Does dissolved organic matter matter?
- Implications for bioavailability of organic chemicals

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**Key words:** bioavailability, humic substances, dissolved organic matter, organic contaminants, *Daphnia magna*, water hardness.

In natural waters, total concentration of a contaminant does not necessarily reflect the actual amount of the chemical that is available for uptake by aquatic organisms, i.e. its bioavailability. Dissolved organic matter (DOM) has been shown to be one of the most important factors controlling the bioavailability of hydrophobic organic contaminants in freshwaters. Several studies have shown that in most cases DOM either decreases or does not affect the bioavailability of organic chemicals. In a few cases, DOM has increased the bioavailability of certain compounds. Most studies, however, have been performed with isolated fractions of DOM or with commercial preparations.

The purpose of the present study was to evaluate the effects of natural DOM on the bioavailability of various contaminants to *Daphnia magna* in freshwaters with various characteristics. The model contaminants used in this thesis were 3,3',4,4'-tetrachlorobiphenyl, benzo[a]pyrene, pyrene, pentachlorophenol, naphthalene and atrazine, arranged according to decreasing lipophilicity. The association of the model compounds with DOM was strongly related to the characteristics of both the chemicals and the DOM. In general, the association constants followed the lipophilicity of the contaminants, higher lipophilicity resulting in greater sorption. In certain cases, however, the association constants were higher for less hydrophobic pyrene than for benzo[a]pyrene. Furthermore, in the case of 3,3',4,4'-tetrachlorobiphenyl, the association with DOM was lower than (or equal to) that of polycyclic aromatic hydrocarbons (PAHs: benzo[a]pyrene and pyrene) with lower lipophilicity. Higher aromaticity of DOM, as measured with a simple UV-spectroscopical method, usually resulted in higher association with the model contaminants. However, this was not the case when bulk DOM and isolated fulvic acid were compared. Furthermore, low water hardness favored the association of the model compounds with DOM.

In DOM-free water, the bioconcentration of model compounds was mainly correlated with the lipophilicity of the contaminants. However, the effects of DOM on bioavailability differed among the compounds studied. Of the model compounds used, DOM had the greatest impact on bioavailability of benzo[a]pyrene. The bioavailability of pyrene was reduced only by high concentrations of DOM, and therefore in most natural freshwaters the bioavailability of pyrene and of PAHs with lower lipophilicity is affected only slightly. In the absence of DOM, water hardness had only a minor effect on bioavailability of the model compounds. In the presence of DOM, however, increasing water hardness may increase their bioavailability. No enhanced bioavailability of organic contaminants due to DOM only was observed. Furthermore, the equilibrium dialysis method was shown to provide a good estimate of the bioavailable fraction in water.

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List of original publications

This thesis is based on the following publications, referred to in the text by the Roman numerals. Some unpublished results are also presented.


### Abbreviations

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<th>Abbreviation</th>
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<tr>
<td>ABS&lt;sub&gt;270&lt;/sub&gt;</td>
<td>specific absorptivity at 270 nm</td>
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<tr>
<td>AFW</td>
<td>artificial freshwater</td>
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<tr>
<td>B&lt;sub&gt;a&lt;/sub&gt;P</td>
<td>benzo[a]pyrene</td>
</tr>
<tr>
<td>BCF</td>
<td>bioconcentration factor</td>
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<tr>
<td>DOC</td>
<td>dissolved organic carbon</td>
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<tr>
<td>DOM</td>
<td>dissolved organic matter</td>
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<tr>
<td>HbA</td>
<td>hydrophobic acids</td>
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<tr>
<td>Hl</td>
<td>hydrophilics</td>
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<tr>
<td>HS</td>
<td>humic substances</td>
</tr>
<tr>
<td>K&lt;sub&gt;DOC&lt;/sub&gt;</td>
<td>partition (association) coefficient</td>
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<tr>
<td>MW</td>
<td>molecular weight</td>
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<tr>
<td>NoFA</td>
<td>Nordic reference fulvic acid</td>
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<tr>
<td>PAH</td>
<td>polycyclic aromatic hydrocarbon</td>
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<tr>
<td>PCB</td>
<td>polychlorinated biphenyl</td>
</tr>
<tr>
<td>PCP</td>
<td>pentachlorophenol</td>
</tr>
<tr>
<td>TCB</td>
<td>3,3',4,4'-tetrachlorobiphenyl</td>
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<td>UV</td>
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1. INTRODUCTION

The history of ecotoxicology is relatively short compared to many other fields of science. Truhaut defined the term “ecotoxicology” in 1969 as studies of the effects of harmful substances on organisms (Connell et al., 1999). Since then the development has been rapid and today numerous scientists work in the various branches of ecotoxicology. Aquatic toxicology is the branch of ecotoxicology that studies the aqueous environments. Due to various natural processes and human activities, an overwhelming number of harmful substances end up in the aquatic environment. Therefore, the characteristics of aqueous systems are important for the environmental fate of the contaminants.

The first step, for environmental chemicals to move in food chains and/or to have an effect on organisms, is to enter the organism. This process is controlled by many different factors, depending on the characteristics of the chemical, ambient medium and the organisms. At this point we come to the concept of bioavailability, which describes the availability of nutrients or, as in this case, pollutants to organisms. Several factors, such as temperature, pH, dissolved organic matter, and concentration and characteristics of the contaminant, control the bioavailability. In this thesis the bioavailable fraction refers to the portion of the total amount of contaminant that is potentially available for organisms.

Although nowadays it is recognized that several factors control the uptake of many contaminants, their potential effects are still largely evaluated on the basis of standardized toxicity tests. In such tests the conditions are fixed, which means that the tests fail to account for diverse biological and physico-chemical features of natural waters. Therefore, much more basic research is needed if we are to understand the behavior and effects of chemicals on the environment.

2. DISSOLVED ORGANIC MATTER IN AQUATIC ENVIRONMENTS

2.1. Occurrence and composition of dissolved organic matter

Although dissolved organic matter (DOM) is present in all natural waters; the concentration varies greatly between locations depending on the geochemistry, season, type of water, etc. In groundwater and seawater the concentrations of dissolved organic carbon (DOC) are in the same range with the average being 0.7 and 1 mg/L, respectively (Thurman, 1985). In groundwater, however, DOC concentrations up to 15 mg/L have been determined, while in seawater the DOC range is usually 0.3-2.0 mg/L. In rivers and lakes the variation in DOC content is far higher; but globally, concentrations below 10 mg/L are common (Thurman, 1985). However, high organic carbon concentrations are typical for waters in boreal regions although also in Finland the variability is high (5-30 mg/L; Lahermo et al., 1995). In aquatic environments, the mass of DOM is greater than the mass of organisms (Thomas, 1997); therefore, it is not surprising that DOM has many ecological implications.

DOM in boreal waters consists mostly of humic substances (HS) that are formed during the decay of biomatter. In boreal region up to 90% of the DOM in the surface waters is considered to be HS, with the global average value being about 50% (Thurman, 1985; Kronberg, 1999). HS are also called refractory organic matter, which refers to the resistance of HS to microbial degradation and chemical reactions. The rest of the DOM, which
is not considered to be humic material, is mainly carbohydrates, amino acids, low molecular weight organic acids and fatty acids. These are considered to constitute the labile fraction of DOM.

The cover photo presents some of the water samples used in the studies on which this thesis is based. The samples from left to right are: Lake Kontiolampi, the Kalliojoki River, the Pielisjoki river and Lake Kuorinka (Photo by Mika Lännenpää). The samples are arranged in order of their DOC concentration and the yellow-brownish color indicates the presence of HS. The DOC concentration in Lake Kontiolampi is about 20 mg/L while in the Lake Kuorinka it is only 2 mg/L.

Roughly 50% of HS is carbon, the other major constituents being oxygen (35-45%), hydrogen (5%), nitrogen (3%) and sulfur (Thurman, 1985; MacCarthy, 2001). The major functional groups include carboxylic and phenolic hydroxyl groups. HS can be divided into three fractions simply according to the solubility of the fractions at different pH. Humin is not soluble at any pH, while fulvic acids (FA) are soluble regardless of pH. Humic acids (HA) precipitate at pH 2, but are soluble at pH values above that. In natural waters, fulvic acids dominate over the two other constituents. For example in several North American natural waters the proportions of FA and HA were reported to be 54-68% and 13-29%, respectively, of the DOC (Ma et al., 2001). FA are considered to be of lower molecular weight and less aromatic than HA (Thurman, 1985; Karanfil et al., 1996).

Numerous methods have been used to characterize DOM and HS. The problem in characterization of HS, especially when functionality is studied, is that one usually has to break the macromolecules into smaller units. These techniques may be difficult and time consuming and at the same time, some essential and unique features in structure and function of DOM may be altered, such as properties that control the sorption of contaminants. Therefore, simple and non-destructive spectroscopical methods have been shown to be useful in structural and functional studies (Chin et al., 1994; Hautala et al., 2000; Chen et al., 2002).

2.2. Ecological implications of DOM

Among their other functions, DOM, and especially the HS, contribute significantly to the acidity of freshwaters. The organic acids of DOM are mainly responsible for the low pH in Finnish lakes; while in North America and Norway, acid rain and other anthropogenic sources are more likely to cause acidification (Kortelainen, 1999). On the other hand, the weak organic acids in DOM also buffer freshwaters against acid deposit.

DOM has also been shown to affect light conditions in natural waters by attenuating both visible and ultraviolet (UV) light (Jones and Arvola, 1984; Huovinen et al., 2000). The importance of this is increased when we consider that the most dangerous wavelengths in the UV region are effectively absorbed by HS (Scully and Lean, 1994). By attenuating light, DOM may decrease the growth of autotrophs but at the same time protects aquatic organisms against harmful UV radiation. In addition, many reactive and hazardous radicals are produced during photochemical degradation of DOM (Frimmel, 1994).

The reactions of DOM with sunlight also influence the oxygen conditions in natural waters. Photochemical degradation of DOM consumes oxygen, and therefore sunlight may lower oxygen conditions at the surface of DOM rich waters (Lindell and Rai, 1994). Although several reactions occur in the presence of DOM, a major part of the energy in sunlight is still
transformed into heat (Münster et al., 1999). Since light cannot reach very deep into brown-water lakes, a relatively narrow surface layer warms up leading to strong thermal stratification of the water. The warm surface layer hampers the transport of oxygen from the air into the deeper layers of water, resulting in oxygen deficiency near the bottom (Eloranta, 1999). Furthermore, if the spring overturn is prevented by rapid warming of the top layer, the oxygen deficiency may get even worse.

Bacteria can use some fractions of DOM as a source of energy (Thomas, 1997). Accordingly, the term assimilable organic carbon refers to the fraction of DOM that can be utilized by bacteria. While the color of the surface waters is mainly related to higher molecular weight fractions of DOM (>10000 Daltons), the assimilable organic carbon is related to the low molecular weight fraction (<1000 Daltons) (Hem and Efraimsen, 2001).

DOM can not be easily removed from water; and often during disinfection of drinking water, toxic chemicals are formed (Kronberg and Christman, 1989; Matsuda et al., 1992). Furthermore, during this disinfection, DOM breaks down into smaller units, which, in turn, are suitable sources of energy for microbes (Miettinen et al., 1996). In boreal regions, however, the DOM content in drinking water may be high; but a low concentration of inorganic nutrients limits the growth of bacteria (Miettinen et al., 1996). In harder waters the opposite may be true.

Association with DOM affects the environmental fate of various contaminants. Processes, such as transport, solubility, degradation, volatilization and bioavailability of hydrophobic organic compounds, can be largely controlled by the presence of DOM (Piccolo, 1994). Since there are vast numbers of contaminated sites, environmental risk assessment is a rapidly growing field. In order to fulfill the needs and to improve the models used in chemical risk assessment, much basic data is needed. New information is needed on the environmental fate of contaminants, and in particular, about the effects of special and relevant environmental factors, such as the quality and quantity of DOM in natural waters.

### 2.3. Macromolecular structure of humic substances and interaction with organic chemicals

No unambiguous structure has been depicted for HS. It may even be that every humic molecule is unique, and therefore, no definitive model can be proposed for the structure. Despite the heterogeneity of HS, many similarities can also be found in samples from different sources. For example, the elemental composition of humic and fulvic acids from various sources seems to be similar, although the function may vary greatly (MacCarthy, 2001). The macromolecular, i.e. secondary, structure of HS has been shown to play a fundamental role in sorption and association with organic chemicals. Regardless of the mechanism, the terms “association” and “sorption” is used in this thesis to describe the interaction of the model compounds with DOM.

The secondary structure of HS is hypothesized to contain “cage-like” pseudomicellar structures (also referred to as hydrophobic domains or aromatic moieties) at various points along the skeletal chain (Wershaw, 1986; Engebretson and von Wandruszka, 1994). The pseudomicelles are considered to be similar to true micelles, but looser and larger, with a hydrophobic core and hydrophilic structures on the surface. Furthermore, the pseudomicelles are assumed to be
responsible for the association with hydrophobic organic chemicals. The secondary structure of HS has been shown to vary, depending on chemistry of the ambient water (Ghosh and Schnitzer, 1980; Aho and Lehto, 1984; Tsutsuki and Kuwatsuka, 1984; Engebretson and von Wandruszka, 1994; Ephraim et al., 1995). At low ionic strength, high pH and low concentration, HS are loose, linear, flexible and high molecular weight molecules. Correspondingly, at high ionic strength, low pH and high DOM concentration, HS are tight, globular-shaped spheres. Furthermore, with increasing ionic strength, the radius of the colloids decreases (Warwick et al., 2001). The varying size and shape of HS caused by changes in solution chemistry have also been visualized spectromicroscopically (Myneni et al., 1999). However, when the formation of the pseudomicelles is considered, the situation is more complex.

Under alkaline conditions, the major functional groups (-COOH, -OH) are dissociated; and repulsion between negative charges hinders the formation of hydrophobic domains (Engebretson et al., 1996). When the solution turns acidic, negative charges are neutralized, hydration occurs and ultimately hydrophobic domains are formed. Therefore, the sorption of organic chemicals to HS is greater at low pH (Kukkonen, 1991b; Schlautman and Morgan, 1993).

Previous studies have also shown that the effects of ionic strength depend on the ions used to manipulate chemistry of the solution and on the properties of DOM. An increase in NaCl concentration has been shown to increase the association between organic chemicals and HS, probably due to the salting-out effect (Gauthier et al., 1986; Jota and Hassett, 1991). However, Schlautman and Morgan (1993) reported decreasing sorption of PAHs to HS with increasing concentrations of NaCl. Other studies have also shown decreased association due to increasing ionic strength (Gauthier et al., 1986; Murphy et al., 1994; Jones and Tiller, 1999). The decrease in sorption is related to the structural changes that occur with increasing ionic strength. As mentioned earlier, increasing ionic concentration in the solution compresses humic molecules into tight spheres, thus hindering the access of organic compounds to hydrophobic domains or even preventing the formation of the domains.

Adding metal ions (like Ca$^{2+}$ and Mg$^{2+}$) to the solution reduces the repulsive forces between dissociated functional groups by neutralizing the negative charges, expanding the hydrophobic domains and subsequently increasing the sorption of organic chemicals (Schlautman and Morgan, 1993; Engebretson et al., 1996). However, under acidic conditions the effect of metal ions may be negligible, since the negative charges are already neutralized.

Increasing temperature leads to intramolecular coiling of HS, resulting in more rigid pseudomicellar structures (Engebretson and von Wandruszka, 1994). For most compounds the association constants have been shown to increase with decreasing temperature (ten Hulscher and Cornelissen, 1996; Lüers and ten Hulscher, 1996); however, the effect is not particularly strong. For some chemicals, such as 2,2',5,5'-tetrachlorobiphenyl, effects of temperature have not been detected (Jota and Hassett, 1991).

Molecular weight (MW) has been suggested to be the most important characteristic affecting the capacity of HS to associate with organic chemicals (Chiou et al., 1986; Engebretson et al., 1996). Smaller molecules do not have the size and flexibility to form
hydrophobic domains, which has been demonstrated by cutting humic acid molecules into smaller segments (Engebretson and von Wandruszka, 1997). Furthermore, it has been shown that the relative number of hydrophobic domains increases with increasing MW of fulvic acids (Chin et al., 1994). MW is also closely related to aromaticity of HS, which, in turn, has been shown to correlate strongly with sorption of organic compounds (Gauthier et al., 1987; Chin et al., 1997; Haitzer et al., 1999). However, Perminova et al. (1999) found no significant correlation between partition coefficients and MW, but they did observe a strong correlation between partition coefficients and several parameters describing aromaticity.

Although this pseudomicellar structure is becoming generally accepted among scientists, it is still questionable whether the association of neutral organic chemicals is controlled by specific binding sites or whether the “binding” is simply a partition between two phases (water and HS). This may even vary with different compounds. Overall, organic chemicals can interact with HS in several ways, including ion exchange, hydrogen bonding, charge transfer, covalent binding or hydrophobic adsorption and partitioning, depending on the type of chemical (Senesi and Chen, 1989; Piccolo, 1994).

Assuming that specific binding sites dominate the interaction, at some chemical concentration all the sites would be occupied and at chemical concentrations above that concentration lower association coefficients would be detected. This would also mean competition between the molecules of different chemicals for binding sites. In the partition model this would not happen. In their review, Krop et al. (2001) indicated that at environmentally relevant DOM concentrations, competition for binding sites does not occur. A few studies have shown that the association coefficients are independent of contaminant concentration below the limits of solubility (Landrum et al., 1984; McCarthy and Jimenez, 1985; Morehead et al., 1986). Recently, evidence has also been found that an increase in contaminant concentration may lead to lower association constants (Laor and Rebhun, 2002).

3. OBJECTIVES
The objectives of this investigation were:
1) To study the role of DOM on bioavailability of organic contaminants in freshwaters with differing characteristics,
2) To compare the effects of DOM on bioavailability of different types of organic contaminants,
3) To study the possible increase in bioavailability due to low levels of DOM, and
4) To compare the association of the model compounds measured by chemical methods to actual bioavailability to Daphnia magna.

4. MATERIALS AND METHODS
4.1. Model compounds
The model compounds were chosen to represent a variety of different chemical classes and different lipophilicities (Figure 1). Polycyclic aromatic hydrocarbons (PAHs), such as benzo[a]pyrene, pyrene and naphthalene, are formed during defective combustion of fossil fuels and other organic matter and are therefore produced by natural processes (e.g. forest fires, volcanic activity and oil seepages). However, a major proportion of PAHs released into the environment is due to human activity (traffic,
combustion of municipal waste, industry, oil spills etc.) (Neff, 1985).

Polychlorinated biphenyls (PCBs), such as 3,3',4,4'-tetrachlorobiphenyl (TCB), have been produced for several industrial uses. PCBs are non-flammable, chemically stable and have low conductivity. Therefore, they have been used as cooling and insulating fluids in transformers and capacitors, hydraulic fluids, surface coatings of copy papers, printing inks, rubbers, paints, waxes, asphalt and pesticides as well as in flame retardants in lubricating oils (Erickson, 1997). The same persistence and chemical stability, that have made PCBs so useful in the products listed above is also the reason for the problems they cause in nature.

Atrazine is a triazine herbicide that is largely used to control weeds on crop and forest lands (Huber, 1993; Solomon et al., 1996). Indeed, atrazine has been one of the most used pesticides in North America and an important herbicide worldwide, used especially in corn cultivation. In most cases atrazine causes no significant risk to aquatic environments, but in areas where it is heavily used the risk is obviously greater (Solomon et al., 1996).

Pentachlorophenol, and other chlorinated phenols, are used in wood preservatives and as pesticides and are also formed during chloro bleaching of kraft pulp (Coulston and Kolbye, 1994). Furthermore, chlorination and combustion of organic matter have increased the amount of chlorophenolics in nature. Even though the chlorinated phenols are somewhat degradable, they are still persistent enough to cause environmental problems (Paasivirta et al., 1985).

In this study, mainly [14C]-labelled chemicals were used, except in the case of benzo[a]pyrene ([3H]-labelled). Since the autoradiolysis of [3H]-benzo[a]pyrene is relatively rapid, before the experiments this compound was always purified by solvent extraction or thin layer chromatography. The chemicals were quantified by liquid scintillation counting by the external standard method and corrected for quench and chemoluminescence. The reagents and equipment used in liquid scintillation counting are described in each article.

4.2. Experimental waters

More detailed descriptions of the samples as well as their characterizations are given in the original publications. Natural water samples were stored in plastic containers in the dark at +4-5°C without any treatment. Before use, all natural waters were filtered (0.45 µm), and all the material remaining in the water was considered to be dissolved.

4.3. Association of the model compounds with DOM

Partition coefficients between water and DOM were determined mainly by the equilibrium dialysis method (Carter and Suffet, 1982). In addition, in Article I the methods used were reverse-phase separation (Landrum et al., 1984) and liquid-liquid extraction (Gjessing and Berglind, 1981). The fundamental difference between the methods is that equilibrium dialysis does not interfere with the DOM contaminant interaction, whereas the latter two do. All the methods are described in Article I.
Figure 1. Chemical structures of the model compounds. References for the chemical characteristics are (De Maagd et al., 1998) for benzo[a]pyrene and naphthalene, (Helweg et al., 1997) for pyrene, (Sabljic et al., 1993) for 3,3',4,4'-tetrachlorobiphenyl and (Solomon et al., 1996) for atrazine. * The log $K_{ow}$ value for pentachlorophenol varies with pH. Here the log $K_{ow}$ is given for the pH range 9.2 to 6.1 (Nowosielski and Fein, 1998).

4.4. Bioavailability experiments
The test organism used in the bioavailability studies was the water flea, Daphnia magna. D. magna is a crustacean in the order Cladocera and it is extensively used in the field of aquatic toxicology. In laboratory
experiments, it has mostly been used as a parthenogenetically reproducing female population in order to minimize the variation between individuals. The reason for this popularity in experimental work is that *D. magna* fulfills the basic requirements for a laboratory test organism: small size, short life-cycle and, most important, suitable for laboratory culturing. Furthermore, water fleas play an important role in aquatic food webs. The daphnids were cultured in Elendt M7 media, in which these animals have been shown to grow and reproduce well (Samel et al., 1999). Furthermore, M7 media is recommended in the OECD guidelines for culturing *D. magna* (OECD, 1998).

Organisms may be exposed to harmful compounds through food or the media. In this work only exposure via the medium, i.e. water, is considered. Bioconcentration is a process in which the compound is taken up directly from the surrounding water. The results are expressed as bioconcentration factors (BCF), which is a value that describes the tendency of a compound to concentrate in the tissues of an organism from the surrounding water, i.e. the concentration of the chemical in the organism divided by concentration of the chemical in the exposure water in conditions of steady state uptake. To demonstrate the differences between treatments, however, such conditions are not necessary (III).

The bioconcentration experiments were conducted primarily with 4- to 6-day-old subadults (sexually immature) and with an organism/ water-ratio of 1/20 mL. In Article II, however, the ratio for the atrazine experiment was 1/6.7 mL. The daphnids were exposed at room temperature and in dark for 24 h without any prior acclimatization to the experimental waters. This exposure period was chosen because pilot studies and investigations by other authors have shown that in *D. magna* many organic chemicals usually reach (at least near) steady state accumulation within 24 h (McCarthy, 1983; Nikkilä and Kukkonen, 2001). Furthermore, the exposure period was also kept short in order to minimize the effects of different water-quality characteristics on the condition of the water fleas and also to avoid the effects of starvation (i.e. dissimilar changes in lipid content of the water fleas in different water samples).

5. RESULTS AND DISCUSSION

5.1. Experimental waters

The natural samples were part of a larger EU-study in which the purpose was to characterize DOM and study its effects on bioavailability of organic compounds in different water samples (II, Table 1). On the basis of water quality, the natural water samples can be divided into two groups, namely, samples from Finnish and Swedish (Scandinavian) waters and samples from Spain and the Netherlands. The waters from Spain and the Netherlands were hard and had high conductivity as well as little color. The Scandinavian waters were soft and had stronger color. Furthermore, pH was lower in the Scandinavian samples, whereas it was highest in the samples from Spain. Overall, the characteristics in this water series were quite uniform in the samples from a particular country but varied greatly between countries.

As already indicated by color, the quality and quantity of DOM also varied greatly between countries (II, Table 1 and 2). In the Scandinavian waters DOM was characterized as humic, as shown by the high absorptivity and the large proportion of hydrophobic acids (HbA%). DOM in waters from the Netherlands had some aromaticity, as indicated by...
absorptivity, but most of the Spanish samples had extremely low absorptivity. The fraction of hydrophobic neutrals, which has been shown to exhibit extremely low aromaticity (Kukkonen et al., 1990), was smallest in the samples from Scandinavia and larger in those from Spain and the Netherlands. Throughout the water series the fraction of hydrophilics (HI%), which contain mostly aliphatic structures (Ma et al., 2001), was the most stable fraction. In Article II, functional groups (phenolic and carboxylic) of DOC were also measured. The phenolic groups (-OH) showed a similar trend with many other characteristics being higher in the more humic waters (II, Table 2). The number of carboxylic groups (-COOH) did not follow exactly the same trend because the number of groups was about the same in samples from Finland, Sweden and Spain, but lower in samples from the Netherlands.

Lake Kontiolampi water was used in every article in this thesis. Probably due to temporal variation, in different experiments the DOC in the samples varied between 17-20 mg/L. Furthermore, the Lake Kontiolampi samples used in Articles I and V were taken from 1 m above the bottom (about 9 m deep), whereas in other studies the water samples were taken from 1 m below the surface. The absorptivity in the untreated Lake Kontiolampi samples ranged from 40 to 46.

5.2. Association of the model compounds with DOM

5.2.1. Differences between chemicals

Based on fluorescence quenching studies, the sorption of PAHs by DOM has been considered to take place very quickly, even within minutes (McCarthy and Jimenez, 1985; Gauthier et al., 1986; Perminova et al., 1999). However, the fluorescence quenching method fails to detect changes in the strength of the interaction. In our studies, equilibrium partitioning took slightly longer but for PAHs it was still usually completed within 24 h, as determined by the reverse-phase and the liquid-liquid extraction methods (I; V). Slower sorption has also been observed for chlorophenols determined by liquid-liquid extraction (Peuravuori et al., 2002). It may be that the weakest interactions are formed instantly, after which the PAH molecules still continue to diffuse deeper into the DOM structure and are thereby bound more strongly. However, this was not obvious for TCB, which suggest that sorption to DOM differs between chemical classes (I).

The association of atrazine with DOM was low. Improved instrumentation made it possible to measure the $K_{DOC}$ for atrazine in Article IV, while in study II the sorption was not detectable. The measured association coefficients were low, but the effect of water hardness on $K_{DOC}$ values for atrazine was similar to that of the other model compounds (IV). This suggested that, although atrazine is much less lipophilic, the sorption still depends, at least partly, on factors similar to those for sorption of the more lipophilic model compounds. Low association of atrazine with DOM was expected because previous studies have shown that sorption to humic acids is extremely low, and furthermore, the sorption is higher in acidic conditions (Saint-Fort and Visser, 1988; Barriuso et al., 1992; Beck and Jones, 1996).

At pH 7 and water hardness of 2.5 mM, it was not possible to detect the $K_{DOC}$ for PCP in the Lake Kontiolampi water (unpublished data). This is due to the strong dependence of $K_{DOC}$ on pH. Kukkonen (1991b) showed that with increasing pH the $K_{DOC}$ for PCP decreased drastically. On the other hand, the ion composition of the medium can affect the octanol-water
partition of chlorophenols. Increasing KCl concentration in water results in an increase of $K_{ow}$ (Westall et al., 1985). Furthermore, the sorption of chlorophenols to natural organic matter is strongly affected by the ionic strength of the water (Schellenberg et al., 1984).

Despite the higher lipophilicity of TCB, its sorption to DOM was lower than that of B[a]P (Figure 2). The $K_{DOC}$ for TCB were somewhat similar to those for pyrene, except in the case of Nordic reference fulvic acid (NoFA), to which the sorption of TCB was minimal (IV). In general, previous studies have shown that the capacity of fulvic acids to sorb organic chemicals is lower than that of humic acids (De Paolis and Kukkonen, 1997; Chin et al., 1997). The lower aromaticity together with greater amount of acidic functional groups and charges offers an explanation for the lower association of neutral organic compounds with fulvic acids (Thurman, 1985; Chin et al., 1997; Kretzschmar and Christl, 2001). Despite that, the $K_{DOC}$ for TCB and NoFA was surprisingly low (IV) when we take into consideration the fact that for the same PCB congener and fulvic acids Uhle et al. (1999) measured $K_{DOC}$s that were more than an order of a magnitude higher. This is probably due to variation in the characteristics of fulvic acids from different sources and also caused by differences in methods (equilibrium dialysis vs. solubility enhancement).

![Figure 2](image-url)  

**Figure 2.** Logarithms of DOM-water partition coefficients ($\log K_{DOC}$) in Lake Kontiolampi for the model compounds, determined by the equilibrium dialysis method at a water hardness of 0.5 mM. The compounds are arranged according to increasing lipophilicity. The different shades represent data from Articles I, II and IV.
Previous studies have also indicated lower sorption for PCBs compared to sorption of the slightly less lipophilic PAH (Kukkonen et al., 1990; Kukkonen and Oikari, 1991). Similarly, Cho et al. (2002) showed that association of the more lipophilic DDT with humic acid is lower than that of pyrene. Furthermore, previous authors have also measured lower $K_{DOC}$s for compounds (DDT and 2,2',5,5'-TCB) with about the same lipophilicity as for B[a]P (Landrum et al., 1984; Kulovaara et al., 1992; Kulovaara, 1993). It has been suggested that different electron densities in the model compounds and DOM fractions are the reason for differences in the association affinity of PAH and PCB (Kukkonen et al., 1990; McCarthy et al., 1994). However, B[a]P exhibits relatively high affinity towards all DOM fractions (Kukkonen et al., 1990).

The interaction with DOM was also weaker for TCB than for B[a]P (1). Both lower and weaker association indicate a difference in the association mechanism. On the other hand, Uhle et al. (1999) hypothesized that the association of PCBs is also driven by the aromaticity of DOM. However, PCBs have been shown to highly associate with hydrophobic neutral (XAD-8 fractionated) fraction of DOM, which has low aromaticity (Kukkonen et al., 1990). Furthermore, in a series of 20 natural waters from Finland the $K_{DOC}$ for B[a]P seemed to increase with increasing DOC concentration (Kukkonen and Oikari, 1991). This was due to higher aromaticity and larger proportion of hydrophobic acids at higher DOC levels. TCB did not follow a similar trend, but the $K_{DOC}$ was higher at low levels of DOC, where the hydrophilic fraction dominated.

This leads to the conclusion that the size and shape, or other steric factors, of the molecules play a role in sorption. The effect of steric factors is supported by the fact that the sorption affinity of PCB congeners depends on the position of chlorine atoms in the aromatic rings. Chlorines in the ortho position hinder free rotation of the phenyl rings around the 1,1’ carbon; this kind of “stiffer” structure may inhibit sorption to DOM, and some studies have shown that non-ortho substituted congeners have higher $K_{DOC}$ values than ortho substituted congeners do (Kukkonen et al., 1990; Uhle et al., 1999).

It may well be that bulkier chlorinated molecules simply do not fit to the same binding sites as smaller molecules do. However, the $K_{DOC}$ for PCB congeners has been shown to correlate positively not only with log $K_{ow}$ and solubility but also with surface area of the molecule (Lara and Ernst, 1989). Furthermore, the dimensions of the model compounds used in this study do not differ greatly (Table 1). The axes used to describe the dimensions of the model compounds are presented in Figure 3 for B[a]P. TCB is longer than B[a]P, but the other dimensions are somewhat smaller and therefore size itself is not necessarily the crucial factor in lower and weaker association of TCB. Instead of size, the presence of substitute atoms and the differences in electron clouding may play a role in this. Chlorine atoms (number and position) may control the

Figure 3. Axes (H, L and D) for the dimensions used to describe the size of the model compounds in Table 1.
hydrophobic interaction between aromatic structures in PCBs and DOM.

It can be hypothesized that the association of TCB with DOM could be mostly a partition process (weaker interaction). In this case hexane, used in the liquid-liquid extraction method, would provide a more favorable phase for TCB, and therefore only extremely low \( K_{DOC} \) would be detected (I). For B[a]P, the association would occur through both phase partitioning and site specific binding (stronger interaction), and thus the difference between \( K_{DOC} \) values measured by equilibrium dialysis and those determined by the methods interfering with equilibrium partitioning (reverse-phase and liquid-liquid extraction) is smaller than for TCB.

### Table 1. Size and volume of the model compounds (Dr. Pipsa Hirva, Department of Chemistry, University of Joensuu, personal communication).

<table>
<thead>
<tr>
<th>Compound</th>
<th>H (Å)</th>
<th>L (Å)</th>
<th>D (Å)</th>
<th>V (Å³)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TCB</td>
<td>9.043</td>
<td>14.354</td>
<td>12.908</td>
<td>203</td>
</tr>
</tbody>
</table>

Å (ångström) = 10⁻¹⁰ m
V = molecular volume
Note: The dimensions are for molecular mechanically optimized free molecules (i.e. the dimensions can change in solution or when the molecules are bound to a substrate)

#### 5.2.2. Effects of water hardness

Despite the great differences in \( K_{DOC} \) values between B[a]P, pyrene, TCB and atrazine, increasing water hardness decreased the sorption of all the model compounds by DOM in Lake Kontiolampi water (IV). The trend was obvious also for atrazine, even though the association with DOM was extremely low. The sorption of atrazine and TCB by NoFA was remarkably low, and due to the high variation between replicates (near the detection limit), no trend was seen.

An increase in ionic concentrations in water compresses the HS into tighter spheres, hampering the access of the model compounds to hydrophobic domains. At the same time, bivalent metal ions (\( Ca^{2+} \) and \( Mg^{2+} \)) are making the conditions more favorable for the hydrophobic domains to emerge and expand (Engebretson and von Wandruszka, 1994; Engebretson et al., 1996). However, the net effect of increasing water hardness (and total ionic concentration) is a decrease in the measured \( K_{DOC} \) values, because coiling of the humic molecules reduces the access of the chemicals to the binding sites or hydrophobic regions (IV). Furthermore, according to theories about the structure of HS, the effect of water hardness should be greater at lower DOM concentrations. At low DOM level, the humic molecules are open and stretched; therefore increasing water hardness can have a stronger influence on the structure. At higher DOM level, humic molecules are already coiled and chemistry of the solution is less important.

Increasing water hardness had the greatest impact on the association between TCB and DOM in Lake Kontiolampi water, where \( K_{DOC} \) differed by an order of a magnitude between the lowest and the highest test hardness. There may be several reasons for this. Since it has been shown that TCB is...
associated more with the hydrophobic neutral fraction of DOM (Kukkonen et al., 1990), it is possible that this fraction was decreased by precipitation of DOM at higher water hardness levels (IV). In addition, if the sorption mechanism is different, increasing water hardness may change the conformation in DOM so that the environment is less favorable for TCB to interact.

5.2.3. Effects of quality and quantity of DOM
Specific absorptivity is an easy and valid measurement, which describes aromacity of DOM and therefore is also a good indicator of the sorption capacity. Furthermore, absorptivity is carbon normalized; and thus natural water samples with different DOC concentrations can readily be compared. However, there may be differences when total natural DOM and isolated fractions of DOM are compared. This is seen in the case of Lake Kontiolampi DOM and NoFA, where the absorptivity for the latter indicated higher aromacity but the effects on bioavailability (KDOC and BCF values) were lower (IV). Furthermore, the difference between KDOCs for the two DOM types was greatest for TCB, because sorption of TCB by NoFA was negligible. Therefore, results obtained for isolated DOM fractions must be extrapolated to natural samples with caution.

In the series of water samples from European rivers the KDOC for B[a]P correlated positively with aromacity (ABS270) of DOM in the water samples (II). This is consistent with the findings of previous studies, which have shown that the sorption of PAHs, including B[a]P and pyrene, is largely controlled by the aromacity of HS (Gauthier et al., 1987; Chin et al., 1997; Haitzer et al., 1999). A strong relationship between aromacity and sorption of B[a]P has also been observed in a series of Finnish freshwaters (Kukkonen and Oikari, 1991). However, the KDOC for pyrene did not completely follow a similar trend (II). Instead of a correlation with aromacity, the KDOC for pyrene correlated with the proportion of hydrophilic fraction and the amount of carboxylic groups (II). This was mainly due to the surprisingly high KDOCs in three samples from Spain and in one sample from the Netherlands. In those samples the KDOCs were at a level similar to the values in the samples from Finland, although the aromacity in Spanish and Dutch samples was low (II, Table 2). Moreover, the KDOC was the same for both PAHs in the Göta Älv sample. In Riera Major and La Solana the KDOC for pyrene even exceeded that determined for B[a]P, which is in contradiction to previous studies, where, within a single class of chemicals, KDOC correlated with Kow.

A few studies have shown that with isolated humic fractions the KDOC values for PAHs increase with increasing aromacity of the humic matter (McCarthy and Jimenez, 1985; Perminova et al., 1999), but the situation may differ in samples with total DOM, where different fractions interact. This is seen in the case of NoFA and Lake Kontiolampi DOM where the aromacity was higher for NoFA but the measured KDOCs were lower (IV). As already mentioned, comparison of isolated fractions and total DOM can be misleading.

Although most studies on the relationship between association of organic contaminants and the properties of natural organic matter have emphasized the importance of aromacity, in recent studies, aliphatic chains have also received attention. For example, it has been hypothesized that aliphatic chains in soil organic matter are able to form domains suitable for sorption of organic chemicals (Hu et al.,
Salloum et al. (Salloum et al., 2002) showed that the sorption of phenanthrene to aliphatic rich organic matter from soil and sediment was higher than to highly aromatic humic acid. There are also other indications that aliphatic structures contribute significantly to the sorption of pyrene and phenanthrene in natural organic matter from various sources (Chefetz et al., 2000; Mao et al., 2002). How these findings apply to DOM is still unknown.

Although the effect of concentration of DOM on association capacity was not drastic, it was still statistically significant (I). The slight decrease is in agreement with previous studies with DOM dilution series of natural water (Kukkonen et al., 1989) as well as with concentration series of isolated humic materials (Landrum et al., 1984; McCarthy and Jimenez, 1985). Even though the effect is quite small, it may be of some importance in transport of chemicals in watersheds where DOM dilutes downstream. It has been stated that DOM can be a significant factor in transport of contaminants in groundwater, where the DOM levels are considerably lower than in surface waters (Thurman, 1985; McCarthy and Zachara, 1989). Therefore, when large volumes of water are considered, changes in association capacity may affect the transport.

5.3. Bioavailability

5.3.1. Bioconcentration of the model compounds in DOM-free conditions

In DOM-free reference water the bioconcentration factors (BCF) followed the increasing \( K_{\text{ow}} \) values despite the fact that the model compounds were from different classes (Figure 4). For atrazine the BCF values were between 2 and 3, which is in agreement with previously reported values for \textit{Daphnia magna} (Ellgehausen et al., 1980; Nikkilä et al., 2001). The BCF values for Naphthalene and PCP ranged from 31 to 55 and from 88 to 156, respectively. These values are in agreement with those reported in the literature for naphthalene (about 40) and PCP (110) (Kukkonen and Oikari, 1991; Geyer et al., 1991; Kukkonen, 1991a). For pyrene the BCF value was about 3000, which is somewhat higher than the values reported earlier (above 2000) (Granier L.K. et al., 1999; Nikkilä and Kukkonen, 2001). The BCF values for \textit{B}[\alpha]P were between 6000 and 10 000. Previous studies have reported values between 6000 and 8000 (McCarthy, 1983; Kukkonen et al., 1990). For the most lipophilic model compound, TCB, the BCF value had quite a high variation (12 000 - 20 000). Kukkonen and Oikari (1991) and Kukkonen (1991) have previously reported values slightly above 20 000.

The BCF values for individual compounds varied substantially between different experiments and also differed from values found in the literature. This is most likely a result of variation in the condition of the test organisms. The size and lipid content can strongly affect the extent to which compounds are accumulated by organisms (Newman, 1995; Landrum and Fisher, 1999). Higher lipid content and smaller size of individuals leads to higher tissue concentration. The differences in condition are partly due to the quality of the algae cultured for food and partly due to some kind of annual cycle of the daphnids, because in mid-winter the condition of both the daphnids and the algae appeared to be weaker.
Figure 4. Bioconcentration of the model compounds in DOM-free conditions. Different bars represent values obtained in different experiments. The model compounds are arranged with respect to their $K_{ow}$ values. The bars represent BCF values obtained in different experiments of the original publications. The BCF values at different water hardness levels (Study IV) are presented as averages, and therefore the effect of water hardness is not shown in this figure.

5.3.2. Effects of quality and quantity of DOM

In waters where DOM is aromatic, the bioavailability of B[α]P is clearly reduced, even at low DOC levels (I; II). In order to decrease significantly the bioavailability of pyrene, which is more hydrophilic than B[α]P, water has to contain a high DOC concentration (I, Nikkilä and Kukkonen, 2001). This was also expected in light of the measured $K_{DOC}$ values.

In Article II, the BCF in DOM-free water is over 2.5 times higher for B[α]P than for pyrene. In several boreal waters, however, the BCF for B[α]P is reduced to the same or an even lower level than the BCF of pyrene (Figure 5). Kukkonen and Oikari (1991), who studied a series of 20 Finnish natural waters (DOC range 2-38 mg/L), showed that in most cases the bioavailability of B[α]P was reduced by DOM. Furthermore, they observed no decrease in BCF of naphthalene due to DOM, but in many cases the BCF in the natural waters exceeded the BCF in the DOM-free reference.

Despite the relatively high $K_{DOCs}$ for pyrene in some samples of the European river water series with low DOM levels, the proportion of bound compound was too low ($\leq 14\%$) to affect bioavailability (II). It seems, that in order for the effect on bioavailability to be significant, at least 40% of the compound should be associated with DOM. The effect of DOM on
bioavailability of PAHs with four rings or less is probably quite insignificant at the DOM levels commonly observed in natural freshwaters. A relatively high concentration of DOM (> 15 mg DOC/L) is needed to reduce the bioavailability of pyrene and the less lipophilic PAHs significantly. In their study with pyrene, Nikkilä and Kukkonen (2001) came to the same conclusion.

For atrazine, neither DOM nor any other water characteristic seemed to have any effect on its bioavailability (II, IV). In their study with daphnids and periphyton communities, Nikkilä et al. (2001) have previously made the same observation. Low levels of DOM did not reduce the bioavailability of naphthalene or PCP (III). This was expected because only low $K_{DOC}$ values (< 5000) have been measured for naphthalene (Kukkonen and Oikari, 1991), and for PCP it was not possible to detect any $K_{DOC}$ in Lake Kontiolampi water at pH 7 (unpublished data). Furthermore, pH seems to play a more important role in bioavailability of PCP than DOM does (Saarikoski and Viluksela, 1982; Kukkonen, 1991b; III).

![Figure 5. Bioconcentration factor (BCF) for Benzo[a]pyrene (B[a]P) and pyrene in different water samples (Data from II).](image)

The BCF for B[a]P in the Ebro sample was significantly lower than in DOM-free water (Figure 5); however, when the BCF experiments were repeated in Study III, no such difference was observed. Similarly, in the repeated experiments the BCF for B[a]P in the Geul sample did not differ but was
considerably lower than in the DOM-free water (II, III). These results are probably due to random variation in the experiments rather than effects of DOM.

5.3.3. Effects of water hardness
As expected on the basis of the measured $K_{DOCS}$, in the water from Lake Kontiolampi the bioavailability of $BaP$, pyrene and TCB increased with increasing hardness of the water (IV). The trend was also obvious for $BaP$ in the presence of Nordic reference fulvic acid (NoFA). Water hardness did not, however, affect the bioavailability of atrazine. Nevertheless, the effect was not drastic, and therefore properties of DOM and the model compounds still played a more important role in bioavailability.

However, the BCF for $BaP$ in Lake Kontiolampi at the highest water hardness was almost 5 times higher than at the lowest hardness, while for pyrene and TCB the difference was only 1.6 and 1.3, respectively. Furthermore, due to aggregation of DOM, the DOC concentration was lower at the higher hardness levels of 2.5 and 4.0 mM, which also had a minor effect on bioavailability. Therefore, in the presence of DOM, water hardness had a pronounced effect only on the bioavailability of $BaP$ (I, IV).

As with the $K_{DOCS}$ values, the effect of water hardness may be greater at lower levels of DOM. Further evidence can be found when the differences in the effects of water hardness on BCF are compared at different DOM levels. In Studies I and III, where the Lake Kontiolampi DOM was diluted to 2 mg DOC/L the BCF for TCB decreased almost 50% at a water hardness of 0.1 mM, while at 2.5 mM the decrease was insignificant. In the undiluted Lake Kontiolampi samples the effect of water hardness on the BCF of TCB was modest.

In DOM-free conditions, the effects of solution chemistry on bioavailability or bioaccumulation of neutral organic compounds are usually considered to be insignificant (Erickson et al., 1994). In general, this also seemed to be the case in the present studies. Pyrene, however, behaved inconsistently in bioconcentration experiments where the effects of water hardness were measured in DOM-free reference water. As seen in the hardness experiment with NoFA, significant differences were observed between hardness levels in the BCF values, while in the Lake Kontiolampi experiment no such differences occurred (IV). Previously, a bioconcentration experiment was conducted for pyrene and $BaP$ (Akkanen et al., 1999). The results showed that the BCF for $BaP$ was not significantly affected by water hardness, while with increasing hardness up to 2.5 mM the increase in BCF was significant for pyrene (Figure 6). In this respect, toxicokinetics were determined for pyrene at hardness levels of 0.1 and 2.5 mM, but no significant difference was observed, although the BCF value was slightly higher in the harder water (Figure 7).

With increasing salinity, Levitan and Taylor (1979) observed an increase in accumulation of naphthalene in killifish ($Fundulus heteroclitus$), they hypothesized that this could be due to an increase in drinking rate or changed osmoregulation of the fishes at higher salinities. The effect of salinity on uptake is similar to some of our observations on effects of hardness (Akkanen et al., 1999; IV). However, the ionic concentrations used by Levitan and Taylor (1979) were considerably higher than ours, and thus the effects could potentially be stronger.
Figure 6. Effect of water hardness on bioconcentration factor (BCF) of benzo[a]pyrene (B[a]P) and pyrene in the absence of DOM (Akkanen et al., 1999). Letters denote significant differences ($p < 0.05$) between water hardness levels. Water hardness is expressed as $[\text{Ca}^{2+}] + [\text{Mg}^{2+}]$ mM.

If we assume that the uptake occurs through passive diffusion, increased filtering rate or, as hypothesized by Levitan and Taylor (1979), in the case of fishes, increased drinking rate could lead to increased uptake. In this situation more chemical will be in contact with the epithelium, and thus the uptake will increase. On the other hand, if the uptake is connected to osmoregulation it could be assumed that osmoregulatory systems are activated by low water hardness because fewer ions are available and that higher activity is needed to maintain the ionic balance. This means that with greater hardness the uptake should be lower, but such is not the case. Since evidence concerning the effects of hardness was found only for pyrene (and not in every experiment), the role of water hardness in controlling bioavailability of hydrophobic organic chemicals still remains unclear but is probably negligible.

5.3.4. Increased bioavailability due to DOM?
In some cases, low levels of DOM have produced higher BCF values and even greater toxicity than in the DOM-free reference conditions (Haitzer et al., 1998). No mechanism nor validation of such effect has yet been established. However, as pointed out by the review by Haitzer et al. (1998), such “enhancement” has occurred in experiments every now and then.
Figure 7. Toxicokinetics of pyrene in artificial freshwater (AFW) at two levels of water hardness ([Ca$^{2+}$] + [Mg$^{2+}$] mM). A one compartment model following first-order kinetics was used to fit the curves (Landrum et al., 1992). COD = coefficient of determination, $k_u$ = uptake clearance, $k_e$ = elimination rate, BCF = bioconcentration factor = $k_u/k_e$.

Nonetheless, neither in the present study (III) nor in those of other authors (McCarthy et al., 1985) has this phenomenon been repeated, even when a large number of different chemicals and DOM of different origins have been used (III). The mechanism behind the “enhancement” has not been studied. The direct effects of DOM (which could be reasons for “enhancement”) on organisms are also largely unknown. Parent and Campbell (1994) showed that adsorption of soil fulvic acid to the surface of algal cells increased the permeability of the cell membrane. Similarly, DOM has been shown to interact with living cells of fish gills and the interaction was shown to be more pronounced at lower pH and higher DOM concentration (Campbell et al., 1997). Furthermore, it has been shown that biotransformation enzymes may be affected by DOM (Wiegand and Steinberg, 2001; Pflugmacher et al., 2001). On the other hand, there are no clear indications that DOM affects the biotransformation of organic compounds (Kukkonen and Oikari, 1988; V). The data still suggest that direct effects of DOM on the physiology or metabolism of organisms could provide some information on “enhancement”.

Perminova et al. (2001) showed that HS from various sources at concentrations of 1.5 - 6.0 mg C/L increased feeding activity (i.e. filtering rate) of *D. magna* when compared to the humic-free control. Feeding activity was measured by a decrease in the concentration algae grazed by the
daphnids. This offers one possible explanation for “enhancement”. If the filtering rate is increased, more contaminant molecules come into contact with epithelial cells, thus increasing the uptake.

This could be an important mechanism, especially for chemicals with low $K_{DOC}$ and for which the bioavailability presumably is only slightly affected by DOM. For example, in a study with samples from 20 freshwater sources the BCF for naphthalene exceeded the DOM-free BCF in several lake waters containing <10 mg DOC/L (Kukkonen and Oikari, 1991). Although, in this study, no significant “enhancement” was observed for naphthalene (III). However, the effects on filtering rate are related to the characteristics (i.e. origin) of HS, and the HS of aquatic origin either increased the filtering rate or had no effect (Perminova et al., 2001). Furthermore, Aldrich humic acid, which in certain cases has been shown to enhance uptake (Leversee et al., 1983; Muir et al., 1994; Traina et al., 1996), increased the filtering rate at low DOC levels. In many cases, when the DOC concentration was further increased, the feeding activity decreased to control level (Perminova et al., 2001).

Despite the indications mentioned above, it is still unclear whether the observed increase in bioavailability due to DOM has environmental implications. Further studies are needed to establish the underlying mechanisms, and only then can we decide whether the phenomenon occurs only in laboratory experiments.

5.3.5. Biotransformation

Despite strong biotransformation, merely measuring total radioactivity seemed to provide a good estimate of the difference in bioavailability of pyrene (V). The accumulation of polar metabolites was surprising because previously PAHs have been detected in daphnids mostly as parent compounds (Herbes and Risi, 1978; McCarthy, 1983). However, in both studies polar metabolites were found in the exposure water, indicating biotransformation by daphnids.

On the basis of the present data it was not possible to make any definitive conclusions as to whether DOM directly affects the biotransformation of pyrene (V). However, in water fleas exposed in Lake Kontiolampi water, the proportion of parent compound was higher. This was probably a result of faster uptake in the DOM-free water (Nikkilä and Kukkonen, 2001) and thus due to higher induction of biotransformation enzymes rather than to an inhibitory effect of DOM in Lake Kontiolampi water. Furthermore, Kukkonen and Oikari (Kukkonen and Oikari, 1988) found no evidence of DOM affecting biotransformation and elimination of PCP in $D. magna$.

There are, however, indications that DOM can directly alter the activity of biotransformation enzymes. Kagan et al. (1987) pointed out that the effects of PBO on biotransformation may have some ecological importance because PBO is structurally similar (to some extent) to many natural compounds such as lignins. Therefore, biotransformation may be inhibited by components of organic matter in natural waters. Wiegand and Steinberg (2001) showed that fulvic acid induced GST activity in the gills of carp ($Cyprinus carpio$), whereas the effect of humic acid and total organic matter was the opposite or non-existent. In other tissues (liver and muscle) the effects were less pronounced, and usually GST activity was inhibited. This indicated that the strong effects detected in the gills were due to direct contact of DOM with the gills, whereas there were no signs of DOM reaching the liver. Furthermore,
for a few aquatic organisms the effects of DOM on biotransformation enzymes have been shown to be variable (Pflugmacher et al., 1999; Pflugmacher et al., 2001). No effects on P450 enzymes have been demonstrated.

In Lake Kontiolampi, DOM decreases bioavailability of pyrene by about 50% (Nikkilä and Kukkonen, 2001; II; IV). However, the acute toxicity (EC$_{50}$ after 24 and 48 h) of pyrene to D. magna was not affected (Nikkilä et al., 1999). The observation that the tissue concentration of parent pyrene was similar in the two exposure waters provides a possible explanation for the equal toxicity (V). However, it has to be noted that the concentration of pyrene used in the toxicity tests is far higher than that used in the bioconcentration experiments. The effects of sub-lethal and lethal levels of pyrene on bioconcentration, bioavailability and biotransformation are unknown. Furthermore, it has been shown that biotransformation of organic contaminants varies greatly among the invertebrate species often used in aquatic toxicology and even between compounds within one species (Verrengia Guererro et al., 2002). Therefore, comparison of the acute toxicity of different compounds in different waters on the basis of chemical concentration in the exposure water is complicated. Knowledge of bioavailability and biotransformation is needed as well.

Despite the intense biotransformation of pyrene in D. magna, we could detect differences in bioavailability between experimental waters. This validates the use of radiolabelled chemicals and liquid scintillation counting in bioavailability studies, without any other analytical methods even with a rapidly biotransformed compound like pyrene. However, when toxicity is concerned the situation may be more complex.

5.4. Linking $K_{DOC}$ values with bioavailability

The equilibrium dialysis method gives a good estimate of the bioavailability of organic chemicals (I). This has also been shown to apply to pulp mill recipient samples and chlorolignin preparations (Kukkonen and Pellinen, 1994). When the equilibrium dialysis method is used, however, certain things should be noted. If the goal is to estimate the bioavailable fraction in natural water samples, the characteristics of the DOM-free reference water should be comparable to the sample. Otherwise, we may either under- or overestimate the bioavailable fraction. Like in Study II where all samples were measured with 0.5 mM AFW (pH 7) as a DOM-free reference, although certain characteristics, such as conductivity, hardness and pH, varied greatly. Therefore, in the hard waters the bioavailable fraction may have been overestimated and slightly underestimated in the soft ones.

The equilibrium dialysis methods failed to give accurate $K_{DOC}$ values for TCB at low DOM levels, whereas the other two methods were effective enough also at low levels of DOM (I). However, the reverse-phase and liquid-liquid extraction methods greatly overestimated the bioavailable fraction of both model compounds tested. At higher levels of DOM, the equilibrium dialysis method estimated the bioavailability of TCB rather well, although there were also indications that a small fraction of DOM-associated TCB could be bioavailable. This is supported by the fact that the interaction of TCB with DOM appeared to be weaker than that of B[a]P (I).
Figure 8. Relationship between the DOM-water partition coefficients ($K_{DOC}$) determined by the equilibrium dialysis method (dial) and biological assay (biol). Data from I, II and IV.

However, when water characteristics vary greatly from one sample to another the situation is more complex. In these cases BCF may be influenced by factors other than DOM, as observed in the case of pyrene where water hardness may affect the BCF. This may be why values for the bioavailable fraction or $K_{DOC}$ determined by biological assay and the equilibrium dialysis do not always agree. At low DOM levels, in particular, slight differences in BCF values in the presence and absence of DOM produce high $K_{DOC}$ values. The correlation between the $K_{DOC}$ values determined biologically and those obtained by equilibrium dialysis is presented for B[a]P (Figure 8). At low levels of DOM, the estimated biological $K_{DOC}$ values can be far too high because even slight differences in BCF values between reference and DOM treatments produce high $K_{DOC}$ values. This is evident, for example, in the Ebro River sample (2.4 mg DOC/L) where the equilibrium dialysis gave only a low $K_{DOC}$ value with high variation (9000±8000); and with this kind of association constant there should not be significant effects on bioavailability. However, the BCF value was significantly lower in the Ebro sample than in the DOM-free reference, and thus produced extremely high $K_{DOC}$ value (415 000).

New methods for the estimation of bioavailability have also been introduced and tested. For example, solid-phase microextraction has been shown to be a useful method for measuring the partition of organic chemicals (Poerschmann et al., 1997).
This method uses a small silica fiber coated with a polymer and is based on the assumption that only the freely dissolved chemical is sorbed by the fiber. The advantages of this method are that it is fast and water samples can be used without any manipulation. The amount of chemical can then be analyzed from the polymer. Ramos et al. (1998) showed that this method correctly estimated the bioavailable fraction of TCB and pentachlorobenzene in the presence of Alrich humic acid.

6. CONCLUDING REMARKS

6.1. Conclusions
Yes, dissolved organic matter matters. However, the magnitude of the effects on bioavailability are largely dependent not only on the quantity but also on the quality of DOM. Furthermore, characteristics, such as hardness of ambient water control the association capacity of DOM and subsequently affect the bioavailability of organic chemicals, even though the same water characteristics would not affect bioavailability in DOM-free water. In boreal region, including in Finland, low water hardness as well as the aromaticity of DOM, favor its association with organic contaminants; therefore bioavailability and transport can be affected, even at low levels of DOM. Any evidence that low levels of DOM could increase the bioavailability of organic contaminants was not found.

The sorption of organic chemicals to DOM is also driven by the characteristics of the chemicals. Within a chemical class, lipophilicity is a major factor in interaction with DOM. In general, higher lipophilicity has been thought to result in greater sorption, although in this study there were also some indications that with natural DOM this is not always the case. When chemicals of different classes are compared the situation becomes more complicated. It seems that the interaction between halogenated compounds, such as PCBs, and DOM is lower and weaker than that of PAHs of lower lipophilicity. Furthermore, the position of chlorine atoms in PCBs play an important role in sorption and bioavailability. In addition, the compounds, whose bioavailability are the most affected by DOM, are in many cases the most harmful ones. This emphasizes even more the importance of DOM as a factor affecting the environmental fate and effects of organic contaminants.

6.2. Future studies
There is still a lot to learn about the interaction between contaminants and DOM. New chemicals are produced all the time and many old ones have not been studied in respect to their sorption and bioavailability in the presence of DOM. An additional aspect that could be added to bioavailability studies is experiments on biotransformation in various test species under different situations. The capacity of invertebrates to metabolize different compounds is in many cases unknown. Tests using toxicant concentration in the exposure water as a measure of toxicity are still routinely used. These include also the standardized daphnid tests. Biotransformation is a fundamental factor when the effects of different chemicals are evaluated and the results extrapolated to another species. Therefore, approaches such as critical body burden have recently received more attention.

This leads us to the direct effects of DOM, which are also largely unknown, although nowadays a few researchers are studying these direct effects. Like the few studies that have shown the effect of DOM on biotransformation, but the results are variable indicating
the complexity of organic matter. Therefore, combining studies on bioavailability and biotransformation could produce important results concerning the role of DOM in the fate and effects of contaminants in organisms.

In addition, sediments are an important sink for many contaminants and pore water may play an important role in bioaccumulation of contaminants in sediments. However, the effects of DOM on the fate of contaminants in sediments are largely unknown. In this respect, studying DOM of sediment origin and its interactions with contaminants may significantly contribute to our knowledge of the environmental fate of harmful substances in aqueous environments.
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