characterized and constant properties. HOC accumulation in passive samplers (PS) is driven by the gradient in chemical activity between the aqueous phase and the PS until thermodynamic equilibrium is attained. The resulting equilibrium concentration in the PS (C_P) has been suggested as a kind of "Chemometer",[12] as C_P in PS equilibrated with environmental media, e.g. water, sediment, and also biota tissue, allows direct comparison of

HOC's thermodynamic level between these media, but also between different ecosystems on a global scale. However, to eliminate differences between the various polymers that can be used, C_P obtained for various environmental media can conveniently be converted to a lipid-based concentration ($C_{L\rightleftharpoons media}$) through multiplication by HOC's lipid–polymer partition coefficient (K_{LP})[12] allowing a simple comparison of HOC levels across media.[10,13-15] This includes fish tissue for which $C_{L\rightleftharpoons tissue}$ can be obtained by equilibrium passive sampling (EPS), performed by placing a PS in fish fillet. EPS provides data equivalent to C_L in fish fillet measured after classical solvent extraction.[16,17] Note that $C_{L\rightleftharpoons water}$ or $C_{L\rightleftharpoons sediment}$ should not be understood as a direct prediction of internal HOC concentration in aquatic organisms, but rather as the thermodynamic HOC level in the monitored habitat the fish is exposed to. The thermodynamic level of persistent HOC, expressed as C_L, is often observed to increase from primary producers, through primary consumers towards predators, and a linear relationship with the trophic level (TL) can be fit to those observations:[5]

$$\log C_L^{TL(x)} = \log C_L^{TL(y)} + \{TL(x) - TL(y)\} \log TMF \quad (1)$$

where a $C_L^{TL(x)}$ is HOC's lipid-based concentration in a fish species of TL(x)=x, the intercept $C_L^{TL(y)}$ is e.g. the C_L for a primary producer (y=1) or other selected TL, and TMF is the lipid-based trophic magnification factor, i.e. the factor by which C_L increases for each unit of TL. The general assumption is that trophic magnification progressively elevates C_L above $C_{L\rightleftharpoons water}$ and $C_{L\rightleftharpoons sediment}$ in the habitat. However, recent literature reports C_L for PCB in fishes of TL up to 4 being lower than $C_{L\rightleftharpoons sediment}$.[12,13,18] To unravel this phenomenon, an HOC's C_L or $C_{L\rightleftharpoons tissue}$ can be compared to $C_{L\rightleftharpoons water}$ in order to position the thermodynamic HOC level in fishes at various TL against that in the water phase. Hereto various fish species are sampled comprising the available range in TL in three selected water bodies in the Czech Republic and Slovakia having different levels of contamination. In the fish fillet and liver, HOC's C_L are determined by solvent extraction and $C_{L\rightleftharpoons tissue}$ by EPS. To estimate $C_{L\rightleftharpoons water}$, PS is applied in water including conventional[19] and dynamic passive sampling.[20] Subsequently, bioaccumulation and biomagnification at various TL are evaluated in relation to HOC's thermodynamic level in the water phase obtained by PS and the observations compared with the current understanding of biomagnification. The outcome is rationalized through modeling the equilibration rate of the passive

HOC uptake by phytoplankton (bottom of aquatic food chains). Finally, implications in relation to chemical biota monitoring practices[5] are discussed and compared with passive sampling.

Experimental

Target HOC included in this research were penta- and hexa-chlorobenzene (PeCB and HCB), polychlorinated biphenyl congeners (PCB 28, 52, 101, 118, 138, 153 and 180), DDx (2,4'-DDE, 4,4'-DDE, 2,4'-DDD, 4,4'-DDD, 2,4'-DDT 4,4'-DDT) and 6 brominated diphenyl ether congeners (BDE 28, 47, 99, 100, 153 and 154).

Fish sampling and aqueous phase PS. Site A is a fishpond (S2-1) where five adult fish species at various trophic levels were sampled: i.e. grass carp, common carp, ide, bronze bream and wels catfish (see S3 for Latin names and acronyms, feeding pattern, numbers, length and weight ranges). Nine adult fish of similar size were collected for each species (S3). Swan and duck mussels were sampled from the fishpond as a baseline reference for primary consumers (TL=2) for estimation of TL of sampled fishes. Because of the large range in size, duck mussels were divided in two size classes (S5). During a six months period prior to fish sampling, stationary PS was applied in the water outlet of the pond and dynamic PS,[20] using forced water flow to enhance HOC's sampler uptake, from the bank of the pond[21] (S4-1). At Site A, two types of silicone were applied for sampling: SSP (SSP-M823, 0.0125 cm thickness, Shielding Solutions Limited, UK) and Altesil (0.05 cm thickness, www.altecweb.com). PS preparation is briefly described in S7-1 and for all sites passive sampling was performed following available guidelines.[19] **Site B** is located in the PCB contaminated river Laborec (S2-2), downstream of a former PCB production plant[22] in eastern Slovakia. Adult fish from the following species were collected by electro-fishing: asp, perch, European chub, common bleak, roach, crucian carp, common nase, bronze bream and common barbel (S3). Only three individual mussels (swollen river mussel) could be sampled, which were combined to one sample (S5). Altesil PS were deployed stationary at three locations (S2) during two consecutive periods between March and August 2017 (S4-2). **Site C** is the mountain river Bečva, which flows in the northeastern part of the Czech Republic (S2-3). In two electro-fishing events, adult fish of only European chub, common

nase and common barbel, could be sampled (S3). No mussels were available from site C. At two selected positions (S2-3), Altesil PS were deployed from the right- and left-hand riverbank for six months (S4-3). All fish and passive samplers were stored at -20ºC until processing.

Fish sample processing. Body lengths and weights were recorded for each individual fish and the ranges are presented in S3. If the total fish weight for a single species was more than 10 kg, individuals were divided into two groups by separating odd and even numbers after ordering them by body weight. If the group consisted of an odd number of fish the last was divided between both groups. Asp, caught at site B, was divided into three size groups due to very large individual size differences. Following the protocol in S6, fillet and liver or hepatopancreas (both referred to as liver), were pooled per fish group. For small fishes, e.g. roach and common bleak, and the fishes from Site C, only fillets were collected (S4). Fillets of each fish group were cut into cubes of ~1.5 cm size and manually mixed. A 300 g portion of fish cubes was homogenized with a kitchen blender. The water content was determined based on the weight loss after drying 5 g homogenate overnight at 105°C (S12). Another 5 g of homogenate was freeze-dried and used for the determination of $\delta^{15}N$ as the fractional difference of the isotope ratio ($^{15}N/\ ^{14}N$) in the sample and nitrogen in air (‰) by the method described in S8-1. For chemical analysis of HOCs internal standards were added to 10-60 g of homogenate followed by solvent extraction with a cyclohexane/isopropanol/water mixture[23] (S8-2). Utilizing a portion of the extract (20-50%), the lipid content was determined as the weight remaining after solvent evaporation and drying at 105 °C until constant weight (S12). Another portion of the extract (20-50%) was concentrated to 1-2 mL hexane and subjected to a destructive cleanup by elution over sulfuric acid loaded silica (S8-5) followed by concentration to 100 µL and instrumental analysis (S8-6). Liver tissue of the corresponding fish species followed the same procedure as for fillets. The chemical analysis provided HOC concentrations in fillet and liver, which were expressed on lipid-basis (C_L).

EPS was applied by equilibrating >1 kg of fillet cubes or >0.2 kg liver tissue pieces and SSP passive samplers (S7-1) as described in S-8.3.[17]. After extraction of the SSP (S8-4), cleanup (S8-5), and instrumental analysis (S8-6), the equilibrium HOC concentrations in the SSP were converted to

lipid-basis ($C_{L\rightleftharpoons tissue}$) by multiplying with the lipid (triolein/fish oil)–polymer partition coefficient (K_{LP}) for SSP. For neutral lipids, these K_{LP} are not lipid specific and are available for PCB, PeCB, HCB and DDx.[24,25] The K_{LP} for BDE were estimated as the ratio of C_L and C_P equilibrated with fillet by EPS for samples from site B and C (highest BDE levels) as described for SSP in S9-1.

Aqueous passive sampling. For HOC with a log $K_{ow} > 6$, equilibrium is not readily attained by PS deployed in surface waters[21] and in such cases the concentration in the sampler polymer at equilibrium ($C_{P\rightleftharpoons water}$) is calculated using a first order uptake kinetics model adapted from Smedes and Booij:[19]

$$C_{P\rightleftharpoons water} = \frac{N_t/m}{1\text{-}\exp\left(-\frac{B\ t}{M^{0.47}\ K_{PW}\ m}\right)} \quad (2)$$

where $\underline{N}_t$ is the HOC mass (ng) accumulated after time t (d) in a sampler of mass m (kg). K_{PW} (L kg^{-1}) is the HOC's polymer–water partition coefficient, M is the HOC's molar mass (g mol^{-1}), and B is a deployment-specific proportionality constant representing the local exposure conditions and sampler geometry. This B is estimated from measured retained fractions of performance reference compounds (PRC) that were dosed to the sampler prior to exposure. PRC fractions retained after exposure were fitted to an exchange model[26] using unweighted non-linear regression,[27] further described in S10. The approach is similar for Altesil and SSP for which required K_{PW} of PRC, PeCB, HCB, PCB, and DDx are available in literature.[28-31] For BDE K_{PW} were only available for Altesil, which were used to derive K_{PW} for SSP as described in S9-4. Finally, $C_{P\rightleftharpoons water}$ were converted to lipid-basis ($C_{L\rightleftharpoons water}$) by multiplication with K_{LP}. All applied partition coefficients are collected in S11.

Results and discussion

Trophic levels of the sampled fishes. The $\delta^{15}N$ in fishes increase with their TL, but also differ between ecosystems, partly because of their dependence on the $\delta^{15}N$ at the base of the food chain. Therefore, the offset of $\delta^{15}N$ in the sampled fish species ($\delta^{15}N_{\text{fish tissue}}$) from $\delta^{15}N$ for primary producers or primary consumers ($\delta^{15}N_{baseline}$) is used to calculate TL:[5]

$$TL = \left(\delta^{15}N_{\text{fish tissue}} - \delta^{15}N_{\text{baseline}}\right) / \Delta^{15}N + TL_{\text{baseline}} \quad (3)$$

where the denominator $\Delta^{15}N$ represents the shift in $\delta^{15}N$ typical for one trophic level (3.4‰).[32] To represent the primary consumers in the food webs, mussels (TL=2 by definition[32]) were sampled and their $\delta^{15}N$ is applied as baseline ($\delta^{15}N_{\text{baseline}}$). At site A (fishpond), the $\delta^{15}N$ of swan mussels, small and larger duck mussels ranged between 10.87-12.62‰ and the lowest value (10.87‰ for the smallest duck mussels) was set as baseline assuming this to be the best representation, on the grounds that the higher $\delta^{15}N$ for the larger mussels may be a result of feeding on excretion of higher TL organisms. The $\delta^{15}N_{\text{mussel}}$ of 9.75‰ for swollen river mussels at site B was used as baseline for site B and C. The final TL values are listed in S12, together with the TL suggested at www.fishbase.org. The obtained TL ranges were 1.2–3.9, 2.3–3.6 and 3.2–3.5, for sites A, B and C, respectively.

Table 1 Mean ratio of lipid-based HOC[a] concentrations by solvent extraction (Ext) and EPS in fish fillet and liver.

Ratios for:	Ext-liver / Ext-fillet	EPS-liver / EPS-fillet	EPS-fillet / Ext-fillet	EPS-liver / Ext-liver
Ratio[a)]	0.98	1.13	1.02	1.18
sd [b)]	0.28	0.24	0.21	0.33
CI0.95 [c)]	0.05	0.04	0.04	0.06

a) Ratios for PeCB, HCB, PCB 28, 52, 101, 118,138, 153 and 180, and 4,4'-DDE.

b) standard deviation of the ratios

c) CI0.95 is 95% ± confidence range of the mean ratio

Comparing C_L in fillet and liver by solvent extraction and EPS. On average, C_L ratios between solvent extraction and EPS as well as between liver and fillet were close to unity (Table 1, S13) with the C_L in liver obtained by EPS being slightly higher on average (factor 1.18) than others. Although some ratios presented in Table 1 statistically differ from unity, the differences themselves were small when compared to differences in C_L occurring between the various species or even between different groups of the same species. For example, C_L values of all analyzed PCB ranged over a factor of 13

between the three asp fish groups from site B, while within each asp fish group the four C_L results by solvent extraction and EPS in both fillet and liver were very similar, ranging within a factor 1.3 (S15).

Observing similar results for C_L and $C_{L\rightleftharpoons fillet}$ for fillets is in agreement with earlier work[17,33] while no application of EPS in liver was found in literature. The $C_{L\rightleftharpoons liver}$ being slightly higher than $C_{L\rightleftharpoons fillet}$ may be explained in various ways: (1) the applied lipid extraction method[23] was not validated for liver tissue and possibly more than only lipid was extracted, resulting in underestimation of C_L, or (2) during the two days tumbling for EPS, degradation of liver tissue could have occurred. This could have reduced liver's HOC uptake capacity, resulting in a higher concentration in the sampler enhancing $C_{L\rightleftharpoons liver}$. On the other hand, some thermodynamic gradient is required for HOC uptake in the fish body. HOC's initial thermodynamic level in food increases in the gastrointestinal tract because digestion reduces food's uptake capacity. The resulting higher thermodynamic level in food processing organs drives the HOC distribution by the bloodstream to the fish body. This transport direction is in agreement with the observation that $C_{L\rightleftharpoons liver}/C_{L\rightleftharpoons fillet}$ ratios larger than unity were predominantly found in predatory fishes, e.g. wels catfish and perch (S14). The considerably higher liver C_L as well as $C_{L\rightleftharpoons liver}$ for one of the wels catfish groups at site A (marked as WC-2 in S13) corresponded with a much higher δ^{15}N in liver compared to fillet, while for most other fish samples δ^{15}N in liver were mostly equal or lower than in fillet (S12).

The overall average factor 1.1 difference in C_L between fillet and liver indicates that the fast pharmacokinetics of HOC quite efficiently limits differences in thermodynamic levels within the fish body. The little differences between solvent extraction and EPS of fillet and liver, and probably also whole fish, indicate that all methods can be interchangeably applied for monitoring of persistent HOC in biota, provided that the results are expressed on lipid-basis.[8,34]

For further evaluation, results by solvent extraction and EPS were averaged as analytical replicates while fillet and liver were considered as two different samples having their own, sometimes different δ^{15}N. This approach provided 18 and 22 datapoints for sites A and B respectively, which were used to compute TMF by linear regression of $\log C_L$ versus TL using eq (1). Regression results and

209 statistical parameters for site A and B are listed in S17. The fishes collected at site C were of similar
210 TL (3.2-3.5), and, consequently, no TMF estimation was possible.

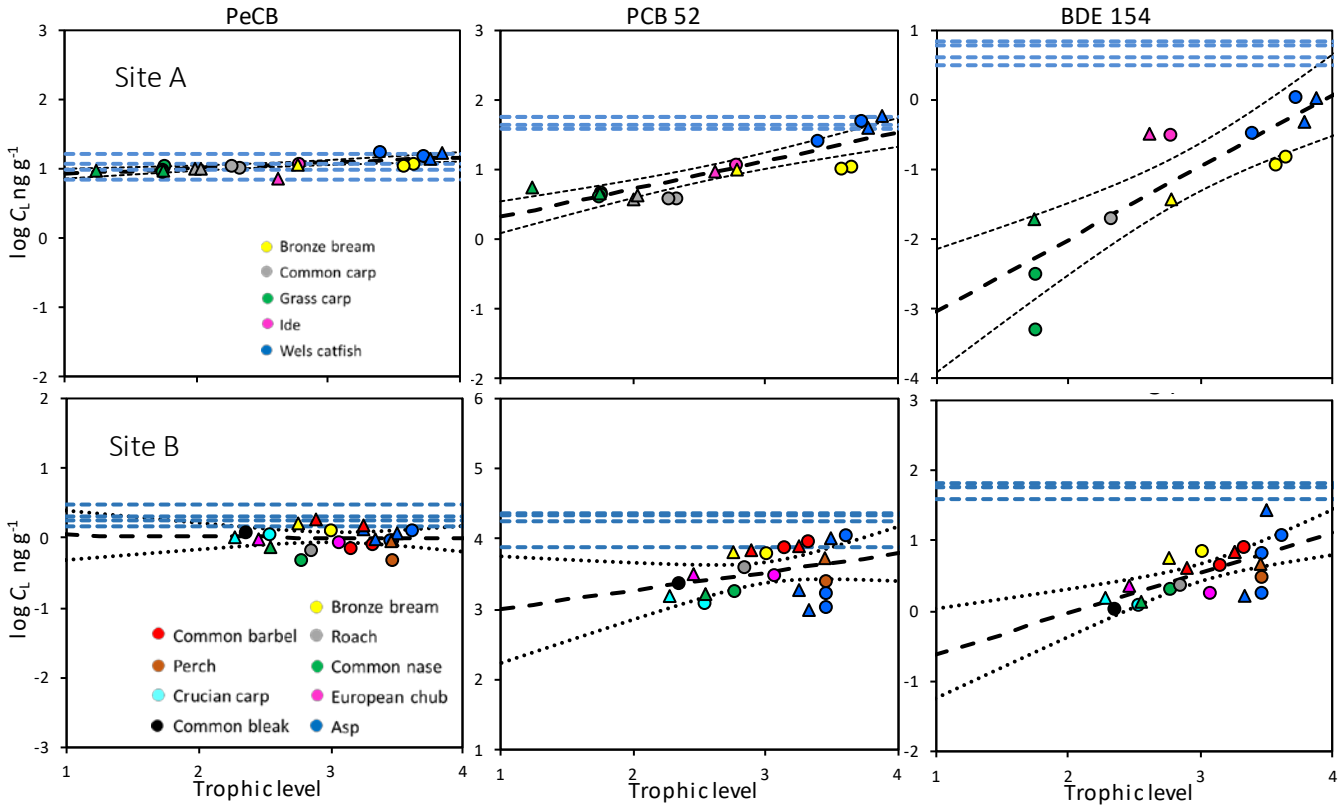


Fig. 1 Lipid-based concentrations (C_L) of three HOC of low, medium and high hydrophobicity in fillet (circles) and liver (triangles) versus the trophic level (TL) estimate at site A (upper row) and site B (lower row). Marker colors indicate fish species. The thick black dashed line represents the regression between $C_L$and TL (eq 1) and the dotted black lines indicate its upper and lower 95% confidence limits. The horizontal dashed blue lines represent the lipid-based concentrations for the water phase ($C_{L\rightleftharpoons water}$) as converted from equilibrium passive sampler (EPS) concentrations (n=4). The different lines indicate individual samplings.

211 **Trophic magnification factors (TMF)**. The HOC's log C_L in the fish fillet and liver samples
212 were nearly constant at all TL for PeCB while a positive log C_L–TL relation was observed for HOC
213 of higher hydrophobicity (Fig. 1 and S16). The slope of this log C_L–TL relation equals log TMF
214 (eq 1), which increased as HOC's hydrophobicity increased. The resulting TMF exhibited a linear
215 relationship with log K_{OW} for the persistent HOC, e.g. the PeCB, HCB and PCB (PCB-group), and
216 most of the BDE (Fig. 2). The TMF–log K_{OW} relationship showed a steeper slope at site A compared
217 to site B for the PCB group and, less pronounced for the BDE ($p>0.4$). The slope of the TMF–log K_{OW}

relationships were higher for the BDE compared to the PCB group, indicating that, in addition to hydrophobicity other unknown properties determine biomagnification. Note that the TMF for BDE 99 is known to be affected by biotransformation[35] and its outlying value was therefore excluded from regressions, as well as BDE 28, a biotransformation product of BDE 99.[35] BDE 47 is also a biotransformation product of BDE 99 but because the BDE 47 concentration is two orders of magnitude higher than BDE 28, an enhancement is not noticeable.

The high R^2 of TMF–log K_{OW} relationships (Fig. 2) confirm the effect of HOC's properties on TMF but do not imply accuracy of the TMF themselves. The individual TMF are barely significant when considering their 95% confidence ranges (see S18). This is because the effect of K_{OW} on the TMF is the same for all species but TMF's uncertainty mainly arises from natural C_L variability between fish species or fish groups (S15, S16). This variability is demonstrated in S19 where the slopes of TMF–log K_{OW} relationships show to vary by a factor five when using all but one fish species, i.e. for every line excluding a different fish species. Note that all regression lines in S19 closely intercept at TMF=1 (PeCB). For site B, with more fish species, the TMF–log K_{OW} slope varies over a factor of 2. A reason for the large TMF variability likely is that available species at the investigated sites, although they belong to the same food web, may not be from a single food chain. Multiple food chains, however, will be the reality in most river catchments.

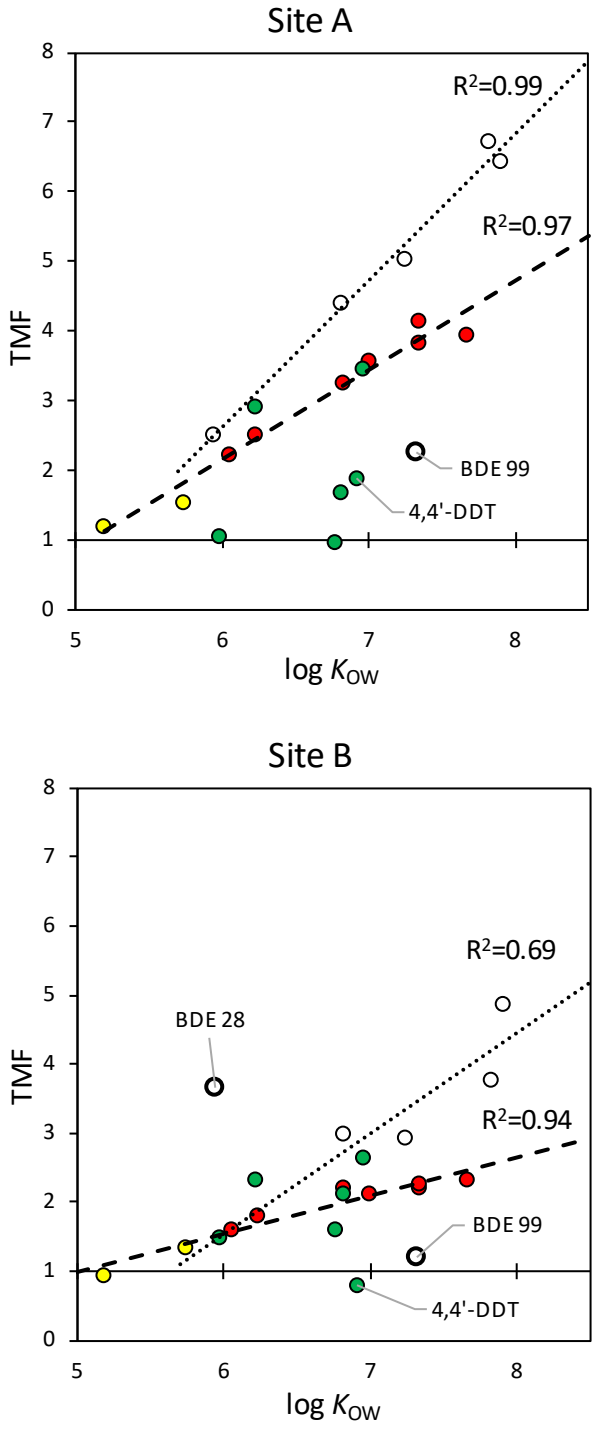


Fig. 2 Trophic magnification factors (TMF, y-axis) plotted versus log K_{OW} (x-axis) for PeCB and HCB (●), PCB (●), DDx (●) and BDE (○) for site A (upper panel) and B (lower panel). Dashed and dotted lines represent linear regression of TMF with log K_{OW} for PeCB, HCB plus PCB, and BDE (excluding bolded markers), respectively. DDx were not included in regression.

236

237 **HOC levels in fish and aqueous phase.** The observed C_L for PeCB in fishes were about equal at

238 all TL and agreed well with $C_{L \rightleftharpoons water}$ (Fig. 1, left panels). However, as HOC's hydrophobicity

increased, their C_L in fishes at lower TL progressively decreased relative to $C_{L\rightleftharpoons water}$. This is visualized in S20 where the ratios between $C_L^{TL(y)}$ and $C_{L\rightleftharpoons water}$ of persistent HOC are plotted as a function of log K_{OW} for TL=y where y varies from 1 to 5. Using the regression data (eq 1) to set $C_L^{TL(y)}$ means that the data points represent a kind of average over all sampled fish species. The graphs in S20 show that at TL=1 the C_L could deviate from thermodynamic equilibrium with the water phase by three orders of magnitude for very high K_{OW}. With increasing TL C_L increased, getting to about the same level as $C_{L\rightleftharpoons water}$ at TL=4. This is not necessarily thermodynamic equilibrium and, assuming trophic magnification continues for organisms of TL>4, HOC's $C_L^{TL(>4)}/C_{L\rightleftharpoons water}$ ratio may exceed unity for HOC of log K_{ow}>~6.

It is illustrative to evaluate lipid-based bioaccumulation factors ($BAF_L^{TL(y)}$) at specific TL values. $BAF_L^{TL(y)}$ are calculated as:

$$BAF_L^{TL(y)} = \frac{C_L^{TL(y)}}{C_W} \quad (4)$$

where C_W were estimated from passive sampling as described in S10. Log $BAF_L^{TL(y)}$ are plotted versus log lipid–water partition coefficients (log K_{LW}, S11) in Fig. 3, showing an increasing deviation from K_{LW} for TL<4 (vertical) and hydrophobicity increases (horizontal). The log BAF–log K_{LW} or log K_{ow} relationships leveling off at higher hydrophobicity (log K_{ow} >5 or 6) is frequently reported in literature and has been associated with a corresponding larger molecular size, which would limit its membrane passage.[36] However, a molecular size effect was not supported by analysis of a large amount of literature data[37] and often can be explained by other factors, including measurement artefacts or non-equilibrium with the surrounding environment[38] and is also shown here.

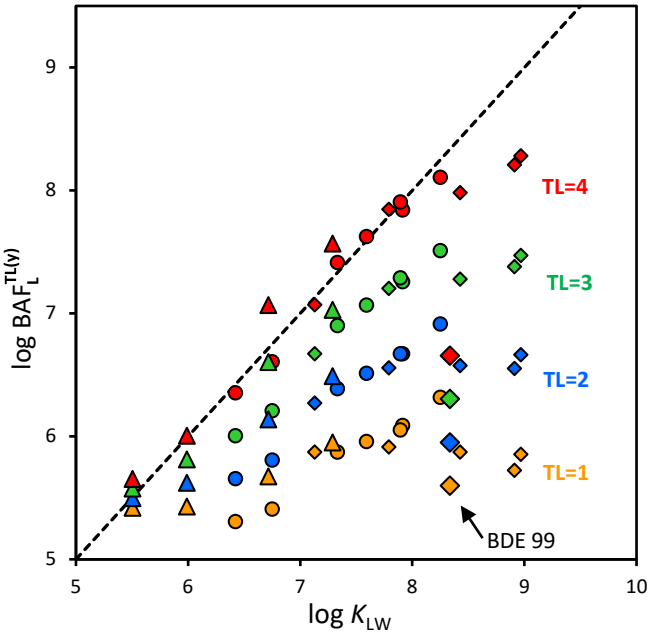


Fig. 3 Lipid-based bioaccumulation factors ($BAF_L^{TL(y)}$) in fishes at site A are plotted versus K_{LW} for different trophic levels (TL). $BAF_L^{TL(y)}$ were calculated as the ratio of the lipid-based concentration at TL=y ($C_L^{TL(y)}$) and the aqueous concentrations obtained by passive sampling (C_W) as described in S10. The $C_L^{TL(y)}$ in fishes at TL(y) for y=1 to 4 (1orange, 2blue, 3green and 4red) were obtained from regression using eq (1) and are actually points on the regression lines in Fig. 1 at the respective TL(y). The dashed line indicates equality between log BAF and log K_{LW}. Symbol shapes indicate (△) Penta- and hexa-chlorobenzene, 4,4'-DDD and 4,4'-DDE, (○) PCB and (◇) BDE.

Thermodynamic level difference between fishes and the water phase. The much lower C_L compared to $C_{L\rightleftharpoons water}$ at TL=1 for high K_{OW} HOC strongly indicate that HOC levels at the bottom of the food web, phytoplankton e.g. algea, are not at thermodynamic equilibrium with the water phase. Evaluation of long-term PCB monitoring in herring gull eggs showed that occasionally lower PCB levels corresponded to seasons with favorable conditions for algal growth, demonstrating how a lack of equilibrium can affect levels higher in the food chain.[39] This is not consistent with exposure of algae to PCB in laboratory conditions, for which equilibration times of one hour,[40] several hours,[41] or a day[42] were observed, while for PCB of $K_{OW} > 10^6$ also non-equilibrium after a week of exposure was reported[43]. Other studies report equilibrium attainment of phytoplankton in the laboratory and the field.[44,45]

At equilibrium, the ratio between the concentration in algae (C_A) and C_W is given by the bioconcentration factor (BCF). Evaluating equilibrium attainment, it should be realized that primary producers such as phytoplankton, phytobenthos, macrophytes dominantly grow from photosynthetic carbon dioxide assimilation and inorganic nutrients. Because these components do not contain HOC, in first instance HOC concentrations in algal cells are minimal and HOC are taken up from the aqueous phase by diffusion through an aqueous boundary layer at the surface (WBL) and a cell wall or a membrane. Previously published models of PCB uptake by phytoplankton and bacteria were not consistent whether the mass transfer is controlled by the biological membrane[46] or the WBL.[47] Here it is important to stress that the mass transfer rate is determined by the product of diffusion coefficient and solubility,[48] i.e. the permeability. Since the BCF also reflects the ratio between solubility of an HOC in an organism and the aqueous phase, slower diffusion in biological material is compensated by a higher solubility than in water, resulting in a high permeability. Consequently, for HOC's of higher hydrophobicity, mass transport in the WBL controls the uptake rate. Additionally, the WBL at the surface of, or actually around the algal cells, is generally much thicker than the biological membrane. Consequently, acknowledging that the transport resistance is dominated by the WBL, the HOC uptake process of primary producers (further referred to as "algae") can be modeled analogously to passive samplers.

Algal HOC uptake modeled as passive sampling. Passive samplers exposed in the laboratory in a defined volume of aqueous HOC solution equilibrate much faster than when exposed in field conditions.[49] This is due to the dependence of the first order kinetic rate constant on the ratio of the sorbent capacity and the water volume used.[50] For WBL controlled partition PS, a model for HOC uptake by a PS from a defined volume of contaminated water is available from Booij et al,[51] which was adapted to provide HOC concentrations in algae (C_A^t, ng kg^{-1}) as a function of exposure time (t, d):

$$C_A^t = \frac{C_W^0 \mathrm{BCF}\left\{1 - \exp\left[-\left(1 + \frac{m_A \mathrm{BCF}}{V_W}\right)\frac{k_W A_A}{m_A \mathrm{BCF}} t\right]\right\}}{1 + \frac{m_A \mathrm{BCF}}{V_W}} \quad (5)$$

where C_W^0 is the dosed HOC concentration in the aqueous solution (ng L^{-1}) at t=0 and a volume V_W (L), the BCF (L kg^{-1}) HOC's bioconcentration factor in algae (replacing K_{PW}), m_A the algae mass (kg), k_W the mass transfer coefficient in the WBL (dm d^{-1}), and A_A the total surface area of the algae (dm^2). Similarly, the time course of the aqueous concentration (C_W^t) is given by:[51]

$$C_W^t = \frac{C_W^0 \left\{1 + \frac{m_A \mathrm{BCF}}{V_W} \exp\left[-\left(1 + \frac{m_A \mathrm{BCF}}{V_W}\right) \frac{k_W A_A}{m_A \mathrm{BCF}} t\right]\right\}}{1 + \frac{m_A \mathrm{BCF}}{V_W}} \quad (6)$$

Note that in these equations the exponent equals $-k_e t$, wherein k_e (d^{-1}) is the first order exchange rate constant. Consequently, k_e is higher if $m_A \mathrm{BCF}/V_W$ (L L^{-1}) is high, explaining the fast equilibration when sorbent capacity (m_ABCF; in L) of the algae is much larger than the water volume V_W (L). Opposite, when V_W is infinitely large, as is the case in field conditions, $m_A \mathrm{BCF}/V_W \rightarrow 0$ and eq (5) reduces to:

$$C_A^t = C_W^0 \mathrm{BCF} \left\{1\text{-}\exp\left[-\frac{k_W A_A}{\mathrm{BCF}\ m_A} t\right]\right\} \quad (7)$$

In such situation C_W^t remains constants and equal to C_W^0.

Using these models, C_A^t and C_W^t were modeled as a function of time for three cases involving HOC uptake by a same algal mass (~25×10^{-6} kg) where (I) V_W=0.05 L and A_A=10 dm^2 by Sijm et al,[40] (II) V_W = 1 L and A_A=16 dm^2 by Koelmans et al[42] and (III) $V_W = \infty$ (field situation) and A_A=10 dm^2, assuming a hypothetical situation that algae do not grow (see S21-1 for more detail). An average k_W of 0.1 µm s^{-1} (0.09 dm d^{-1}) was derived by fitting k_e to the rate constants reported for the cases (I)[40] and (II).[42] This k_W was used in all three cases providing sampling rates (R_S=$k_W A_A$) of 0.86, 1.35 and 0.86 L d^{-1}, for (I), (II) and (III), respectively.

Modeling results: laboratory versus field. Modeling showed that equilibration times for HOC observed in laboratory conditions generally do not apply to the field situation and are expected to increasingly deviate with increasing BCF. Modeling C_A^t and C_W^t profiles versus time for HOC of log BCF 3 to 8, showed that algae equilibrate in less than a day at laboratory conditions where a limited water volume is applied (S21-2 and S21-3), while in the field for HOC of log BCF 5 to 8 equilibration times of 10 to 10000 days would apply (S21-4).

To evaluate and explain this, instead of full equilibrium requiring infinite time, we calculated the time at which C_A^t and C_W^t are within 10% of their final equilibrium values, i.e. t_A^{90} and t_W^{90} for algae and water phase, respectively (S21). These timepoints are marked in S21-2 and S21-3, and listed in S22. For all three cases these t^{90} are plotted versus log BCF in Fig. 4, applying a logarithmic and a temporal scale in panel A and B, respectively. Panel A in Fig. 4 shows that for the field situation (III), t_A^{90} increases proportionally to BCF. The t_A^{90} exceeds the lifespan of algae at log BCF 4.5 to 5, which is evident when t^{90} are plotted at a linear scale (Fig. 4, panel B). In contrast, t_A^{90} is relatively short in laboratory exposures and constant when log BCF is higher than 4 and 5 for case (I), V_W=0.05 L and case (II), V_W=1 L, respectively. This is because for equilibration in a defined water volume with $m_A \mathrm{BCF}/V_W \gg 1$, t_A^{90} is determined by V_W, rather than BCF.[40]

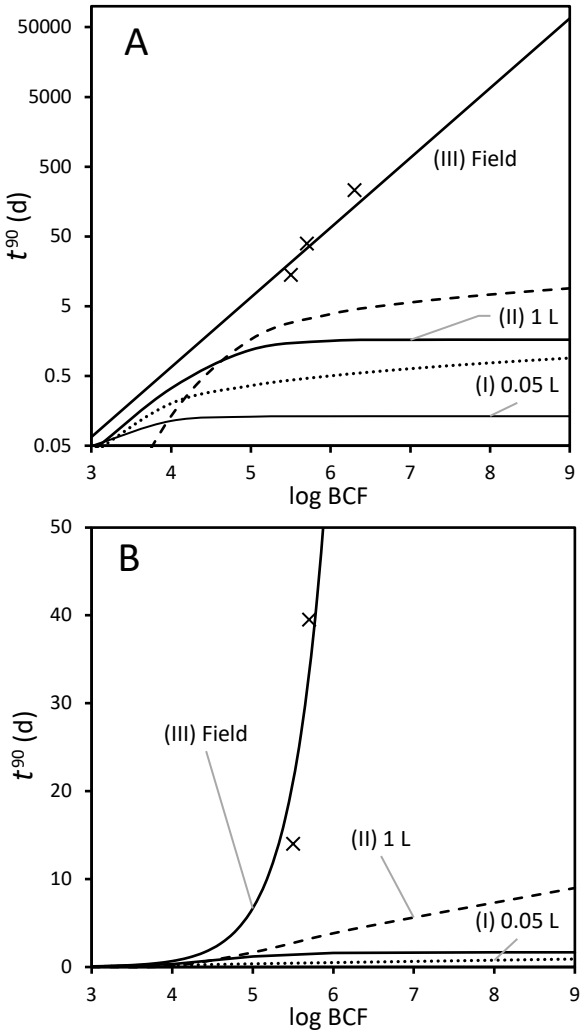
For the aqueous phase, the profiles in panel A of Fig. 4 and S21-2 and S21-3, show that towards higher BCF, t_W^{90} becomes increasingly larger than t_A^{90}. The t_W^{90} equals t_A^{90} when $m_A \mathrm{BCF}/V_W$=1 and for higher ratios the relative difference between t_W^{90} and t_A^{90} is determined by the phase capacity factor log ($m_A \mathrm{BCF}/V_W$), by which C_W has to decrease to attain a level within 10% from equilibrium:

$$\frac{t_W^{90}-t_A^{90}}{t_A^{90}} = \log\left(\frac{\mathrm{BCF}\, m_A}{V_W}\right) \qquad \text{and} \qquad t_W^{90} = t_A^{90} \log\left(\frac{\mathrm{BCF}\, m_A}{V_W}\right) + t_A^{90} \tag{8}$$

Note that this equation fails if the algal uptake does not affect C_W^0 by more than 10%, i.e. t_W^{90}=0.

In the graphs in S21-2 and S21-3, the extent C_W at t_A^{90} is higher than C_W at t_W^{90} is shown by the length of a vertical line. At a log BCF of 7 the ratio $C_W(t_A^{90})/C_W(t_W^{90})$ amounts 6000 and 250 for case (I) and (II), respectively. Although this factor is higher when V_W is very small, the phenomenon is more relevant for larger V_W because of the associated longer absolute time between t_A^{90} and t_W^{90}. The extent t_W^{90} is longer than t_A^{90}, is relevant for in laboratory BCF estimations, where equilibrium attainment is generally assessed by monitoring the concentration in the algae. Ending exposure earlier than t_W^{90} causes an increasing underestimation of BCF towards HOC of higher hydrophobicity. Such underestimation may contribute to the log BCF–log K_{OW} relation for algae leveling off around log $K_{OW} \approx 6$[43]. We write "contributing", as such effect is indistinguishable from overestimation of

349 aqueous concentrations by insufficiently excluding HOC fractions bound to DOC or exudate when
350 quantifying C_W.
351



352

353 Fig. 4 Modeled time required for algae to attain the concentration within 10% equilibrium (t^{90})
354 for three cases where ~25×10^{-6} kg algae was exposed to (I) 0.05 L,[40] (II) 1 L,[42] or (III) infinite volume
355 of water, plotted versus log BCF with the y-axis at a logarithmic scale (panel A) and at a linear scale
356 for 0–50 days (panel B). Solid and dashed lines indicate the t^{90} for algal uptake and water phase
357 decrease, respectively. In the field the aqueous concentration was considered constant and t_W^{90} does
358 not apply. The (×) markers represent experimental t^{90} from gas purge elimination of three PCB
359 congeners by Koelmans et al.[42]
360

361 **Evaluation of model application.** When applying passive sampling theory for modeling WBL
362 controlled algal uptake, the key parameter is the mass transfer coefficient (k_W) which depends on the

water turbulence near the algal surface. The k_W derived above corresponds to a sampling rate of 0.09 L d^{-1} dm^{-2}, which is very low compared to what is observed for passive sampling, where even in quiescent conditions sampling rates are commonly higher by more than an order of magnitude. However, the position of a passive sampler is typically fixed in the water current, whereas algae float in/with the streaming water and may reside in the same water causing a thick water boundary layer and low k_W. Except for algae growing on rocks, there is no reason to believe that k_W in field would be higher than estimated for stirred laboratory exposures, and the timescale of the modeled equilibration times (Fig.4) for HOC of higher hydrophobicity (log BCF > 5) can be considered realistic. Due to the long equilibration timescale for HOC of high log K_{OW}, combined with an inevitable algal growth, it is not feasible to validate the model results in field conditions. However, elimination rate estimation in the laboratory by gas purging of HOC simulates an aqueous phase of infinite size.[42] Assuming that, similar to WBL controlled passive sampling, the algal uptake process is also isotropic, the elimination process of HOC from dosed algae mirrors the uptake process. Inserting the t^{90} release times from the gas purge elimination curves by Koelmans et al[42] in Fig. 4 shows that these are in good agreement with the t_A^{90} modeled for field uptake by algae. This observation supports that laboratory results can be used to predict passive uptake using the presented modeling approach. In field conditions, both slow HOC uptake and growth dilution are the main obstacles that prevent algae to achieve the same thermodynamic levels as the aqueous phase. Consumer organisms on the other hand, take up HOC via food, which is only partly converted to biomass. The accumulation from food uptake, together with respiratory uptake, increases HOC's concentration more than growth dilution will decrease it. Accumulation above the thermodynamic level of the aqueous phase will end when the respiratory HOC flux driven by a thermodynamic gradient between the organism and the water phase, equals the uptake by the food route.

Implications. The C_L in fishes of TL <4 being lower than $C_{L\rightleftharpoons water}$ is not compatible with the general belief that trophic magnification typically amplifies levels of persistent HOC in e.g. fish above that of the surrounding environment. Although C_L in fish and invertebrates being lower than $C_{L\rightleftharpoons water}$ or $C_{L\rightleftharpoons sediment}$, has been frequently observed,[10,12-14,52] possible reasons, including the above,

were discussed without firm conclusions, except that $C_{L\rightleftharpoons water}$ and $C_{L\rightleftharpoons sediment}$ are useful in bioaccumulation research and a viable alternative to chemical monitoring in biota. The latter could apply for the OSPAR Coordinated Environmental Monitoring Program (CEMP)[2] or the chemical biota monitoring under the WFD.[1] Differences in concentrations in fishes of different TL were recognized by the WFD requiring compliance to Environmental Quality Standards (EQS) of priority substances in fishes at TL=4, and if such fishes are not available, inter- or extrapolation (eq 1) has to be applied using data of available fish species.[5] The necessary TMF are ecosystem, food web and HOC-specific. Guidance is provided for selecting and determination of TMF for application under the EU WFD,[4] but it could not be resolved which TMF would apply to the river systems and the fishpond in this study. The most robust way to correct for trophic magnification is in-situ TMF determination, i.e. regressing HOC's $C_L^{TL(x)}$ measured in various sampled fish species versus TL, preferably comprising at least two TL units.[4] Nevertheless, the $C_L^{TL(4)}$resulting from extrapolation at site A and B, each using twenty fish samples, on average showed a two times larger 95% confidence range compared to $C_{L\rightleftharpoons water}$ for which four samplings were performed (S24, S25). Apart from the 95% confidence range $C_L^{TL(4)}$ may still be biased if by chance the sampled fish species are not from the same food chain and/or the reference $\delta^{15}N$ as well as the 3.4 ‰ for $\Delta^{15}N$ do not apply for the local situation. The concept of PS, on the other hand, is straightforward. PS essentially adds an artificial medium (a polymeric sampler of constant properties without biotransformation) to the ecosystem. The accuracy of $C_{L\rightleftharpoons water}$ relates to uncertainties of HOC quantification in the sampler, polymer–water and lipid–sampler partition coefficients, and to that of the model used for in-situ sampler calibration. The partition coefficients are physical constants that have no natural variability and are as accurate as analytically determined. Moreover, $C_{L\rightleftharpoons water}$ also is a better reflection of the actual HOC exposure of organisms, as HOC's C_L may be reduced or diminished due to bio-transformation in fish, e.g. some DDx and BDE 99, while the exposure to HOC undetected in the tissue may have harmed the organism. To bridge both approaches it was suggested to routinely apply PS and, in case the EQS is approached or exceeded, apply chemical monitoring in biota.[53]

For HOC of K_{OW} >10^6, uptake and equilibration rates for primary producers need further attention and such research should include the use of PS and/or passive dosing. Moreover, the thermodynamic levels for HOC in primary producers and primary consumers being lower than in the aqueous phase need to be considered when setting the food chain/web bottom reference point in modeling of real field conditions with realistic growth.

The authors declare no competing financial interest.

Supporting information

Supporting information contains additional procedure descriptions, tables and figures presented in the order they are referred to in the text. This information is available free of charge via the internet at http://pubs.acs.org.

Acknowledgements

This work was supported by the Czech Science Foundation grant No. GACR 15-16512S "Investigation of accumulation of persistent bioaccumulative toxic organic substances into aquatic organisms". The research activities were carried out in the RECETOX Research Infrastructure supported by the Czech Ministry of Education, Youth and Sports (LM2015051) and the European Structural and Investment Funds, Operational Programme Research, Development, Education (CZ.02.1.01/0.0/0.0/16_013/0001761). We thank Petra Přibylová, Petr Kukučka, Jakub Martiník, Ondřej Audy, Krzysztof Okonski and Roman Prokeš from RECETOX, Masaryk University for their assistance with sampling, sample preparation and instrumental analysis of samples and Ondřej Sáňka from RECETOX, Masaryk University for his assistance with the preparation of maps. We also thank Dr. Daniel Gruľa and his colleagues from the ichthyological survey company ARAR s.r.o. and Dr. Pavel Jurajda and his colleagues from the Institute of Vertebrate Biology of the Czech Academy of Sciences for providing fish samples for the study from the Laborec and Bečva rivers. Advice of an unknown reviewer was highly appreciated.

Literature cited

1 EU WFD Directive 2013/39/EU of the European Parliament and of the Council of 12 August 2013 amending Directives 2000/60/EC and 2008/105/EC as regards priority substances in the field of water policy. *Off. J. Eur. Union* **2013,** L226, 1-17.

2 OSPAR Coordinated Environmental Monitoring Programme (CEMP), OSPAR Agreement 2016-01, updated 2018. **2016,** http://www.ospar.org/documents?d=32943

3 OSPAR Background Document on CEMP assessment criteria for the QSR 2010. **2009,** *Document nr: 461/209; ISBN 978-1-907390-08-1;* https://qsr2010.ospar.org/media/assessments/p00390_supplements/p00461_Background_Doc_CEMP_Assessmt_Criteria_Haz_Subs.pdf

4 Kidd, K.A.; Burkhard, L.P.; Babut, M.; Borgå, K.; Muir, D.C.G.; Perceval, O.; Ruedel, H.; Woodburn, K.; Embry, M.R. Practical advice for selecting or determining trophic magnification factors for application under the European Union Water Framework Directive. *Integr. Environ. Assess. Manag.* **2018,** 15 266-277; DOI 10.1002/ieam.4102

5 Deutsch, K.; Leroy, D.; Belpaire, C.; den Haan, K.; Vrana, B.; Clayton, H.; Hanke, G.; Ricci, M.; Held, A.; Gawlik, B.; Babut, M.; Perceval, O.; Lepom, P.; Heiss, C.; Koschorreck, J.; O'Toole, S.; Valsecchi, S.; Polesello, S.; Carere, M.; ten Hulscher, D.; Verbruggen, E.; Dulio, V.; Green, N.; Viñas, L.; Bellas, J.; Lilja, K.; Bignert, A.; Whitehouse, P.; Sumner, K.; Law, R.; Brant, J.; Leverett, D.; Merrington, G. Guidance document no. 32 on biota monitoring (the implementation of EQS biota) under the water framework directive. *Common Implementation Strategy for the Water Framework Directive (2000/60/EC)* **2014,** Technical Report - 2014 - 083 DOI: 10.2779/833200

6 OSPAR CEMP Guidelines for Monitoring Contaminants in Biota (updated 2018). **1999,** https://www.ospar.org/convention/agreements?q=1999-02&t=&a=&s=

7 Fliedner, A.; Rüdel, H.; Teubner, D.; Buchmeier, G.; Lowis, J.; Heiss, C.; Wellmitz, J.; Koschorreck, J. Biota monitoring and the Water Framework Directive---can normalization

overcome shortcomings in sampling strategies? *Environ. Sci. Pollut. Res.* **2016,** 23, 21927-21939; DOI 10.1007/s11356-016-7442-2"

8 Fliedner, A.; Rüdel, H.; Lohmann, N.; Buchmeier, G.; Koschorreck, J. Biota monitoring under the Water Framework Directive: On tissue choice and fish species selection. *Environ. Pollut.* **2018,** 235, 129-140; DOI 10.1016/j.envpol.2017.12.052

9 Khairy, M.A.; Noonan, G.O.; Lohmann, R. Uptake of hydrophobic organic compounds, including organochlorine pesticides, polybrominated diphenyl ethers, and perfluoroalkyl acids in fish and blue crabs of the lower Passaic River, New Jersey, USA. *Environ. Toxicol. Chem.* **2019,** 38, 872-882; DOI 10.1002/etc.4354

10 Mäenpää, K.; Leppänen, M.; Figueiredo, K.; Mayer, P.; Gilbert, D.; Jahnke, A.; Gil-Allué, C.; Akkanen, J.; Nybom, I.; Herve, S. Fate of polychlorinated biphenyls in a contaminated lake ecosystem: combining equilibrium passive sampling of sediment and water with total concentration measurements of biota. *Environ. Toxicol. Chem.* **2015,** 34, 2463-2474; DOI 10.1002/etc.3099

11 Reichenberg, F.; Smedes, F.; Jonsson, J.; Mayer, P. Determining the chemical activity of hydrophobic organic compounds in soil using polymer coated vials. *Chem. Cent. J.* **2008,** 2, 8; DOI 10.1186/1752-153X-2-8

12 Jahnke, A.; Mayer, P.; McLachlan, M.S.; Wickstrom, H.; Gilbert, D.; MacLeod, M. Silicone passive equilibrium samplers as 'chemometers' in eels and sediments of a Swedish lake. *Environ. Sci. Processes Impacts* **2014,** 16, 464-472; DOI 10.1039/C3EM00589E

13 Jahnke, A.; MacLeod, M.; Wickstrom, H.; Mayer, P. Equilibrium sampling to determine the thermodynamic potential for bioaccumulation of persistent organic pollutants from sediment. *Environ. Sci. Technol.* **2014,** 48, 11352-11359; DOI 10.1021/es503336w

14 Figueiredo, K.; Mäenpää, K.; Lyytikäinen, M.; Taskinen, J.; Leppänen, M.T. Assessing the influence of confounding biological factors when estimating bioaccumulation of PCBs with passive samplers in aquatic ecosystems. *Sci. Total Environ.* **2017,** 601–602, 340-345; DOI 10.1016/j.scitotenv.2017.05.140

15 Figueiredo, K.; Mäenpää, K.; Leppänen, M.T.; Kiljunen, M.; Lyytikäinen, M.; Kukkonen, J.V.K.; Koponen, H.; Biasi, C.; Martikainen, P.J. Trophic transfer of polychlorinated biphenyls (PCB) in a boreal lake ecosystem: Testing of bioaccumulation models. *Sci. Total Environ.* **2014,** 466–467, 690-698; DOI 10.1016/j.scitotenv.2013.07.033

16 Jahnke, A.; Mayer, P.; Adolfsson-Erici, M.; McLachlan, M.S. Equilibrium sampling of environmental pollutants in fish: comparison with lipid-normalized concentrations and homogenization effects on chemical activity. *Environ. Toxicol. Chem.* **2011,** 30, 1515-1521; DOI 10.1002/etc.534

17 Rusina, T.P.; Carlsson, P.; Vrana, B.; Smedes, F. Equilibrium passive sampling of POP in lipid-rich and lean fish tissue: quality control using performance reference compounds. *Environ. Sci. Technol.* **2017,** 51, 11250-11257; DOI 10.1021/acs.est.7b03113

18 Mäenpää, K.; Leppänen, M.T.; Reichenberg, F.; Figueiredo, K.; Mayer, P. Equilibrium sampling of persistent and bioaccumulative compounds in soil and sediment: comparison of two approaches to determine equilibrium partitioning concentrations in lipids. *Environ. Sci. Technol.* **2011,** 45, 1041-1047; DOI 10.1021/es1029969

19 Smedes, F.; Booij, K. Guidelines for passive sampling of hydrophobic contaminants in water using silicone rubber samplers. *ICES Techniques in Marine Environmental Sciences* **2012,** No. 52 (20pp); DOI:10.17895/ices.pub.5077

20 Vrana, B.; Smedes, F.; Allan, I.; Rusina, T.; Okonski, K.; Hilscherová, K.; Novák, J.; Tarábek, P.; Slobodník, J. Mobile dynamic passive sampling of trace organic compounds: Evaluation of sampler performance in the Danube River. *Sci. Total Environ.* **2018,** 636, 1597-1607; DOI 10.1016/j.scitotenv.2018.03.242

21 Vrana, B.; Rusina, T.; Okonski, K.; Prokeš, R.; Carlsson, P.; Kopp, R.; Smedes, F. Chasing equilibrium passive sampling of hydrophobic organic compounds in water. *Sci. Total Environ.* **2019,** 664, 424-435; DOI 10.1016/j.scitotenv.2019.01.242

22 Kocan, A.; Petrik, J.; Jursa, S.; Chovancova, J.; Drobna, B. Environmental contamination with polychlorinated biphenyls in the area of their former manufacture in Slovakia. *Chemosphere* **2001,** 43, 595-600; DOI 10.1016/S0045-6535(00)00411-2

23 Smedes, F. Determination of total lipid using non-chlorinated solvents. *Analyst* **1999,** 124, 1711-1718; DOI 10.1039/a905904k

24 Smedes, F.; Rusina, T.P.; Beeltje, H.; Mayer, P. Partitioning of hydrophobic organic contaminants between polymer and lipids for two silicones and low density polyethylene. *Chemosphere* **2017,** 186, 948-957; DOI 10.1016/j.chemosphere.2017.08.044

25 Jahnke, A.; McLachlan, M.S.; Mayer, P. Equilibrium sampling: Partitioning of organochlorine compounds from lipids into polydimethylsiloxane. *Chemosphere* **2008,** 73, 1575-1581; DOI 10.1016/j.chemosphere.2008.09.017

26 Rusina, T.P.; Smedes, F.; Koblizkova, M.; Klanova, J. Calibration of silicone rubber passive samplers: experimental and modeled relations between sampling rate and compound properties. *Environ. Sci. Technol.* **2010,** 44, 362-367; DOI 10.1021/es900938r

27 Booij, K.; Smedes, F. An Improved method for estimating in situ sampling rates of nonpolar passive samplers. *Environ. Sci. Technol.* **2010,** 44, 6789-6794; DOI 10.1021/es101321v

28 Smedes, F.; Geertsma, R.W.; van der Zande, T.; Booij, K. Polymer-water partition coefficients of hydrophobic compounds for passive sampling: application of cosolvent models for validation. *Environ. Sci. Technol.* **2009,** 43, 7047-7054; DOI 10.1021/es9009376

29 Smedes, F. Silicone–water partition coefficients determined by cosolvent method for chlorinated pesticides, musks, organo phosphates, phthalates and more. *Chemosphere* **2018,** 210, 662-671; DOI 10.1016/j.chemosphere.2018.07.054

30 Smedes, F. Corrigendum to “Silicone–water partition coefficients determined by cosolvent method for chlorinated pesticides, musks, organo phosphates, phthalates and more” [Chemosphere 210 (2018) 662–671]. *Chemosphere* **2018,** 212, 1180; DOI 10.1016/j.chemosphere.2018.09.094 "

547 31 Smedes, F. SSP silicone–, lipid– and SPMD–water partition coefficients of seventy hydrophobic
548 organic contaminants and evaluation of the water concentration calculator for SPMD.
549 *Chemosphere* **2019,** 223, 748-757; DOI 10.1016/j.chemosphere.2019.01.164
550 32 Post, D.M. Using stable isotopes to estimate trophic position: models, methods, and assumptions.
551 *Ecology* **2002,** 83, 703-718; DOI 10.1890/0012-9658(2002)083[0703:USITET]2.0.CO;2
552 33 Jahnke, A.; Mayer, P.; Broman, D.; McLachlan, M.S. Possibilities and limitations of equilibrium
553 sampling using polydimethylsiloxane in fish tissue. *Chemosphere* **2009,** 77, 764-770; DOI
554 10.1016/j.chemosphere.2009.08.025
555 34 Schneider, R. Polychlorinated biphenyls (PCBs) in cod tissues from the Western Baltic:
556 significance of equilibrium partitioning and lipid composition in the bioaccumulation of
557 lipophilic pollutants ingill-breathing animals. *Meeresforsch.* **1982,** 29, 69-79.
558 35 Noyes, P.D.; Kelly, S.M.; Mitchelmore, C.L.; Stapleton, H.M. Characterizing the in vitro hepatic
559 biotransformation of the flame retardant BDE 99 by common carp. *Aquat. Toxicol.* **2010,** 97,
560 142-150; DOI 10.1016/j.aquatox.2009.12.013
561 36 Opperhuizen, A.; van der Velde, E.W.; Gobas, F.A.P.C.; Liem, D.A.K.; van der Steen, J.M.D.
562 Relationship between bioconcentration in fish and steric factors of hydrophobic chemicals.
563 *Chemosphere* **1985,** 14, 1871-1896; DOI 10.1016/0045-6535(85)90129-8
564 37 Arnot, J.A.; Arnot, M.I.; Mackay, D.; Couillard, Y.; MacDonald, D.; Bonnell, M.; Doyle, P.
565 Molecular size cutoff criteria for screening bioaccumulation potential: Fact or fiction? *Integr.*
566 *Environ. Assess. Manag.* **2010,** 6, 210-224; DOI 10.1897/IEAM_2009-051.1
567 38 Jonker, M.T.O.; van der Heijden, S.A. Bioconcentration factor hydrophobicity cutoff: an
568 artificial phenomenon reconstructed. *Environ. Sci. Technol.* **2007,** 41, 7363-7369; DOI
569 10.1021/es0709977
570 39 Smith, D. Synchronous response of hydrophobic chemicals in herring gull eggs from the Great-
571 Lakes. *Environ. Sci. Technol.* **1995,** 29, 740-750; DOI 10.1021/es00003a023

572 40 Sijm, D.; Broersen, K.; de Roode, D.; Mayer, P. Bioconcentration kinetics of hydrophobic
573 chemicals in different densities of Chlorella pyrenoidosa. *Environ. Toxicol. Chem.* **1998,** 17,
574 1695-1704; DOI 10.1002/etc.5620170908
575 41 Gerofke, A.; Komp, P.; McLachlan, M. Bioconcentration of persistent organic pollutants in four
576 species of marine phytoplankton. *Environ. Toxicol. Chem.* **2005,** 24, 2908-2917; DOI
577 10.1897/04-566R.1
578 42 Koelmans, A.; van der Woude, H.; Hattink, J.; Niesten, D. Long-term bioconcentration kinetics
579 of hydrophobic chemicals in Selenastrum capricornutum and Microcystis aeruginosa. *Environ.*
580 *Toxicol. Chem.* **1999,** 18, 1164-1172; DOI 10.1002/etc.5620180614
581 43 Swackhamer, D.L.; Skoglund, R.S. Bioaccumulation of PCBs by algae: kinetics versus
582 equilibrium. *Environ. Toxicol. Chem.* **1993,** 12, 831-838; DOI 10.1002/etc.5620120506
583 44 Sobek, A.; Gustafsson, O.; Hajdu, S.; Larsson, U. Particle-water partitioning of PCBs in the
584 photic zone: A 25-month study in the open Baltic Sea. *Environ. Sci. Technol.* **2004,** 38, 1375-
585 1382; DOI 10.1021/es034447u
586 45 Sobek, A.; Cornelissen, G.; Tiselius, P.; Gustafsson, O. Passive partitioning of polychlorinated
587 biphenyls between seawater and zooplankton, a study comparing observed field distributions to
588 equilibrium sorption experiments. *Environ. Sci. Technol.* **2006,** 40, 6703-6708; DOI
589 10.1021/es061248c
590 46 Del Vento, S.; Dachs, J. Prediction of uptake dynamics of persistent organic pollutants by
591 bacteria and phytoplankton. *Environ. Toxicol. Chem.* **2002,** 21, 2099-2107; DOI
592 10.1002/etc.5620211013
593 47 Gobas, F.A.P.C.; Opperhuizen, A.; Hutzinger, O. Bioconcentration of hydrophobic chemicals in
594 fish: relationship with membrane permeation. *Environ. Toxicol. Chem.* **1986,** 5, 637-646; DOI
595 10.1002/etc.5620050704
596 48 Sijm, D.T.H.M.; Van der Linde, A. Size-dependent bioconcentration kinetics of hydrophobic
597 organic chemicals in fish based on diffusive mass transfer and allometric relationships. *Environ.*
598 *Sci. Technol.* **1995,** 29, 2769-2777; DOI 10.1021/es00011a011

599 49 Booij, K.; Tucca, F. Passive samplers of hydrophobic organic chemicals reach equilibrium faster
600 in the laboratory than in the field. *Mar. Pollut. Bull.* **2015,** 98, 365-367; DOI
601 10.1016/j.marpolbul.2015.07.007
602 50 Banerjee, S.; Sugatt, R.H.; O'Grady, D.P. A simple method for determining bioconcentration
603 parameters of hydrophobic compounds. *Environ. Sci. Technol.* **1984,** 18, 79-81.
604 51 Booij, K.; Vrana, B.; Huckins, J.N.; Greenwood, R.; Mills, G.A.; Vrana, B. Theory, modelling
605 and calibration of passive samplers used in water monitoring, In *Passive sampling techniques in*
606 *environmental monitoring*; Greenwood, R., Mills, G., Vrana, B., Eds.; Elsevier: Amsterdam,
607 2007; pp. 141-169.
608 52 Schäfer, S.; Antoni, C.; Möhlenkamp, C.; Claus, E.; Reifferscheid, G.; Heininger, P.; Mayer, P.
609 Equilibrium sampling of polychlorinated biphenyls in River Elbe sediments – Linking
610 bioaccumulation in fish to sediment contamination. *Chemosphere* **2015,** 138, 856-862; DOI
611 10.1016/j.chemosphere.2015.08.032
612 53 Miège, C.; Mazzella, N.; Allan, I.; Dulio, V.; Smedes, F.; Tixier, C.; Vermeirssen, E.; Brant, J.;
613 O'Toole, S.; Budzinski, H.; Ghestem, J.; Staub, P.; Lardy-Fontan, S.; Gonzalez, J.; Coquery, M.;
614 Vrana, B. Position paper on passive sampling techniques for the monitoring of contaminants in
615 the aquatic environment – Achievements to date and perspectives. *Trends Environ. Anal. Chem.*
616 **2015,** 8, 20-26; DOI 10.1016/j.teac.2015.07.001
617