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Sorption of Highly Hydrophobic Organic Chemicals to Organic Matter Relevant for Fish Bioconcentration Studies

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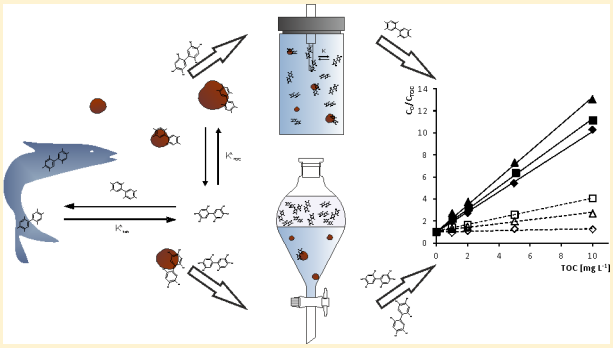
Supporting Information

ABSTRACT: With regard to a potential underestimation of bioconcentration factors (BCF) in flow-through fish tests, sorption of 11 highly hydrophobic organic chemicals (HOCs) (log K_{OW} 5.5−7.8) from different substance classes was systematically investigated for the first time in the presence of fish feed (FF) and filter residues (FR), the organic matter (OM) most relevant for fish bioconcentration studies. Sorption was investigated in batch-equilibrium experiments by solid-phase microextraction (SPME) resulting in partitioning coefficients of solid−water (K_d), total organic carbon−water (K_{TOC}), and dissolved organic carbon−water (K_{DOC}). Results prove a high affinity of HOCs for FF and FR supporting a significant impact on BCF studies and differing from sorption to Aldrich-humic acid (AHA) utilized as reference sorbent. Sorption is influenced by interactions between HOCs and OM characteristics. For FF, K_{DOC} values were higher than K_{TOC} values. Results help to assess the relevance of interaction of HOCs from different substance classes with OM relevant for BCF studies.



INTRODUCTION

The identification of persistent, bioaccumulative, and toxic (PBT) substances is fundamental for regulatory chemical safety assessment. The bioaccumulation potential of chemicals is usually evaluated in fish bioconcentration studies carried out according to the OECD Test Guideline 305 (OECD TG 305).[1] Through these studies, a bioconcentration factor (BCF) is determined as a ratio of the test substance's concentrations in fish and water. OECD TG 305 does not specify methods for the extraction of test substances from the aqueous phase, but the most commonly used is solvent extraction by liquid−liquid extraction (LLE). This is considered as an exhaustive extraction method for the determination of total analyte concentrations.[2,3] Results of BCF studies may be influenced by many factors such as temperature, salinity, pH, or organic matter (OM) content of the water phase.[4] In this review, the authors observed that about 45% of BCF values are subject to at least one major source of uncertainty and that measurement errors generally result in an underestimation of actual BCF values. Especially OM in the test water can have a high impact on the bioavailability of hydrophobic organic chemicals (HOCs) in bioconcentration studies.[5−9] The use of total analyte concentrations from exhaustive extraction methods like LLE, which do not discriminate between freely dissolved and bound analytes, can potentially lead to an underestimation of BCF values. Only freely dissolved substances are bioavailable, but significant amounts of the test substance which are bound by sorption processes are extracted as well from the aqueous phase.[4,10−12] While the determination of freely dissolved analyte concentrations was challenging and laborious for a long time, the use of nonexhaustive solid-phase microextraction (SPME) is straightforward.[13−15] It allows discrimination between bound and freely dissolved test substances, with the freely dissolved analyte concentrations corresponding to the bioavailable concentrations for organisms.[16−21] In addition to physiological explanations, the impact of sorption processes on the bioavailability of test substances has been discussed as a major reason for the phenomenon of the "hydrophobicity cutoff". This means that the correlation between measured BCF values and *n*-octanol−water partition coefficients (K_{OW}) of HOCs levels off or even declines above log K_{OW} 5−6, which indicates that those substances bioconcentrate less than expected on the basis of their hydrophobicity.[4,11,12] The influence of OM on the sorption of several (highly) HOCs (log K_{OW} > 5−6) was studied with a broad range of different qualities and origins of suspended and dissolved organic matter (DOM).[7,22−27] Laboratory BCF systems are less complex compared to environmental systems. Nevertheless, a systematic examination of the interaction of (highly) hydrophobic test

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substances with natural OM occurring in flow-through fish test systems is missing. However, these systems are most relevant for regulation purposes and for decision-making regarding the evaluation and classification of substance characteristics. Further investigations on the influence of OM on the sorption of HOCs are thus required to ensure the estimation of correct BCFs essential for the regulatory evaluation and classification of chemicals as well as for the understanding of environmental processes. With regard to the potential impact of sorption on bioavailable concentrations in the test system and the related risk to underestimate BCF values, the objectives of this study were (i) to identify differences in sorption strength depending on OM quality relevant for BCF studies, (ii) to assess the impact of sorption with regard to low analyte concentrations and within a very low OM range of below 10 mg L^{-1} total organic carbon (TOC), which is the maximum TOC concentration allowed according to OECD TG 305, and (iii) to compare the two methods LLE and SPME, with respect to their potential and limitations for the extraction of HOCs from aqueous phases containing OM. In the present study, sorption of 11 HOCs with varying potential for bioaccumulation (log K_{OW} 5.5−7.8) from different substance classes was systematically investigated in aqueous phases containing defined concentrations of total OM and DOM from different origins. Selected test substances (Figure S1) included persistent organic pollutants (POPs) according to the Stockholm Convention (hexachlorobenzene, HCB; polychlorinated biphenyls, PCB 138, PCB 153, PCB 156; polybrominated diphenyl ethers, BDE 15 [non-Stockholm BDE], BDE 47) as well as emerging contaminants such as plasticizers (diethylhexyl adipate, DEHA; diethylhexyl phthalate, DEHP), UV filters (octocrylene, OCR), and chemicals used in industrial processes (*o*-terphenyl, OTP; nonylphenol, NP). The OM utilized in this study was fish feed for rainbow trout (FF) and filter residues (FR) taken from an experimental tank used as control for an OECD TG 305 study with rainbow trout. FR, which contains fish feces and feed residues, represents the main components of natural OM occurring in bioconcentration test systems. Aldrich-humic acid (AHA) was used as reference OM.

EXPERIMENTAL SECTION

Chemicals. All test substances were purchased from Dr. Ehrenstorfer GmbH (Augsburg, Germany) (purity >98%). Molecular structures of the test substances are given in Figure S1, and physicochemical properties are given in Table S1. DEHP was purchased as a deuterated compound (DEHP D_4) to deal with contamination of the system by this ubiquitous plasticizer. For ease of chromatography analysis, 4-*n*-nonylphenol was used as a reference for nonylphenols instead of the technical mix.[28] 1,2,3,4-Tetrachloronaphthalene (TCN) was used as internal standard for LLE. A stock solution of all test substances was prepared in methanol (details in the Supporting Information). Solvents and inorganic salts (methanol, *n*-hexane, *n*-heptane, sodium sulfate) were all obtained in p.a. quality (≥99%) from Carl Roth GmbH (Karlsruhe, Germany). All water used was of Milli-Q quality (Milli-Q Advantage A10 System, Millipore).

Organic Matter. FF and FR were chosen according to their presence in test systems for bioconcentration studies. FF was purchased from Biomar (INICIO PLUS, 0.8 mm, micro pellets). FR was collected from a mechanical filter connected to the outlet pipe of a flow-through tank used as control for a bioconcentration study with rainbow trout. AHA was obtained as reference OM from Sigma-Aldrich as sodium salt (CAS-RN 68131-04-4). FF and AHA were used as supplied; FR was used after drying at 105 °C and grinding to a fine powder by ball mill. Dry matter of all matrices was determined after heating for 24 h at 105 °C (AHA: 92%; FF: 94%; FR: 94%). Elemental and nutrient composition of OM is given in Table S2. Elemental composition was determined by CNS Analyzer (VarioEL III Elementar, Hanau, Germany). No inorganic carbon was detected; therefore, C_t and TOC are equal. Nutrients were determined according to Weende analysis (crude nutrient analysis). For partitioning coefficients of solid−water (K_d) and total organic carbon−water (K_{TOC}) experiments, different amounts of OM were weighed into 250 mL glass bottles (Schott Duran screw neck bottles). Afterward, 250 mL of water was added, resulting in concentrations of 1, 2, 5, and 10 mg L^{-1} TOC with the OM being both suspended and dissolved. Initial weights of the sorbents are given in Table S3. OM suspensions were equilibrated for at least 2 h before spiking with the analytes. DOM fractions for the partitioning coefficient of dissolved organic carbon−water (K_{DOC}) experiments were prepared by separately suspending FF and FR in water (initial weights 0.5−3 g L^{-1}). Dissolution of DOM was improved by ultrasonic treatment. DOM stock solutions were then obtained by pressure filtration of suspensions with cellulose-acetate filters of 0.45 μm pore size (Sartorius AG, Göttingen, Germany) to separate dissolved and particulate fractions.[29] Dissolved organic carbon (DOC) concentrations in DOM stock solutions were measured as TOC by photometric cuvette tests (Hach-Lange, Düsseldorf, Germany).[30,31] After filtration with 0.45 μm pore size, TOC and DOC are assumed to be equal.[29] DOM stock solutions were diluted on the basis of measured DOC concentrations (35−400 mg L^{-1} DOC) with water to concentrations of 1, 2, 5, and 10 mg L^{-1} DOC (±0.05 mg L^{-1} DOC by calculation). For each sample, 250 mL of DOM solution was filled to 250 mL glass bottles.

Experimental Design. Determination of sorption was performed according to a quotient approach. Freely dissolved analyte concentrations in the presence of OM were compared to the analyte concentrations in the absence of OM. Analysis was carried out by automated SPME (compare section Instrumental Analysis). Two independent types of experiments were performed. K_d and K_{TOC} values were determined from the same samples whereas K_{DOC} values were derived from a separate experiment. Except for sample preparation (compare section Organic Matter), the procedure for both experiments was the same. Samples with 0, 1, 2, 5, and 10 mg L^{-1} TOC (K_d/K_{TOC} experiments) or DOC (K_{DOC} experiments) were spiked each with 7.5 μL of the analyte stock solution (eVol dispensing system, Thermo scientific). For 0 mg L^{-1} TOC, two bottles were prepared. Concentrations of a selected analyte were adjusted in all samples to 0.6−5.3 μg L^{-1}, depending on the water solubility of the respective analyte. All sorption studies were performed with test substance concentrations within their water-soluble range (Table S1). Therefore, they conform to relevant environmental concentrations as well as to exposure conditions given in OECD TG 305. The content of methanol (0.003%) as solubilizing agent was below 0.01% in accordance to OECD TG 305. The spiked samples were equilibrated prior to extraction for 24 h on an overhead shaker to accelerate the distribution of analytes between aqueous phase and sorbents. The equilibration time was defined according to results from preliminary kinetic experiments with AHA (Figure S2). An experiment which included 100 mg

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L^{-1} sodium azide (NaN_3)[32] was compared with an untreated experiment to assess possible degradation of test substances over time but revealed no significant differences (t test, p = 0.203 to 0.883). After equilibration, aliquots of 20 mL were transferred with volumetric glass pipettes to brown glass vials (20 mL headspace vials with magnetic caps, CS Chromatographie Service, Langerwehe, Germany). Pipettes were washed twice with 20 mL of sample prior to use. Aliquots from samples with 1, 2, 5, and 10 mg L^{-1} OC were transferred in duplicates. For 0 mg L^{-1} OC, eight aliquots were transferred. After filling, samples in brown glass vials were subsequently analyzed by SPME. In comparison to sorption measurement by SPME, the quotient approach was intended to be applied as well after LLE to check for TOC-independent extraction of test substances. Therefore, the remaining 170 mL of all samples was extracted by LLE as a reference method for exhaustive extraction. After pipet washing and aliquoting, 170 mL remained for samples with 1, 2, 5, and 10 mg L^{-1} OC. Also, 170 mL remained for the 0 mg L^{-1} OC sample after disposal of 80 mL from the second bottle. The aqueous phases were extracted three times with *n*-hexane (50, 20, and 10 mL) followed by centrifugation to enhance separation of liquid phases. The extract was dried with sodium sulfate (Na_2SO_4) and concentrated by rotary evaporator after adding 100 μL of *n*-heptane. Extracts were adjusted to 200 μL with *n*-heptane and measured by GC/MS. All materials used in experiments were of glass or polytetrafluoroethylene to minimize the risk of sorption to materials. However, sorption to glassware cannot be prevented for HOCs,[33,34] and the extent to which this occurred was examined in a separate experiment and remained below 5% for all analytes. Detailed procedures and results are given in Table S4.

Instrumental Analysis. Samples of a specific OM type were measured in a sequence with four sorbent concentrations (1, 2, 5, and 10 mg L^{-1} OC) in duplicate (n = 8). Before and after each duplicated concentration, a sample with 0 mg L^{-1} OC was measured. Samples with 0 mg L^{-1} OC were used both for the quotient approach and as reference samples to assess the sorption of HOCs to glassware. They were also used to assess variability of the instrument and SPME fiber during the measurement period. Sequences (N) were prepared and measured up to seven times per OM type. Extraction of freely dissolved analyte concentrations was performed by negligible depletion-SPME in immersed mode.[18] Information on the actual depletion is given in Table S5. Processing of the sample sequence was performed with an autosampler (CTC-Analytics, Combi Pal PAL-System) controlled by CHRONOS software (Axel Semrau, Sprockhövel, Germany). The samples were thermally equilibrated (5 min) prior to extraction (30 min) with 100 μm PDMS fibers (Supelco). Samples were kept at 30 °C and agitated (250 rpm) during equilibration and extraction in a heating device within the autosampler (CTC-Agitator). After extraction, the fiber was thermally desorbed in the injection system for 3 min in splitless mode at 280 °C. Before extraction as well as after thermodesorption, the fiber was additionally desorbed in a needle heater at 280 °C for 4 min, respectively, to avoid analyte residues on the fiber. This was ensured by frequently measuring pure water samples (blanks). Separation and quantification were carried out by GC/MS. Details of the instruments and methods (SPME and LLE samples) as well as on quality assurance and quality control are given in Tables S6 and S7.

Data Analysis. Sorption was determined according to a quotient approach similar to the Stern–Volmer relationship.[35−37] Freely dissolved analyte concentrations in the absence of OM were compared to reduced freely dissolved analyte concentrations in the presence of OM. In the absence of sorbents, the free analyte concentration corresponds to the total analyte concentration. Sorption coefficients K_d, K_{TOC}, and K_{DOC} result from the slope of a linear regression made up as a function of quotients plotted against sorbent concentrations. Quotients were calculated as a ratio of the GC/MS response of total concentrations (C_0) in the absence of sorbents (0 mg L^{-1} OC) and the GC/MS response of freely dissolved concentrations (C_{OM}, C_{TOC}, and C_{DOC}) in the presence of sorbents (eqs 1−3).

$$\frac{C_0}{C_{OM}} = K_d \times OM[kgL^{-1}] + 1 \quad (1)$$

$$\frac{C_0}{C_{TOC}} = K_{TOC} \times TOC[kgL^{-1}] + 1 \quad (2)$$

$$\frac{C_0}{C_{DOC}} = K_{DOC} \times DOC[kgL^{-1}] + 1 \quad (3)$$

In K_d/K_{OC} experiments, sorbents were quantified by their initial weights (OM) or rather by their TOC contents (Table S3). In K_{DOC} experiments, DOM was quantified by its DOC content. The sorption coefficient K_d results from the slope of a linear regression made up as a function of quotients C_0/C_{OM} plotted against OM concentrations. The regression of C_0/C_{TOC} plotted against TOC concentrations yields the sorption coefficient K_{TOC}, and the regression of C_0/C_{DOC} plotted against DOC concentrations results in the sorption coefficient K_{DOC}.[35,36] The slope of this linear regression was calculated with mean values of cumulated quotients obtained from all measured sequences/replications. Extreme outliers were determined in box plots as values outside the 3-fold distance of median and upper/lower quartile[38] and excluded from the calculation of sorption coefficients, when at least four quotients per sorbent concentration existed. Extreme outliers are given in the Supporting Information. Independent from the determination of sorption by SPME, quotients of the ratio C_0/C_{TOC} were calculated as well for results from LLE extracts. However, in the case of the exhaustive extraction method LLE, extracted amounts are intended to always be total concentrations and should not differ depending on absence/presence of sorbents nor on sorbent concentration. Therefore, resulting quotients should always be equal to 1. In the case of extraction by SPME, increasing quotients with increasing sorbent concentration indicate higher sorption; however, for LLE, increasing quotients with increasing sorbent concentration indicate an insufficient extraction of analytes by the solvent used.

RESULTS AND DISCUSSION

Sorption of HOCs to OM Relevant for Fish BCF Studies. Results of sorption studies confirm the hydrophobic characteristics of the investigated test substances as shown by their high affinity to bind to the OM present. Analogous to the wide range of K_{OW} values of the analytes, sorption resulted in K_d values between approximately 28 000 (OTP with FR) and 730 000 (PCB 156 with AHA) and K_{TOC} values between approximately 75 000 (HCB with AHA) and 1 900 000 (PCB 156 with AHA) for all sorbents (Tables 1 and S8−S10).

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Table 1. Measured Partitioning Coefficients K_d, K_{TOC}, and K_{DOC} for Aldrich Humic Acid (AHA), Fish Feed (FF), and Filter Residue (FR) as Sorbents[a]

	AHA		FF			FR	
analyte	log K_d	log K_{TOC}	log K_d	log K_{TOC}	log K_{DOC}	log K_d	log K_{TOC}
OTP	4.48 ± 0.11	4.89 ± 0.11	4.76 ± 0.11	5.06 ± 0.11	5.43 ± 0.14	4.45 ± 0.06	4.99 ± 0.06
NP	5.11 ± 0.09	5.53 ± 0.09	5.01 ± 0.11	5.31 ± 0.11	5.54 ± 0.14	4.98 ± 0.09	5.52 ± 0.09
HCB	4.46 ± 0.06	4.88 ± 0.06	4.65 ± 0.05	4.95 ± 0.05	5.38 ± 0.13	4.51 ± 0.12	5.05 ± 0.12
BDE 15	4.85 ± 0.10	5.26 ± 0.10	4.88 ± 0.08	5.18 ± 0.08	5.55 ± 0.06	4.52 ± 0.06	5.06 ± 0.06
DEHA	5.59 ± 0.05	6.00 ± 0.05	5.64 ± 0.09	5.94 ± 0.09	6.57 ± 0.16	5.08 ± 0.09	5.63 ± 0.09
OCR	5.53 ± 0.13	5.95 ± 0.13	5.37 ± 0.05	5.67 ± 0.05	6.17 ± 0.10	5.00 ± 0.01	5.55 ± 0.01
DEHP	5.62 ± 0.06	6.04 ± 0.06	5.65 ± 0.11	5.95 ± 0.11	6.57 ± 0.04	4.87 ± 0.11	5.42 ± 0.11
BDE 47	5.86 ± 0.10	6.28 ± 0.10	5.67 ± 0.15	5.97 ± 0.15	6.55 ± 0.04	5.46 ± 0.13	6.01 ± 0.13
PCB 138	5.77 ± 0.06	6.18 ± 0.06	5.69 ± 0.08	6.00 ± 0.08	6.56 ± 0.15	5.42 ± 0.08	5.96 ± 0.08
PCB 156	5.86 ± 0.15	6.28 ± 0.15	5.76 ± 0.12	6.07 ± 0.12	6.61 ± 0.19	5.43 ± 0.08	5.98 ± 0.08
PCB 153	5.67 ± 0.06	6.08 ± 0.06	5.71 ± 0.08	6.01 ± 0.08	6.63 ± 0.19	5.42 ± 0.10	5.96 ± 0.10

[a]Further information (unlogged values, r^2, RSD, N) is given in Tables S8–S11.

K_{TOC} values for AHA from this study match well with K_{OC} values in the literature if they are available (Table S1). Possible methodical constraints regarding the measured sorption are discussed below (compare section Critical Considerations on Sorption Coefficients). Absolute sorption (K_d values) differed significantly between the three sorbents. Most test substances showed a much weaker (up to factor 6) affinity for FR than for AHA and FF, which can only be partly explained by the smaller TOC content of 28.5% for FR compared to 38.4% and 49.9% for AHA and FF, respectively. Further, for several substances, K_d values for FF are smaller than for AHA despite the significantly higher TOC content of FF. While the content of OC is commonly regarded to be the main parameter to affect the extent of sorption, the amount of sorption is not solely explainable by OC content. It depends as well on the interaction of both analyte characteristics and composition of OM,[39−42] which is supported by the present results. Apparently, FF and FR are not comparable with AHA regarding their composition. For humic substances like AHA which have a complex macromolecular structure, sorption increases with increasing complexity of OM (from fulvic acids to humic acids to humin).[41,43] In contrast, the main source of carbon relevant for sorption in FF is lipids with a less complex composition. However, lipids are per se an attractive medium for HOCs. In addition, results for FR suggest a less complex macromolecular structure of its OM. Besides the composition of OM, molecular characteristics such as the hydrophobicity of test substances are a significant factor influencing the sorption of HOCs. The impact of OM composition is also reflected by K_{TOC} values. After normalization for TOC, K_{TOC} values of all HOCs (except OTP and HCB) determined with AHA as sorbent were higher than for FF and FR as sorbents. Differences for the less hydrophobic analytes are not significant for the most part. For most HOCs, K_{TOC} values determined with FF and FR converge, suggesting that differences in K_d values for FF and FR were adjusted by TOC normalization. This highlights again differences in OM structure between AHA and the sorbents relevant for BCF studies, FF and FR. However, depending on sorbate characteristics, significant differences between FF and FR are seen for K_{TOC} values of NP, DEHA, and DEHP. Comparative extraction by LLE generally proved a total extraction independent of TOC concentration. However, results partly indicated that extraction efficiency of *n*-hexane could be reduced by OM resulting in an underestimation of total concentrations but still overestimating free concentrations (Figure S3).

Sorption of HOCs to Dissolved Organic Matter. K_{DOC} values were expected to be smaller than K_{TOC} values because sorption usually increases with increasing molecular size of sorbents, which is smaller for dissolved molecules after a filtering process than for particulate OM.[36,39,43] Nevertheless, DOM from FF could be identified as a very strong sorbent since K_{DOC} values for FF are significantly higher than their K_{TOC} values. K_{DOC} values for FF-DOC are in a range of approximately 240 000 (HCB) to 4 300 000 (PCB 153), being up to more than four times greater than K_{TOC} values for FF (Tables 1 and S11). This is explained by the structural composition of FF. Since the feed consists of fiber, ash, protein, and fat, it is assumed, that fiber and ash do not contribute significantly to sorption but remain particulate. However, the lipids are assumed to dissolve within the process of DOM preparation and should thus contribute most to the sorption of HOCs. Proteins have a lower impact on sorption of neutral HOCs, especially in the presence of significant lipid concentrations.[16,44−46] However, depending on the type of protein, dissolved fractions might also have contributed to sorption. Sorption of the test substances to FR-DOC as sorbent is significantly smaller than to FF-DOC. Sorption of HCB estimated from raw data had a K_{DOC} of around 10 000. However, in the low concentration range of 0−10 mg L^{-1}, the FR-DOC sorption induced decrease of the analytes freely dissolved concentrations was in the range of variation in raw data. Therefore, it was not possible to determine K_{DOC} values for FR-DOC. The variation of raw data resulted from the SPME-GC/MS method and is attributed to variation of SPME fiber sensitivity and variation of the MS not being sensitive and robust enough to detect moderate sorption in a low DOC range.

Critical Considerations on Sorption Coefficients. The plotting of experimentally determined sorption coefficients K_{TOC} (AHA) against K_{OW} values of the respective test substances from the literature[47,48] matches a linear correlation within ±0.5 log units (Figure S4). Sorption within the concentration range of 1−10 mg L^{-1} TOC was predominantly linear. For AHA, deviation of K_{TOC} values for single TOC concentrations (calculated according to eq 2) from the K_{TOC} value for the whole concentration range was within 0.1 log units for 9 of the 11 substances. For DEHA and DEHP, deviation was higher (0.3 and 0.4 log units, respectively, at 1 mg L^{-1}

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TOC and 0.1 and 0.2 log units, respectively, at 2 mg L^{-1}). K_{TOC} values for PCBs, DEHA, and DEHP indicate a systematic increase of K_{TOC} with decreasing TOC content. This phenomenon was stronger for the sorbent FF (Figures S5−S7). However, significant scattering inhibits explicit conclusions and quantitation of this phenomenon, especially in the very low OM range of 1 and 2 mg L^{-1} TOC. A deviation of K_{TOC} values depending on sorbent concentration has already been reported[49,50] but was suggested as being due to analytical artifacts.[39] Potential explanations include methodology as well as sorbent inherent reasons. Because SPME measurements were performed under nonequilibrium conditions of fiber and aqueous phase, diffusion layer effects cannot be excluded, i.e., the effect of an accelerated transport of analytes in the presence of DOM to the fiber compared to pure water samples.[18,37,51−54] This effect was mainly observed for polycyclic aromatic hydrocarbons (PAH) but also for PCBs and PBDEs when extraction time was not sufficient. This effect would usually be stronger for higher DOM amounts in the samples and could result in an underestimation of K values. In such cases, K values would increase with increased extraction times. Nevertheless, in their study on OM-PAH interactions, de Perre and colleagues found no statistical significant differences between 30 and 120 min extraction times.[37] Likewise in the present study, no systematic differences in K_{TOC} values were detected between 12 and 180 min (Figure S8). In this case, an adverse effect due to depletion has to be considered as a possibility. Depletion increases with increasing sampling time (Table S5) and could disturb the equilibrium between analyte and sorbent if depleted analytes stimulate the desorption of sorbates to form a new equilibrium. However, an additional screening of sorption with a 7 μm PDMS fiber revealed an increase of K_{TOC} values for some analytes compared to the results obtained with the common 100 μm fibers (Table S12). Smaller coating volumes reduce the equilibration time between fiber and aqueous phase. These results indicate that it is not sufficient to consider for facilitated transport within a limited period of extraction time. Still, an increase of K_{TOC} values with decreasing TOC remained for data measured with the 7 μm fiber. The discrepancy between K_{TOC} values measured with 7 and 100 μm fibers is relativized if compared to K_{OC} values from the literature obtained with different methods and sorbents. Exemplarily, log K_{TOC} values for PCB 153 measured with 100 and 7 μm PDMS fibers are 6.1 and 6.3, respectively, while measured log K_{OC} values for PCB 153 range between 5.3 and 7.7 in the literature.[47] Extraction by automated SPME in immersed mode is beneficial due to its quick handling, fast processing, and the small sample volumes needed. However, a compromise is required with respect to the extraction time. If it is too short, diffusion layer effects might affect results. If it is too long, results can be affected by depletion. On the basis of the results of the present study, it seems acceptable to use the method when potential artifacts are considered, at least for a screening of sorption coefficients with several matrices. For some highly HOCs, the use of a fiber with smaller coating diameter (e.g., 7 μm PDMS) could be beneficial (Table S12). Sorption can be further influenced by changes of ionic strength and pH. Both have not been adjusted in this study to consider test conditions with a continuous variation in OM concentrations without adjustment of those parameters. Although variations in pH and ionic strength have been moderate (pH: AHA 5.8−6.9, FF 5.8−7.1, FR 5.8−6.1; ionic strength [μS cm^{-1}]: AHA 2.5−9.1, FF 6.1−8.2, FR 4.8−12.3), systematic variation of K_{TOC} values seems to be caused by a decrease of pH with decreasing TOC. Results from a subsequent screening of sorption of PCBs at a pH of 7.1 in buffered AHA (1−10 mg L^{-1} TOC, 5 mM PO_4) were in the same range as K_{TOC} values given in Table 1 but did not show a systematic increase of K_{TOC} values with decreasing TOC.

Consequences for the Conduct of BCF Studies. Results of the SPME measurements show that decreased bioavailability of hydrophobic test substances may occur even in the presence of very low concentrations of OM as found in fish bioconcentration studies carried out according to OECD TG 305. High sorption of HOCs to OM relevant for BCF studies (FF, FR) support the conclusion of an underestimation of BCF values if total water concentrations are determined and if freely dissolved test substance concentrations are not taken into account for OM in the test system. However, the sorption coefficients presented in this study represent a worst case scenario for BCF studies as they are determined under equilibrium conditions in a closed system. On the basis of K values for FF and FR from this study, BCF values for exemplary selected HCB and PCB 153 would be underestimated by 50−70% and >90%, respectively, at a TOC concentration of 10 mg L^{-1} in the test vessel (Figure 1; theory and calculations are given in the Supporting Information). On the basis of the procedure according to OECD TG 305, a daily input of >2 g of FF to the test vessels is a realistic scenario, which correlates to >14 mg L^{-1} TOC in a 70 L aquarium solely from FF (before food uptake by fish) (details are given in the Supporting Information). However, tests according to OECD TG 305 are flow-through tests, providing a constant delivery of freely dissolved test substance. Combined with the careful removal of feed residues and feces, this should result in higher free (bioavailable) concentrations of the test substances than under closed equilibrium conditions. For many relevant substances, K_{OC} values are not available in the literature. If available at all, nonequilibrium conditions together with significant differences in the amount of sorption depending on sorbent quality inhibit the estimation of the actual amount of sorption in a test system based on K_{OC} values from the literature. While Linear Solvation Energy Relationship (LSER) and Polyparameter Linear Free Energy Relationship (PP-LFER) models yield relatively exact results, models have to be fitted and validated for new sorbents with a set of experimentally derived data.[55−57] If possible influences like diffusion layer effect and depletion are considered, automated SPME could be beneficial for a screening of sorption coefficients for test substances with several matrices to estimate the amount of sorption for OM occurring in BCF tests. Further, it seems to be appropriate to use SPME to monitor bioavailable concentrations during BCF tests to cover the impact of specific OM present in the test system. OM is expected to vary between test vessels and is especially dependent on the fish species used in the test. For example, carp and rainbow trout differ significantly in feeding habits and consistency of fecal matter which have consequences for the amount and structure of OM. If differences between total and freely dissolved concentrations of the test substance are observed, water quality, especially TOC content, could be corrected with minimum delay if required. However, the impact of TOC on the bioavailable fraction and the results of a BCF study with HOCs has to be reflected under experimental conditions in a flow-through test system according to OECD TG 305 to be able to estimate the influence of TOC under

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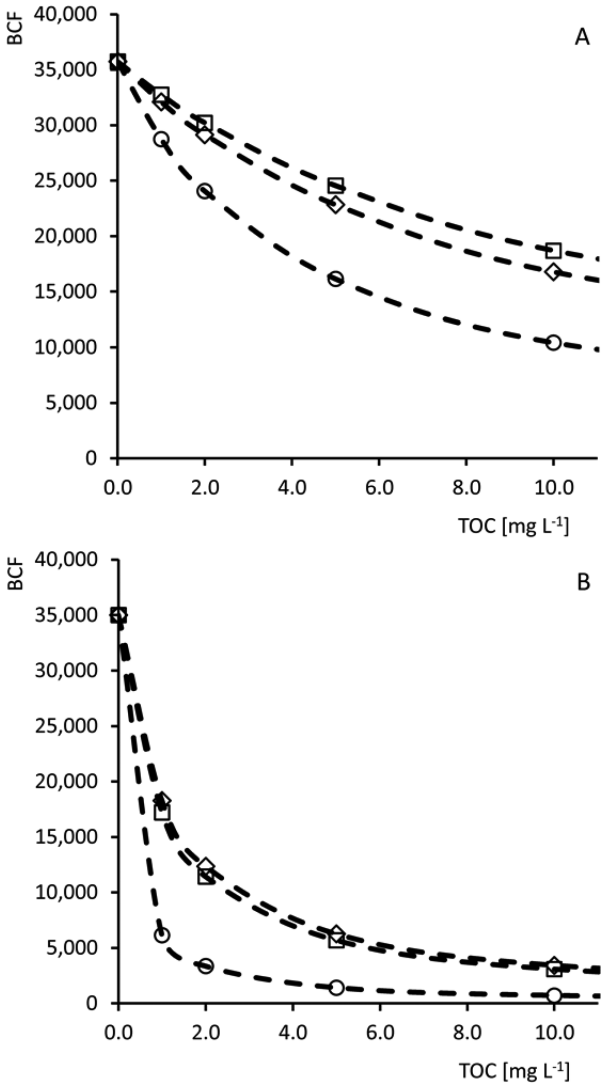

Figure 1. Estimated BCF values for (A) HCB and (B) PCB 153 in the presence of TOC from fish feed (rectangles), filter residue (diamonds), and DOC from fish feed (circles).

nonequilibrium conditions and to check for monitoring with SPME.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.est.6b01778.

Detailed information on materials and methods as well as additional figures and tables as referenced throughout the manuscript (PDF)

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The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

Notes

The authors declare no competing financial interest.

Results from the project provoked the recommendation of SPME for HOCs within the revised OECD TG 305. Results of this study will further be integrated into an upcoming Guidance Document for the revised OECD TG 305.

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