Bioavailability and toxicity of chemicals in inland waters
-the importance of prevailing water chemistry and
implications for risk assessment

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ABSTRACT

Physicochemical characterization of freshwater samples from Finland, the Netherlands, Spain, and Sweden revealed that water hardness and pH decreased and the quantity and quality of humic substances changed considerably in this geographical series from south to north. Since the ambient water chemistry may affect the availability of chemicals, the total aqueous concentration of a chemical may be insufficient to predict the bioconcentration, subsequent biological response, and thus risk. In addition, organisms could be affected directly by water quality characteristics. In this context the main objective of this thesis was to investigate the bioavailability of selected ecotoxicologically relevant chemicals (cadmium, benzo(a)pyrene, and pyrene) in various European surface waters and to show the importance of certain water chemistry characteristics in interpreting the bioavailability and toxicity results.

The bioavailability of cadmium to Daphnia magna was examined in very soft humic lake water. Humic substances as natural ligands decreased the free and bioavailable proportion of cadmium in soft lake water. As a consequence the uptake rate and the acute toxicity decreased compared with the humic-free reference. When the hardness of humic lake water was artificially elevated, the acute toxicity of cadmium decreased, although the proportion of free cadmium increased. The decreased bioavailability of cadmium in hard water was a result of effective competition for uptake by the hardness cations, especially calcium ions. The protective role of humic substances and water hardness against cadmium toxicity was also observed in Lumbriculus variegatus, although D. magna was more sensitive to cadmium.

The bioavailability of two polycyclic aromatic hydrocarbons (PAHs), pyrene and benzo(a)pyrene, was studied in European surface waters of varying water chemistry. Humic substances acted as complexing ligands with both PAHs, but the bioavailability of the more lipophilic benzo(a)pyrene to D. magna was affected more by humic substances than that of pyrene. In addition, not only the quantity of humic substances, but also their quality affected the bioavailability of benzo(a)pyrene. Nevertheless, the humic substances played a protective role in the photo-enhanced toxicity of pyrene under UV-B radiation. Water hardness had no effect on pyrene toxicity.

Results indicate that the typical physicochemical characteristics of boreal freshwaters should be considered carefully in local and regional risk assessment of chemicals concerning the Fennoscandian region.
LIST OF ORIGINAL PAPERS

This thesis is based on the following papers, which in the text are referred to by their Roman numerals:


III Penttinen, S., Malk V., Väisänen A. & Penttinen, O.-P. 2010: Using the critical body residue approach to determine the acute toxicity of cadmium at varying levels of water hardness and dissolved organic carbon concentrations and to test a potential mode of action. Manuscript.


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THE AUTHOR’S CONTRIBUTION

I  The study was planned by JK, AO, and SP. SP performed the experimental work, analyzed the data, interpreted the results, and wrote the paper under supervision of JK and AO.

II  The study was planned by SP, AK, and JK. AK performed the experimental work. SP analyzed the data, interpreted the results, and wrote the paper under supervision of JK.

III The study was planned by SP and OPP. VM performed the experimental work. SP and OPP analyzed the data. SP interpreted the results and wrote the manuscript with contributions from AV (chemical analysis) and OPP (metabolic rate).

IV The study was planned by AN, SP, and JK. AN performed the toxicity testing with the help of SP. SP characterized the experimental waters and determined the partition coefficients of pyrene. AN and SP calculated their own data and AN performed the statistical analyses. AN interpreted the results and wrote the paper under supervision of JK.

V The study was planned by JK, SP, and JA. SP performed the physiochemical characterization of experimental waters, determined the total phenolic content and modified the carboxylic group determination method with R. Halkko, determined the partition coefficients of benzo(a)pyrene and pyrene and initiated the XAD fractionation. JA performed the rest of the experimental work. JA and SP calculated their own data, JA and MH performed 3-D modeling, and JA performed the statistical analyses. JA and MH interpreted the results and wrote the paper under supervision of JK.

In addition to the results of the original papers, the thesis also includes unpublished additional material analyzed by the author.
ABBREVIATIONS USED IN THE THESIS AND THEIR DEFINITIONS:

ABS$_{270}$ specific absorptivity at 270 nm
BaP benzo(a)pyrene
BCF bioconcentration factor
BL Biotic Ligand
BLM Biotic Ligand Model
CBR critical body residue
DOC dissolved organic carbon
FIAM Free Ion Activity Model
GSIM Gill Surface Interaction Model
HbA hydrophobic acid fraction
HbN hydrophobic neutral fraction
HI hydrophilic fraction
IC inorganic carbon
K Freundlich adsorption constant
$K_{DOC}$ partition coefficient
k$_u$ uptake rate constant
k$_d$ depuration rate constant
LC$_{50}$ median lethal concentration for 50% of the test population
LR$_{50}$ median lethal body residue for 50% of the test population
PAH polycyclic aromatic hydrocarbon
PNEC predicted no-effect concentration
TOC total organic carbon
UV ultraviolet light
WHAM Windermere Humic Aqueous Model
1. INTRODUCTION

When chemicals are released into the aquatic environment, they participate in a variety of physical, chemical, and biological processes. As a result, they are distributed among the environmental compartments of water, sediment, and biota. Within abiotic compartments the chemicals could be partitioned further, e.g. between the water phase and humic substances or between sediment particles and interstitial water. Partitioning is a physicochemical process.

In the distribution of chemicals between abiotic compartments and biota, not only the physics and chemistry but also biological processes are involved. Bioconcentration and bioaccumulation describe processes by which chemicals are taken up by aquatic organisms from water only or from water, sediment, and food (Rand et al. 1995). Typically, the first step in uptake is adsorption onto the permeable surface of an organism. The uptake itself could be active or passive, depending on the chemical.

Uptake is a link between the environment and the biota: to elicit a biological response, the chemical must enter the organism. Metals can be taken up passively or actively, whereas hydrophobic organic chemicals are mainly taken up passively. The lipophilicity of compounds and the lipid contents of organisms are the main determinants in the bioconcentration of organic chemicals.

As distinct from the previous, physiology plays an important role in the bioconcentration of metals, since active uptake and elimination mechanisms exist to allow organisms to regulate the internal concentration of essential metals. Occasionally, nonessential toxic metals may follow the pathways of essential metals (Roesijadi and Unger 1993).

The total aqueous concentrations of chemicals are usually inadequate to predict their bioconcentration and subsequent biological responses sufficiently, since only a fraction of the total concentration is available to biota. In the aqueous phase, ambient water characteristics, especially water hardness or salinity and pH, and the presence of naturally occurring ligands, such as humic substances or inorganic ligands, modify the bioavailability of chemicals (e.g. Blust et al. 1992, Haider et al. 1998, Heijerick et al. 2003). Usually, the concentrations of uncomplexed and freely dissolved chemicals are considered to be the fraction most bioavailable to aquatic organisms for uptake through gills and skin.

There is no single definition for bioavailability, since bioavailability is a complex and dynamic process in which various physical, chemical, and biological interactions are involved, and different disciplines have their own perspectives (Dickinson et al. 1994). Bioavailability incorporates both the properties and speciation of chemicals, and behavior and physiology of organisms (Rand et al. 1995).

The following components are essential parts of bioavailability (Dickinson et al. 1994, Peijenburg and Jager 2003, Vijver 2005):

1) environmental availability, also referred to as chemical availability, which is a physicochemically driven desorption process,

2) environmental bioavailability, also referred to as biological availability, which is a physiologically driven uptake process, and

3) toxicological availability, a process in which the distribution of accumulated chemicals inside organisms affects the dose at the site of toxic action.

The concentration of chemicals in organisms are distinct indication of their bioavailability, since all external factors modifying bioavailability are intrinsically integrated in the body residue of an exposed organisms (Lanno et al. 2004, Chapman 2008). The critical body residue
(CBR) is a specific body residue that is associated with adverse biological response (McCarty and Mackay 1993), e.g. the median lethal body residue (LRₜₐ). Usually, the actual dose of a chemical at the site of toxic action remains unrevealed, but the body residue reflects more accurately that dose than does the exposure concentration (Meador et al. 2008, Rosen et al. 2008).

However, as a direct measure of chemical bioavailability (Lanno et al. 2004), CBR also has its weaknesses, and the straightforward application of the CBR approach, especially to metal toxicity, is challenged by internal bio(un)availability. Some species can store a portion of the internal metal dose in a biologically inactive form and concurrently the body residue increases without observable adverse response (reviewed by Vijver et al., 2004).

2. HUMIC SUBSTANCES IN SURFACE WATER

2.1 Origin and characteristics

Humic substances are omnipresent in terrestrial and aquatic ecosystems. They are organic breakdown products of dead plants and animals, partially or wholly decomposed, and probably also resynthesized by microorganisms (McKnight and Aiken 1998). In addition to biological mechanisms, humic substances may be altered by abiotic degradation, e.g. under ultraviolet (UV) radiation. The molecular size and macromolecular structures of humic substances reflect the characteristics of their indigenous organisms, as well as the prevailing atmospheric and environmental conditions. Thus, humic substances originating from diverse environments may differ not only in quantity but also in quality, such as molecular size, shape, content of aromatic, aliphatic, and carbohydrate components, number of functional groups, and elemental contents (Myneni et al. 1999, Peuravuori and Pihlaja 1999).

The various origins explain for the most part why an exact chemical or structural formula could not be represented. Instead of having an identifiable structure, aquatic humic substances are heterogeneous mixtures of high-molecular weight organic macromolecules with aromatic, acidic, and polyelectrolytic characteristics (e.g. Thurman 1985). The polyelectrolytic characteristics as well as aqueous solubility and acidity, are due to carboxylic and phenolic groups, which are the major functional groups of aquatic humic substances.

The most important source of aquatic humic substances is terrestrial ecosystems, although primary production and decomposition of aquatic organisms produce organic macromolecules as well (Melit 1992, Kortelainen 1993, Mattsson et al. 2005, Bade et al. 2007). In natural waters, organic matter exists as dissolved, colloidal, and particulate matter (Rand et al. 1995). Particulate matter usually sediments to the bottom of bodies of water. The dissolved phase has the main impact on the chemistry and biology of water (Kronberg 1999).

In the laboratory, aquatic organic matter is divided operationally into dissolved (< 0.45 μm) and particulate (> 0.45 μm) phase by membrane filtering. Although high molecular weight organic macromolecules account for the majority of dissolved phase species, the filtrate contains also low molecular weight organic compounds with identifiable chemical structure such as organic acids, carbohydrates, proteins, carboxyl and amino acids (Thurman 1985). However, the dissolved organic carbon (DOC) pool as a whole is referred to as aquatic humic substances in this study.
2.2 Humic substances in European surface waters

The Boreal Vegetation Zone is predominantly a puzzle formed of coniferous forests and peatlands (Meili 1992, Mattson et al. 2007). The cold climate in Northern Europe favors humic matter accumulation in soils (Kortelainen 1999). However, significant amounts of humic substances leach from catchments and in consequence many Fennoscandian surface waters are acidic and brown-stained. The concentrations of colored DOC in boreal lakes and rivers are typically higher than in other climate and vegetation zones in Europe (< 5 mg/l, Thurman 1985), but vary within wide limits (Hongve 1999). For instance, the median total organic carbon (TOC) and color of 987 randomly selected lakes throughout Finland were 12 (range 0.5–47) mg/l and 100 (range 0–600) mg Pt/l, respectively (Kortelainen 1993). The average TOC concentration of the 31 rivers that discharge to the coast of Finland, was 13 (range 6.1–20) mg/l (Kortelainen et al. 2004). In the surface waters in Finland, DOC constitutes a major fraction of TOC on average (Mattson et al. 2005, Vuorenmaa et al. 2006).

DOC leaching is an important flux of carbon in boreal catchments (Vuorenmaa et al. 2006), in which annual differences exist (Jager et al. 2009). Nevertheless, during the last two decades DOC concentrations in Nordic surface waters have increased (Skjelkvåle et al. 2005, Vuorenmaa et al. 2006). The reason for these elevated DOC concentrations is not currently known, but among others climate change has been proposed (Futter and de Wit 2008).

In addition to the moderately high concentrations of DOC, surface waters in Norway, Sweden and Finland are usually soft or very soft (Meili 1992, Källqvist 2009), due to regional bedrock. For example, half of the surface waters in the Northern Europe have a water hardness below 0.10 mmol/l Ca (very soft water), while in the Western Europe almost 50% of the surface waters have a hardness above 2.0 mmol/l Ca (hard water, European Chemicals Bureau 2007).

2.3 Role in ecotoxicology

Researchers representing various disciplines of the natural sciences are fascinated by humic substances. From the ecotoxicological point of view, the most interesting aspect is their effect on the environmental fate of chemicals; they are involved in the transport, distribution, sequestration, and bioavailability of elements and compounds in the environment. Due to their wide occurrence, humic substances may play an important role in the distribution and bioavailability of chemicals in boreal bodies of water.

Humic substances often reduce the bioavailability of chemicals, due to complexation or partitioning. However, the literature also reveals discrepancies, particularly on the effects of DOC on cadmium (Cd) bioavailability to aquatic organisms. For instance, in the presence of DOC the toxicity (e.g. Winner 1984, Oikari et al. 1992, Stuijtszand et al. 1999) and the bioaccumulation (Kozuch and Pempkowski 1996, Guo et al. 2001, Voets et al. 2004) of Cd has been reported to increase.

3. AIMS OF THE STUDY

Regional catchment and atmospheric processes govern the chemistry of surface waters, causing considerable variation in the characteristics of surface waters in Europe. Moreover, ambient water characteristics may modify the bioavailability and thus toxicity of chemicals in surface waters.
Within the chemical and water legislation of the European Union, the risks of chemicals at local, regional, and continental scales are assessed. In environmental management the results of standardized toxicity tests (e.g. acute toxicity tests for Daphnia magna OECD 1984 and ISO 1996) are preferred. The ability of toxicity test results to predict the toxicity of chemicals in natural waters of different geographical regions of Europe may be limited if the chemicals are tested in standardized reconstituted water only. The starting point of this study was to use natural waters not only in bioavailability experiments but also in toxicity testing.

The main objectives of this thesis were to investigate the bioavailability of chemicals in various surface waters of Europe from a dynamic point of view (Dickinson et al. 1994) and to show the importance of certain water chemistry characteristics in interpreting toxicity test results.

The specific objectives were:

1) to examine the chemical availability of selected ecotoxicologically relevant model compounds (cadmium, benzo(a)pyrene and pyrene) in freshwater by determining their partitioning to DOC of natural origin and to evaluate how partitioning is affected by quantity and quality of DOC and/or water hardness,

2) to examine the biological availability of the model compounds in freshwater with the aid of uptake kinetics, bioconcentration, and/or acute toxicity to the water flea Daphnia magna (Straus) and to evaluate how the biological responses measured are linked with the chemical availability measured, and

3) to test the applicability of critical body residue approach by determining the acute toxicity of cadmium to the oligochaete Lumbricus variegatus (Müller) at varying levels of water hardness and DOC concentration.

4. MATERIAL AND METHODS

An overview of the chemical measures of experimental waters and biological assessments is presented in Table 1.

4.1 Chemicals

Cadmium (Cd) is one of the most toxic heavy metals, having no known biological function. A radioactive isotope (115Cd) was used in the bioavailability studies, in which cadmium sulfate (CdSO₄·8H₂O) was added when needed. Appropriate corrections to the concentration of 115Cd were taken into account, due to the short half-life of the radioactive isotope. In toxicity tests and partitioning experiments, Cd was added as a chloride (CdCl₂·H₂O). The stock solutions were prepared in distilled water. The purity and manufacturers of each Cd compound are given in I, II, and III and the specific activity of 115Cd in I. The concentrations of Cd in water and in organisms were analyzed with atomic absorption spectroscopy (II, III) or liquid scintillation counting (I).

Pyrene and benzo(a)pyrene (BaP) are polycyclic aromatic hydrocarbons (PAHs). Pyrene has four aromatic rings and is less lipophilic than BaP with five aromatic rings. In acute toxicity tests the stock solution of pyrene was prepared in acetone (IV). In chemical and biological availability studies, 14C-labeled pyrene and 3H-labeled BaP were used and the stock solutions were prepared in ethanol (IV, V). The purity, specific activity, and manufacturers of the radiolabeled PAHs are given in IV and V. The concentrations of PAHs in water and in organisms were analyzed with a liquid scintillation counter.
Table 1. Overview of material and methods used in this thesis.

<table>
<thead>
<tr>
<th>Paper</th>
<th>Chemicals</th>
<th>Organism</th>
<th>Natural DOC source</th>
<th>Water chemistry measures (^1)</th>
<th>Chemical measure of bioavailability</th>
<th>Biological measure of bioavailability</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Cd</td>
<td><em>D. magna</em></td>
<td>2</td>
<td>basic</td>
<td>-</td>
<td>(k_a^a) body residue (^a)</td>
</tr>
<tr>
<td>II</td>
<td>Cd</td>
<td><em>D. magna</em></td>
<td>1</td>
<td>basic</td>
<td>(K_{DOC}, K)</td>
<td>(LC_{50}^b)</td>
</tr>
<tr>
<td>III</td>
<td>Cd</td>
<td><em>L. variegatus</em></td>
<td>1</td>
<td>DOC, pH</td>
<td>-</td>
<td>(LC_{50}^b), (LR_{50}^a)</td>
</tr>
<tr>
<td>IV</td>
<td>pyrene</td>
<td><em>D. magna</em></td>
<td>6</td>
<td>basic, (ABS_{270})</td>
<td>(K_{DOC})</td>
<td>(LC_{50}^b)</td>
</tr>
<tr>
<td>V</td>
<td>pyrene, BaP</td>
<td><em>D. magna</em></td>
<td>14</td>
<td>basic, (ABS_{270}), IC, XAD-8, functional groups</td>
<td>(K_{DOC})</td>
<td>BCF (^a)</td>
</tr>
</tbody>
</table>

\(^1\) The basic water chemistry measures include DOC, pH, color, and conductivity.
\(^a\) A direct biological measure of bioavailability.
\(^b\) An indirect biological measure of bioavailability.

4.2 Experimental waters and their characterization

The surface waters used in the experiments were representatives of geographically and atmospherically different areas in Europe. Surface water samples were collected from Eastern and Southern Finland, Western Sweden, from the border of the Netherlands and Belgium, and from Catalonia, Spain. Water samples from Finland were from rivers and lakes (I–V), while the other European samples were river waters (IV, V). Description of the pretreatment and storage of the samples is given in the original publications (I–V). Basic water chemistry measures e.g., pH, color, conductivity and the concentration of DOC were determined immediately in the laboratory (I–V, Table 1). In addition, some of the experimental waters were analyzed for further inorganic characteristics, such as the concentration of phosphate, nitrate, and chloride and the elements Na, K, Ca, Mg, Al, Fe, and Mn (Buyxk et al. 1999).

Reconstituted freshwater (DOC < 0.2 mg/l) was prepared for reference or for diluting the natural waters according to the techniques then used by the Finnish Standards Associations (SFS 5062:1984) or international standards (OECD 1984 and ISO 1999). If the standardized composition (such as pH or water hardness) was modified during the experiments, the description of the procedure was given in the original publication.

One aim in IV and V was to apply relatively simple methods to describe the quality of humic substances in untreated water samples for a comparative characterization. The absorbances of the samples were measured at wavelengths of 250, 270, 365, 465 and 665 nm (Hitachi U-2000 spectrophotometer, Hitachi Ltd., Tokyo, Japan) and the relations \(E_2/E_6(A_{250}/A_{365})\) and \(E_2/E_6(A_{465}/A_{665})\) were calculated (Chen et al. 1977, Schnitzer 1977, De Haan 1983); these results were not published previously. In addition, the aromaticity of humic substances was estimated by calculating
the absorptivity at 270 nm (ABS$_{270}$) of samples (IV, V) (Gauthier et al. 1987, Traina et al. 1990): 

$$\text{ABS}_{270} = (A_{270}/\text{DOC}) \cdot 1000$$

where $A_{270}$ is the absorbance of the water sample measured at wavelength 270 nm and DOC is the concentration of dissolved organic carbon (mg/l).

To study possible photodegradation of humic substances during the UV-B exposure (0–72 h, IV) the DOC, pH, and absorbances at wavelengths 250, 365, 465, and 665 nm were measured and the ABS$_{270}$ calculated.

The functional groups were determined spectrophotometrically (V). The aim was to apply rapid, as simple as possible but sufficiently accurate methods to determine two major functional groups of humic substances, the phenolic and carboxylic groups, without destructive pretreatment of samples. The total phenolic contents of the samples were determined as in Kukkonen (1992), in which the method of Singleton and Rossi (1965) was applied to water samples. In the present study, the method of De Nobili et al. (1990), originally designed for samples with very high concentrations of DOC (about 100 mg/l) was applied to natural waters. However, it was not possible to measure the phenolic content and carboxylic groups, if the DOC concentration was less than 5 mg/l and 10 mg/l, respectively. Thus, water samples with low concentration of DOC were concentrated. A more detailed description of the concentration procedure is given in V.

The DOC pool of the water samples was fractionated with XAD-8 resin into three fractions (Leenheer and Huffman 1979) (V): the hydrophobic acid (HbA), hydrophilic (HI), and hydrophobic neutral fractions (HbN). When an acidic (pH 2) water sample passed through the XAD-8 resin, the HbA fraction was absorbed by the resin and the HI fraction passed through. The HbA fraction was eluted by base. The fraction remaining in the resin was defined as the HbN fraction. The concentrations of DOC in the HbA and HI fractions were determined while the concentration of DOC in the HbN fraction was calculated. Finally, the relative proportions (%) of fractions were calculated. The HbA, HI, and HbN fractions are operationally defined isolates of DOC without exact chemical characterization. However, they can be used in the comparative characterization of humic substances of different origins.

### 4.3 Equilibrium dialysis

The organic carbon normalized partition coefficient ($K_{\text{DOC}}$) was determined (II, IV, V) by the equilibrium dialysis method (Carter and Suffet 1982, McCarthy and Jimenez 1985), which does not interfere with the interaction between chemical and DOC. The water sample was added to a semipermeable dialysis tube that was placed in a jar containing the aqueous solution of a chemical. After the jar was sealed, the equilibrium in partitioning between water and DOC was allowed to be reached. The basis for this method is that the dialysis tube is permeable for the chemical studied, but not for humic macromolecules. The outside concentration ($C_o$) of the chemical is considered to be the freely dissolved fraction, and the difference between the inside and outside concentration ($C_b$) is the chemical bound to DOC inside the tube. The $K_{\text{DOC}}$ ($l/kg$) was calculated as

$$K_{\text{DOC}} = C_b / (C_o \cdot \text{DOC})$$

where DOC is the concentration of dissolved organic carbon (kg/l).

The Freundlich adsorption constant ($K$) was determined for Cd with Freundlich isotherm, which is an empirical equation that describes the adsorption
3) \[ C_b = K \cdot C_c^a \]

where \( C_b \) is the concentration of bound Cd (mg/kg C) and \( C_c \) is the concentration of freely dissolved Cd, when the equilibrium is reached.

The proportion (%) of bound chemical was calculated with formula (4) and the proportion of free chemical with formula (5):

\[
\text{(4) } \% \text{ bound} = \frac{\text{DOC} \cdot K_{\text{DOC}}}{1 + \text{DOC} \cdot K_{\text{DOC}}} \cdot 100
\]

\[
\text{(5) } \% \text{ free} = \frac{1}{1 + \text{DOC} \cdot K_{\text{DOC}}} \cdot 100
\]

where \( K_{\text{DOC}} \) (l/kg) is the partition coefficient and DOC is the concentration of dissolved organic carbon (kg/l).

A more detailed description of the method is given in the original publications (II, IV, V). Concentrated samples were used when the partitioning of both PAHs was determined in Spanish waters and the partitioning of pyrene was determined in Dutch waters.

4.4 Bioassays

4.4.1 Experimental organisms

A parthenogenic \textit{Daphnia magna} culture in reconstituted freshwater was maintained in the laboratory as described in I, II, IV, and V. During the studies I and II, the culturing medium (hardness 0.5 mmol/l, soft) was prepared according to then used Finnish standard (SFS 5062:1984). As sensitive species, daphnids are affected by certain water characteristics, such as pH and water hardness. To avoid culturing difficulties, Elendt M7 medium (hardness 2.5 mmol/l, very hard) was used during later studies (IV, V), as recommended in the OECD guidelines for culturing \textit{D. magna} (OECD 1998).

\textit{Lumbriculus variegatus} population (III) was cultured in the laboratory in accordance with the procedure given by Leppinen and Kukkonen (1998).

4.4.2 Bioconcentration experiments

The bioconcentration of the model compounds was determined in treatments differing in concentration of DOC and/or water hardness (I, III, V) to determine their effects on the bioavailability of chemicals and body residue. The daphnids in bioavailability studies were sexually immature: 6–7 (I) and 4–5 (V) days old. In the latter studies the animals were younger, since waterfleas developed more rapidly in Elendt M7 medium than in the previously used soft culturing medium. The bioaccumulation of Cd was also analyzed in the surviving \textit{L variegatus} associated with 0–50% lethality in the acute toxicity tests (III). At the end of exposure the concentration of the chemical was determined in the animals and water. The results are reported as the concentration of Cd in animals (ng/mg wet weight in daphnids and µmol/kg wet weight in oligochaetes) and for the organic model compounds as the bioconcentration factor (BCF). The BCF is the ratio of tissue chemical residue to chemical concentration in the water at the steady state.

\textit{Cd} accumulation kinetics in daphnids were studied in further detail with one compartment model, which is the simplest mathematical model that describes the accumulation (Spacie and Hamelink 1995). One compartment model is adequate if the distribution of the chemical within the organism is homogenous. The exposed organism is regarded as one compartment in which a chemical is taken up and simultaneously eliminated. However, at the beginning of the exposure the elimination is negligible. When the rate of uptake of a chemical by the organism exceeds its rate of elimination, bioaccumulation can occur.
The rate of uptake and depuration follow the first-order kinetics. The change in chemical concentration in an organism over time is described as

$$\frac{dc}{dt} = k_u C_w - k_d C$$

where $k_u$ is the uptake rate constant, $C_w$ the concentration of a chemical in water, $k_d$ the rate constant for depuration, and $C$ the chemical concentration in the exposed organism.

In the steady state, the rate of uptake balances the rate of elimination. If the exposed organism is transferred to clean water, the body residue decreases, due to still continuing elimination. Elimination in a clean environment is called depuration.

4.4.3 Acute toxicity tests

Acute toxicity was tested with neonate water fleas (D. magna) according to standardized methods (SFS 5062:1984 in II, ISO 6341:1996, and OECD Guideline 202 in IV). To determine the effects of humic substances and water hardness, the acute toxicity of Cd and pyrene was studied in humic lake water of original and elevated hardness. The pH was adjusted to constant value (6.5 or 7.0) to perform toxicity experiments with the laboratory-reared daphnids at optimal pH and to prevent the possible confounding effect of variable pH. When phosphate buffering was used, the pH remained steady ($\pm 0.1$) during the experiments. Hardness levels of 0.5 mmol/l and 2.5 mmol/l were chosen, because they were the hardness levels recommended for reconstituted freshwater in standards SFS 5062:1984 and ISO 6341:1996, respectively. Intermediate hardness levels (0.2 mmol/l and 1.5 mmol/l) were also prepared for acute toxicity tests of Cd. Reconstituted freshwater with comparable hardness levels was prepared for reference. The acute toxicity of pyrene was tested in the dark and under UV-B radiation. Lethality was observed after 24 and 48 h.

The acute toxicity test using L. variegatus (III) was performed according to ISO 6341:1996, originally designed for D. magna. The hardness levels tested at pH 7 were 0.2 mmol/l, 0.5 mmol/l, and 2.5 mmol/l in both humic and reference water. Four worms were added to 10 ml of experimental water in a 20 ml glass vessel. Three replicates of each concentration were prepared. The test vessels were kept in the dark at 20 °C. Lethality was observed after 24 and 48 h.

The results of acute aquatic toxicity tests are conventionally expressed in external toxicity metrics as the median lethal concentration (LC$_{50}$) of a chemical in the water. The LC$_{50}$ calculation methods are given in the original publications (II, III, V). To estimate the median lethal body residues (LR$_{50}$) in Cd-exposed oligochaetes (III) corresponding to the LC$_{50}$ measured, analyzed body burdens (mg/kg dry weight) were converted into appropriate units (mol/kg wet weight) and a linear regression equation was applied to establish the relationship between internal (µmol/kg wet weight) and external Cd concentrations (µmol/l). The LC$_{50}$ determined was substituted in the regression equation to estimate the corresponding LR$_{50}$.

5. RESULTS AND DISCUSSION

5.1 Characteristics of experimental waters

Water samples from Lake Louhilampi, Liuhapuro River, and Lake Mekkojärvi (I–III) were characterized for key physicochemical water quality characteristics such as pH and water hardness (Table 2). In addition the quantity of DOC was determined, but no further characterization of DOC was performed. However, humic substances
Table 2. Some physicochemical characteristics of surface water samples originated from geographically and atmospherically different areas in Europe. The Finnish bodies of water Louhijärvi, Mekkojärvi, and Kontiolampi are lakes, while the others are rivers.

<table>
<thead>
<tr>
<th>Water sample</th>
<th>Color (mg Pt/l)</th>
<th>DOC (mg/l)</th>
<th>IC (mg/l)</th>
<th>pH</th>
<th>Hardness (mmol/l)</th>
<th>Conductivity (mS/m)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Louhijärvi (I)</td>
<td>160</td>
<td>14.2</td>
<td>na</td>
<td>4.8</td>
<td>0.10</td>
<td>2.8</td>
</tr>
<tr>
<td>Louhijärvi (II)</td>
<td>180</td>
<td>19.6</td>
<td>na</td>
<td>5.2</td>
<td>0.10</td>
<td>2.4</td>
</tr>
<tr>
<td>Liuhapuro (I)</td>
<td>375</td>
<td>23.7</td>
<td>na</td>
<td>4.3</td>
<td>0.05</td>
<td>2.0</td>
</tr>
<tr>
<td>Mekkojärvi (III)</td>
<td>na</td>
<td>24.9</td>
<td>na</td>
<td>5.8</td>
<td>0.24</td>
<td>na</td>
</tr>
<tr>
<td>Kontiolampi (V,IV)</td>
<td>300</td>
<td>18.5</td>
<td>0.4</td>
<td>5.2</td>
<td>0.07</td>
<td>3.3</td>
</tr>
<tr>
<td>Kalliojoki (V,IV)</td>
<td>160</td>
<td>15.8</td>
<td>0.6</td>
<td>5.9</td>
<td>0.11</td>
<td>3.2</td>
</tr>
<tr>
<td>Koitajoki (IV)</td>
<td>60</td>
<td>8.4</td>
<td>1.4</td>
<td>7.0</td>
<td>0.09</td>
<td>3.5</td>
</tr>
<tr>
<td>Pielisjoki (V,IV)</td>
<td>50</td>
<td>7.3</td>
<td>1.1</td>
<td>6.8</td>
<td>0.09</td>
<td>2.5</td>
</tr>
<tr>
<td>Lärjänä (IV)</td>
<td>80</td>
<td>7.1</td>
<td>6.1</td>
<td>7.2</td>
<td>0.42</td>
<td>19.1</td>
</tr>
<tr>
<td>Hultabäcken (V,IV)</td>
<td>40</td>
<td>7.0</td>
<td>3.0</td>
<td>7.1</td>
<td>0.29</td>
<td>10.0</td>
</tr>
<tr>
<td>Maas (K) (V,IV)</td>
<td>10</td>
<td>4.6</td>
<td>26.3</td>
<td>8.1</td>
<td>1.99</td>
<td>61.3</td>
</tr>
<tr>
<td>Maas (E) (IV)</td>
<td>10</td>
<td>4.4</td>
<td>29.0</td>
<td>7.8</td>
<td>2.06</td>
<td>55.0</td>
</tr>
<tr>
<td>Göta älv (V,IV)</td>
<td>15</td>
<td>4.1</td>
<td>3.4</td>
<td>7.3</td>
<td>0.26</td>
<td>10.0</td>
</tr>
<tr>
<td>Geul (IV)</td>
<td>10</td>
<td>3.1</td>
<td>42.2</td>
<td>7.8</td>
<td>2.56</td>
<td>56.5</td>
</tr>
<tr>
<td>L’Avenco (IV)</td>
<td>10</td>
<td>2.5</td>
<td>27.8</td>
<td>8.2</td>
<td>1.41</td>
<td>29.2</td>
</tr>
<tr>
<td>Ebro (IV)</td>
<td>5</td>
<td>2.4</td>
<td>33.3</td>
<td>8.1</td>
<td>4.20</td>
<td>114.2</td>
</tr>
<tr>
<td>La Solana (IV)</td>
<td>5</td>
<td>2.0</td>
<td>43.7</td>
<td>8.3</td>
<td>2.31</td>
<td>40.3</td>
</tr>
<tr>
<td>Riera Major (V,IV)</td>
<td>5</td>
<td>1.4</td>
<td>22.1</td>
<td>8.1</td>
<td>0.97</td>
<td>21.8</td>
</tr>
</tbody>
</table>

Origin of samples: aFinland, bSweden, cthe Netherlands, and dSpain. The color is a subjective and rough estimate of humic substances, while the concentration of dissolved organic carbon (DOC) was regarded as a quantitative measure of humic substances in this study. Inorganic carbon (IC) consists of inorganic carbon species, e.g., carbonate and bicarbonate. Hardness of water was determined as a sum of [Ca^{2+}] and [Mg^{2+}] (mmol/l). Not analyzed = na.

from these waters were characterized in other studies (Kukkonen and Oikari 1991, Peuravuori and Pihlaja 1998, Peuravuori 2001). Despite the possible annual variation in composition of humic substances, these studies were considered sufficient to describe the aromatic humic characteristics of DOC in the experimental waters.

The physicochemical characteristics of the river water samples from Europe (Table 2) reflected the geochemical characteristics of different climate zones and geographical areas. Spain is located in the Mediterranean climate zone. The soils and rock foundations in the catchments of Ebro, L’Avenco, La Solana, and Riera Major are calcareous or siliceous, and the vegetation is dominated by oaks, alders, and other deciduous trees (Marti and Sabater 1996, Guasch et al. 1997). Water samples from Spain typically showed high concentrations of inorganic carbon (IC), hard water, high conductivity, and moderate pH. The water samples were colorless and the concentration of DOC was low.

The water samples from Finland (Kontiolampi, Kalliojoki, Koitajoki, and Pielisjoki) were representative of the Boreal Zone, where soils and surface waters are naturally acidic and contain humic substances. These samples were characterized by low IC, low or neutral pH, low conductivity, very soft water, dark color, and high concentrations of DOC. The characteristics of water samples from the Netherlands (Geul, Maas Eysden, and Maas Keizerweer) and Sweden (Göta älv, Hultabäcken, and...
Lärjeän) were between the characteristics of Spanish and Finnish samples, forming a geographical continuum from south to north.

The characteristics describing the quality of humic substances (Table 3) revealed that aromatic humic substances (humic and fulvic acids) were present in all aqueous samples from Finland and in two samples (Hultabäcken and Lärjeän) from Sweden. High ABS270 and high proportions of the HbA-fraction are typical for natural waters rich in humic substances (Kukkonen et al. 1990, Kukkonen and Oikari 1991). The proportion of the HbN fraction was low. The E2/E3 ratios of the Finnish samples varied between 3.8 and 5.3, while in the Swedish samples (Lärjeän and Hultabäcken) the ratios E2/E3 were 4.0 and 5.4. De Haan (1983) showed that fulvic acids of strongly humic and oligotrophic bodies of water are characterized by ratios of E2/E4 about 4. The water sample from Göta älven was almost colorless and the concentration of DOC was low, but XAD-8 fractioning showed that the quality of DOC corresponded to that in other Swedish samples. Rivers Hultabäcken and Lärjeän discharge to Göta älven, but since the catchment area of Göta älven is large, nonhumic rivers also discharge there. In addition, humic substances in Göta älven are less aromatic than in Hultabäcken and in Lärjeän, which is seen in the lower ABS270 and higher E2/E3 ratio (7.4).

The DOC in the Spanish and Dutch water samples deviated not only in quantity but also in quality from the Nordic samples. The proportion of the HbA fraction was low and the proportions of the HbN and HI fractions high, implying that the DOC pool is dominated by low-molecular weight molecules, not by acidic and aromatic humic substances (Leenheer and Huffman 1979). It is also seen in the E2/E3 ratios, which were 7.2–8.6 and 4.9–7.2 for Dutch and Spanish samples, respectively. When the E2/E3 ratio increases, the aromaticity and the molecular weight of humic substances decrease (Peuravuori and Pihlaja 1997). In addition, the molecular weight of DOC in these Western European samples were on average lower than that in the Nordic samples (J. Kukkonen, personal communication). The water samples came from areas where regional climate, vegetation, and soil conditions did not favor humification.

Determining the E2/E6 ratios was not successful (original data not presented), since in some samples the absorbance at wavelength 665 nm was zero or negative and no relationships or trends were seen between the E2/E6 ratio and other physicochemical properties of the water samples. Peuravuori and Pihlaja (1997) found no relationship between aromaticity and the E2/E6 ratio, which according to them supports the statement of De Haan (1983) that this ratio originally used for characterization of humic substances in soil is not applicable to limnology.

The total phenolic contents of the Nordic samples (V), which were 1.2–1.5 mmol/g C, corresponded to the typical concentration of phenolic hydroxyl groups in humic substances, which is averages 1.5 mmol/g C (Thurman 1985, Watt et al. 1996). However, the concentrations of carboxylic groups were moderately high (13.6–14.5 mmol/g C), compared with the values reported previously in the literature, usually about 4–6 mmol/g C (Thurman 1985, Watt 1996). Otto et al. (2001) determined slightly higher concentrations (6.1–8.6 mmol/g C) and the carboxylic concentrations obtained in this study were within the results (11.2–16.6 mmol/g C) of Newcombe et al. (1997).

The complete characterization of humic substances is difficult, time-consuming, expensive, and easily out of the usual ecotoxicological laboratory routines, often including isolation, concentration, fractionation, purification,
Table 3. Concentrations and characteristics of DOC affecting chemical and biological measures of PAH bioavailability in surface water samples from different geographical regions of Europe.

<table>
<thead>
<tr>
<th>Water sample</th>
<th>DOC (mg/l)</th>
<th>Characteristics of DOC</th>
<th>Pyrene</th>
<th>Benzo(a)pyrene</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>$K_{D,OC} \cdot 10^4$</td>
<td>bound (%)</td>
</tr>
<tr>
<td>Kontiolampi(V, IV)</td>
<td>18.5</td>
<td>46.0</td>
<td>51</td>
<td>40</td>
</tr>
<tr>
<td>Kalliojoki(V, IV)</td>
<td>15.8</td>
<td>38.7</td>
<td>58</td>
<td>36</td>
</tr>
<tr>
<td>Koitajoki(V, IV)</td>
<td>8.4</td>
<td>35.8</td>
<td>59</td>
<td>31</td>
</tr>
<tr>
<td>Pielisjoki(V, IV)</td>
<td>7.3</td>
<td>34.7</td>
<td>56</td>
<td>40</td>
</tr>
<tr>
<td>Lärjeån(V, IV)</td>
<td>7.1</td>
<td>37.8</td>
<td>46</td>
<td>39</td>
</tr>
<tr>
<td>Hultbäcken(V, IV)</td>
<td>7.0</td>
<td>28.2</td>
<td>46</td>
<td>40</td>
</tr>
<tr>
<td>Maas (Kf, V, IV)</td>
<td>4.6</td>
<td>18.5</td>
<td>19</td>
<td>32</td>
</tr>
<tr>
<td>Maas (E fry, IV)</td>
<td>4.4</td>
<td>19.3</td>
<td>22</td>
<td>38</td>
</tr>
<tr>
<td>Göta älv(V, IV)</td>
<td>4.1</td>
<td>20.6</td>
<td>41</td>
<td>42</td>
</tr>
<tr>
<td>Geuf(V, IV)</td>
<td>3.1</td>
<td>18.6</td>
<td>29</td>
<td>37</td>
</tr>
<tr>
<td>L’Avence(V, IV)</td>
<td>2.5</td>
<td>14.0</td>
<td>17</td>
<td>39</td>
</tr>
<tr>
<td>Ebro(V, IV)</td>
<td>2.4</td>
<td>8.7</td>
<td>21</td>
<td>41</td>
</tr>
<tr>
<td>La Soland(V, IV)</td>
<td>2.0</td>
<td>8.1</td>
<td>16</td>
<td>63</td>
</tr>
<tr>
<td>Riera Major(V, IV)</td>
<td>1.4</td>
<td>5.9</td>
<td>55</td>
<td>28</td>
</tr>
</tbody>
</table>

Origin of samples: aFinland, bSweden, cthe Netherlands, dSpain. Not detected = nd. Concentrated water samples were used when the partitioning of both PAHs was determined in Spanish waters and the partitioning of pyrene was determined in Dutch waters. The BCF of pyrene and benzo(a)pyrene in reference water was 3035±297 and 8212±2249, respectively. The asterisk denotes a statistical difference between surface water sample and reference water.
freeze-drying and redissolving of humic substances (Milne et al. 2001, Kalbitz et al. 2000). An overview of the various characterization methods is provided by Abbt-Braun et al. (2004). If a humic water sample is treated strongly during characterization, the structure and composition of humic substances may change (Peuravuori and Pihlaja 1999). In the comparative characterization of humic substances, rapid spectroscopic methods (e.g. fluorescence and UV spectroscopy, the E3/E4 ratios, and ABS270) of untreated water samples are useful, and their applicability has been proven by thorough characterization (Peuravuori and Pihlaja 1997, Kalbitz et al. 2000). It has been evident in several other studies that basic chemical and optical characterizations are sufficiently accurate to reflect biologically and ecotoxicologically significant differences in the quality of humic substances (Haitzer et al. 1999a, Richards et al. 2001, Akkanen et al. 2004). In addition, spectroscopic characterization of DOC improved the accuracy of the computed speciation model of Cd (Cornu et al. 2009).

5.2 Chemical availability

5.2.1. Cadmium

The KDOC of Cd decreased when the total aqueous concentration of Cd increased. At the same time, the proportion of free Cd increased (Table 4). In addition, at every Cd concentration tested the KDOC and the K decreased by an order of magnitude when the hardness of the humic water was elevated from very soft (0.1 mmol/l) to very hard (2.5 mmol/l) (II), and simultaneously the proportion of free Cd increased from 39–55% to 86–99%, respectively (Table 4).

The carboxylic and phenolic hydroxyl functional groups in humic substances usually participate in binding metal ions (Cameron and Sohn 1992, Cao et al. 1995, Li et al. 1998). Hardness cations (Ca2+ and Mg2+) compete with Cd2+ for binding at the same sites in humic molecules (Otto et al. 2001). However, Ca2+ is more effective than Mg2+ in interfering the binding of Cd2+ (O’Shea and Mancy 1978). There was an excess of Ca2+, even in very soft water, compared with the Cd2+ added, but the explanation for why binding to DOC was not prevented, may be that Cd2+ has a higher affinity for binding to DOC than Ca2+ (O’Shea and Mancy 1978, Meinelt et al. 2001).

When the water hardness was elevated to hard or very hard, the excess in hardness cations increased, which compensated for their moderately weak affinity for binding to DOC (Tipping 1993). Thus, the hardness cations effectively replaced Cd2+ at potential binding sites. Other divalent cations, such as Zn2+ and Cu2+, also compete with Ca2+ for binding sites, but the competition is most pronounced with Cd2+ (O’Shea and Mancy 1978), because Ca2+ is chemically similar to Cd2+ in ionic size and charge (Otto et al. 2001). In addition, the competition between Cd2+ and hardness cations in this study was demonstrated with WHAM modeling (E. Tipping, personal communication July 8, 1997). WHAM (Windermere Humic Aqueous Model) is designed to calculate equilibrium chemical speciation in surface and ground waters, sediments, and soils, especially when the chemical speciation is dominated by humic substances (Tipping 1994).

In addition, changes in water hardness (and in general ionic strength) govern the swelling and shrinking of humic macromolecules and thus set the actual spatial conformation (Berbel et al. 2001). Steric hindrance may also be involved in decreased binding by humic substances, but it is more probable in the case of organic compounds, in which the shrinking of humic macromolecules prevents the partitioning of organic compounds into hydrophobic
Table 4. Proportion (%) of the free cadmium in humic water of Lake Louhilampi. Calculated from data (II).

<table>
<thead>
<tr>
<th>Cadmium concentration</th>
<th>Water hardness 0.1 mmol/l</th>
<th>0.5 mmol/l</th>
<th>1.5 mmol/l</th>
</tr>
</thead>
<tbody>
<tr>
<td>40</td>
<td>39%</td>
<td>74%</td>
<td>86%</td>
</tr>
<tr>
<td>100</td>
<td>37%</td>
<td>76%</td>
<td>89%</td>
</tr>
<tr>
<td>300</td>
<td>50%</td>
<td>78%</td>
<td>91%</td>
</tr>
<tr>
<td>600</td>
<td>55%</td>
<td>80%</td>
<td>92%</td>
</tr>
<tr>
<td>850</td>
<td>55%</td>
<td>82%</td>
<td>99%</td>
</tr>
</tbody>
</table>

microenvironments in humic substances. For instance, in the study of Akkanen and Kukkonen (2001) the binding of pyrene and BaP by humic substances was decreased when water hardness was increased.

5.2.2 Model PAHs

The $K_{DOC}$ of pyrene represented in IV were part of a larger experiment series, which was published as a whole in V. In general the $K_{DOC}$ of pyrene were smaller than those of the more lipophilic BaP (Table 3), but were within the same order of magnitude as those published in Nikkilä and Kukkonen (2001). At best, only 47% of the pyrene was bound to humic substances (Kontiolampi, Table 3). In the present study the $K_{DOC}$ of pyrene was not directly comparable to the concentration of DOC or to the aromaticity, as previously observed by Gauthier et al. (1987), Chin et al. (1997), and Peuravuori (2001). In most previous studies, isolated humic or fulvic acids have been used instead of the entire DOC pool, and in addition, Peuravuori (2001) stated that the refinement of aquatic humic solutes strengthens their binding affinities for pyrene compared with the original conditions predominating in the water.

The $K_{DOC}$ of pyrene in three samples (Kontiolampi, Riera Major, and Göta älv) were significantly ($P < 0.05$) higher than others ($4.4 \times 10^4$, $4.2 \times 10^4$, and $3.9 \times 10^4$ l/kg, respectively), but still only 5% and 14% of the pyrene added was associated with humic substances in the samples from Riera Major and Göta älv (Table 3). This was due to the low DOC concentration of the water samples.

The binding of BaP generally increased when the concentration of DOC increased (Table 3). The $K_{DOC}$ in the water sample from Lake Louhilampi (48.1 $\times 10^4$ l/kg) was significantly ($P < 0.05$) higher than other coefficients. As much as 90% of the BaP added was bound to humic substances. In the water samples from Pielisjoki, Kalliojoki, Koitajoki, and Lärjeån, the $K_{DOC}$ were similar and differed significantly from other coefficients determined. The proportion of bound BaP was 59–76%.

The $K_{DOC}$ of BaP in the water sample from Hultabäcken (7.2 $\times 10^4$ l/kg) sparked interest, because it was significantly ($P < 0.05$) smaller than in the sample from Lärjeån (21.3 $\times 10^4$ l/kg), although the concentration of DOC was similar (7.0 and 7.1 mg/l, respectively). As a consequence, the proportion of BaP bound was only 33% in Hultabäcken, while 60% was bound in Lärjeån. The $K_{DOC}$ in the water sample from Göta älv was the lowest ($P < 0.05$) in the Nordic samples and within the range of the Spanish and Dutch water samples, where only 1–15% of added BaP was bound.

The $K_{DOC}$ of BaP was correlated with aromaticity and other characteristics typical for humic substances (V). Abundant data confirming this phenomenon are found in the literature.
(e.g. McCarthy et al. 1989, Kukkonen and Oikari 1991, De Paolis and Kukkonen 1997, Haitzer et al. 1999a,b,c, and Gourlay et al. 2005).

5.3 Bioavailability of cadmium

5.3.1 Kinetics, bioconcentration, and toxicity in Daphnia magna

The increased uptake rate and therefore bioconcentration and toxicity of Cd to D. magna in Lake Louhilampi humic water (hardness 0.10 mmol/l, very soft) was a consequence of lower water hardness than in the reference water (I) and not a result of humic substances, as earlier assumed (Oikari et al. 1992). Although the reference water was prepared according to then used Finnish standard (SFS 5062:1984, hardness 0.5 mmol/l, soft), in which the recommended reference water was substantially softer than in the comparable international standard (ISO 6341:1996, hardness 2.5 mmol/l, very hard), the difference in water hardness between reference and experimental humic water was significant enough to be seen in the biological response measured.

After this phenomenon was detected, the standardized composition of the reference water was modified to be comparable with the hardness of the natural water studied. However, the ratio between Ca$^{2+}$ and Mg$^{2+}$ remained 4:1 (I) as in the reference water (SFS 5062:1984). In later studies (II) the ratio between Ca$^{2+}$ and Mg$^{2+}$ was 5:3, corresponding to the natural ratio of these ions in Lake Louhilampi water, and also when the hardness was artificially elevated. Water hardness is a sum of the concentration of Ca$^{2+}$ and Mg$^{2+}$ ions in water, but waters with the same calculated hardness can contain significantly different ratios of these ions (Davies and Hall 2007). When the bioavailability or toxicity of chemicals is studied, it is important to have the measure of both the total hardness and the ratio of the hardness cations, since not only the total hardness but also the ratio affects metal binding to DOC (O’Shea and Mancy 1978), adsorption to bacteria (Slaveykova et al. 2009), toxicity to fish and aquatic invertebrates (Welsh et al. 2000, Naddy et al. 2002), and also sulfate toxicity to aquatic invertebrates (Davies and Hall 2007).

The uptake rate of Cd in reference water (21.290 ml·g$^{-1}$·h$^{-1}$) was significantly (P < 0.05) faster than in humic lake water (10.935 ml·g$^{-1}$·h$^{-1}$), when water hardness was at natural level (0.1 mmol/l, soft, I). This is in good agreement with the toxicity test results (Figures 1 and 2), in which Cd was significantly (P < 0.05) more toxic in reference than in humic water at water hardness of 0.1 mmol/l. The proportion of free Cd was 39% in humic lake water at natural hardness (Table 4), when the total experimental concentration of Cd (40 µg/l) in equilibrium dialysis was close to the acutely toxic level observed in toxicity testing (37.8 µg/l and 58.3 µg/l in soft reference and humic water, respectively).

The concentration of free Cd was decreased by binding to DOC in humic lake water, which was reflected in a significantly (P < 0.05) slower uptake rate and significantly (P < 0.05) higher LC$^{50}$ in humic than in reference water. Due to the faster uptake rate, acutely toxic concentrations of Cd in daphnids were attained earlier in reference than in humic water, since the depuration rate was similar in both experimental waters (0.011 and 0.009 h$^{-1}$), regardless of the differences in water hardness (I). Burnison et al. (2006) also reported that Cd accumulation in zebrafish (Danio rerio) eggs was decreased by DOC.

In addition, another LC$^{50}$ in Lake Louhilampi was calculated, using the free concentration of Cd determined experientially by equilibrium dialysis. This recalculated LC$^{50}$ was comparable to the LC$^{50}$ determined in the reference
water (II). This supports the conceptual basis of FIAM (Free Ion Activity Model, Morel 1983) that the free ion is the most bioavailable metal species. Van Ginneken et al. (2001) and Voets et al. (2004) studied the bioavailability of Cd to carp (Cyprinus carpio) and to zebra mussel (Dreissena polymorpha) in humic water and demonstrated that the presence of DOC slowed the uptake rate by decreasing the activity of free Cd$^{2+}$ ion. However, in the study of Voets et al. (2004) Cd accumulation was higher than expected, based on the free ion activity. They concluded that zebra mussels can to some extent accumulate Cd complexed with humic substances and that this partial bioavailability of complexed Cd could be the explanation for the enhanced bioaccumulation of Cd commonly observed in mollusks (Kozuch and Pempkowiak 1996, Guo et al. 2001). In addition, this partial bioavailability of organically complexed Cd by filter feeders may also explain the results of Stuijfzand et al. (1999), in which midge larvae (Chironomus riparius) were protected from copper toxicity by humic acids, while copper toxicity was not decreased and Cd toxicity was even increased in the presence of humic acids for the zebra mussel.

In this study (II), even a 10% dilution (DOC 2.0 mg/l) of the humic lake water was sufficient to significantly (P < 0.05) decrease the acute toxicity after 48 h at original hardness level of the lake water (Figure 2). One possible explanation could be the changes in the macromolecular structures of humic substances that modify the surface area of humic molecules and alter the functional group chemistry (Myneni et al. 1999), thus favoring the binding of Cd to humic substances. In addition, Saar and Weber (1979) noted that Cd binding to fulvic acid is strongest when fulvic acid is most dilute. Rosen et al. (2008) also noted that relatively small changes in concentrations of DOC (from 1.30 to 2.24 mg/l) in natural water samples significantly decreased the adverse effects of copper toxicity.

Figure 1. Acute toxicity (LC$_{50}$, 48 h) of cadmium in humic and reference water to neonatal Daphnia magna as affected by water hardness. The error bar denotes the 95% confidence limits of the LC$_{50}$ value, and the asterisk denotes a statistical difference between humic and reference water.
on embryo-larval development for the Mediterranean mussel, *Mytilus galloprovincialis*. In the study of Stackhouse and Benson (1989), the bioaccumulation of Cd in *D. magna* was significantly reduced in a 50 mg/l humic acid concentration in water, although no influence of lower concentrations of humic acid (0.5 or 5.0 mg/l) was observed after a 48 h exposure. However, their study was performed in moderately hard reconstituted water in the presence of Aldrich humic acid at pH 8, so straightforward comparison with the results of the present study is not justified, due to the possible confounding effects of different levels of water hardness and pH.

An exception to the concepts of FIAM was revealed when the hardness of water was elevated. Relatively small increases in water hardness level (to 0.2 mmol/l, very soft) decreased the toxicity of Cd in humic and reference water compared with lower hardness levels (0.1 mmol/l, very soft) (Figure 1). Still, Cd was significantly (*P* < 0.05) more toxic in reference than in humic water. As a result, in very soft water the humic substances showed a protective effect against Cd toxicity. However, when the hardness of the experimental waters was elevated further (to 0.5, 1.5, and 2.5 mmol/l), the toxicity decreased compared with soft water and there was no significant difference between Cd toxicity in reference and humic water, despite the fact that the *K*<sub>DOC</sub> also decreased (II) and the proportion of free Cd was 74–99 % (Table 4). The general trend was a decrease in Cd toxicity until the hardness was elevated to 1.5 mmol/l (hard), but the elevation of hardness from 1.5 mmol/l (hard) to 2.5 mmol/l (very hard) had no further effect on toxicity.

Nonessential and toxic cadmium has no uptake pathway of its own, and it was revealed that Cd<sup>2+</sup> could be partly taken up through the existing mechanisms for Ca<sup>2+</sup> (Roesijadi and Unger 1993), due to their similar chemistries. In aquatic organisms the transport of Cd<sup>2+</sup> ion across

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**Figure 2.** Effects of dilution of natural humic water on the acute toxicity (LC<sub>50</sub>, 48 h) of cadmium to neonatal *Daphnia magna* at a natural hardness of lake water (0.1 mmol/l). The dissolved organic carbon concentration is expressed both as a percentage of maximal and as milligrams per liter. The error bar denotes the 95% confidence limits of LC<sub>50</sub> value, and the asterisk denotes a statistical difference.
the permeable surface of an organism, e.g. the gills, may occur accidentally by passive diffusion through Ca\(^{2+}\) channels and/or actively via Ca\(^{2+}\) pumps (Rainbow et al. 1993, Pedersen and Bjerregaard 2000, Bondgaard and Bjerregaard 2005). Since the activity of the Ca\(^{2+}\) pumps in crustaceans with high calcium requirement increases at low salinity (Rainbow et al. 1993, Rainbow 1995), the Ca\(^{2+}\) in very soft experimental water (hardness 0.1 and 0.2 mmol/l) may have been taken up actively by daphnids, which led to the significantly (P < 0.05) faster uptake of Cd in soft and low-salinity water than in hard or high-salinity water, as also observed in the studies of Blust et al. (1992) and Winter (1996).

When the water hardness was elevated to hard or very hard, the excess of Ca\(^{2+}\) increased compared with the concentration of Cd\(^{2+}\) ions, and Ca\(^{2+}\) effectively competed with Cd\(^{2+}\) ions for binding sites in humic molecules. As a result, most of the added Cd\(^{2+}\) was not bound to DOC. At the same time, however, the acute toxicity of Cd was reduced, compared with that in soft water. The uptake of Cd\(^{2+}\) was strongly affected by the excess Ca\(^{2+}\), either by competing for binding sites in the uptake system or by reducing cell permeability (as in fish gills in Pagenkopf (1983) and Gundersten and Curtis (1995), respectively). These results are in good accordance with the study of Meinelt et al. (2001), in which the relative importance of Ca and humic substances on the toxicity of Cd to zebra fish embryos was determined.

The usefulness of FIAM in aquatic environments of complex physiochemistry was limited in several studies, and therefore an updated conceptual FIAM was presented by Brown and Markich (2000). In addition, the current widely applied Biotic Ligand Model (BLM) was developed to explain and predict the effects of complex water chemistry on the acute toxicity of metals to aquatic organisms (Di Toro et al. 2001). The BLM incorporates several previous models used to explain the toxicity of metals, such as FIAM and the GSIM (Gill Surface Interaction Model) of Pagenkopf (1983), the partitioning of metals with DOC (WHAM), and some inorganic speciation models. A historical overview of the development of the BLM is provided by Paquin et al. 2002. The BLM is based on the hypothesis that the metal-BL interaction is comparable to any other reaction of a metal species with an organic or inorganic ligand and that mortality occurs when the metal-BL complex reaches a critical concentration (Di Toro et al. 2001). The critical BL concentration corresponds to a metal concentration on the BL that results in 50% lethality. The BLM has been criticized, mainly because it is a steady-state model, lacks biological and physiological points of view (Hassler et al. 2004), and considers the aqueous phase as the only source of chemical (Steen Redeker and Blust 2004).

In the present study, the chemical and biological availability of Cd was measured in various water chemistries and no predictive bioavailability modeling was performed. However, Di Toro et al. (2005) used the experimental data in II to calibrate the BLM of Cd and determined the critical BL concentration of Cd in D. magna and Ceriodyphnia dubia (7.53 mmol/g wet weight).

5.3.2 Toxicity and the lethal body residue in Lumbriculus variegatus

As an extension of previous studies, the CBR approach was applied to determining the acute toxicity of Cd in varying water chemistries, using the oligochaete worm L. variegatus (III). The acute toxicity of Cd decreased significantly (P < 0.05) when the hardness increased from very soft (0.2 mmol/l) to soft (0.5 mmol/l) and very hard (2.5 mmol/l) in both humic and reference water (Table 5). Significant
decreases in toxicity were also noted when another oligochaete species *Tubifex tubifex* was exposed to cadmium in soft (0.45 mmol/l), hard (1.7 mmol/l), and very hard (3.3 mmol/l) reconstituted water, in which the pH was 7.3, 7.8 and 8.2, respectively (Rathore and Khangarat 2003). Previous data are mainly in agreement with accumulation kinetics and acute toxicity data obtained in I and II, although *D. magna* was more sensitive to Cd than oligochaetes. In addition, the protective role of humic substances against Cd toxicity to *L. variegatus* was observed not only in very soft, as in *D. magna*, but also in hard and very hard water. This could be due to differences in physiology between the studied species but most probably to the higher concentration of DOC in Lake Mekkojärvi water.

The LC$_{50}$ measured varied from 2.4 to 66.1 μmol/l at a ratio of 28, while the estimated LR$_{50}$ varied from 225 to 413 μmol/kg wet weight with a ratio of only 1.8 (Table 5). In the studies of Borgmann et al. (1991) and Meyer et al. (2002), the LR$_{50}$ varied in only the 3- and 2-fold range, while the LC$_{50}$ varied in the 50- and 14-fold range, when the amphipod *Hyalopella azteca* was exposed to Cd in tap water with the complexing agents EDTA, Aldrich humic acid, and two sediment samples and *L. variegatus* to copper under different combinations of pH and water hardness, respectively. In the data above there is clearly less variability in metal internal toxicity metrics (LR$_{50}$) than in the external toxicity metrics (LC$_{50}$).

The LR$_{50}$ (324 ± 78 μmol/kg wet weight) obtained was fairly constant in all hardness and DOC treatments (Table 5). Steen Redeker and Blust (2004) exposed *T. tubifex* to Cd (0.5–100 μmol/l) and determined the LC$_{50}$ at different time points up to 17 d. The LC$_{50}$ varied from 70.8 μmol/l at 24 h to 1.7 μmol/l at 17 d. The bioaccumulation of cadmium was studied in parallel with the toxicity test. As a result they determined an experimental LR$_{50}$ (370 ± 70 μmol/kg wet weight), which was essentially constant and within range of the LR$_{50}$ measured in present study. Since such repeatability among the LR$_{50}$ of Cd has been observed at various water chemistries and time points, the Cd critical body burden, at least in oligochaetes, appeared to be independent of exposure conditions and time.

5.3.3 Synthesis

Uptake kinetics determine the body residue of a chemical. The bioavailability of aqueous Cd to *Daphnia magna* was directly measured by uptake kinetics. The uptake rate constant ($k_a$) was influenced by water hardness, and the concentration of DOC, but the depuration rate constant ($k_d$) was not (Figure 3).

For example, in very soft water the uptake rate was significantly faster in reference than in humic water. Therefore, if the prerequisite for toxic effects is that the concentration at the site of toxic action exceeds a certain critical concentration, as assumed in the BLM and CBR approaches, the critical concentration was attained faster in the reference water than in the lake water rich in complexing humic substances. When the acute toxicity was expressed conventionally in external toxicity metrics based on total exposure concentration at a fixed time (e.g. LC$_{50}$, 48 h), the LC$_{50}$ obtained in the reference water was significantly lower than the LC$_{50}$ determined in humic water. Second, due to identical uptake rates in hard water, there was no difference between the LC$_{50}$ in the reference and humic water. However, the LC$_{50}$ in hard water were about 20-fold higher than the LC$_{50}$ in soft water. In addition, the LC$_{50}$ determined with oligochaetes in an identical experimental setup varied 28-fold.
Table 5. Acute toxicities (LC₅₀ with 95% confidence limits) and corresponding lethal residues (LR₅₀) of cadmium in *Lumbriculus variegatus* in varying levels of water hardness and DOC concentration at pH 7. Lethal residues were estimated by linear regression. Regression equations for the relationship between internal chemical concentrations of organisms (y = μmol/kg wet weight) and nominal exposure concentration in water (x = μmol/l).

<table>
<thead>
<tr>
<th>Water sample</th>
<th>Ca+Mg (mmol/l)</th>
<th>LC₅₀ (μg/l)</th>
<th>LC₅₀ (μmol/l)</th>
<th>LR₅₀ (μmol/kg)</th>
<th>Regression equation</th>
<th>r²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reference water</td>
<td>0.2</td>
<td>270 (232–258)</td>
<td>2.4 (2.1–2.3)</td>
<td>307</td>
<td>y = 108.850x + 45.529</td>
<td>0.56</td>
</tr>
<tr>
<td>DOC &lt; 0.02 mg/l</td>
<td>0.5</td>
<td>410 (370–434)</td>
<td>3.6 (3.3–3.9)</td>
<td>253</td>
<td>y = 57.006x + 47.903</td>
<td>0.64</td>
</tr>
<tr>
<td></td>
<td>2.5</td>
<td>2161 (1939–2400)</td>
<td>19.2 (17.3–21.3)</td>
<td>405</td>
<td>y = 18.657x + 46.675</td>
<td>0.84</td>
</tr>
<tr>
<td>Lake Mekkojärvi</td>
<td>0.2</td>
<td>1272 (1149–1391)</td>
<td>11.3 (10.2–12.4)</td>
<td>338</td>
<td>y = 27.950x + 22.395</td>
<td>0.96</td>
</tr>
<tr>
<td>DOC 24.9 mg/l</td>
<td>0.5</td>
<td>1726 (1551–1937)</td>
<td>15.3 (13.8–17.2)</td>
<td>226</td>
<td>y = 11.623x + 48.112</td>
<td>0.75</td>
</tr>
<tr>
<td></td>
<td>2.5</td>
<td>7431 (6435–9067)</td>
<td>66.1 (57.2–80.7)</td>
<td>413</td>
<td>y = 5.3796x + 57.249</td>
<td>0.74</td>
</tr>
</tbody>
</table>
The LC$_{50}$s in daphnids and oligochaetes varied, depending on the water chemistry and the sensitivity of the species; generally, a LC$_{50}$ should not be considered as a constant indicator of toxicity. Body residue, as a result of bioconcentration, is an explicit expression of a chemical’s bioavailability, and it is thus a better surrogate for doses at the site of toxic action and a preferable measure of toxicity than the conventionally used exposure concentration, which is sensitive to bioavailability-modifying factors (Mackay et al. 2001, Lanno et al. 2004, Chapman 2008, Meador et al. 2008). For example, the LC$_{50}$s of Cd determined with oligochaetes varied at a ratio of 28, while the estimated LR$_{50}$s varied at a ratio of only 1.8.

Despite the identical exposure conditions, the LR$_{50}$ of Cd determined in the present study (Table 5) for oligochaetes (324 ± 78 μmol/kg wet weight) was app. 40-fold higher than the critical BL concentration for daphnids (7.53 μmol/kg wet weight) calculated by Di Toro et al. (2005). Unfortunately, the whole-body residue associated with acute toxicity was not directly measured in daphnids, which makes it difficult to perform meaningful comparison between the LR$_{50}$ and the critical BL concentration. However, Meyer et al. (2002) and Rosen et al. (2008) found that the critical whole-body residues of copper in oligochaetes and marine bivalves were also considerably higher than the critical BL concentration for daphnids and fish determined by Di Toro et al. (2001) and MacRae et al. (1999). In both fish and daphnids, gills are the uptake sites of metals and also possible sites of their toxic action, regarded as BLs according to Janssen et al. (2003), whereas in nonchitinous oligochaetes with no gills, the uptake and respiratory physiology is different and all metal can be assumed to have accumulated in the soft tissues (Meyer et al. 2002) of the body through the skin.

In some other cases, e.g. when internal sequestration is probable, measured specific tissue residues may be more accurate surrogates for dose than whole-body residues (Mackay et al. 2001, Vijver et al., 2004, Rosen et al. 2008), although generally the body burdens are analytically more appropriate for experimental organisms of small size (Mackay et al. 2001). Nonetheless, for small aqueous organisms at short-term exposures, the whole-body residue could mostly be regarded as a valid approach, if modeling is not preferred. The measured body residues of oligochaetes were assumed to represent the concentration at the site of toxic action and also to be bioavailable as a whole without any confounding physiological processes.

5.4 Bioavailability of model PAHs

The bioconcentration of pyrene decreased, but not significantly, when the concentration of DOC increased in natural surface waters with varying physicochemistry, which reflects the weak binding capacity of pyrene to DOC (Table 2). The only exception was found in the water sample of Lake Kontiolampi in which a moderate binding capacity (K$_{DOC}$ 4.4·10$^4$ l/kg, bound 47%) led to statistically lower bioconcentration of pyrene in humic (BCF 1693) than in reference water (BCF 3035). These results are comparable with the results of Nikkilä and Kuukkonen (2001). However, there was no significant difference between surface waters and reference water in pyrene toxicity in the dark (IV), not even in the sample from Lake Kontiolampi.

The hardness of the water samples varied between 0.07 and 4.20 mmol/l. Akkanen and Kuukkonen (2001) studied the bioconcentration of pyrene and BaP in humic-free water in nearly the same hardness range (0.1–4.0 mmol/l), in which the hardness did not affect the bioconcentration of the PAHs. However,
Figure 3. Schematic diagram of bioavailability mechanisms of cadmium studied and discussed in this thesis. Oval figure with solid line represents an exposed organism with a distinct body residue of cadmium. The BL on the external surface of an organism stands for a biotic ligand. Within the solid line rectangle the total aqueous concentration of cadmium in boreal lake water is divided into DOC bound and freely dissolved fractions. In general, the solid lines and arrows illustrate the determined concentration, parameter, or mechanism, while the dashed lines are known to be present but not determined in this study.
they found that the bioconcentration of pyrene increased with artificially elevated water hardness in humic lake water. In the present study, the toxicity of pyrene was not influenced significantly when the hardness of humic-free water was elevated from very soft (0.1 mmol/l) to soft or very hard (0.5 or 2.5 mmol/l) (IV). In conclusion, the reconstituted water (0.5 mmol/l) used in this study was appropriate reference water for all natural water samples and both organic model compounds.

The phototoxicity of PAHs has been demonstrated in the laboratory for many standard aquatic test species, as reviewed by McDonald and Chapman (2002). In all treatments of the present study pyrene was more toxic to D. magna under UV-B exposure than in the dark (IV). However, under UV-B exposure the toxicity of pyrene decreased when DOC increased and the significant difference between toxicity in the UV-B and dark treatments was no longer observed in humic-rich Kalliojoki River and Lake Kontiolampi samples after 48 h (IV). Unfortunately, the KDOCs were not determined, but it is unlikely that this phenomenon would have been due to stronger binding to DOC under UV-B radiation than in the dark. Rather, it could have been due to the attenuation of UV-B radiation in dark humic waters (Huovinen et al. 2003), which in turn prevented the photomodification of pyrene. Since the UV radiation and soft water act as mutual stressors among calcium-demanding zooplankton species such as daphnids (Hessen and Alstad Rukke 2000), this attenuating role of humic substances could have more future ecological relevance, when the amount of UV radiation will increase gradually in the Northern Hemisphere due to ozone depletion. During a 72 hour UV-B exposure, no photodegradation of DOC was observed. This is in accordance with the results of Naganuma et al. (1996), while other wavelengths of UV radiation may photodegrade dissolved organic material (Corin et al. 1996, Dahlen et al. 1996, Kulovaara et al. 1996).

The bioconcentration of BaP mainly decreased when the concentration of DOC, the ABS270 and the proportion of hydrophobic acids increased, while the binding to DOC concomitantly increased (Table 2). The bioconcentration decreased significantly, compared with the reference water (BCF 8212) when the proportion of BaP bound was 59% or more (BCF 292–3336, Lärjän and all Finnish samples).

An exception to the above was the significant difference between the reference water and the sample from the Ebro River, in which the BCF was 4238, although only 2% of BaP added was bound (Table 2). The very hard water of the Ebro River (4.2 mmol/l) could have been an explanation if the reduced permeability of the cell surface were assumed to cause the effect, but when Haitzer et al. (2001) redetermined the bioconcentration of BaP in the Ebro River water, this difference was not observed. This BCF obtained in the sample from the River Ebro is not explicable with DOC or water hardness and probably is an artifact.

Relationships between the BCF of BaP and the concentration of DOC similar to those observed here were previously demonstrated by Kukkonen and Oikari (1991) in natural waters with varying concentrations of DOC and by Haitzer et al. (1999a,c) in dilution series of isolated fulvic and humic acids from different locations. The negative correlation between BCF and the characteristics describing distinct humic attributes such as ABS270 and HbA% (V) reflect the same phenomenon.

There is also a strong negative correlation between BCF and KDOC, indicating that the BaP bound is not bioavailable, as seen clearly in the BaP binding and bioconcentration comparison between water samples from Hultabäcken.
and Lärjeän. The respective binding percentages and BCFs in the water samples were 33% and 5451 for Hultabäcken, and 60% and 2487 for Lärjeän with a significant (P < 0.05) difference between the BCF values. While the concentration of DOC was similar in both water samples, the difference between the BCF values is not explained by quantity but rather by the quality of DOC, indicating an important role of aromaticity.

In conclusion, the difference in bioavailability of the PAHs studied in natural surface waters are due to their different lipophilicities, affecting both bioaccumulation and binding to humic substances, while the role of water hardness is negligible. In addition, not only the quantity of humic substances, but also their characteristics had a significant effect on the binding and bioaccumulation of the more lipophilic BaP.

5.5 Implications for risk assessment

In setting environmental quality criteria and in risk assessments, ecotoxicological data are applied and in this context standardized toxicity tests are preferred. The benefit of using standardized toxicity tests is that the toxicities of different chemicals can easily be compared and classified according to their degree of toxicity. At the same time, results usually are of limited ecological relevance. For instance, in standardized toxicity test protocols, reconstituted media are recommended as experimental and dilution water, but the characteristics of reconstituted freshwater (e.g. in OECD 1984 and ISO 1996) usually reflect better the quality of freshwater in Western Europe than in Finland and Scandinavia. In the former Finnish standard for performing the Daphnia toxicity test (SFS 5062:1984), the hardness and pH were adjusted to better match the average water chemistry in Finland, but still without including humic substances.

Reconstituted water, however well matched, is only a simplified representative of natural surface water. For example, even the SFS standard water (soft) was an inappropriate reference for humic lake water (very soft) when the effect of DOC on Cd toxicity and kinetics was studied (Oikari et al. 1992, I), since the difference in hardness influenced the results. More accurate site-specific reconstituted water with correct natural concentration and ratio of hardness cations was needed to study the effects of DOC. If only standardized reconstituted water is used in determination of water quality criteria or the predicted no-effect concentrations (PNECs), they may be either over- or underprotective, compared with the situation in natural waters with differing water chemistry (Welsh et al. 2001, Heijerick et al. 2003).

In current risk assessment procedures, the results of the toxicity test are more easily applied than the direct measures of bioavailability, e.g. kinetic parameters or body residues. The incorporation of bioavailability usually reduces the uncertainty. Indirect measures of bioavailability could be obtained by modifying the experimental water in otherwise standardized toxicity test protocols (e.g. II). The effects of all possible bioavailability-affecting factors should then be carefully considered when variable toxicity test results are compared, interpreted, or assessed in risk assessments. To do this, the physicochemical characteristics of both reconstituted and natural experimental waters should be sufficiently determined and reported. In addition, even a few simple quality parameters of DOC and the ratio of calcium and magnesium ions are certainly useful to include in the physicochemical characteristics in
addition to the total concentration of DOC and total hardness of water.

A water hardness correction (US EPA, 2001) has already been incorporated in the equation of the regional PNEC for Cd in European Union risk assessment, but this equation should not be extrapolated in very soft waters (below 0.4 mmol/1 Ca, European Chemicals Bureau 2007). However, Källqvist (2009) recently demonstrated that the proposed PNEC at a hardness of 0.4 mmol/1 Ca was also protective for the chronic effects of Cd to the green alga Pseudokirchneriella subcapitata in very soft water, although the sensitivity of P. subcapitata to Cd is lower than that of the most sensitive invertebrates, such as D. magna. The hardness equation only accounts for water hardness and certain hardness-interrelated characteristics, such as pH (Ryan et al. 2009), but not for the concentration of DOC, although DOC was observed to protect D. magna from Cd toxicity in very soft water (II) and L. variegatus at every hardness level tested (III). According to Ryan et al. (2009) adoption of the BLM for use as a regulatory tool may be particularly suitable for regions with very soft waters, where the concentration of competing hardness cations is low compared to the concentration of complexing DOC.

Body residues could be useful in predicting toxicity in monitoring-based risk assessment studies (Rosen et al., 2008), since the bioavailability-modifying effect of ambient water chemistry is avoided. However, the application of median lethal body residues for chronic metal exposure in the environment is more complex than for acute metal exposure in the laboratory, because in long-term exposures the organisms are not only able to inactivate accumulated metals by binding or sequestering them (Perceval et al., 2002; Voets et al., 2009), but also acclimation and/or adaptation (De Wolf et al., 2004) may happen. In the case of essential metals, hormesis and homeostasis could also have confounding effects on the metal body burden (Ma, 2005).

6. CONCLUDING REMARKS

Natural waters have different combinations of DOC concentration, hardness, and pH, depending on the ambient environment. The high concentrations of DOC and naturally low pH levels of soft boreal surface waters are mostly contributed by acidic humic substances, while nonhumic hard inland waters on the European continent are neutral or slightly basic, due to the interrelationship between hardness cations and a buffering capacity based on IC species. Humic substances and water hardness proved to be two key water quality characteristics influencing bioavailability and toxicity of the chemicals studied, Cd, BaP, and pyrene, in European surface waters. However, being the third important factor modifying the bioavailability of chemicals in the aqueous environment, pH was not studied thoroughly in this thesis.

The extent of water hardness and humic substances as bioavailability-modifying factors in surface waters depended not only on their relative aqueous concentrations, but also on their quality and on the characteristics of these chemicals and on the physiology of organisms. Humic substances acted as complexing ligands with both Cd and PAHs, but the hardness cations, especially calcium, affected only the bioavailability of Cd due to competing effects on uptake. The bioavailability of the more lipophilic BaP was affected more by humic substances than that of pyrene. In addition, the specific characteristics of aqueous humic substances, such as aromaticity, had significant effects on the binding and bioconcentration of BaP. Under UV-B
radiation the humic substances had a protective role on the photo-enhanced toxicity of pyrene.

Differences in bioavailability due to ambient water chemistry and species were reflected in the variability in acute toxicity measures of Cd, when the toxicity test results were expressed conventionally in external toxicity metrics. The LC50 increased significantly when the hardness of water increased stepwise from very soft to very hard in both humic and nonhumic water in both experimental species, although the waterflea Daphnia magna was more sensitive to Cd than the oligochaete Lumbriculus variegatus. Humic substances played a protective role against Cd toxicity to both species in very soft water and to L. variegatus in hard and very hard water.

Based on the results of this thesis, the environmental fates of chemicals in the various European surface waters may differ, depending on the geographical area and water characteristics mirroring the properties of the bedrock and vegetation in each area. The high concentration of aromatic humic substances and low hardness of water, typical characteristics of boreal freshwaters, may increase or decrease bioavailability of chemicals, and thus their toxicity, compared with those in standardized reconstituted freshwater or to natural surface waters elsewhere in Europe. As a consequence, they should be considered carefully in local and regional risk assessment concerning the Fennoscandian region.

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