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Anna Nikkilä

Effects of Organic Material on the Bioavailability, Toxicokinetics and Toxicity of Xenobiotics in Freshwater Organisms

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ABSTRACT

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The effects of organic carbon, as dissolved in water (DOC) or in sediment (SOC), on the binding, bioavailability, toxicokinetics and toxicity of organic xenobiotics were studied. The binding of pyrene (log K_{ow} 5.2) to DOC was fairly low, but mainly related to the amount of DOC. Pyrene uptake by the waterflea *Daphnia magna* decreased by 17 % in the high-DOC water (17.4 mg DOC/l) compared to that in DOC-free water. Pyrene also became less bioconcentrated in *D. magna* in DOC-rich waters than in the DOC-free reference water. Atrazine (log K_{ow} 2.7) uptake and elimination, in turn, were not affected by the amount of DOC. Both the uptake and elimination of atrazine were higher in the multispecies periphyton communities than in *D. magna*. The atrazine bioconcentration was low, both in the periphyton and *D. magna*, but again, higher in the periphyton. A substantial concentration of SOC also decreased the bioavailability and uptake of retene (log K_{ow} ~ 6), 2,4,5-trichlorophenol (2,4,5-TCP) and pentachlorophenol (PCP) (log K_{ow} values 3.8 and 5.0, respectively) for the oligochaete worm *Lumbriculus variegatus*. Retene was chronically non-toxic to the worms at 200 µg retene/g sediment dry weight. DOC and SOC can thus decrease the bioavailability of the lipophilic pyrene, retene and PCP as well as the relatively lipophilic 2,4,5-TCP. The bioavailability of a more hydrophilic atrazine is not affected by DOC. The periphyton community structure and factors related to organisms affected the bioconcentration of atrazine more than the quantity or quality of DOC did. The lethal body residues for both the chlorophenols in *L. variegatus* were practically the same in freshwater and sediments, expressing the usefulness of this end point for more accurate predictions on the bioavailability and toxicity of chemicals. Coexposure to UV-B radiation enhanced the acute toxicity of pyrene to *D. magna* but DOC decreased the photomodification of the freely dissolved pyrene by diminishing the amount of light coming into contact with the parent compound.

Key words: Atrazine; bioaccumulation; chlorophenols; dissolved organic carbon; PAHs; sediment organic carbon; ultraviolet radiation.

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CONTENTS

LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following papers, referred to in the text by their Roman numerals I-V. The studies in papers I and V, and most of the studies in papers II, III and IV, have been carried out by myself. Jussi Kukkonen was fully responsible for the study idea and practical methodology in paper IV. The studies and / or their practical methodology in other papers have been planned by all co-authors with myself being mainly responsible for their final form and realisation. I compiled, processed and wrote the material in all the papers.

I Nikkilä, A. & Kukkonen, J.V.K. 2001: Effects of dissolved organic material on binding and toxicokinetics of pyrene in the waterflea *Daphnia magna*. - Archives of Environmental Contamination and Toxicology 40: 333-338.

II Nikkilä, A., Penttinen, S. & Kukkonen, J.V.K. 1999: UV-B-induced acute toxicity of pyrene to the waterflea *Daphnia magna* in natural freshwaters. - Ecotoxicology and Environmental Safety 44: 271-279.

III Nikkilä, A., Paulsson, M., Almgren, K., Blanck, H. & Kukkonen, J.V.K. 2001: Atrazine uptake, elimination and bioconcentration by periphyton communities and *Daphnia magna*: effects of dissolved organic carbon. - Environmental Toxicology and Chemistry 20: 1003-1011.

IV Nikkilä, A., Halme, A & Kukkonen, J.V.K. 2001: Toxicokinetics, toxicity, and lethal body residues of two chlorophenols in the oligochaete worm, *Lumbriculus variegatus*, in varying sediments. (submitted to Chemosphere)

V Nikkilä, A., Kukkonen, J.V.K. & Oikari, A. 2001: Bioavailability of sediment-associated retene to an oligochaete worm *Lumbriculus variegatus*: effects of sediment organic carbon and retene concentration. - Journal of Soils and Sediments 1: 137-145.

The studies in this thesis were mainly carried out in two universities:

University of Jyväskylä, Department of Biological and Environmental Science
and
University of Joensuu, Department of Biology.

ABBREVIATIONS

AI	absorptivity at 270 nm, also called ABS_{270}
BAF	bioaccumulation factor
BCF	bioconcentration factor
BSAF	biota-sediment accumulation factor
DOC	dissolved organic carbon
DOM	dissolved organic material
EC_{50}	effective concentration immobilising 50 % of the test population
K_{DOC}	partition coefficient to DOC
k_d	depuration rate constant
k_{dA}	depuration rate constant for the first fast phase
k_{dB}	depuration rate constant for the slower phase
k_e	elimination rate constant
k_s	uptake rate constant from sediment
k_u	uptake rate constant from water
LBR_{50}	lethal body residue for 50 % of the test population
LC_{50}	lethal concentration for 50 % of the test population
log K_{ow}	logarithmic octanol-water partition coefficient
PAH	polycyclic aromatic hydrocarbon
PCP	pentachlorophenol
SOC	sediment organic carbon
2,4,5-TCP	2,4,5-trichlorophenol
$t_{½}$	elimination half-life
t_{eff}	effective (depuration) half-life
UV	ultraviolet light, consisting of:
UV-A	ultraviolet A radiation (wave lengths between 320-400 nm)
UV-B	ultraviolet B radiation (wave lengths between 280-320 nm)
UV-C	ultraviolet C radiation (wave lengths between 180-280 nm)
visible light	wave lengths between 400-760 nm
WS	water solubility

1 INTRODUCTION

Aquatic organisms are exposed to many xenobiotics due to anthropogenic contamination. Many environmental factors typical to northern nature can affect the fate and effects of organic contaminants in freshwater ecosystems. Factors of this kind examined in this thesis are the quantity and quality of organic carbon in sediment or dissolved in water, as well as solar ultraviolet radiation. The majority of lakes and rivers in boreal regions receive a great deal of dissolved organic material (DOM) from their watersheds, and thus contain high concentrations of dissolved organic carbon (DOC). The concentrations of DOC in boreal freshwaters usually vary from 3 to 30 mg/l, whereas in other parts of Europe they typically contain less than 5 mg/l (Thurman 1985). Similarly to DOC, organic carbon in the sediments (SOC) varies over a substantial range in Nordic lakes due to differing loads, the sedimentation of organic material, and the relatively low degradation rates of the SOC.

DOM has for long been regarded as of great importance, because of its ability to interact with many xenobiotics (McCarthy & Jimenez 1985a & b, Gauthier et al. 1987, Kukkonen et al. 1991, Alberts et al. 1994, Harkey et al. 1994, Suffet et al. 1994, Chin et al. 1997, Haitzer et al. 1999a). This interaction may consist of binding of the xenobiotics, which, in turn, leads to reduced bioavailability to aquatic organisms. This is caused by the fact that the DOM-xenobiotic complex can be too large to penetrate biomembranes (McCarthy et al. 1985). The bound fraction of a xenobiotic is therefore not available to aquatic organisms for accumulation, whereas the freely dissolved compound is bioavailable (McCarthy et al. 1985, Kukkonen & Oikari 1989; Kukkonen et al. 1990, Kukkonen & Oikari 1991, Chin et al. 1997, Haitzer et al. 1998, Haitzer et al. 1999b).

Most of the organic material in freshwaters does not correspond to any single or particular chemical entity and has no readily identifiable structure (Aiken et al. 1985). This heterogeneous group of polyelectrolytes, which consists of carboxylic, hydroxyl and phenolic functional groups, is the main class of organic compounds in natural waters and is often referred to as humic substances (Vik & Eikebrokk 1989). In most cases, DOC consists predominantly

of humic substances (Aiken et al. 1985, Steinberg & Muenster 1985, Vik & Eikebrokk 1989) and in this study DOC is used as a measure of these substances.

Similarly to DOC, SOC often serves as a binding site for many xenobiotics and thus alters their fate in freshwater ecosystems. Many hydrophobic compounds are adsorbed and/or absorbed by the organic matrix of sediment particles and tend to remain associated with sediments, this resulting on the one hand in reduced bioavailability and on the other in longer lasting contamination of recipient areas (Landrum & Faust 1991, McCarthy et al. 1991).

Polycyclic aromatic hydrocarbons (PAHs) like pyrene and retene (7-isopropyl-1-methylphenanthrene) used as model compounds in this study have anthropogenic background in respect of their occurrence in nature, and are ubiquitous contaminants found throughout the world (Helfrich & Armstrong 1986, Wikström & Tolonen 1987, Garcia et al. 1993, Tavendale et al. 1995, Masclet et al. 1995, Wakeham 1996, Menzie et al. 1992). PAHs are commonly formed by the thermal decomposition of organic molecules and subsequent recombination of the organic particles (pyrosynthesis). At high temperatures (500-800°C) incomplete combustion of organic matter produces PAHs. Retene also originates microbially under anaerobic conditions from the resin acid-constituent of coniferous trees (spruce and pine), such as dehydroabietic acid (Ramdahl 1983).

Aquatic contamination by PAHs is caused by petroleum spills, discharges and seepages, industrial and municipal wastewater, urban and suburban surface run-off, and atmospheric deposition (Albers 1995). Ultimately, aquatic ecosystems receive their PAH load through deposition and runoff processes (Gao et al. 1998). Lipophilic PAHs are expected to be relatively quickly sorbed into abiotic compartments such as DOM, and to remain associated with this for prolonged periods. On the other hand, when desorbed and dissolved in water, interstitial or overlaying, they can be expected to accumulate in living organisms and in many cases to cause toxicity. For instance, McCarthy & Jimenez (1985b) found that the benzo(*a*)pyrene (B*a*P) accumulation was reduced by 90 % by the binding to dissolved humic material, while the relatively low binding of naphthalene, a much less lipophilic two-ringed PAH, was to all practical purposes not affected by the presence of DOM. Although some PAHs are known as carcinogens, many others are still undergoing evaluation for toxicity (Menzie et al. 1992). Nevertheless, both model PAHs, pyrene and retene, can cause severe toxicity to aquatic animals (Billiard et al. 1999).

Furthermore, sunlight, especially the atmospheric ultraviolet (UV) portion of the electromagnetic spectrum, significantly enhances the toxicity of several PAHs (Oris et al. 1990, Huang et al. 1993, Mekenyan et al. 1994, Ren et al. 1994, Monson et al. 1995, Veith et al. 1995, Huang et al. 1997, Krylov et al. 1997, Little et al. 2000). Depletion of the stratospheric ozone, and the subsequent increase in solar ultraviolet B (UV-B) irradiation reaching aquatic systems, can profoundly affect the fate of PAHs in those systems, especially when a unit loss in ozone will result in an approximately two unit increase in harmful UV irradiation. An increase in the toxicity of pyrene is linked mainly to absorbance of the most harmful range of solar UV radiation reaching the Earth's surface, namely UV-B,

rather than to UV-A or visible light only (e.g. Ren et al. 1994). This energy absorbance from UV-B leads to changes in the structure of the compound. On the other hand, variable or even low amounts of dissolved humic substances, and the consequent light to deep brown colour of the water, are known to reduce UV transmittance drastically (Wetzel 1983, Barron et al. 2000, Huovinen et al. 2000a) and so diminish the harmful effects of UV irradiation.

The triazine compound atrazine has been used as a major herbicide worldwide, which has resulted in widespread residues (Solomon et al. 1996, Holland 1999). It is a polar, non-ionic compound, which is relatively stable and highly selective in its pure state (Huber 1993, Tang et al. 1997). Atrazine inhibits photosynthetic electron transport (Bowyer et al. 1991), and is thus most harmful and toxic to plants but can also be accumulated by benthic invertebrates, zooplankton and fish (Ney 1990, Nikunen et al. 1990, Huber 1993, Tang et al. 1997). Due to the relatively low hydrophobicity and thus low bioconcentration and rapid elimination of atrazine, it is assumed to cause only non-permanent damage to aquatic ecosystems at concentrations of up to 20 µg/l (Huber 1993). Although the interaction between atrazine and humic substances is relatively low, atrazine has been shown to become bound to DOM to some extent (Haniff et al. 1985, Saint-Fort & Visser 1988, Zhang et al. 1990). This binding is also shown to be correlated to the aromaticity of dissolved humic substances (Piccolo et al. 1992).

The third group of compounds studied, chlorophenols, are also harmful to aquatic life because of their capability to bioaccumulate and cause toxicity (Coulston & Kolbye 1994, IV). Due to their relatively high lipophilicity, they are also sorbed to the organic matrix of particles and tend to remain associated with sediments. Various kinds of use of chlorophenols in the 20th century over many decades raised concern over their potentially adverse effects on freshwater biota and the environment some twenty years ago. Chlorophenols, including the two used as model compounds in this study, 2,4,5-trichlorophenol (2,4,5-TCP) and pentachlorophenol (PCP), have been used as fungicides and other pesticides, and wood preservatives, as well as for other industrial, agricultural and commercial uses (Coulston & Kolbye 1994, Lampi 1996). They can also be formed during the chlorine bleaching of pulp in pulp and paper mills (Coulston & Kolbye 1994). Although the use of the most harmful chlorophenols is nowadays banned in many European countries or the consumption and thus contamination in many other regions has decreased, the global use has led to their detectable presence in air, water and soil throughout Europe (Muir & Eduljee 1999). For instance, in Finland in certain soils (Knuutinen et al. 1990) and sediments (Lampi et al. 1992, Palm & Lammi 1995) chlorophenols are still an important group of pollutants due to their use in the past and their strong tendency to become sorbed to the organic matrix.

Organic material in natural freshwaters and freshwater sediments thus plays a significant – although complex – role in the fate of xenobiotics. This role of organic material and some other water and sediment characteristics in the bioavailability, toxicokinetics and toxicity of organic contaminants, however, has not yet been established with certainty. Further, the physico-chemical properties of a contaminant and the physiology, lifestyle, behaviour or habitat

of the organisms all affect the final outcome of the bioavailability, toxicokinetics and toxicity of organic xenobiotics.

Freshwater organisms used in this study were selected on the basis of their ecological importance in aquatic ecosystems. Mainly autotrophic phytoplankton are in key position in aquatic ecosystems, because they convert solar energy (using inorganic compounds and water) into organic matter by photosynthesis (primary producers). The phytoplankton is therefore an essential source of nourishment for most aquatic crustaceans such as the waterflea. Waterfleas and oligochaete worms, on the other hand, are central species in freshwater systems, because they, as consumers, convert phytoplankton, bacteria and detritus into animal biomass, and comprise a significant part of the diet of numerous fish species. In this thesis, these organisms were used in laboratory-scale studies, and therefore any comprehensive ecosystem-level conclusions cannot be made. Still, the used experimental setups give valuable information on the fate of some xenobiotics in freshwater environments.

2 OBJECTIVES

The objective of this thesis was to determine the effects of two environmental factors on the bioavailability, toxicokinetics and toxicity of a few globally common xenobiotics from different compound groups. The environmental factors under study were:

1) quantity but also quality of organic material,
    a) both dissolved in freshwater and
    b) occuring within freshwater sediment, and
2) solar ultraviolet-B radiation.

The effects of UV-B radiation were studied with regard to its impact on the toxicity of pyrene (polycyclic aromatic hydrocarbon, PAH) in freshwaters with various concentrations of DOC (II). The effects of DOC on the toxicokinetics and bioconcentration of pyrene (I) and the triazine-herbicide atrazine (III) were also examined. The toxicokinetics of atrazine and pyrene were studied in the water phase using a planktonic cladoceran (*Daphnia magna*, Crustacea). Toxicokinetic rates among intact periphytic algal communities established *in situ* were examined for atrazine. The use of these multispecies periphyton communities, in place of the single algal species approach used so far (Tang et al. 1997, Tang et al. 1998), can contribute significantly to our knowledge of the toxicokinetics of atrazine, especially when combined with *D. magna*, a single species that has been studied a great deal.

The role of SOC on the toxicokinetics, toxicity, and lethal body residues of two sediment-associated chlorophenols, 2,4,5-TCP and PCP, as well as on the bioavailability of sediment-associated retene (PAH-compound) to the subsurface deposit feeder, *Lumbriculus variegatus* was studied in two publications (IV, V, respectively).

3 MATERIALS AND METHODS

3.1 Chemicals

Compounds studied were atrazine (a heterocyclic triazine), chlorophenols 2,4,5-trichlorophenol (2,4,5-TCP) and pentachlorophenol (PCP), pyrene (an unsubstituted polycyclic aromatic hydrocarbon), and retene (a substituted PAH compound). The physico-chemical properties and molecular structures of these compounds (Table 1, Fig. 1) indicate that their bioavailability, toxicity and toxicokinetics can lead to different patterns in aquatic organisms.

TABLE 1 Some physico-chemical characteristics of the compounds studied. Water solubility is expressed at mg/l at ~ 25°C, and Log K_{ow} is the logarithmic partition coefficient between octanol and water phases.

Properties	Atrazine	2,4,5-TCP	PCP	Retene	Pyrene	References
Molecular formula	$C_8H_{14}ClN_5$	$C_6H_3Cl_3O$	C_6HCl_5O	$C_{18}H_{18}$	$C_{16}H_{10}$	Mackay et al. 1992 Manahan 1994 Shiu et al. 1994 Budavari 1996
Molecular weight	215.72	197.45	266.32	234.14	202.26	Mackay et al. 1992 Nikunen et al. 1990 Shiu et al. 1994
Water solubility	33.0	948	14	—	0.13	Mackay et al. 1992 Nikunen et al. 1990 Shiu et al. 1994 Burnison et al. 1996
Log K_{ow}	2.7	3.8	5.0	ca. 6	5.2	Mackay et al. 1992 Ney 1990 Shiu et al. 1994

— not precisely known

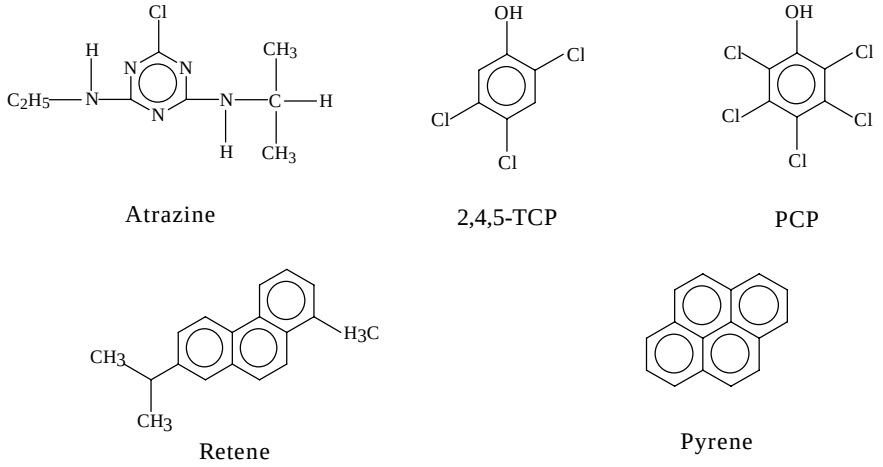

FIGURE 1 Molecular structures of the studied compounds.

Pyrene toxicokinetics (I) and binding capacities (I, II) as well as atrazine toxicokinetics (III) were evaluated using ^{14}C-labeled compounds and liquid scintillation counting. For pyrene toxicity (II) and retene bioavailability (V) determinations nonlabeled compounds were used at nominal concentrations (II) or at concentrations determined by gas chromatography combined with mass spectrometry (V). The toxicokinetics and toxicity of chlorophenols (IV) were measured using both ^{14}C-labeled and nonlabeled compounds with the justified assumption that the radioisotopes bioaccumulated and partitioned identically to stable isotopes of the same atoms (since their chemical nature is the same due to their having the same amount of protons and electrons).

3.2 Water and sediment samples

The natural waters used in the experiments were obtained from relatively uncontaminated areas close to the cities of Joensuu (Finland), Gothenburg (Sweden), Barcelona (Spain), and near the southeast border of The Netherlands. They were filtered (0.45 µm), and are characterised in each original publication (I, II, III). The artificial freshwater used as a DOC-free reference water in the experiments was made according to the ISO (1996) and Finnish (1984) standards. The following hardness values were used in the reference waters: 0.1 mmol/l served as a reference for Finnish waters, 0.5 mmol/l was according to the Finnish standard, and 2.5 mmol/l represented the culturing conditions as well as the hardest test waters. The pH and hardness of these waters were designed to match the respective conditions in each experiment. A more precise characterisation of the study waters is given in each original work.

Pure sediments used in the chlorophenol (IV) and retene (V) exposures were obtained from relatively unpolluted Lake Höytiäinen in Eastern Finland

(IV) and Lake Palosjärvi in central Finland (V). The sediments were processed and characterised as explained in both publications. Both were found by analysis to be fairly uncontaminated (Ristola et al. 1999). The DOC-free reference water was used as the overlying water in the sediment assays.

3.3 Test species

A parthenogenetic *Daphnia magna* Straus population was cultured in the laboratory as described in publications I, II and III. Neonate daphnids (age < 24 hrs) for the toxicity experiments (II) were obtained by separating adult females ready to reproduce. Neonates were always taken from the second and following clutches, as in the first brood the *D. magna* neonates are usually of the smallest size (Lampert 1993). The toxicokinetic experiments were conducted with juvenile (III) or subadult (I) individuals. The term juvenile here refers to individuals smaller than the smallest adults without visible oil droplets or ovary development (Berberovic 1990). Newly developed adult animals without eggs in the brood chamber are defined as subadults (Berberovic 1990). These juveniles and subadults were raised from birth separately from the rest of the stock.

Periphyton (algal microbenthic communities) used in the atrazine toxicokinetic experiments (III) were colonised *in situ* in Göta Älv River, Lärjeån Stream and Hultabäcken Brook in southwest Sweden close to the city of Gothenburg. Periphyton was colonised on glass discs mounted on polyethylene sampling devices (Fig. 2), as described previously by Blanck & Wängberg (1988), Guash & Tubbing (1996), and Ivorra et al. (1999). The devices were placed vertically at a depth of approximately 0.5-m parallel to the main current, and kept there for 3 to 6 weeks, depending on the colonisation and growth rates, in order to obtain an adequate periphyton biomass.

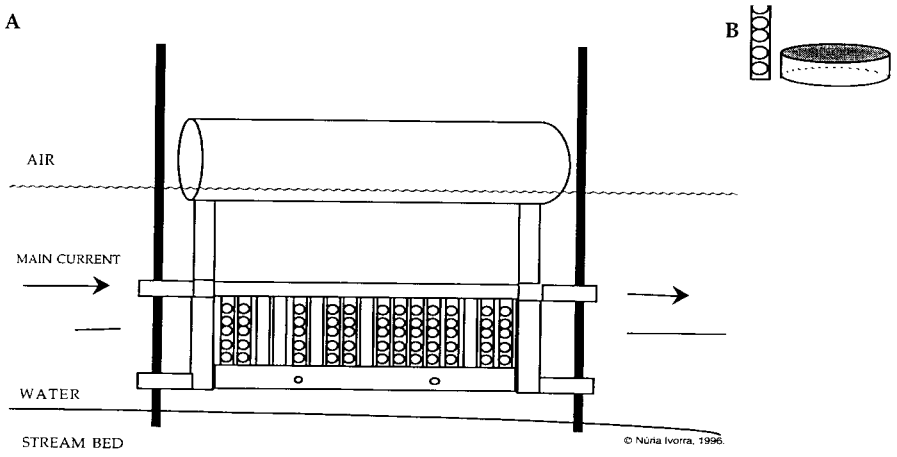

FIGURE 2 A) A device for periphyton sampling according to Ivorra et al. (1999). B) Periphyton is colonised on the glass discs. Each holder takes five glass discs.

A stock of *Lumbriculus variegatus* Müller was cultured as described in publications IV and V. Clumps of adult worms were transferred from the culture aquaria, and exposed to the sediment-associated or water-borne compounds under study as described (IV, V).

3.4 Determination of partition coefficients

An equilibrium dialysis method (Carter & Suffet 1982, McCarthy & Jimenez 1985a) was used to determine the partition coefficients (K_{DOC}) of ^{14}C-pyrene in waters containing DOC in the papers I and II. This method is based on the use of semipermeable tubes, which are permeable to most xenobiotics, but not to the larger humic macromolecules. It thus gives the binding capacity or affinity of the total humic content in the test water to the xenobiotic in question. The dialysis tubes were filled with a humic water sample, closed with clamps, placed in a DOC-free aqueous solution of the radio-labeled compound, and left to reach equilibrium. Samples inside and outside the tubes were taken and measured by liquid scintillation counting. Please see the original publications for more precise descriptions of the method.

3.5 Measuring toxicity

The role of DOC in water in the toxicity of pyrene to juvenile *D. magna* in the dark and under UV-B radiation was evaluated in publication II. An intensity of twice the theoretical daily maximum UV-B at latitudes corresponding to Finland in July was used at a distance of 50 cm from the open test vials. Two humic waters were tested at a time together with the reference water. The toxicity of 2,4,5-TCP and PCP to *L. variegatus* and the corresponding tissue concentrations, i.e. the lethal body residues causing death of the worms, were determined in freshwater and in sediments with varying SOC concentrations (IV). The effective (II) or lethal (IV) concentrations, as well as the lethal body residues (IV) for half of the test population (EC_{50}, LC_{50}, and LBR_{50}, respectively) and their 95% confidence limits were estimated using the general linear model with probit transformation by Kerr & Meador (1996). Immobility of juvenile *D. magna* (observed by prolonged lack of swimming movement and proper orientation even if the animals were still able to move their antennae) was used as an endpoint for effective concentrations in paper II.

3.6 Modelling toxicokinetics: some principal concepts

Most often, the movements of xenobiotics in aquatic environments follow first-order rate-kinetics (Spacie & Hamelink 1985; Newman 1995; Landrum et al. 1996), where the rate of movement of a xenobiotic is dependent on the concentration of the xenobiotic in the source compartment (Landrum & Lydy 1991). An organism is considered simply as a single, homogeneous compartment surrounded by a biological membrane. Uptake, the movement of the bioavailable fraction of a xenobiotic into or onto the organism, can occur directly from water (Newman 1995) or from ingested material in the gut. Although the possible uptake mechanisms are numerous, most neutral xenobiotics are taken into the body by passive diffusion across semipermeable membranes (Spacie & Hamelink 1985). This physical process requires no metabolic energy by the organism (Spacie & Hamelink 1985), and takes place as a movement of solute down an electrochemical or activity gradient (Newman 1995). The rate of this movement into an organism is directly proportional to the difference between xenobiotic activity in the source and that in the organism.

Elimination is a general term for a loss of a xenobiotic from the organism. It takes place during uptake, and includes metabolic, excretory, physiochemical, and radioactive decay processes (Newman 1995). Once the organisms have taken up a xenobiotic, the time at which elimination starts is variable. It depends on the xenobiotic and the type and age of an organism. Net uptake represents a situation where the rates of the two opposed phenomena, uptake and elimination, have reached the so-called dynamic or apparent steady state (i.e. the sum of influx and outflux rates is constant and equal). Uptake will thus occur if the rate of uptake of a xenobiotic by an organism exceeds the rate of elimination (Spacie & Hamelink 1985). Depuration is the loss of a xenobiotic from the organism after it has been placed in an environment devoid of the xenobiotic (Newman 1995). This term refers to a particular experimental design where the organisms are exposed to a xenobiotic before being transferred to a xenobiotic-free depuration solution. The xenobiotic concentration inside an organism thus starts to decline by a flux into the xenobiotic-free depuration solution, and asymptotically approaches zero.

The dynamic equilibrium between source and organism is difficult to determine. Most components in nature behave unpredictably and are continuously proceeding from one stage to another. As time approaches infinity, organisms can attain a steady state in experimental conditions where both the exposure and environmental/physiological factors can be kept constant (Landrum et al. 1992). Although the equilibrium or steady state are widely used conceptual terms in ecotoxicology, they are seldom reached in the long term in nature. To remind us of this fact the steady state is called an apparent steady state (I, III, IV).

The two models presented (I, III, IV) are commonly used for predicting accumulation in ecotoxicology (i.e. the xenobiotic movement between compartments), and are based on several assumptions according to Newman &

Jagoe (1996): (1) the xenobiotic concentration in the source compartment is constant and available to uptake, (2) there is instantaneous mixing in one homogeneous compartment, (3) the probability of elimination for any molecule or atom is independent of its residence time in that compartment, and (4) elimination from the compartment during uptake conforms to first-order kinetics. The formulations of the models also assume no biotransformation or organism growth, and ignore the importance of organism physiology (Landrum et al. 1992, Landrum et al. 1996). During short-term experiments it is reasonable to assume that any growth dilution effect is not significant.

Processes that control the movements of a xenobiotic inside and outside organisms (e.g. uptake, detoxication and elimination processes) are rate dependent (Landrum & Lydy 1991). Consequently, in the end all essential effects of a xenobiotic will depend on those uptake and elimination rates (Landrum & Lydy 1991) that are again, according to the formulations, unchangeable over the course of an exposure (Landrum et al. 1996). Despite the simplifications of this first-order rate modelling, it is a very useful tool with which the fluxes of xenobiotics by aquatic biota can be predicted with reasonable cost-effectiveness, because experimental designs can be kept relatively simple. Furthermore, the more complex a kinetic model is, the higher too are its requirements in regard to the basic data. The resulting rates, however, must be viewed as conditional only (Landrum et al. 1996).

3.7 Measuring toxicokinetics

3.7.1 Accumulation, elimination and half-lives

Accumulation and simultaneous elimination by *D. magna*, periphyton and *L. variegatus* were determined by exposing a suitable amount of individuals as a group (*D. magna* and *L. variegatus*) or a periphyton disc to the compound under study. *D. magna* were measured for pyrene (I) and atrazine (III) toxicokinetics, and *L. variegatus* for 2,4,5-TCP and PCP (IV) toxicokinetics, and the periphyton discs for atrazine (III) toxicokinetics. Initial concentrations in these exposures were chosen as a compromise between the acute toxicity, the expected concentrations in the organisms, and the analytical detection limits. The effects of dissolved as well as sediment organic carbon on the toxicokinetic parameters were examined.

Two first-order rate accumulation models (I, III, IV) according to Newman (1995) were used to predict an increase in concentration within an organism with time until an apparent steady state concentration was reached. The experimental design and methods are described in more detail in each of the original publication. Atrazine (III), 2,4,5-TCP and PCP (IV) depuration was measured after a 24-hour accumulation period by transferring the organisms to compound-free test waters. Elimination and/or depuration half-lives were determined as well (I, III, IV). The bioavailability of sediment-associated retene as affected by SOC concentration was measured by means of *L. variegatus* (V).

3.7.2 Bioconcentration and bioaccumulation

Bioconcentration is the accumulation of the freely dissolved contaminant in water by aquatic organisms through nondietary routes (Landrum et al. 1996). Bioaccumulation, on the other hand, refers to uptake from ingested material in the gut but in many cases includes also some uptake from water — most often of interstitial origin due to the sediment-dwelling behaviour of many benthic organisms. The bioconcentration and bioaccumulation factors (BCF and BAF), as they are calculated at an apparent steady state, thus represent an apparent steady state between the organism and source compartments (Landrum et al. 1992). The BCF of pyrene in *D. magna* (I) and BCF of atrazine in *D. magna* and periphyton (III) were studied in natural freshwaters with various DOC concentrations. The bioaccumulation factors (BAFs) of 2,4,5-TCP and PCP (IV), as well as retene (V), in *L. variegatus* were determined in sediments with various SOC concentrations.

3.8 General conditions in the exposures

Cool fluorescent light ($\lambda > 500$ nm) was applied or the use of artificial lighting otherwise minimised during the preparations and exposures to prevent the possible chemical photodegradation. A light:dark regime of 16:8 h was usually applied. The glassware used was either autoclaved or after normal washing procedures rinsed with 96 % acetone and ethanol, followed by deionized water to avoid contamination. The pH of the waters in the assays was buffered to either 6.5 (I, IV, V) or 7.0 (II, III), and the temperature was kept steady at approximately 20°C, except for the periphyton exposed to atrazine (III). Temperature, pH and saturation of dissolved oxygen were monitored in the water at the beginning of the exposures and at their termination. The organisms were not given any additional nourishment during the exposures.

The exposure concentrations used were chosen as compromise between the evaluated clearance rates, the analytical detection limits and the toxicities of the compounds, while also taking into account the capabilities of sampling during the time required, and obtaining to reasonable exposure conditions (e.g. densities of organisms). Usually, appropriate preliminary experiments were required in order to clarify (some of) these factors. Appropriate positive and negative controls were always employed parallel to the exposures under question. Besides of water temperature, pH, and saturation with dissolved oxygen, the test units were verified for animal behaviour and survival, and often also for chemical contamination, which all remained within acceptable limits (OECD 1981, 1993, 1998; Finnish standard 1984; ISO 1996; ASTM 1995, 1997a, b).

Exposures were carried out in a statistically sound manner, for example by appropriate test units and random processing. The possible effect of temporal pseudoreplication was also taken into account when possible: for instance, in pyrene toxicity assays by randomly choosing over half of the waters to be tested

twice, in random order, and exposing each test vial (i.e. one replicate per one test concentration per one test water of the the three waters to be tested at a time) to UV-B radiation in a randomised order under the tubes.

The studied chemicals, organisms and organic carbon sources as well as the measured toxicokinetic variables for each orginal paper are summarized in Table 2. The used treatments and replicates are included in the right-hand column.

TABLE 2 Experimental design and the toxicokinetic variables studied in original papers. Please see abbreviations in page 7 for variable explanations.

Paper	Chemical	Organism	OC source	Toxicokinetic variables	Treatment and replicate numbers[a]
I	Pyrene	*D. magna*	DOC[b]	k_u, k_e, $t_{½}$, BCF	3 × 1 × 3 × 10
				K_{DOC}	3 × 1 × 5 ×—
II	Pyrene	*D. magna*	DOC	EC_{50}	7 × 6-7 × 3 × 5
				K_{DOC}	7 × 1 × 2-4 ×—
III	Atrazine	*D. magna*	DOC	k_u, k_e, BCF	4 × 1 × 3 × 15
				k_{dA}, k_{dB}, $t_{½}$, t_{eff}	4 ×—× 3 × 15
	Atrazine	periphyton	DOC	k_u, k_e, BCF	4 × 1 × 1-5 × 1[c]
				k_{dA}, k_{dB}, $t_{½}$, t_{eff}	4 ×—× 1-5 × 1
IV	2,4,5-TCP[d]	*L. variegatus*	FW[e] + SOC[f]	k_u, k_s, BCF, BAF	3 × 1 × 3 × 10
				k_d, $t_{½}$	3 ×—× 5 × 5
				LC_{50}, LBR_{50}	3 × 4-7 × 3 × 10
	PCP[g]	*L. variegatus*	FW + SOC	k_u, k_s, BCF, BAF	4 × 1 × 3 × 10
				k_d, $t_{½}$	3 ×—× 5 × 5
				LC_{50}, LBR_{50}	3 × 5 × 3 × 10
V	Retene	*L. variegatus*	SOC	BAF, BSAF	3 × 2 × 3 × 100

[a] Orcanic carbon concn. × compound concn. × replicates per one time × individuals per one replicate

[b] DOC = dissolved organic carbon (includes also artificial organic-free freshwater, FW)

[c] one periphyton disc (a sample of a periphyton community attached onto the disc)

[d] 2,4,5-TCP = 2,4,5-trichlorophenol

[e] FW = artificial organic-free freshwater

[f] SOC = sediment organic carbon

[g] PCP = pentachlorophenol

— not included

4 RESULTS AND DISCUSSION

4.1 Bioavailability

4.1.1 Binding of pyrene to dissolved organic material

The partition coefficients of pyrene (K_{DOC}) determined in I and II indicated that pyrene affinity to DOC appeared in relation to the concentration of DOC, water colour and absorptivity at 270 nm (Table 3). Yet, water from the River Riera Major in II showed almost as high affinity of pyrene to DOC as Lake Kontiolampi water with its fourteen times higher DOC concentration. The concentration carried out to the R. Riera Major and R. Maas water samples caused some loss of DOC, which can result in slight overestimation in the binding capacity calculation. On the other hand, other characteristics of the water in the R. Riera Major (like the high inorganic carbon concentration) may have resulted in higher affinity than could be predicted from the concentration of DOC.

Observed differences in the affinities of pyrene between different waters can be thus be mainly explained by the amount and aromaticity of DOC. Not only the quantity but also the quality of the DOC may affect the binding capacity of a hydrophobic, planar ring compound, pyrene, to DOC (Chin et al. 1997, I, II). Further, the magnitude of binding is usually linearly related to the lipophilicity of the compound (e.g. McCarthy & Jimenez 1985a). Thus, the binding of pyrene to DOC was considerably lower than that, for instance, observed with a more hydrophobic PAH, benzo(*a*)pyrene (B*a*P) in other studies (Kukkonen 1991; Kukkonen & Oikari 1991; Kukkonen & Pellinen 1994; De Paolis & Kukkonen 1997, Akkanen et al. 2001).

TABLE 3 Dissolved organic carbon concentrations, colour, absorptivity, and pyrene binding to DOC ($K_{DOC} \times 10^3 \pm$ SD, n = 2-5) in the test waters in papers I and II.

Water (publication)	**DOC** (mg/l)	**Colour** (mg Pt/l)	**AI**[a]	**$K_{DOC} \times 10^3 \pm$ SD** (l/kg)	**% Bound**
DOC-free reference water (I, II)	< 0.2	0	nd	0	0
R. Riera Major (II)	1.4	5	5.9	42.1 ± 3.6[b]	6
R. Maas (II)	4.6	10	18.5	5.7 ± 3.9[b]	3
R. Hultabäcken (II)	6.9	40	28.2	12.7 ± 8.6	8
R. Pielisjoki (II)	7.9	50	35.3	21.3 ± 3.2	14
R. Pielisjoki (I)	9.4	60	38.8	37.1 ± 7.4	26
R. Kalliojoki (II)	15.8	160	38.7	22.4 ± 5.5	26
L. Kontiolampi (I)	17.4	200	43.9	34.9 ± 8.0	38
L. Kontiolampi (II)	19.8	300	46.0	44.0 ± 5.8	47

[a] A measure of the humic aromaticity, AI = A_{270}/(mg DOC/l) × 1000, according to Gauthier et al. (1987) and Traina et al. (1990)

[b] Concentrated water sample

4.1.2 Effects of organic material on contaminant bioavailability and bioconcentration

The results suggested that the quantity or quality of DOC in natural waters do not particularly affect the toxicokinetics of atrazine. A wide range of natural DOC concentrations (0.04-21.4 mg/l) with different qualities were used in the experiments but no effect on the bioavailability of atrazine could be found during short-term exposures (24-h) for either *Daphnia magna* (Fig. 3) or periphyton. The interaction between atrazine and humic substances extracted from soil is reported to be relatively low (Saint-Fort & Visser 1988, Zhang et al. 1990), and it seems that this also applies for river and lake water DOC as well. This is due to the relatively low hydrophobicity of the compound. This in turn is reflected by the low BCFs in our experiments and is similar to results observed by others (Tang et al. 1998, Kümmerlin 1986, Akkanen & Kukkonen 2001, Akkanen et al. 2001).

Pyrene bioavailability, on the other hand, decreased with the high DOC concentration in L. Kontiolampi water (17.4 mg/l), but notably, the moderate DOC concentration in R. Pielisjoki water (9.4 mg/l) did not slow the rate of uptake compared to that in DOC-free reference water (Fig 3). In L. Kontiolampi water, the DOC concentration was high enough to reduce the bioavailable fraction, and the uptake suggested that the binding of pyrene to abundant DOC resulted in its decreased bioavailability to *D. magna*.

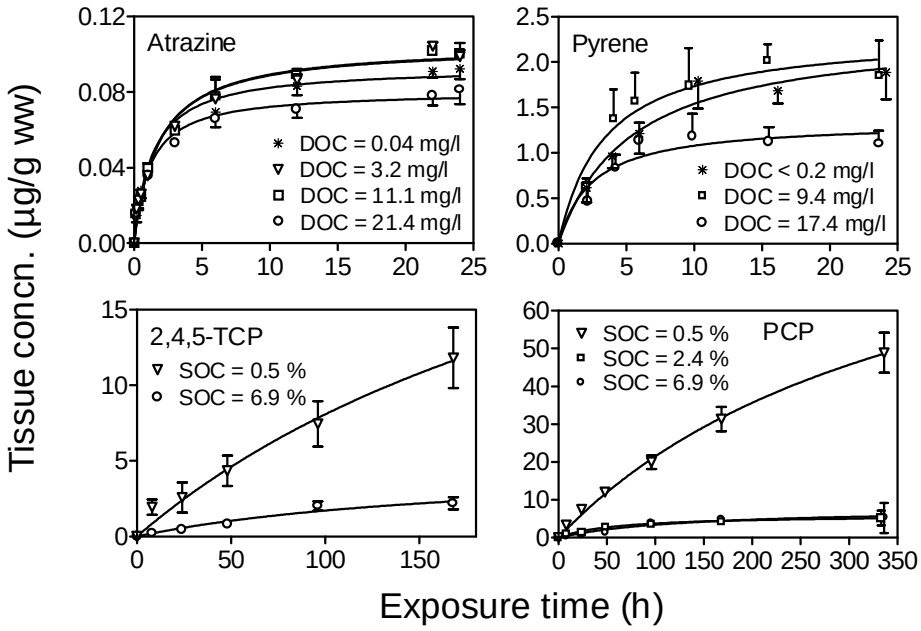

FIGURE 3 Atrazine and pyrene accumulation in *D. magna* (± SD, n = 3), and 2,4,5-TCP and PCP bioaccumulation in *L. variegatus* (± SD, n = 3) in various organic carbon (DOC or SOC) concentrations. Curves are fitted using non-linear regression according to Newman (1995).

For 2,4,5-TCP and PCP, a clearly higher bioavailability was attained in the sandy sediment (SOC = 0.5 %) compared to sediments with medium (measured only for PCP) and high SOC concentrations (Fig. 3; analysis of variance, Tukey's HSD, $p<0.001$), suggesting a significant chlorophenol binding capacity for the latter two L. Höytiäinen sediments. No remarkable difference in the bioavailability of PCP between sediments with SOC concentrations of 2.4 and 6.9 % of dry weight (dw) was measured (ANOVA, Tukey's HSD, $p=0.987$).

Pyrene also became less bioconcentrated in *D. magna* in R. Pielisjoki and L. Kontiolampi waters with moderate and high concentrations of DOC (9.4 and 17.4 mg/l, respectively) than in the DOC-free reference water (Fig. 4). The difference in the observed BCFs, again, was significant only for water with a high DOC concentration as a homogeneous subset of the DOC-free reference water and water with moderate DOC concentration was observed in multiple comparisons (ANOVA, Tukey's HSD, $p < 0.05$). Results of the same kind have been reported also quite recently (Akkanen et al. 2001). Indeed, it seems obvious that a high concentration of DOC is needed to reduce the amount of pyrene bioavailable for bioconcentration in *D. magna*.

The estimated BCFs yielded similar values to the observed BCFs (Fig. 4). According to the same principle, the BCFs in the DOC containing waters based on the freely dissolved pyrene water concentrations yielded practically the same values of 2,148 as those in the DOC-free reference water. This indicates

that when the presence of DOC in the system is disregarded, the system will show the same balance, or distribution ratio, between the two compartments as in the DOC-free reference water. Above all, it shows that only the freely dissolved compound can be accumulated, and that the freely dissolved concentration of a compound can be estimated in this way.

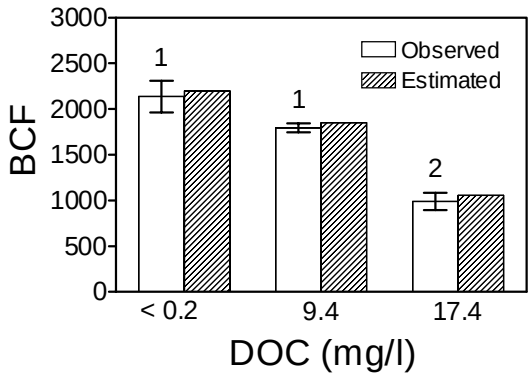

FIGURE 4 Observed (± SD, n = 3) and estimated bioconcentration factors (BCFs) of pyrene in *D. magna* in test waters with three DOC concentrations. Estimation is based on a quotient of the measured k_u and k_e according to publication I. Homogeneous subsets in oneway ANOVA by Tukey's HSD ($p < 0.05$) are denoted by numerals 1 and 2.

The triazine-herbicide atrazine, being much less lipophilic than pyrene, shows only little affinity for DOC (Haniff et al. 1985; Saint-Fort & Visser 1988; Zhang et al. 1990) and was thus almost as bioavailable in all waters, without any dependence on DOC concentrations (Fig. 5). The bioconcentration of atrazine was low, both in the periphyton and *D. magna* due to the low hydrophobicity of atrazine, but the BCFs were higher in the periphyton communities than in juvenile *D. magna*.

Bioconcentration of atrazine in *D. magna* was enhanced in the waters having low to moderate DOC concentrations of between 3.2 and 11.1 mg/l, compared to DOC-free reference water or to a high DOC concentration (Kruskall-Wallis, $p < 0.001$). Mechanisms behind this phenomenon are unclear. Slower depuration measured in waters with low to moderate DOC concentrations in comparison to those of high (21.4 mg/l) and DOC-free reference waters, however, may have resulted in a higher body residue in the former two waters. In relation to the simultaneous uptake, *D. magna* was able to eliminate atrazine much faster than the periphyton. Obviously, this is one reason behind the lower BCF in *D. magna*. Another possible reason may be the carapace, which could act as a barrier.

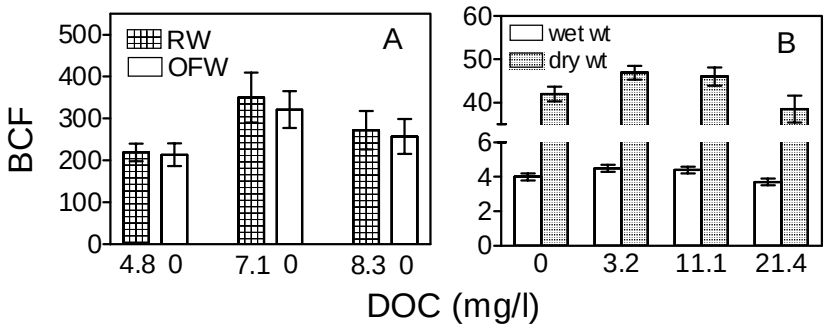

FIGURE 5 Bioconcentration factors (BCF ± SD) of atrazine (A) by different periphyton communities on a dry weight basis in river waters (RW) with varying dissolved organic carbon concentrations (DOC) and in organic-free freshwaters (OFW), and (B) by *D. magna* on a wet weight and dry weight basis in test waters with different DOC concentrations.

The periphyton community structure (not studied here) was observed to influence the bioconcentration of atrazine more than DOC and water quality in general. No differences between DOC-free and river waters in the BCFs by the same communities were observed (Kruskall-Wallis, $p < 0.001$). The BCFs on a dw basis ranged from 213 to 350 between the periphyton communities in this study. The range is similar to those of different species of green algae (Chlorophyta): BCFs ranged from about 175 to 320 (Tang et al. 1998). Considering that periphyton is a very complex matrix composed of different species, detritus and other organic matter, these values are, in fact, surprisingly similar.

On the other hand, diatoms have much lower BCFs than green algae (Tang et al. 1998). This lower bioconcentration of atrazine in diatoms compared to green algae may be due to the silica frustule, which may act as a barrier, like the carapace in *D. magna.* Additionally, many other characteristics that differ between green algae and diatoms may affect the bioconcentration, these including surface area, biovolume and lipid content (Tang et al. 1998). These factors may also be a reason for the differences seen between algae and *D. magna* and the similarity between the periphyton community and single-species green algal test systems. The BCFs of the Lärjeån periphyton were highest in both river (DOC = 7.1 mg/l) and DOC-free waters tested. This is also probably due to some structural qualities like the dominance of green algae (visual observation) in the Lärjeån periphyton assemblage.

The bioaccumulation factor (BAF) of sediment-associated 2,4,5-TCP was 8, which is one quarter of that of PCP in the same Lake Höytiäinen sediment (Fig. 6). A slightly lower BAF was measured for PCP in sediment with a lower SOC concentration (IV).

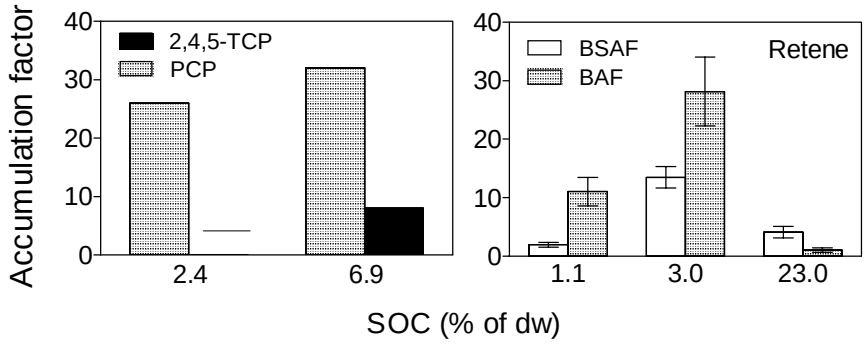

FIGURE 6 Bioaccumulation factors (BAF) of 2,4,5-TCP and PCP, and BAFs (± SD), as well as biota-sediment accumulation factors (BSAF ± SD) of retene, in different sediment organic carbon concentrations (SOC) in *L. variegatus* (IV).

Retene BAFs varied from 1.1 to 28.2 but normalising the bioaccumulation on an organic material basis, as total lipids in the worms and to OC in the sediments, reduced the variation so that the biota-sediment accumulation factors (BSAF) varied from 0.8 to 13.5 (Fig. 6). In theory, carbon normalised BSAFs should not vary between individuals or SOC concentrations. However, the complexity of sorption-desorption net rates playing a role during the exposures is such that it is unlikely that simple normalisation to the hydrophobic carbon-containing targets of retene in the worms and sediments would diminish all the variation observed in the BSAFs. For instance, some sorption also to dissolved or colloidal organic carbon in the sediment interstitial water may have taken place especially in the SOC-rich (23.0 %) sediment.

4.2 Accumulation and elimination kinetics

Uptake of pyrene by *D. magna* as studied in I was slower in L. Kontiolampi water rich in DOC (17.4 mg DOC/l) than in R. Pielisjoki water (9.4 mg DOC/l) or in the practically DOC-free freshwaters. The measured uptake rate constants were 346, 396 and 290 $ml/g_{ww}/h$ in DOC-free, R. Pielisjoki and L. Kontiolampi waters, respectively, with a significant decrease observed in the uptake rates with R. Pielisjoki and DOC-rich L. Kontiolampi waters (non-linear regression, $p < 0.05$, Fig. 7).

Atrazine was taken up by the periphyton extremely quickly, with k_u-values ranging from 48 to 375 $ml/g_{dw}/h$ (Fig. 7), and leading rapidly to apparent steady-state concentrations within three hours in most of the treatments. Although the uptake of atrazine in the periphyton was faster than in *D. magna*, the compound was also quickly taken up by the latter. The k_u-values were about 2 $ml/g_{ww}/h$ (Fig. 7) and an apparent steady state was reached within 22 to 24 hours. There was a surprisingly similar pattern of uptake and

elimination between *D. magna* and the multispecies periphyton community. In both groups a fast uptake reaching an apparent steady state within a couple of hours, and a fast depuration phase followed by slower depuration, were observed. Earlier studies confirm these findings but also suggest a bi-phasic uptake (Ulrich et al. 1994, Ellgehausen et al. 1980, Tang et al. 1998, Kümmerlin 1986). First the compound, independent of environmental conditions, will quickly be adsorbed to the algal cell surface (Tang et al. 1998). This is followed by slow diffusion deeper into the periphyton matrix and trans-membrane transport into the cells (Tang et al. 1998). Although this is the most likely uptake pattern, our data fitted well to a one-source two-compartment model (the compartments being water and organisms), and we could not separate adsorption and absorption. The fast uptake, fast elimination and the fact that periphyton and *D. magna* showed similar kinetic patterns suggest that atrazine is a very mobile molecule and its transportation from water to periphyton or *D. magna* cells is independent of water chemistry.

Further, despite the wide DOC range from less than 0.04 to 21.4 mg/l, the DOC concentration or water quality in general did not affect the amount of atrazine taken up by the periphyton (Fig. 7). The uptake and elimination rates were similar within each community in both waters tested (NLR, $p > 0.05$). Instead, the significant differences in atrazine uptake (NLR, $p < 0.001$) must be attributed to properties of the periphyton communities *per se* and not the quality of the surrounding medium. The uptake was fastest in Lärjeån periphyton in both waters tested. This was probably due to structural qualities of the Lärjeån periphyton assembly, like the dominance of green algae (visual observation). The uptake was slowest in the thickest Göta Älv community, which indicates that diffusion into the biofilm is a rate limiting step in atrazine uptake. Similarly in *D. magna* the atrazine uptake rate constants suggested no difference in relation to the quantity or quality of DOC (NLR, $p > 0.05$, Fig. 7).

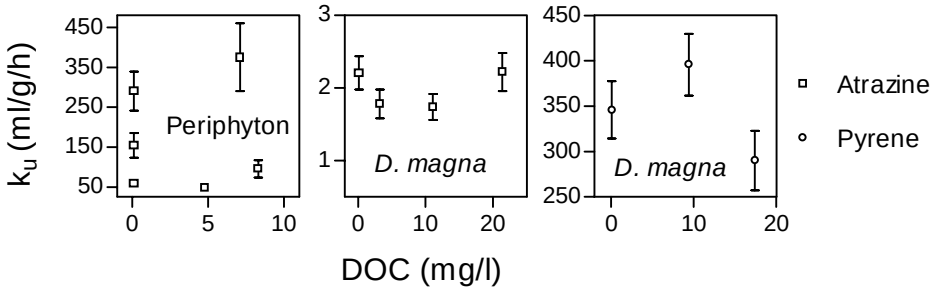

FIGURE 7 Uptake rate constants ($k_u \pm SE$) for atrazine and pyrene in various dissolved organic carbon (DOC) concentrations in *D. magna* (I, III) and the periphyton (III).

The elimination rate constant (k_e) estimated for pyrene in *D. magna* from the uptake data (I) was on average 0.2 1/h (Fig. 8) with no differences found between the waters (NLR, $p > 0.05$). The k_e values for atrazine by *D. magna* (III)

did not differ either (NLR, $p > 0.05$, Fig. 8) but indicated a slightly faster elimination of atrazine than pyrene. The remarkably higher uptake of pyrene than atrazine by *D. magna*, and only a small difference in the elimination rates was reflected by the much lower bioconcentration of atrazine than pyrene. Whereas no difference in the periphyton k_e values were found in regard to the concentration of DOC (NLR, $p > 0.001$), the non-linear regression modelling revealed differences between the three periphyton communities (NLR, $p < 0.001$). The highest k_e values were again found in the Lärjeån periphyton.

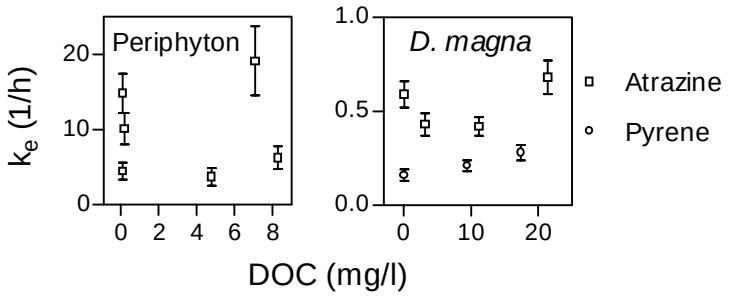

FIGURE 8 Elimination rate constants (k_e ± SE) for atrazine and pyrene in various DOC concentrations in *D. magna* (I, III) and the periphyton.

The measured atrazine depuration (k_{dA} and k_{dB}) by *D. magna* in the studies of the third publication was rapid and even faster in the case of the periphyton. Depuration was most rapid in the periphyton, with k_{dA} -values ranging from 11.7 to 28.6 1/h compared to 2.5 - 6.2 1/h for *D. magna*. The slow depuration of atrazine (k_{dB}) was similar for *D. magna* and periphyton, but the difference between the slow and the fast phases was sharper in the periphyton. The measured slow depuration indicated no influence of DOC on the slow outflux of atrazine from periphyton or *D. magna* (NLR, $p > 0.05$). Even so, the outflux in general for periphyton seemed to be somewhat faster in the natural waters with DOC compared to the DOC-free reference water.

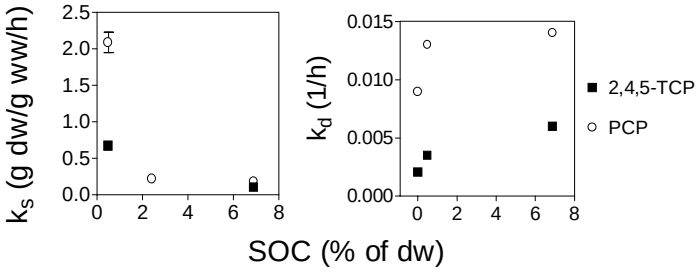

FIGURE 9 Uptake and depuration rate constants (k_s ± SE, k_d, respectively) for 2,4,5-TCP and PCP in *L. variegatus* in various sediment organic carbon (SOC) concentrations (IV).

A clearly higher bioavailability of 2,4,5-TCP and PCP was attained in the sandy sediment compared to sediments with medium (measured only for PCP) and the highest SOC concentrations (Fig. 3). The same increase was observed in the uptake rates in the sandy sediment compared to the other two (Fig. 9). No remarkable difference in the bioavailability of PCP between sediments with moderate and high SOC content was measured. This was also seen in the measured uptake rate coefficients (k_s) in these two sediments, which differed only slightly.

The intermediate and high SOC sediments also mainly consisted of significantly smaller particles of < 43 µm in diameter compared to the sandy sediment, where almost 60 % of the particles had a diameter in the 63-200 µm size class. This partly also explains the different bioavailability and bioaccumulation observed in the sediments, because most of the organic material in sediments and soils usually resides on the surface of the smallest particles (Kukkonen & Landrum 1996). When exposed to xenobiotics, *L. variegatus* might select the particles on which it feeds on the basis of how toxicant binding to various particle sizes has occurred, because it is capable of sensing (and avoiding) contaminated environments. On the other hand, the feeding habits of *L. variegatus* cannot be expected to influence the bioaccumulation of xenobiotics to any large extent, because the species is fairly generalistic in terms of its feeding habits and does not select the edible particles on the basis of their carbon content but feeds on all the fine grain portions of sediments similarly (Kukkonen & Landrum 1995).

In the sediments, natural habitats for *L. variegatus*, the depuration of both 2,4,5-TCP and PCP was faster than in water-only depuration (Fig. 9). It has been stated that specifically purification through the gut contents is one of the most important means in regard to depuration by sediment-burrowing invertebrates (Landrum & Scavia 1983, Kukkonen & Landrum 1995). The results, too, imply that the gut epithelium of *L. variegatus*, by transferring the compounds taken in (possibly partly as metabolised to a more hydrophilic form) to the pure gut contents, plays a significant role also in the depuration of these two chlorophenols. Consequently, the slowest depuration in a water only situation arises from a lower total body area usable for elimination because of fasting due to the lack of sediment.

Both periphyton and *D. magna* are able to quickly halve the amount of atrazine in their tissues. Depending on the calculation method used, the half-life in *D. magna* varied from less than a quarter of an hour to about an hour and a half (Table 4). In the periphyton, atrazine was halved within a few minutes. Accordingly, atrazine may be eliminated at 20°C during the normal average life span of *D. magna* of 56 d (U.S. EPA 1991). In the periphyton, a residue left in the algal cells of the communities is most likely, because 10 to 25 % of atrazine is shown to be irreversibly bound in algae (Böhm 1976, Kümmerlin 1986). Pyrene, on the other hand, might be eliminated at 20°C during the life time of *D. magna* (U.S. EPA 1991), if no pyrene were irreversibly bound to stable compartments. Benthic crustaceans like the amphipod (*Pontopereia hoyi*) and the isopod (*A. aquaticus*), for instance, cannot be expected to eliminate pyrene in water this

rapidly (Landrum 1988, Van Hattum & Montañés 1999). As anticipated, the elimination half-lives were smaller for atrazine than for pyrene (Table 4).

TABLE 4 Pyrene and atrazine half-lives (h) in *D. magna* and the periphyton communities (I, III). Artificial freshwater (DOC < 0.2) was used as a practically DOC-free reference.

Organism	DOC (mg/l)	Pyrene	Atrazine	
		$t_{½}$ [a]	$t_{½}$ [a]	t_{eff} [b]
R. Göta Älv periphyton	< 0.04	—	0.16	0.06
	4.8	—	0.19	0.04
R. Lärjeån periphyton	< 0.04	—	0.04	0.03
	7.1	—	0.04	0.02
R. Hultabäcken periphyton	< 0.04	—	0.07	—
	8.3	—	0.11	—
D. magna	< 0.2	4.4	1.2	0.1
	3.2	—	1.6	0.3
	9.4	3.2	—	—
	11.1	—	1.7	0.2
	17.4	2.5	—	—
	21.4	—	1.0	0.1

[a] Elimination half-life, $t_{½} = \ln 2 / k_e$

[b] Effective (depuration) half-life, $t_{eff} = \ln 2 / k_{eff}$, where $k_{eff} = \sum_{i=1}^{n} k_{ei}$

— Not determined

The decay of both 2,4,5-TCP and PCP was accelerated in SOC-rich media, because the depuration of both chlorophenols was the fastest in the SOC-rich sediment (SOC = 6.9 %) and the slowest in water-only depuration (Table 5).

TABLE 5 2,4,5-trichlorophenol and pentachlorophenol depuration half-lives ($t_{½}$) in *L. variegatus* (IV). Artificial freshwater (DOC < 0.2) was used as a practically organic-free reference.

Exposure media	SOC (% of dw)	$t_{½}$ (h)	
		2,4,5-TCP	PCP
Freshwater	0	365	79
Lake Höytiäinen sediment	0.5 ± 0.02	259	53
Lake Höytiäinen sediment	6.9 ±0.02	121	50

4.3 Toxicity

4.3.1 Role of organic material on toxicity and lethal body residues

Even though pyrene was found to become bound to DOC, this binding was not sufficient to decrease the acute toxicity of the compound in the dark (Fig. 10). Pyrene bioavailability and bioconcentration in *D. magna* was decreased only by a high concentration of DOC in L. Kontiolampi water (Fig. 3, 4). Still, one could have expected some DOC impact on the pyrene toxicity as well, at least in this high-DOC water of the same origin (although the water samples were different). In the binding and bioavailability assays the concentration of pyrene was between ca. 1-5 µg/l, whereas the effective concentration of pyrene appeared approximately between 20-55 µg/l (measured at 24 and 48 h; Fig. 10, 11). DOC of about 19 mg/l in L. Kontiolampi water in the binding and bioavailability assays resulted in about 40 % decrease in the bioavailable fraction of pyrene. Would have been the impact of DOC in the toxicity assays within the same range (40 %), it would have yielded roughly in about 10 to 20 µg/l increase in the EC_{50} values in L. Kontiolampi water. This increase would have been within the confidence limits of the observed EC_{50} values. Furthermore, in these higher effective concentrations of pyrene, 40 % of the total amount of pyrene in the system has been hardly bound by the DOC concentration of about 19 mg/l.

In the sediment, however, SOC seemed to decrease the short-term toxicity of 2,4,5-trichlorophenol and pentachlorophenol (Fig. 10). Similarly to the higher bioavailability of 2,4,5-TCP in sediment with a low SOC concentration (0.5 %), the LC_{50} values indicate that the compound was acutely slightly more toxic in that sediment than in SOC-rich sediment (SOC = 6.9 %). For PCP, this was not as evident. The higher toxicity of PCP compared to 2,4,5-TCP, on the other hand, results in part from higher uptake rates for PCP, which in turn results from a higher chlorination state and lipophilicity of PCP than 2,4,5-TCP and therefore its higher tendency to become bioaccumulated. In other words, the time at which the effect-concentration in an organism is reached, is hastened by the higher uptake rates for PCP.

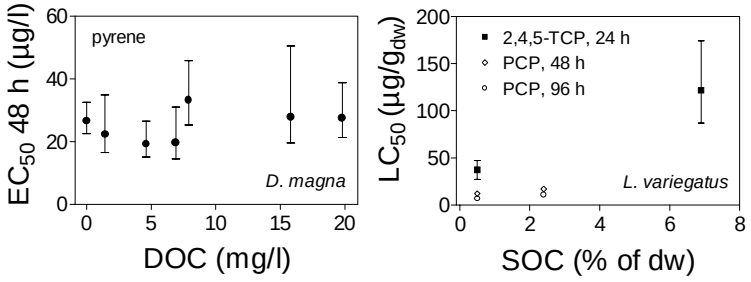

FIGURE 10 Toxicity of pyrene and chlorophenols in various dissolved or sediment organic carbon concentrations. Vertical lines represent 95% confidence limits.

Some time dependence was obvious in the EC_{50} and LC_{50} values as the response is always a result of both the exposure concentration and time (Fig. 10, 11). The appearance of toxicity for most xenobiotics is highly dependent on the environmental conditions, which may alter the compounds' bioavailability. The LC_{50} value only gives the ambient concentration in various sources which causes toxicity, while the lethal body residue approach gives a better measure of the bioavailable concentration that actually reaches the target site(s) within the organism and causes toxic effects, regardless of the environmental conditions present at the time of the exposure. Thus, the lethal body residues for *L. variegatus* were practically the same in the different media, most notably for 2,4,5-TCP (Table 6). This demonstrates the usefulness of this method for accurate, and more comparable, measure of toxicity of chemicals with the same mode of toxic action in varying conditions.

TABLE 6 Lethal body residues (LBR_{50}) of 2,4,5-TCP and PCP in organic-free freshwater (SOC = 0) and in sediments with varying SOC concentrations followed by the 95% confidence limits.

Congener	**SOC** (% of dw)	**LBR_{50}** (µmol/g ww)	**95% CL**
2,4,5-TCP	0	1.18	1.05-1.56
	0.5 ± 0.02	1.10	0.98-1.25
	6.9 ± 0.02	1.07	0.99-1.16
PCP	0	0.83	0.77-0.90
	0.5 ± 0.02	0.62	0.57-0.70
	2.4 ± 0.02	0.43	0.39-0.47

According to McCarty & Mackay (1993) and Penttinen & Kukkonen (1998) it appears that both chlorophenols have the same suggested mode of toxic action, namely uncoupling of oxidative phosphorylation. Penttinen & Kukkonen (1998) have discussed that at critical threshold concentrations for *L. variegatus,* 2,4,5-TCP causes the same heat output response as PCP, a reference compound for the respiratory uncoupling. Additionally, 2,4,5-TCP and PCP caused lethal effects in this study at tissue concentrations equal to the sublethal energetic response in their study. The lethal body residues still indicate what was also found by Penttinen & Kukkonen (1998) that PCP has a higher uncoupling potency in the mitochondrion membrane than 2,4,5-TCP. Indeed, Escher et al. (1996) have stated that chlorophenols have clear differences in uncoupling activities (i.e. critical body residues).

4.3.2 UV-B-enhanced toxicity of pyrene

The acute toxicity of pyrene in publication II was higher under UV-B radiation than in the dark. Under UV-B, high DOC and thus water colour decreased the toxicity of pyrene (ANOVA, $p < 0.05$), which was not observed in the dark

(ANOVA, $p > 0.05$). EC_{50} values of pyrene for daphnids under UV-B radiation were positively correlated with DOC ($r^2 = 0.97$), colour ($r^2 = 0.91$) and A_{250} ($r^2 = 0.96$) as well as with AI ($r^2 = 0.73$), in the test waters. Under UV-B radiation DOC, especially in L. Kontiolampi and R. Kalliojoki (> 15.8 mg DOC / l) but also in R. Pielisjoki and R. Hultabäcken (> 6.9 mg DOC / l), reduced the toxicity of pyrene. The total hardness of the water (Ca + Mg) had no effect on the estimated EC_{50} values tested with the reference waters (Fig. 11).

Since the binding of pyrene to humic macromolecules in the dark had no effect on the observed acute toxicity, it was concluded that waters with abundant humic substances decreased the photomodification of the freely dissolved parent compound simply by diminishing the amount of light in contact with the chemical. A variable amount of dissolved humics and, by implication, the light to deep brown colour of the water, are known to reduce UV transmittance drastically (Wetzel 1983, Barron et al. 2000, Huovinen et al. 2000a). In this case, the higher toxicity of pyrene in the other waters was probably a partial result of increased toxicity of photoaltered compound(s) due to a greater penetration of UV-B into the deeper layers of the test vials.

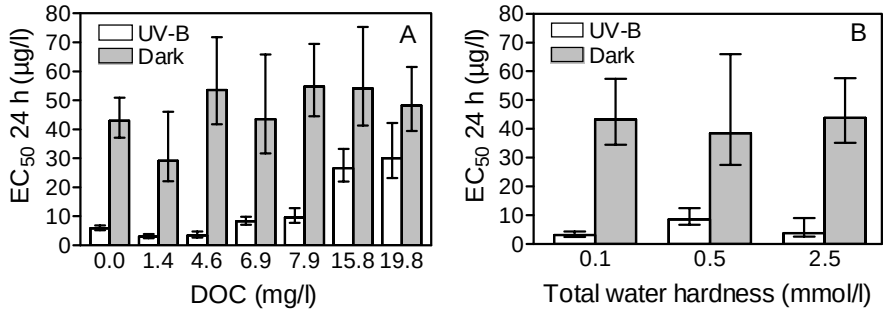

FIGURE 11 Acute toxicity of pyrene in relation to the concentration of dissolved organic carbon, DOC (A) and to the total hardness (Ca + Mg) of the water (B) in the dark and under UV-B radiation. Vertical lines represent 95% confidence limits.

Unlike many earlier observations with mainly UV-A, but also some UV-B radiation (Dahlén et al. 1996), with mainly UV-C radiation only (Corin et al. 1996), or with simulated solar radiation and UV-C only (Kulovaara 1995, Kulovaara et al. 1996), the UV-B radiation used did not photodegrade DOM, not even after 72 continuous hours of irradiation at the same intensity of 0.63 Wh / m^2 as in the *Daphnia* exposures (2 × 2 hrs / d). It is worth noting, however, that the disintegration of the humic macromolecules due to UV is certainly dependent on the efficiency of the light received, and thus is generally achieved with higher radiation doses than those used in this study. Previously, Naganuma et al. (1996) used about the same UV-B dosages as were used here (II) and found no decrease in DOC.

The quenching effect of humic waters not only decreased the formation of more toxic breakdown products of pyrene but also gave shelter to daphnids from stressing and harsh light conditions. Yet, the survival of *D. magna* in the

test water controls did not differ between the dark and the UV-B treatments (ANOVA, $p > 0.05$), which demonstrated that UV radiation alone did not cause mortality of daphnids in the assays. It is well known, on the other hand, that UV light increases activity levels and immunosuppression in fish (Salo et al. 1998) triggering many complex physiological processes which can contribute indirectly to mortality under stress conditions caused by xenobiotic contamination (Bowling et al. 1983). UV radiation also inhibits *in situ* phytoplankton production in the short term (Huovinen & Goldman 2000), and can disturb and delay the early development of the giant kelp (*Macrocystis pyrifera*) gametophytes (Huovinen et al. 2000b). Temporary immunosuppression by solar UV-B irradiation has been detected also in humans (Jansén et al. 2000), and UV-B radiation-induced oxidative stress in cultured human skin fibroblasts (Guochang 1993). The given UV-B radiation with its higher energy content compared to that of visible light may thus have been an additional stress factor for the daphnids during the exposure. Consequently, the higher toxicity of pyrene under UV-B was probably a result of either an additive or a synergistic effect of photoinduced toxicity of pyrene and stress due to UV-B light.

5 CONCLUSIONS

The results suggested that the binding of pyrene to abundant dissolved organic carbon and retene to abundant sediment organic carbon can result in decreased bioavailability in freshwater systems. However, the variation in the retene BSAFs suggests that there are also other factors beside the SOC and worm lipid contents that can affect the bioaccumulation dynamics of sediment-associated retene and the final outcome of its bioavailability. With atrazine, on the other hand, DOC, independent of quantity or quality, has no effect on uptake, elimination or bioconcentration in periphyton communities and *D. magna*. In fact, this appears to be applicable to water chemistry in general. What appears to affect the toxicokinetics of atrazine are instead factors related to the organisms themselves.

SOC also decreased the bioavailability of both chlorophenols, 2,4,5-TCP and PCP, used in this study. The LBR_{50} values were practically the same in freshwater and sediments, demonstrating the usefulness of this method for more accurate predictions on the bioavailability of chemicals. It appeared that both chlorophenols have the same mode of toxic action, i.e. respiratory uncoupling.

Our work has shown that the two PAHs, pyrene and retene, as well as the two chlorophenols, 2,4,5-TCP and PCP, can cause a threat to aquatic organisms, because they were taken up by representative species of freshwater systems and important prey of fish, *D. magna* and *L. variegatus*. Sediment-spiked retene was chronically non-toxic to *L. variegatus* at the environmentally realistic concentrations used, whereas waterborne pyrene caused severe toxicity to *D. magna*, as did both chlorophenols to *L. variegatus*. Atrazine, on the other hand, although taken up extremely rapidly by intact periphyton communities and *D. magna*, was also fairly quickly eliminated by the organisms examined. It remains, however, to be demonstrated whether the low atrazine residue left in organisms has any implication for the viability of freshwater ecosystems.

The results of the present study also demonstrate that the acute toxicity of one model PAH, pyrene, to *D. magna* is not affected by the amount of DOC in the dark. Coexposure to UV-B radiation enhanced the toxicity of pyrene and,

unlike in the dark, this occurred with an inverse relationship to the amount of DOC in the waters. Obviously, the presence of DOC caused UV-B attenuation in the test waters and thereby significantly reduced the UV-B induced toxicity of pyrene to *D. magna*. Further experimentation, preferably with simulated solar radiation, however, should be conducted in order to evaluate the numerous factors that affect that the photoinduced toxicity of PAHs in freshwaters. Nevertheless, it appears likely that photoenhanced toxicity of certain PAHs along with the continuing worldwide use of fossil fuels in different combustion processes will pose a notable threat to aquatic ecosystems.

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YHTEENVETO (Summary in Finnish)

Orgaanisen aineksen vaikutus vierasaineiden biosaatavuuteen, toksikokinetiikkaan ja toksisuuteen vesieliöillä

Pohjoisille ekosysteemeille tyypillinen ominaisuus, runsas orgaanisen aineksen määrä, joko liuenneena vedessä tai järvien pohjasedimenteissä esiintyen, voi sitoa useita kemiallisia saasteita (vierasaineita) ja siten ainakin tietyn aikaa vähentää eliöiden altistumista niille (biosaatavuutta) sekä siitä syntyviä haitallisia vaikutuksia – ei yksinomaan kyseisille eliöille vaan myös koko ekosysteemille. Tutkimuksen yhtenä tavoitteena oli selvittää vedessä olevan liukoisen orgaanisen aineksen vaikutusta pyreenin (polyaromaattinen hiilivety, PAH) biosaatavuuteen, kinetiikkaan ja myrkyllisyyteen *Daphnia magna* -vesikirpulla. Pyreenin myrkyllisyys vesikirpuille määritettiin pimeässä ja ultravioletti-B (UV-B) säteilyssä, koska useilla PAH-yhdisteillä mukaan lukien pyreeni on taipumus absorboida tehokkaasti auringon valoenergiaa spektrin ultraviolettialueella, joista energialtaan tehokkain maan pinnalle pääsevä on UV-B. Tämä energian absorptio voi aiheuttaa alkuperäisen yhdisteen muuntumisen valoaktivoituneeksi molekyyliksi, joka usein on alkuperäistä yhdistettä myrkyllisempi. Liukoisen orgaanisen aineksen vaikutusta triatsiini-tyyppisen kasvimyrkyn, atratsiinin, biokertymiseen ja kinetiikkaan *D. magna* -vesikirpulla sekä päällysleväyhteisöillä selvitettiin myös. Niinikään sedimentin ominaisuuksien, mukaan lukien sen orgaanisen hiilen pitoisuuden, vaikutusta reteenin (PAH-yhdiste) biosaatavuuteen, sekä kahden kloorifenolin, 2,4,5-trikloorifenolin ja pentakloorifenolin biosaatavuuteen, kinetiikkaan, myrkyllisyyteen sekä vaikuttaviin kudospitoisuuksiin selvitettiin.

Kyseiset vierasaineet ovat kaikki olleet ja useimmat edelleenkin ovat merkittäviä ympäristömme, etenkin vesiekosysteemien vierasaineita, jotka vaikutuksiltaan ovat haitallisia sisävesistöjemme eliöille. Lisäksi, vaikka niiden aiheuttama saastuminen on useimmiten enimmäkseen paikallista ja ongelma siten lähinnä tietyillä alueilla, näitä kohteita löytyy kaikkien tutkittujen aineiden osalta runsaasti sekä Suomesta että maailmanlaajuisesti. Tutkimuksessa käytetyt eliöt on valittu niiden ekologiseen tärkeyteen perustuen. Pääosin autotrofinen kasviplankton (päällysleväyhteisöt) on avainasemassa vesiekosysteemeissä, koska ne muuntavat auringon valoenergian orgaaniseksi ainekseksi fotosynteesissä (ensimmäisen asteen tuottajia). Kasviplankton on siten välttämätön ravintolähde useimmille vesiäyriäisille kuten vesikirpuille. Vedessä elävä vesikirppu sekä sedimentissä elävä harvasukamato sitä vastoin ovat keskeisessä asemassa makeissa vesistöissä, koska ne kuluttajina muuntavat kasviplanktonista, bakteereista ja detrituksesta (osittain hajonnut eloperäinen aines) saamansa energian eläinbiomassaksi, ja muodostavat merkittävän osan useiden kalalajien ravinnosta.

Pyreeni sitoutui liukoiseen orgaaniseen ainekseen vähäisessä määrin, mutta pääosin suhteessa sen pitoisuuteen. Pyreenin bioakkumulaatio oli siten noin 17 % vähäisempää humusvedessä (17.4 mg C/l) kuin liukoisesta orgaani-

sesta aineksesta vapaassa referenssivedessä, koska lähinnä vain vapaana liukoisessa muodossa oleva yhdiste on biosaatavaa. Liukoinen orgaaninen aines sitoi osan pyreenistä ja vähensi siten yhdisteen kertymistä vesikirppuun.

Pyreeni oli myrkyllisempää vesikirpulle UV-B-valossa. Liukoinen orgaaninen aines vähensi pyreenin valoindusoitunutta myrkyllisyyttä. Liukoisen orgaanisen aineksen pitoisuus ei vaikuttanut pimeässä mitattuun myrkyllisyyteen kun taas UV-B-säteilyssä pyreenin myrkyllisyys väheni suhteessa liukoisen orgaanisen aineksen määrään. Koska liukoisessa orgaanisessa aineksessa ei havaittu hajoamista käytetyillä UV-B-säteilyn annoksilla, tulkitsimme sen sekä veden korkean värin vähentäneen alkuperäisen liukoisen pyreenin valohajoamista aiheuttamalla UV-B-säteilyn vaimenemista vesissä. UV-B-indusoitunut myrkyllisyys oli joko valomuuntuneen pyreenin ja UV-B-stressin yhdessä aiheuttama lisäävä tai yhteisvaikutus.

Runsas sedimentin orgaanisen aineksen määrä vähensi myös reteenin, 2,4,5-trikloorofenolin sekä pentakloorifenolin biosaatavuutta ja akkumulaatiota harvasukamatoon. Madot lisääntyivät reteenialtistuksessa, eikä reteeni ollut kroonisesti myrkyllistä alkuperäisessä altistuspitoisuudessa 200 µg / g sedimentin kuiva-ainetta. Sen sijaan kummankin tutkitun kloorifenolin myrkyllisyys vaihteli huomattavasti eri sedimenttien sekä sedimenttien ja orgaanisesta aineksesta vapaana olevan referenssiveden välillä. Kuitenkin vaikuttavat pitoisuudet matojen kudoksissa olivat käytännössä samat eri sedimenteissä ja vedessä, mikä osoitti menetelmän käyttökelpoisuuden haluttaessa saada tarkempia arvioita kemikaalien biosaatavuudesta ja niiden myrkyllisyydestä. Samoin kuin vierasaineiden biosaatavuus ja kinetiikka, myös myrkyllisyys on suuresti riippuvainen ympäristön olosuhteista. Tavanomaiset myrkyllisyysmääritykset antavat tiedon ympäristön pitoisuudesta, joka on eliöille myrkyllinen kun taas määritettäessä kudospitoisuuksia, joissa myrkyllisyysoireet ovat ilmenneet, saadaan tieto toisaalta eliölle biosaatavilla olevasta, mutta myös tehollisesti myrkyllisestä pitoisuudesta kudoksessa (siis kyseisen yhdisteen vaikutuspaikassa kudoksessa), joka on kullekin eliölle yhdistekohtaisesti aina jokseenkin sama riippumatta ulkoisista olosuhteista.

Atratsiinilla liukoisen orgaanisen aineksen määrä ei vaikuttanut yhdisteen toksikokinetiikkaan. Atratsiinin akkumulaatio ja eliminaatio olivat aluksi erittäin nopeita ja tapahtuivat hyvin samankaltaisesti vesikirpulla ja monilajisilla päällysleväyhteisöillä. Atratsiinin akkumulaatio- sekä eliminaationopeusvakiot olivat kuitenkin suurempia päällyslevillä kuin vesikirpulla. Atratsiinin kertyminen vesikirppuun ja päällysleväyhteisöihin oli vähäistä, mutta suurempaa päällysleväyhteisöissä, joiden rakenteelliset ominaisuudet vaikuttivat biokertymiseen enemmän kuin liukoinen orgaaninen aines tai veden laatu yleensä.

Liukoinen orgaaninen aines voi vähentää rasvaliukoisen pyreenin samoin kuin sedimentin orgaaninen aines rasvaliukoisten reteenin ja pentakloorifenolin sekä suhteellisen rasvahakuisen 2,4,5-trikloorifenolin biosaatavuutta. Vesiliukoisen atratsiinin biosaatavuuteen orgaanisella aineksella ei ole vaikutusta. Päällyslevien yhteisörakenne ja eliöihin liittyvät ominaisuudet vaikuttavat atratsiinin biokertymiseen enemmän kuin liukoisen orgaanisen aineksen määrä tai laatu.

UV-B käsittely lisäsi pyreenin myrkyllisyyttä, mutta liukoinen orgaaninen aines vähensi sitä aiheuttamalla säteilyn vaimenemista humuspitoisissa vesissä.

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