MEASURING THE BIOAVAILABILITY OF TWO HYDROPHOBIC ORGANIC COMPOUNDS IN THE PRESENCE OF DISSOLVED ORGANIC MATTER

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Abstract—Bioavailability of benzo[a]pyrene (BaP) and 3,3′,4,4′-tetrachlorobiphenyl (TCB) was studied in natural lake water containing dissolved organic matter (DOM). Lake water was diluted to give a dissolved organic carbon (DOC) range of 1 to 20 mg/L. Partition coefficients for the model compounds were assessed at different DOM concentrations and over time with three different methods, namely equilibrium dialysis and reverse-phase and liquid-liquid extraction. In addition, biological partition coefficients were estimated from the difference in the bioconcentration of the model compounds in Daphnia magna in the presence and absence of DOM. Results showed that bioavailability of the model compounds was reduced by the presence of DOM. The equilibrium dialysis method gave the best estimates for bioavailability of the model compounds when compared with biologically determined values. Both the reverse-phase and the liquid-liquid extraction overestimated the bioavailable fraction. The more pronounced overestimation of bioavailable fraction of TCB suggested that the sorption of TCB was not only lower but the interaction was also weaker than that of BaP. Increasing DOM concentration produced lower partition coefficients and the effect seemed to be more pronounced when measured by the reverse-phase and the extraction methods.

Keywords—Dissolved organic matter Humic substances Sorption Bioconcentration Bioavailability

INTRODUCTION

Dissolved organic matter (DOM) is present in all surface freshwaters and contains up to 90% of humic substances [1]. A change in parameters like pH, ionic conditions, and DOM concentration causes reorganization in the macromolecular structure of dissolved humic substances [2–5]. In other words, molecular size, shape, and weight of humic material in solution are affected by these variables. The changes in conformation of humic material are probably responsible for the alteration of their association capacity with hydrophobic organic chemicals. Increase in pH, ionic strength, water hardness, or concentration of humic matter has been reported to yield lower partition coefficients for hydrophobic organic compounds [6–9].

A number of methods have been used to measure the association of organic chemicals with DOM [10]. These include commonly used methods like fluorescence quenching [6], equilibrium dialysis [11], and reverse-phase separation [12] as well as less frequently used methods like liquid–liquid extraction [13] and biological assay [14]. Most of these methods have some disadvantages or limitations, and the results have been shown to vary greatly between the methods [10,15]. Basically, these methods can be divided into two groups, those that interfere and those that do not interfere with the DOM-contaminant interaction.

Several experiments conducted by different methods, like the equilibrium dialysis and reverse phase [11,12,16], adsorption to glass [17], or increase of solubility [18], have indicated that the capacity of humic substances to sorb contaminants depends on its concentration. In those studies, increasing DOM concentration led usually to detection of lower partition coefficients. On the other hand, Krop et al. [10] questioned the statistical significance of the effects in these studies. Furthermore, Gauthier et al. [6] did not notice such a relationship when measuring the association of polycyclic aromatic hydrocarbons (PAHs) with humic materials by the fluorescence quenching method. In addition, DOM concentrations far higher than environmentally relevant levels have been used in some of these studies. Due to this inconsistency in data, Krop et al. [10] concluded in their review that changes in the quantity of DOM do not affect its association capacity within environmental levels of DOC (below 100 mg/L).

Bioconcentration of organic chemicals follows a nonlinear relationship with DOM concentration. The effect of a similar increase in DOM concentration on bioavailability is higher for lower DOM levels (DOC < 10 mg/L) but is less pronounced for higher DOM levels. This relationship has been shown to apply not only for isolated humic material dissolved in different concentrations [19,20] but also for dilution series of a natural water sample [21] as well as for series of natural freshwaters with various DOM concentrations [22].

In this study, we evaluated the effects of environmentally relevant DOM levels on sorption and bioavailability of benzo[a]pyrene (BaP) and 3,3′,4,4′-tetrachlorobiphenyl (TCB) by four different methods. Furthermore, we tested the applicability of a nonlinear function to describe the relationship between bioconcentration and DOM concentration.

MATERIALS AND METHODS

Model compounds

The radiolabeled model compounds used in this study were [G-3H]-benzo[a]pyrene (specific activity 81 Ci/mmol, radiochemical purity > 98%; Amersham, Little Chalfont, UK) and [14C]-3,3′,4,4′-tetrachlorobiphenyl (specific activity 104.0 mCi/mmol, radiochemical purity > 97%; Sigma, St. Louis, MO, USA) (Fig. 1). Original solvents were evaporated under a stream of nitrogen, and the chemicals were redissolved in
Bioavailability of BaP and 3,3′,4,4′-TCB

**Experimental waters**

Soft artificial freshwater (AFW) was prepared in Milli-Q®-grade water (Bedford, MA, USA) by adding the following salts: CaCl₂·2H₂O, 11.8 mg/L; MgSO₄·2H₂O, 4.9 mg/L; NaHCO₃, 2.6 mg/L; and KCl 0.2 mg/L (Table 1). Hardness of the AFW was 0.1 mM expressed as concentration of Ca²⁺ + Mg²⁺ with an ionic ratio of 4:1. The AFW was used to dilute the natural water in the equilibrium dialysis experiments and as DOM-free exposure water in the bioconcentration experiments. The concentration of DOC, which was used to quantify the DOM, was analyzed with a total organic carbon analyzer (TOC 5000-A; Shimadzu, Kyoto, Japan).

Soft natural freshwater from Lake Kontiolampi (Finland, 62°44′N, 29°52′E) was used as the source of DOM. Lake Kontiolampi is a small, brown-water lake with low pH (original pH 5.4). This particular water was chosen because recent studies have shown that the DOM in this lake efficiently reduces the bioavailability of organic compounds [9,25,26]. The Lake Kontiolampi water was filtered twice, first through combusted glass fiber filters (Schleicher & Schuell GF 52, Dassel, Germany) and then through 0.45-μm filters (sterile membrane filter, Schleicher & Schuell). Furthermore, the Lake Kontiolampi water was diluted with AFW to give a series of six different DOM concentrations (five dilutions and the original) (Table 1).

Water–DOM partition coefficients (KDOC) were measured over time using a period from 1 to 7 d for undiluted and 1:20 diluted DOM samples with the equilibrium dialysis and from 4 h to 7 d with reverse-phase and liquid–liquid extraction methods. Furthermore, KDOC was measured in all six DOM concentrations with the equilibrium dialysis and the biological assay.

**Table 1. The experimental waters (pH adjusted to 7)**

<table>
<thead>
<tr>
<th>DOM dilution series</th>
<th>DOC (mg/L)</th>
<th>IC (mg/L)</th>
<th>Conductivity (mS/m)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AFW</td>
<td>&lt;0.2</td>
<td>&lt;0.7</td>
<td>3.1</td>
</tr>
<tr>
<td>1:20</td>
<td>1.1</td>
<td>0.6</td>
<td>3.1</td>
</tr>
<tr>
<td>1:10</td>
<td>1.9</td>
<td>0.6</td>
<td>3.1</td>
</tr>
<tr>
<td>1:4</td>
<td>4.8</td>
<td>0.5</td>
<td>3.2</td>
</tr>
<tr>
<td>1:2</td>
<td>9.7</td>
<td>0.4</td>
<td>3.3</td>
</tr>
<tr>
<td>3:4</td>
<td>15.2</td>
<td>0.4</td>
<td>3.6</td>
</tr>
<tr>
<td>Original</td>
<td>19.9</td>
<td>0.4</td>
<td>3.8</td>
</tr>
</tbody>
</table>

*DOM = dissolved organic matter; DOC = dissolved organic carbon; IC = inorganic carbon; AFW = artificial freshwater.

**Equilibrium dialysis**

Association of the model compounds with DOM was measured with the equilibrium dialysis technique using Spectra/Por 6 dialysis tubing (mol wt cut-off 1,000 Daltons, volume/length 1 ml/cm; Spectrum, Houston, TX, USA) [11,16]. Stock solutions of the model compounds were dosed individually to the bottom of 210-ml glass jars. After the carrier had evaporated, 200 ml of AFW was decanted to the jars. Before use, the dialysis tubes were cut to a length of 11 to 12 cm and washed in Milli-Q-grade water. The dialysis tubes were then closed with a clip from the other end and a 6-ml DOM sample was added to the tubes. The tubes were closed and placed into the jars, which were closed with screw caps. To verify free diffusion of the model compounds, control samples were made correspondingly except that AFW (DOM free) was added also inside the tubes. At least three replicates were made for each sample. The jars were incubated on a shaker (45 rpm) in the dark at 20 ± 1°C until sampling.

In the equilibrium dialysis method, DOM is assumed to remain inside the dialysis tubes while the chemicals can freely diffuse through the semipermeable membrane. During dialysis, hydrophobic chemicals can also adsorb to the tubing and glassware. Because the method is based on the fact that the whole system is at equilibrium, any chemical adsorbed to the tubing and glassware does not interfere with the equilibrium between DOM-associated and the freely dissolved fraction of the chemical. The concentration of chemical outside the tubes is considered as freely dissolved and the concentration inside the tubes is the sum of freely dissolved and DOM-associated chemical. Based on this, the partition coefficients are calculated as $K_{DOC} = C_l / (C_l - DOC)$, where $K_{DOC}$ is the partition coefficient (L/kg), $C_l$ is the concentration of DOM-associated chemical (difference between the aqueous concentrations inside and outside the dialysis tubing), and $DOC$ is the concentration of dissolved organic carbon (kg/L). Concentration of the chemicals was quantified by taking a 5-ml sample inside and duplicate 5-ml samples outside the dialysis tubes into scintillation vials. After adding 5 ml of scintillation cocktail (Insta-Gel Plus, Packard, Groningen, The Netherlands), the samples were left for 24 h before the analysis of radioactivity with a liquid scintillation counter (Wallac Winspectral 1414; Wallac, Turku, Finland).

Furthermore, possible leaking of DOM from the dialysis tubes was tested for undiluted and 1:20 diluted Lake Kontiolampi water in a separate test without radioactive chemicals added. The leakage was determined by ultraviolet spectroscopy and directly by measuring the concentration of DOC inside the dialysis tubes. Wavelengths between 250 and 280 nm have been used to measure relative aromaticity in dissolved organic matter [27–29]. This absorbance is related to π–π* transitions in carbon double bonds in aromatic structures. Moreover, aromaticity is strongly related to the association of organic chemicals with DOM, higher aromaticity leading to higher sorption [22,27,30–32].

After 4 d of dialysis, DOC measurement revealed a 15.9 ± 5.0% (n = 4) decrease in organic carbon content of undiluted Lake Kontiolampi sample inside the dialysis tubes. The spectrosopically determined decrease in absorbance at 270 nm was 5.0 ± 0.8% (n = 4). After 7 d, the loss was 22.0 ± 4.8% (n = 3) for DOC and 10.6 ± 2.2% (n = 3) for absorbance.
For the 1:20 diluted sample, it was not possible to obtain any accurate estimates.

Reverse-phase separation

In the reverse-phase separation method, water samples containing DOM and the model compound were passed through a Sep-Pak C-18 cartridge (Millipore, Milford, MA, USA) by using a 10-ml glass syringe [12]. In this method, the freely dissolved compound is retained by the C-18 column while the compound associated with DOM passes through. The DOM-associated fraction is calculated as a ratio of concentration of the compound passing through the column and total concentration of the compound. The $K_{\text{DOM}}$ is calculated as described earlier.

Two-hundred-milliliter samples (four replicates) were spiked similarly as in the equilibrium dialysis method ($n = 4$). The closed jars were kept on a shaker (45 rpm) in the dark at 20 ± 1°C until sampling. Before sampling, 20 ml of Milli-Q-grade water was passed through the column. At each sampling time, two 5-ml samples were taken from the test solution, and then 5 ml of test solution was passed through the column at a flow rate of 1 ml/min. This flow rate was chosen because, at higher flow rates, TCB exhibited a considerable breakthrough when tested with DOM-free AFW. The radioactivity in the samples was analyzed as described earlier.

The Sep-Pak cartridges retained 3.4 ± 0.9% ($n = 3$) of the DOC in the undiluted lake water sample and the absorbance (270 nm) in the undiluted sample decreased 2.2 ± 0.8% ($n = 3$) when passed through the cartridge. These values indicate that the sorption of DOM onto the C-18 matrix is low and probably will not significantly affect the measurement of partition coefficients.

Liquid-liquid extraction

Sorption of the model compounds to DOM was also measured by n-hexane extraction. The method assumes that the compound sorbed by DOM remains in the water phase while the freely dissolved fraction is extracted by the solvent. Samples (5 ml) were taken from the same jars as the reverse-phase samples. The samples were extracted with 1 ml of n-hexane by vortexing for 30 s. Before removing the hexane layer, the samples were centrifuged for 2 min at 380 g. The extraction was repeated and the hexane extracts were combined and analyzed for radioactivity. A sample (1 ml) was also taken from the extracted water and analyzed for radioactivity. The DOM-associated fraction was calculated as a ratio of the chemical concentration remaining in the extracted water and total chemical concentration. The extraction efficiencies from DOM-free AFW were 95.2 ± 0.2% ($n = 3$) and 96.9 ± 2.3% ($n = 3$) for BaP and TCB, respectively.

Bioconcentration experiments

The Daphnia magna population was cultured in Elendt’s M7 water (at 20 ± 1°C, 16L:8D photoperiod) as recommended by the Organization for Economic Cooperation and Development guideline [33] and fed three times a week with green algae (mainly Scenedesmus sp.). Four- to five-day-old subadults, without eggs in the brood chamber, were used in the bioconcentration experiments.

The model compounds were dosed individually to the test solutions and were left overnight (16 h) in the dark at room temperature. At least an hour before the exposure, daphnids were brought to clean culture water to clear their gut contents. The daphnids were exposed in groups of five individuals. The exposure was made in glass jars (closed with a cap) containing 100 ml of test solution for 24 h in the dark at a temperature of 20 ± 1°C. Three replicate jars were made for every treatment. After the exposure, the daphnids were collected on a filter paper, rinsed with 1 ml of clean water, blotted dry, and weighed. The daphnids were then transferred into scintillation vials, 0.3 ml of tissue solubilizer (Soluene-350; Packard) was added, and the vials were incubated overnight at 50°C. After a few minutes of cooling at room temperature, 6 ml of scintillation cocktail (UltimaGold; Packard) was added to the vials. Radioactivity in the exposure water was analyzed by taking duplicate 1-ml samples from each jar and adding 10 ml of scintillation cocktail (Insta-Gel Plus). Radioactivity was analyzed with a liquid scintillation counter (Wallac Winspectral 1414). The results are calculated as 24-h bioconcentration factors (BCF), where $BCF = C_f/C_w$, with $C_f$ being the concentration of the chemical in daphnids (ng/g wet wt) and $C_w$ being the concentration of the chemical in the exposure water (ng/ml) after 24 h of exposure. The concentrations were calculated from the specific activity of the radiolabeled compounds.

A nonlinear function (Eqn. 1) was used to describe the relationship between BCF and DOM concentration [15,34] as

$$BCF_{\text{DOM}} = \frac{BCF_{0} \left(1 + K_{\text{DOC}} (\text{DOC})\right)}{(1 + K_{\text{DOC}} (\text{DOC}))}$$

where $BCF_{\text{DOM}}$ and $BCF_0$ are the bioconcentration factors in the presence and absence of DOM, respectively. Further, the variable $K_{\text{DOC}}$ is used here to fit the experimental data and is a representative value for average partition coefficient across the DOM concentration series. The fraction of freely dissolved compound ($f_{\text{free}}$) is calculated from the $BCF_{\text{DOM}} = BCF_f/f_{\text{free}}$. The $f_{\text{free}}$ was then used to calculate the biological $K_{\text{DOC}}$ as described earlier.

Statistics

The results are expressed as mean ± standard deviation. The data were tested for normal distribution (Kolmogorov–Smirnov one-sample test) and for homogeneity of variances (Levene test). In cases of unequal variances, a common logarithm transformation was used to fulfill the assumptions of normal distribution and equal variances. If the assumptions were not fulfilled after transformation, the nonparametric Kruskal–Wallis test was used. For data with equal variances (and normal distribution), one-way analysis of variance was used to test differences between treatment means. Tukey’s honestly significant difference test was used to carry out multiple pairwise comparisons. The F test was used to study the significance of the slopes in linear regressions. All analyses were conducted with SPSS 10.1.4 (SPSS, Chicago, IL, USA) software.

RESULTS

Association of the model compounds with DOM

The $K_{\text{DOC}}$ for both chemicals was measured over time in 1: 20 (1 mg DOC/L) diluted and undiluted (20 mg DOC/L) DOM samples. In equilibrium dialysis, the $K_{\text{DOC}}$ for BaP with the level of 1 mg DOC/L reached its highest value faster (after 2 d) than in the undiluted DOM, where the $K_{\text{DOC}}$ increased until 4 d (Fig. 2A). Between 4 and 7 d, the sorption of BaP to 1 mg/L of DOM decreased, whereas the sorption to undiluted sample remained constant. In the equilibrium dialysis, the association of TCB with undiluted DOM increased throughout
the time series, but the changes were not significant \((p > 0.05)\) between 3, 4, and 7 d (Fig. 2A). At 1 mg DOC/L, it was not possible to obtain accurate \(K_{\text{DOC}}\) values for TCB. Overall, the \(K_{\text{DOC}}\)s were higher for BaP than for TCB.

The reverse-phase technique gave substantially lower \(K_{\text{DOC}}\)s than the equilibrium dialysis for both model compounds (Fig. 2B). For BaP, in the sample with 1:20 diluted DOM, the \(K_{\text{DOC}}\) increased up to 4 d and then remained constant between 4 and 7 d, while in the undiluted DOM, the \(K_{\text{DOC}}\) remained constant after 1 d (no significant differences, \(p > 0.05\)). In the 1:20 diluted sample, there were no significant differences \((p > 0.05)\) between the \(K_{\text{DOC}}\)s for TCB up to 4 d, but significantly higher \((p < 0.05)\) \(K_{\text{DOC}}\) was measured after 7 d. In the undiluted DOM, however, there were no significant differences \((p < 0.05)\) between \(K_{\text{DOC}}\)s for TCB.

The liquid–liquid extraction gave \(K_{\text{DOC}}\)s that were comparable with those determined by the reverse-phase method (Fig. 2C). The \(K_{\text{DOC}}\) for BaP increased up to 3 d, and after that exhibited a slight downward trend in the 1:20 diluted DOM. In the undiluted DOM sample, the \(K_{\text{DOC}}\) for BaP remained fairly constant (no significant differences, \(p > 0.05\)) between 1 and 7 d with the exception of day 3, where the \(K_{\text{DOC}}\) was significantly higher \((p < 0.05)\) than in the other time points. In the 1:20 diluted DOM, the \(K_{\text{DOC}}\) for TCB remained constant (no significant differences, \(p > 0.05\)) throughout the time series. No significant differences were found in the \(K_{\text{DOC}}\) for TCB in the undiluted DOM between 1 and 4 d either, but at 7 d, the \(K_{\text{DOC}}\) was significantly \((p < 0.05)\) higher than between 1 and 3 d.

On the basis of the data presented in Figure 2A, 4 d was chosen as a suitable dialysis period for measuring the \(K_{\text{DOC}}\)s over the DOM concentration range because an apparent equilibrium was reached. The \(K_{\text{DOC}}\)s for both BaP and TCB showed a significant \((p < 0.05)\) downward trend with increasing DOC concentration (Fig. 3). For TCB, the \(K_{\text{DOC}}\) value at the lowest DOC level was omitted because, due to a large standard deviation between replicates, it was not possible to obtain any accurate value \((173,460 \pm 114,242)\). The \(K_{\text{DOC}}\) for BaP was twice as high at a DOC concentration of 1 mg/L compared with that at 20 mg/L after 4 d of dialysis.

**Bioconcentration**

Bioconcentration of both model compounds was significantly \((p < 0.05)\) lower in the Lake Kontiolampi dilution series than in the DOM-free AFW (Fig. 4). As expected on the basis of higher \(K_{\text{DOC}}\) values, the reduction of BCF in the presence of DOM was more pronounced for BaP. The bioconcentration of BaP was 18 times higher in the DOM-free AFW than in undiluted Lake Kontiolampi water, while the same difference for TCB was only 3. Furthermore, the relationship between bioconcentration and DOM concentration was nonlinear for both model compounds.

Bioconcentration of the model compounds was also calculated against freely dissolved concentration of the compounds (referred to here as BCF\(_{\text{free}}\)) (Fig. 4). The freely dissolved concentration was estimated from the \(K_{\text{DOC}}\)s measured by equilibrium dialysis (sampled at 4 d). For BaP, the BCF\(_{\text{free}}\) remained fairly constant (no significant differences \(p > 0.05\)) throughout the DOM concentration series (DOC 0–20 mg/L). For TCB, the BCF\(_{\text{free}}\) was lower at the DOC concentration of 2 mg/L (at 1 mg/L, the value was not calculated) than in the AFW or in the higher DOM concentrations. However, differences were not significant \((p > 0.05)\). The lower value at 2 mg/L is probably due to inaccuracy in estimation of the freely dissolved carbon.

\[ \text{DOC (mg/L)} \]

\[ \text{Log } K_{\text{DOC}} \]

\[ \text{DOC (mg/L)} \]

Fig. 3. Partition coefficients \((K_{\text{DOC}})\) for benzo[a]pyrene (BaP) and 3,3',4,4'-tetrachlorobiphenyl with increasing dissolved organic matter (DOM) concentration determined by equilibrium dialysis and biological assay. DOC = dissolved organic carbon; \(\bullet\) = BaP, equilibrium dialysis (solid line), \(r^2 = 0.77\); \(\circ\) = BaP, biological assay (dotted line), \(r^2 = 0.93\); \(\square\) = TCB, equilibrium dialysis (solid line), \(r^2 = 0.91\); \(\Box\) = TCB, biological assay (dotted line), \(r^2 = 0.85\). All slopes were significantly different from zero \((p < 0.05)\).
dissolved concentration of the TCB by dialysis at low DOM levels.

For both model compounds, the biological $K_{\text{DOC}}$s were in agreement with those measured by dialysis (Fig. 3). The biological $K_{\text{DOC}}$s declined significantly ($p < 0.05$) with increasing DOM concentration, showing a similar trend to the $K_{\text{DOC}}$s measured by dialysis. The proportions of sorbed compounds at varying DOM levels estimated by the different methods are listed in Table 2. As already shown by the calculated $K_{\text{DOC}}$s, the DOM-associated fraction was substantially lower when estimated by the reverse-phase and liquid-liquid extraction.

![Fig. 4. Effect of dissolved organic matter concentration on bioconcentration of benzo[a]pyrene (BaP) and 3,3',4,4'-tetrachlorobiphenyl (TCB).](Image)

**Table 2. The proportions (%) of the chemicals associated with dissolved organic matter estimated by four different methods**

<table>
<thead>
<tr>
<th>DOC (mg/L)</th>
<th>BaP</th>
<th>TCB</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ED</td>
<td>BA</td>
</tr>
<tr>
<td>1</td>
<td>68.0</td>
<td>64.6</td>
</tr>
<tr>
<td>2</td>
<td>76.1</td>
<td>77.9</td>
</tr>
<tr>
<td>5</td>
<td>87.7</td>
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</tr>
<tr>
<td>10</td>
<td>93.8</td>
<td>92.3</td>
</tr>
<tr>
<td>15</td>
<td>93.1</td>
<td>93.6</td>
</tr>
<tr>
<td>20</td>
<td>95.5</td>
<td>94.6</td>
</tr>
</tbody>
</table>

$^a$DOC = dissolved organic carbon; BaP = benzo[a]pyrene; TCB = 3,3',4,4'-tetrachlorobiphenyl; ED = equilibrium dialysis; BA = biological assay; RP = reverse-phase extraction; LLE = liquid-liquid extraction.

**DISCUSSION**

The bioavailability of both BaP and TCB was reduced by the presence of DOM, although the effect was less pronounced for the more lipophilic TCB. The behavior of the two model compounds exhibited different patterns in equilibrium dialysis (Fig. 2A). In a sample with 1 mg DOC/L, the $K_{\text{DOC}}$ for BaP no longer changed after 2 d. For TCB, it was not possible to obtain any accurate values because the difference between the TCB concentration inside and outside the dialysis tubes was not detectable and/or deviation between the replicates was considerably large. In undiluted DOM samples, the sorption of BaP seemed to reach equilibrium in 4 d, but the sorption of TCB, instead, increased throughout the time, although the last three time points did not differ significantly ($p > 0.05$) from each other. The reverse-phase and extraction methods showed that the equilibrium in sorption was generally reached after 1 d. When compared with equilibrium dialysis, both the reverse-phase and extraction methods gave substantially lower $K_{\text{DOC}}$s. All methods showed higher $K_{\text{DOC}}$s with lower DOM concentration, but the difference was more pronounced when measured with the reverse-phase and the extraction methods. Furthermore, the bioconcentration of both model compounds decreased nonlinearly with increasing DOM concentration (Fig. 4).

In this study, the $K_{\text{DOC}}$ values decreased with increasing DOM concentration (Fig. 3). This is in agreement with previous studies, which have shown a downward trend with increasing humic acid concentration [11,12]. This phenomenon can be attributed to the macromolecular structure of humic substances. Increasing humic acid concentration diminishes the space and, therefore, the humic molecules cannot maintain their linear and loose structure [2,4]. On the contrary, the humic molecules tend to aggregate or coil at higher concentration. This rearrangement in macromolecular structure probably hinders the access of the contaminants to the hydrophobic areas in humic acid molecules where the interaction would occur. Our results indicate that similar changes occur also in natural samples containing also other than humic fractions of DOM.

It has been pointed out that smaller fractions of DOM (<1,000 D) may diffuse through the dialysis membrane and, subsequently, lead to detection of lower $K_{\text{DOC}}$s [35]. On the other hand, it has been shown that the sorption of organic chemicals is greater to high molecular weight fractions of DOM [30,35,36]. In this study, for the undiluted DOM sample, a 15% decrease in DOC inside the dialysis tubes was observed after 4 d of dialysis, while the spectrophotometrically determined loss in aromatic structure, which is mainly responsible for sorption of hydrophobic contaminants, was only 5%. Carter and Suffet also reported similar 5% losses with the Aldrich humic acid [11]. Moreover, the loss was even greater after 7 d, indicating that continuing the dialysis could lead to questionable results. The loss of organic material during dialysis may vary from one natural water sample to another because of the differences in the molecular weight profiles. It is not known to what extent the sorption of organic chemicals to DOM smaller than 1,000 D affects the bioavailability of these chemicals. Furthermore, in this study, the $K_{\text{DOC}}$s derived by equilibrium dialysis (after 4 d) were in good agreement with the biological $K_{\text{DOC}}$s.

As already mentioned, the $K_{\text{DOC}}$ values are lower for TCB than for BaP. In addition, it seems that there is also a difference
in the strength of the interaction. This observation is made on the basis that the methods that interfere the DOM-contaminant interaction (the reverse-phase and extraction methods) produced considerably lower $K_{\text{DOC}}$ values than the equilibrium dialysis method. In other words, during the liquid–liquid extraction and reverse-phase separation, weaker interactions break, resulting in detection of lower association constant. The interaction of TCB with DOM seemed to be more sensitive to this interference than that of BaP. For BaP, the liquid-liquid extraction and reverse-phase methods underestimated the sorbed fraction by 20% compared with values determined by the equilibrium dialysis or the biological assay. For TCB, the difference was 50%. Therefore, the overestimation of the bioavailable fraction by the reverse-phase and the extraction methods was even more pronounced in the case of TCB.

For BaP, the $K_{\text{DOC}}$s measured by the reverse-phase and extraction methods for both high and low DOM concentrations indicated that the sorption was not yet completed in 4 h. However, this was not observed for TCB. Previous studies conducted by fluorescence quenching have shown that the sorption of PAHs to DOM is completed after few minutes or changes are not detectable with this method [6,16]. It is possible that weaker interactions are formed instantaneously, while some of the BaP molecules continue to diffuse deeper into the DOM structure to form stronger interactions. This was not observed for TCB, which is in agreement with the fact that the interaction with DOM seemed to be much weaker.

In the experiments conducted in this study, the concentration of BaP (0.04 nM) was lower than that of TCB (2.58 nM). It is not clear whether the concentration of chemical affects the interaction with DOM or not [10]. For example, McCarthy and Jimenez [16] demonstrated linear association and dissociation between Aldrich humic acid and BaP up to the solubility limit of the chemical. There are also other indications that the chemical concentration does not affect the measured $K_{\text{DOC}}$s of a variety of organic chemicals by Aldrich humic acid as well as by DOM in few natural waters [12,37]. On the other hand, Laor and Rebhun [38] showed nonlinearity in isotherms describing the sorption of PAH at different concentrations to peat and soil humic acids. Therefore, we cannot exclude the possibility that chemical concentration could cause some difference in $K_{\text{DOC}}$s for BaP and TCB. Probably the lower sorption of TCB is, at least partly, due to steric factors. As pointed out previously, the electron density is different in these two compounds, and this may be the reason that BaP and TCB have been shown to associate with different fractions of DOM [39,40]. Furthermore, chlorine atoms in the TCB molecules may hinder the hydrophobic interactions between aromatic structures of TCB and DOM. In addition to this, the size and shape of the compounds may play a role as factors affecting sorption.

In this study, the BCF values were substantially higher for TCB than for BaP, which is in accordance with previous findings [15]. This is most probably related to the higher lipophilicity of TCB and differences in biotransformation of the model compounds in $D.\ magnifica$. As expected based on our former studies, the bioconcentration of both model compounds was decreased by the DOM from Lake Kontiolampi [9,26]. The decrease in bioconcentration with increasing DOM concentration was nonlinear, and this kind of relationship has been found for several organic chemicals in series of natural waters and also in diluted natural waters as well as in dilution series of isolated DOM samples [19,21,22,34]. However, the coefficient of determination (COD) for the relationship was much higher for BaP (COD = 0.997) than for TCB (COD = 0.653), showing that the model explained totally the variation in the BCF of BaP (Fig. 4). For the BCF of TCB, the fit was not as good as for BaP, probably because the BCFs showed a more linear relationship in the DOM concentration series when the BCF in the DOM-free water was excluded. The linear regression (excluding the BCFs in DOM-free control) gave $r^2$ values of 0.64 and 0.92 for the BCFs of BaP and TCB, respectively.

In general, the BCF$_{\text{free}}$ values showed that the compounds associated with DOM are not bioavailable. However, it seems that, for TCB, the BCF$_{\text{free}}$ (DOC 5–20 mg/L) was slightly higher (not statistically significant) than BCF in the DOM-free water, suggesting that a small fraction of the sorbed compound, measured by the equilibrium dialysis, may still be bioavailable. Therefore, it is possible that the weakest interactions between DOM and TCB do not limit the bioavailability of the compound but are still detectable by the equilibrium dialysis method. On the other hand, high standard deviations in BCF$_{\text{free}}$ values complicates the interpretation of the data, and furthermore, this trend was not as obvious for BaP.

**CONCLUSIONS**

In conclusion, all methods showed a decrease in carbon normalized association of BaP and TCB with increasing DOM concentration. In this case, the effect is distinguishable already at environmentally relevant levels of DOM. The equilibrium dialysis method gave the best estimates for bioavailability of the model compounds. The bioconcentration experiments showed that the fraction of compounds associated with DOM was mostly not bioavailable to $D.\ magnifica$. Furthermore, the bioconcentration of BaP followed a nonlinear relationship with DOM concentration, and this relationship could be represented by a simple equation, assuming that chemical associated with DOM is not bioavailable. The model did not fit as accurately for TBC as for BaP.

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