University of Joensuu, PhD Dissertations in Biology

No: 53

Kimmo Mäenpää

The toxicity of xenobiotics in an aquatic environment: connecting body residues with adverse effects

ACADEMIC DISSERTATION

To be presented, with the permission of the Faculty of Biosciences of the University of Joensuu, for public criticism in the Auditorium B1 of the University, Yliopistokatu 7, on 23rd November, 2007, at 12 noon

Pre-examiners
Professor Albert A. Koelmans
Department of Environmental Sciences,
Wageningen University, The Netherlands

Docent Matti Viluksela
National Public Health Institute
Kuopio, Finland

Examiner
Professor Valery E. Forbes
Department of Environmental, Social and Spatial Change,
Roskilde University, Denmark

Supervisors
Academy Professor Jussi V. K. Kukkonen,
Professor Matti T. Leppänen and
Professor Ismo J. Holopainen
Faculty of Biosciences,
University of Joensuu, Finland

University of Joensuu
Joensuu 2007

Julkaisija Joensuun yliopisto, Biotieteiden tiedekunta
PL 111, 80101 Joensuu
Publisher University of Joensuu, Faculty of Biosciences
P.O.Box 111, FI-80101 Joensuu, Finland

Toimittaja FT Heikki Simola
Editor Dr

Jakelu Joensuun yliopiston kirjasto / Julkaisujen myynti
PL 107, 80101 Joensuu
puh. 013-251 2652, fax 013-251 2691
email: *joepub@joensuu.fi*
Distribution Joensuu University Library / Sales of publications
P.O.Box 107, FI-80101 Joensuu, Finland
tel. +358-13-251 2652, fax +358-13-251 2691
email: *joepub@joensuu.fi*

Verkkojulkaisu *http://joypub.joensuu.fi/joypub/faculties.php?selF=11*
väitöskirjan yhteenveto-osa; toim. Markku A. Huttunen
and Tomi Rosti
ISBN 978-952-219-057-4 (PDF)
Internet version *http://joypub.joensuu.fi/joypub/faculties.php?selF=11*
summary of the dissertation; ed. by Markku A. Huttunen
and Tomi Rosti
ISBN 978-952-219-057-4 (PDF)

Sarjan edeltäjä Joensuun yliopiston Luonnontieteellisiä julkaisuja (vuoteen 1999)
Predecessor Univ. Joensuu, Publications in Sciences (discontinued 1999)

ISSN 1795-7257 (printed); ISSN 1457-2486 (PDF)
ISBN 978-952-219-056-7 (printed)

Joensuun Yliopistopaino
2007

Mäenpää, Kimmo A.
Toxicity of xenobiotics in an aquatic environment: connecting body residues with adverse effects. - University of Joensuu, 2007, 115 pp.
University of Joensuu, PhD Dissertations in Biology, n:o 53. ISSN 1795-7257 (printed), 1457-2486 (PDF)

ISBN 978-952-219-056-7 (printed), ISBN 978-952-219-057-4 (PDF)

Key words: aquatic toxicology, bioavailability, *Chironomus riparius*, critical body residue, *Lumbriculus variegatus*, *Salmo salar* m. *sebago*, sediment, toxicity, xenobiotic

Many xenobiotics that are released into the environment pose a hazard to the organisms that are exposed to them, thus necessitating studies on the bioavailability and toxicity of xenobiotics. Bioavailability depends on the characteristics of the chemical, the organism and the environment. The toxicity of any xenobiotic is related to the bioaccumulated chemical residue in the organism. The evaluation of the xenobiotic-derived hazard has traditionally been related to the chemical concentration in the ambient media. However, body residue of the organism has been proposed as a better dose-metric, since it provides a more relevant measure for chemical exposure. The critical body residue (CBR) approach hypothesizes that the body residues within a defined mode-of-action category are relatively constant for different chemicals, organisms and exposure conditions. The body residue approach therefore has many advantages, which include the use of internal chemical concentrations in the risk assessment.

The aim of this study was to investigate the bioavailability and toxicity of different model compounds (bentazone, ioxynil, pendimethalin, 4-nonylphenol (4-NP), C12-linear alkylbenzene sulfonate (C12-LAS), pentachlorophenol (PCP) and pyrene) for two invertebrates (*Lumbriculus variegatus*, Oligochaeta and *Chironomus riparius*, Insecta) and one vertebrate (*Salmo salar*, Osteichtyes). Acute and sublethal toxicity tests were set up to determine the chemical body residues associated with adverse effects, to measure critical body residues and to test the hypotheses of the CBR approach. The biotransformation of PCP and pyrene was also studied in order to consider the implications of chemical transformation on toxic body residue.

The bioavailability of the chemicals was shown to depend on the characteristics of the chemical and the sediment. The sediment organic carbon (OC) content was found to modify the chemical bioavailability. The results for bentazone and PCP showed that the toxicity values based on the organisms' body residue were relatively constant, while the toxicity estimates based on the exposure media were not constant. Hence the results were in accordance with the hypotheses of the CBR approach. On the other hand, the toxicity studies showed that for ioxynil, 4-NP and C12-LAS, body residues are affected by characteristics associated with organism or exposure conditions. The biotransformation studies showed that *L. variegatus* is able to metabolize PCP and pyrene moderately well. Due to the metabolites and the unextractable chemical pool in *L. variegatus*, biotransformation of the xenobiotics complicated the toxicity analysis. The results indicated that body residue is a more precise metric for exposure than concentration in the ambient medium when chemical toxicity is evaluated. The critical body residue approach could therefore be useful in the risk assessment of environmental pollutants.

Kimmo A. Mäenpää, Faculty of Biosciences, University of Joensuu, P.O.Box 111, FI-80101 Joensuu, Finland

Table of contents

List of abbreviations

AFW	Artificial Fresh Water
ASTM	American Society for Testing and Materials
BAF	Bioaccumulation Factor
BSAF	Biota to Sediment Accumulation Factor
CBR_{50}	Critical Body Residue for 50% response in the toxic effect.
DW	Dry Weight
EC_{50}	Effective Concentration in the exposure medium for 50% response in the toxic effect.
EqP	Equilibrium Partitioning
ERA	Ecological Risk Assessment
EU	European Union
EUREF-FIN	European Reference Frame - Finland
1-HP	1-hydroxypyrene
1-HPG	1-hydroxypyrene glucuronide
HPLC	High Performance Liquid Chromatography
K_{ow}	n-Octanol/Water Partition Coefficient
LAS	Linear alkyl benzene sulphonate (herein: 4-(2-dodecyl)-benzene sulphonate)
LBR_{50}	Lethal Body Residue for 50% mortality.
LC_{50}	Lethal Concentration in the exposure medium for 50% mortality.
LOI	Loss of Ignition
LSC	Liquid Scintillation Counting
N	Nitrogen
4-NP	4-Nonylphenol
OC	Organic Carbon
PCP	Pentachlorophenol
PNEC	Predicted No-Effect Concentration
SPME	Solid-Phase Microextraction
U.S.EPA	United States Environmental Protection Agency
WFD	European Union Water Framework Directive

List of the original publications

The thesis is based on the following articles and the manuscript:

I Mäenpää, K.A., Sormunen, A.J. and Kukkonen, J.V.K. 2003. Bioaccumulation and toxicity of sediment associated herbicides (ioxynil, pendimethalin, and bentazone) in *Lumbriculus variegatus* (Oligochaeta) and *Chironomus riparius* (Insecta). Ecotoxicology and Environmental Safety 56: 398-410.

II Mäenpää, K.A., Penttinen, O-P. and Kukkonen, J.V.K. 2004. Pentachlorophenol (PCP) bioaccumulation and effect on heat production on salmon eggs at different stages of development. Aquatic Toxicology 68: 75-85.

III Mäenpää, K.A. and Kukkonen, J.V.K. 2006. Bioaccumulation and toxicity of 4-nonylphenol (4-NP) and 4-(2-dodecyl)-benzene sulfonate (LAS) in *Lumbriculus variegatus* (Oligochaeta) and *Chironomus riparius* (Insecta). Aquatic Toxicology 77: 329-338.

IV Mäenpää, K.A., Sorsa, K., Lyytikäinen, M, Leppänen, M.T. and Kukkonen, J.V.K. Bioaccumulation, sublethal toxicity, and biotransformation of sediment-associated pentachlorophenol in *Lumbriculus variegatus* (Oligochaeta). Ecotoxicology and Environmental Safety, in press.

V Mäenpää KA, Leppänen MT and Kukkonen JVK. Sublethal toxicity and biotransformation of pyrene in *Lumbriculus variegatus* (Oligochaeta). Manuscript.

I participated in the design of the studies and was mainly responsible for conducting the exposures, the sampling, the data collection, the data analysis and the preparation of the manuscripts. The general layout of the studies conducted in the publications I, III and IV was given, as they were part of the collaboration projects (NordTest, OECD ring test). The processing of the articles was carried out in collaboration with the co-authors.

1. Introduction

Many hydrophobic pollutants that are present in aquatic environments are eventually stored in sediments. Consequently, all benthic organisms are potentially exposed to them. Any exposure to sediment-borne xenobiotics may cause adverse effects at lower trophic levels and/or lead to biomagnification and more serious adverse toxic effects at higher trophic levels (Landrum and Robbins 1990, Lee II 1992, Streit 1992, Newman 1998). Thus, the study of bioavailability and toxicity of the sediment-associated xenobiotics is important in understanding the possible hazards associated with contaminated sediments in aquatic systems.

Several biological and physico-chemical factors affect the bioavailability of contaminants. Considering the great variability of these factors in aquatic environments, the prediction of chemical bioavailability is challenging. For example, the organism's behavioural characteristics (Spacie et al. 1995) and sediment organic carbon content (Landrum 1989) may modify the bioavailability and toxicity of xenobiotics. This makes the risk assessment of xenobiotics-contaminated sediments difficult when it is based only on measurements of chemical concentrations in sediments. However, limit values are commonly set for chemical concentrations in waters, soils or sediments.

At the end of 2006, the Finnish Council of State enacted an Act concerning the restriction and prohibition of the discharge of hazardous and harmful chemicals into the aquatic environment (Suomen säädöskokoelma 1022/2006). The act was a consequence of the tightening of EU legislation (Water Framework Directive 2000, Directive 2006/11/EC) aimed at improving the quality of the aquatic environment. The quality norms were set for 33 chemicals, including polycyclic aromatic hydrocarbons, pentachlorophenol and nonylphenol, which were specified as slowly degrading, having a potential to bioaccumulate in organisms and being toxic. The environmental quality standards were set for the chemicals as the maximum acceptable concentrations in the surface water.

Concentrations in sediments and organisms were excluded from the EU directive, and the quality norms were set only for concentration of the chemical in water. The importance of studying sediments and organisms was recognized as a means to obtain a more reliable general view of the hydrophobic chemicals. The lack of existing data was one reason that the quality norms were not applied to sediments and organisms as well, and thus a need was noted to develop analytical techniques and toxicity testing in this field.

In 1989-1993 and 1999-2000 the Finnish environmental administration surveyed contaminated sites throughout Finland in order to develop limit values for the chemicals with a view to the categorization and restoration of contaminated sites (SAMASE project) (Puolanne et al. 1994, Petejä-Ronkainen and Suokas 2000). On the basis of this work, new limit values were set according to present knowledge in an Act passed in 2007 (Suomen säädöskokoelma 214/2007). The limit values are based on soil concentrations of chemicals, and hence the risk assessment excludes any measures of the chemical residues in organisms.

The critical body residue (CBR) approach has been advocated as an improvement to the risk assessment of environmental pollutants (McCarty and Mackay 1993, Connell et al. 1999). In the

approach, the toxic effects are compared with the chemical body residue, which takes the bioavailability of the chemical into account explicitly.

1.1. Critical body residue (CBR)

Fundamentally, the toxicity of any chemical is a consequence of the chemical concentration at the target site receptors, and the measured body residue is an estimate for the target site concentration. The measurement of chemical concentration in the receptor is often not feasible, but body residue provides a practical approach for exposure assessment (Escher and Hermens 2004, Landrum and Meador 2002).

The use of organism body residue as a measure of chemical exposure was introduced and then developed into the critical body residue approach (CBR) by McCarty (1986, 1992) and McCarty and Mackay (1993). The CBR approach has aroused great interest, and several evaluations of the concept have been published since its introduction (Penttinen and Kukkonen 1998, Connell et al. 1999, Barron et al. 2002, Landrum and Meador 2002, Escher and Hermens 2004).

The CBR concept is defined as the molar body residue, which consistently causes the specific toxic effect, such as the death or a decrease in growth of an organism (McCarty and Mackay 1993). It should be independent of the ambient chemical concentration, environmental characteristics, and the body residue should also be constant for chemical or classes of chemicals that have the same mode of action. Further, it is argued that CBR is constant for different species.

The mode of toxic action is defined as the set of measured alterations in organism physiology and/or behaviour that characterize the adverse biological response (Rand et al. 1995). The toxic body residue is dependent on the chemical mode of toxic action (McCarty and Mackay 1993). Hence the chemicals or chemical categories should be considered independently according to their mode of toxic action (Table 1.).

The modes of toxic action are traditionally divided into two main categories: baseline toxicity (narcosis) and specific mode of toxic action (Rand et al. 1995). A total of 60-70% of environmental pollutants have been estimated to be narcotics, which constitute the minimal toxicity for any hydrophobic contaminant (Veith et al. 1983, Escher and Schwarzenbach 2002). The main hypothesis is that narcotic substances act nonspecifically, incorporating reversibly into the lipid membranes and disturbing the normal cell functions by loss of selective permeability (Mullins 1954, van Wezel and Opperhuizen 1995a, van Wezel et al. 1996). The narcotics may alter membrane protein functions, change membrane fluidity and disrupt the maintenance of the membrane potential, altering nerve function. An alternative hypothesis suggests that narcotics act by binding to ligand-gated ion channels (Simkiss 1996, Franks and Lieb 1994). The respiratory uncouplers have structural similarities with the narcotic substances, but the number and type of the electron-withdrawing groups determine the mode of action (Barron et al. 2002).

Table 1. Critical body residues for lethal and sublethal endpoints in different mode-of-toxic-action or chemical categories.

Mode of action/ Chemical category	Lethal (mmol/kg ww)	Sublethal (mmol/kg ww)
Non-polar narcosis	1-12	0.01-6
Polar narcosis	0.2-5	0.1-1
Respiratory uncoupler/ Excitatory agent	0.1-0.3	0.00015-0.094
Acetylcholinesterase inhibition	0.0025-8	0.0001-0.5
Reactives/Irritants	0.0094-13	-
Central nervous system seizure agents	$4.8x10^{-5}$-0.017	0.0005-0.015
TCDD	0.0009-0.008	$1x10^{-6}$-0.0001
Ah-receptor agonists	$1.5x10^{-7}$-$4x10^{-5}$	$3x10^{-7}$-$8x10^{-6}$

Data collected from: McCarty and Mackay 1993, Connell et al. 1999 and Barron et al. 2002.
TCDD: 2,3,7,8-tetrachlorodibenzo-p-dioxin
Ah-receptor: Aryl-hydrocarbon receptor

It has been argued that, due to the toxicokinetics of the compounds, the toxicity of the chemicals may increase until an equilibrium is reached in concentrations between tissues and ambient medium (Vaal et al. 1997a,b). Chronic toxicity may thus be elicited by other modes of toxic action than acute toxicity. It has been suggested that virtually all of the chemicals act through narcosis, eliciting toxic effects at high body residues, with the more specific modes of toxic action appearing at lower body residues (Rand et al. 1995, Escher and Schwarzenbach 2002).

According to the hypotheses of the CBR approach, the bioavailability and the accumulation kinetics of the chemical are taken into account, as well as the different uptake routes. The modes of toxic action of the different chemicals or chemical classes, and the effects of metabolism on accumulation are also considered (McCarty and Mackay 1993). The CBRs for the each mode-of-action class (e.g. narcotic chemicals) should therefore be constant across different chemicals, species and exposure conditions (Table 1.). Hence, body residue is proposed as a more reliable estimate of the adverse toxic effects in aquatic biota than external concentrations. Finally, it should be easier to extrapolate the test results from laboratory to field in this approach.

There are some uncertainties associated with the CBR approach, mainly attributable to its novelty and the lack of comprehensive testing of the

approach. Firstly, the body residues associated with each mode of action or chemical category are rather broad (Barron et al 2002). The approach has been developed on the basis of acute fish experiments with narcotic chemicals (McCarty and Mackay 1993) and there are not much data concerning CBRs of sublethal effects (Thompson and Stewart 2003). The approach has not been evaluated throughout for a broad range of different chemicals, and the extrapolation of CBR over different species, exposure conditions and chemicals belonging to a certain chemical class has been little studied.

Further, the mode of toxic action of the chemical is difficult to define and the distribution of the chemical in the organism's tissues affects the manifestation of toxicity. It has been argued that the lipid content of the organisms affects critical body residues, weakening the hypothesis that CBRs are not species-specific (van Wezel et al. 1995a, van Wezel et al. 1995b, Escher and Schwarzenbach 2002). The approach is, however, worthy of closer examination, which may help to explain the uncertainties and specify more accurately the CBRs behind different mode-of-action categories.

The CBR approach may be helpful when combined with ecological risk assessment (ERA), which has generally used the chemical concentrations of the ambient environment and various safety factors to estimate e.g. predicted no-effect concentrations (PNEC) (Suter II 1995). The usual estimates using ambient concentrations include uncertainties associated with the spatial and temporal bioavailability of the chemical in the environment. The toxicity data based on body residues can be compared, even when varying chemical bioavailability is assumed (Escher and Hermens 2004).

Exploitation of chemical body residue data in chemical risk assessment still awaits application in decision-making, although Di Toro et al. (2000) and Di Toro and McGrath (2000) have prepared a first proposal for the use of body residues in risk assessment. They have attempted to establish water and sediment quality criteria for non-polar narcotic chemicals, using critical body residues.

2. Objectives and hypotheses

The first objective of this thesis was to study the bioavailability of the test compounds in the freshwater oligochaete worm *Lumbriculus variegatus*. The organisms were exposed to each compound in different sediments, and the hypothesis was that bioavailability is dependent on chemical characteristics and the characteristics of the sediment. In particular the organic carbon content of the sediment was followed as a bioavailability-modifying factor.

The second objective was to test the hypotheses of the critical body residue approach (McCarty and Mackay 1993). This was done by conducting acute (2 days) and chronic (> 10 days) toxicity tests with *Lumbriculus variegatus*, *Chironomus riparius* and *Salmo salar* m. *sebago* and by measuring lethal sediment and water concentrations (LC_{50}), lethal body residues (LBR_{50}), effective sediment and water concentrations (EC_{50}) and critical body residues (CBR_{50}). It was hypothesized that the LC_{50} and EC_{50} concentrations vary by species, compounds and exposure conditions, but the LBR_{50} and CBR_{50} values are more consistent across different variables, as described in the CBR approach.

The third objective was to examine the biotransformation capability of *L. variegatus*, and to consider it as a modifying factor of toxicity, as well as the implications it may have on CBRs.

3. Materials and methods

3.1. Test species

The oligochaete worm, *Lumbriculus variegatus* Müller was used in sediment bioavailability experiments, and in both acute and chronic toxicity experiments (I, III and IV). *L. variegatus* meets most of the criteria for a suitable test species for ecotoxicological testing of the environmental pollutants (U.S.EPA 2000). *L. variegatus* burrows into the sediment and feeds deposits, which makes it a particularly good organism for sediment bioavailability and toxicity studies. *L. variegatus* are easily cultured in the laboratory, which is necessary to ensure an adequate supply of the organisms for the experiments. It is tolerant to a wide range of different sediment characteristics and provides sufficient mass for the chemical analyses. Besides observing the lethal endpoint, it is possible to determine the chemical stress in *L. variegatus* by measuring different sublethal endpoints, such as growth, reproduction, faecal pellet production and heat dissipation (IV, Penttinen et al. 1996, Penttinen and Kukkonen 1998). *L. variegatus* also has ecological relevance as a test species, because it has a wide distribution geographically in freshwater environments (Brinkhurst and Jamieson 1971) and it is an important vector in food webs. Thus it is not surprising that aquatic oligochaetes, including *L. variegatus*, have been adopted for use in ecological risk assessment (Chapman 2001), and that it has been validated as a standard organism in ecotoxicological tests. The American Society of Testing and Materials (ASTM) have published a standard guide for the determination of chemical bioaccumulation in *L. variegatus* (ASTM 2006a). Similarly, the United States Environmental Protection Agency (U.S.EPA) has also published guidance on toxicity and bioaccumulation tests for this species (U.S.EPA 2000). Moreover, international ring tests have recently been performed in developing OECD guidelines for assessing the bioavailability and toxicity of sediment-associated chemicals in *L. variegatus* (Egeler et al. 2005, 2006). Our laboratory participated in this effort.

The larvae of *Chironomus riparius* Meigen, (Insecta) were used in both acute and chronic toxicity experiments (I and III). *C. riparius* is widespread in the northern hemisphere (Hirvenoja and Michailova 1991). Like *L. variegatus*, *C. riparius* is also easily cultivated in the laboratory and thus these organisms are commonly used species for sediment toxicity tests (Ingersol 1995). *C. riparius* is used as the standard species in measuring the toxicity of sediment-associated contaminants (U.S.EPA 2000, ASTM 2006b). *C. riparius* has a complete life-cycle from egg to larva (with four instars), to pupal stage and finally to adult midge. Only the adult is terrestrial, and the other stages of the life-cycle of *C. riparius* are associated with a water body or sediment. Similarly with *L. variegatus*, the larval stage inhabits the sediment and feeds on detritus, and is consequently exposed to sediment-associated pollutants through the cuticula and via the alimentary canal.

Table 2. Dry weight (DW%), organic carbon (OC%) content and small particle fraction ($F_{<37\mu m}$%) of the test sediments.

Sediment	DW	OC	$F_{<37\mu m}$ [a]
Mekrijärvi	10	24	48 (17)
Höytiäinen, open lake	21	3.2	78 (4.1)
Kuorinka	53	1.6	43 (2.6)
Höytiäinen, Varparanta	66	0.5	30 (0.9)
Artificial	59	1.8	-

[a] OC% of the fraction <37μm is shown in brackets.

The eggs of *Salmo salar* m. *sebago* Girard, (Vertebrata) were used to study the sublethal effects of PCP by measuring heat dissipation (II). *S. salar* m. *sebago* is a landlocked salmon of Lake Saimaa and is currently endangered by hydropower stations and dams, which have destroyed the habitats and blocked access to the remaining breeding areas. Only a few other ecotoxicological studies have been conducted with the landlocked salmon of Lake Saimaa (Honkanen et al. 2001, Honkanen et al. 2004). However, related species, especially rainbow trout (*Oncorhynchus mykiss*), have been used extensively in aquatic toxicological testing (Cooney 1995).

3.2. Exposure media

The natural pristine sediments that were used in the research were collected from the freshwater lakes in the province of North Carelia, Finland. The different sediments were chosen to represent a range of characteristics. The sediments were typically characterized by their organic matter content and particle size distribution (Table 2.). Lake Mekrijärvi (EUREF-FIN lat:6965493, lon:701842) sediment was the most organic rich sediment used in the study, followed by the sediment collected from the open part of Lake Höytiäinen (EUREF-FIN lat:6963643, lon:638368) and sediment from Lake Kuorinka (EUREF-FIN lat:6944919, lon:623359). The sediment collected from Lake Höytiäinen at Varparanta was the most inorganic sediment.

The artificial sediment was prepared according to the OECD guideline proposal (2002) to conduct bioavailability and toxicity tests together with several other laboratories in the international ring test.

Artificial fresh water (AFW) was used on top of the sediment column in the test beakers. The toxicity tests with salmon eggs and those with pyrene were conducted solely in AFW. The AFW was prepared according to standard of the Finnish Standards Association (1984).

3.3. Chemicals

Bentazone (IUPAC name: 3-isopropyl-1H-2,1,3-benzothiadiazin-4(3H)-one 2,2-dioxide) has sulphonamide group that makes it a strong acid. It is a widely used herbicide that is used in fields and orchards to control weeds (Tomlin 1994, Li et al. 2003, WHO 2004). The mode of action of bentazone is to inhibit photosynthesis. The World Health Organization (WHO) (WHO 2005) classifies bentazone as slightly hazardous, and the Food and Agriculture Organisation (FAO) has reported that bentazone is not considered hazardous to aquatic organisms under normal conditions of use (FAO 1991) (Figure 1., Table 3).

Table 3. Characteristics of the model compounds.

Chemical (Reference)	**MW (Da)**	**Octanol-water partitioning coefficient (log K_{ow})**	**Water solubility (mg/L)**	**Vapour pressure (mPa)**	**pK_a**
Bentazone (Tomlin 1994)	240.3	0.77 (pH 5) -0.46 (pH 7) -0.55 (pH 9)	570 (pH 7, 20°C)	0.46 (20°C)	2.92-3.3 (24 °C)
Ioxynil (Tomlin 1994, Tomlin 1997, Linders et al. 1994, Nicholls 1994, EC 2004)	370.9	0.89-3.94 2.2 (pH 5) 0.23 (pH 8.7)	50 (25 °C) 539 (pH 5) 5.53 (pH 9)	<1 (20°C)	3.96 4.1
Pendimethalin (Tomlin 1994, Junghans et al. 2006)	281.3	4.82, 5.18	0.3 (20°C)	4.0 (25°C)	-
4-nonylphenol (Müller and Schlatter 1998, Canadian Environmental Quality Guidelines 2002)	220.3	4.48	5.4 (20.5°C)	4.55 (25°C)	10.28
LAS 4-(2-dodecyl)-benzene sulfonate (Larson and Woltering 1995)	348.5	2.0-3.0	>1000	0.00001	-
Pentachlorophenol (Nowosielski and Fein 1998, Arcand et al. 1995, Crosby 1981)	266.3	3.25 (pH 9.2) 3.69 (pH 7.2) 4.60 (pH 5.0) 4.74 (pH 3.0)	9.3-18.6 (pH 4.8-6.3) (23°C)	5.9 (20°C)	4.2-4.9 (23 °C)
Pyrene (ATSDR 1995)	202.3	4.88	0.077 (25°C)	0.33 (25°C)	-

Ioxynil (IUPAC name: 4-hydroxy-3,5-diiodobenzonitrile) is a relatively strong acid and it is also a widely used herbicide that inhibits photosynthesis and uncouples oxidative phosphorylation. It is used for post-emergence control of weeds (Tomlin 1994, Junghans et al. 2006). WHO has rated ioxynil as moderately hazardous (WHO 2005).

Pendimethalin (IUPAC name: N-(1-ethylpropyl)-2,6-dinitro-3,4-xylidine) is a highly hydrophobic herbicide that inhibits cell division and cell elongation and is used for the control of most annual grasses and broad-leaved weeds (Tomlin 1994). Even in common use, the toxic effects of pendimethalin on, e.g. organisms in the aquatic environment are very poorly known, and further examination has been requested (Sandberg and Scott-Fordsmand 2004) (Figure 1., Table 3).

The environmentally widespread 4-nonylphenol is a degradation product of alkylphenol polyethoxylates, which are commonly used in industry and in many consumer goods, in addition to which it is used directly, e.g. as a plasticizer (Müller and Schlatter 1998, Naylor et al. 1992). 4-nonylphenol is an endocrine disruptor exerting oestrogenic effects by binding to

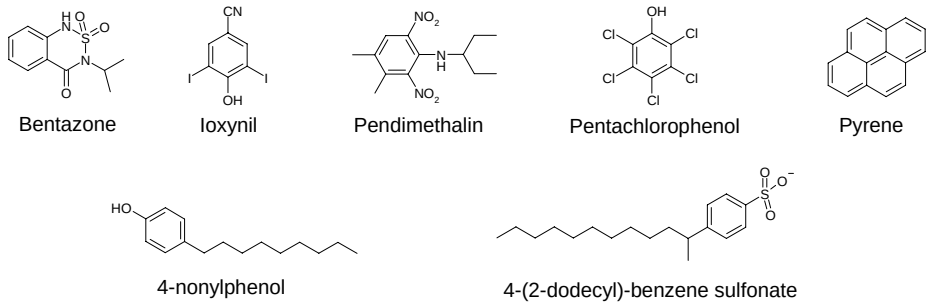

Figure 1. The chemical structures of the model compounds.

the oestrogenic receptors and acting as a low potency oestrogen receptor agonist.

Linear alkylbenzene sulphonate (LAS) is a mixture of homologues that have alkyl chain lengths from C10 to C14, the average for commercial products being C12 (Belanger et al. 2002, Larson and Woltering 1995) as the alkyl chain length for the model compound, 4-(2-dodecyl)-benzene sulphonate (C12-LAS), used in this research. The alkyl chain length and the positions of phenyl have a great influence on homologue physico-chemical characteristics. C12-LAS constitutes the majority of the surfactants used in commercial laundry detergents worldwide. In spite of the efficient biodegradation of C12-LAS during wastewater management, the large volume of C12-LAS released into the environment and its narcotic mode of toxic action constitute the problem associated with this compound (Holmstrup and Krogh 1996) (Figure 1., Table 3).

Pentachlorophenol (PCP) has in the past been used as a pesticide and for the protection of timber, but due to its toxicity, its use was reduced and banned in many countries in the 1990's (Crosby 1981, Muir and Eduljee 1999). However, because of the historical use of PCP and its strong adsorptive nature in soil and sediments, the problems associated with PCP have persisted in many areas. PCP is listed as a priority pollutant in the EU's WFD and by the U.S. EPA. As a weak acid, the bioavailability and toxicity of PCP is strongly affected by the ambient pH (Saarikoski and Viluksela 1981, Mackay et al. 1998, Fisher et al. 1999, Czaplicka 2004). PCP uncouples oxidative phosphorylation, inhibiting adenosinetriphosphate (ATP) synthesis in the mitochondria (Terada 1990), and consequently affecting organism energy metabolism (Penttinen and Kukkonen 2006) (Figure 1., Table 3).

Pyrene belongs to the group of polyaromatic hydrocarbons (PAH), which are released into the environment by the incomplete combustion of organic material, both in natural processes (e.g. forest fires, volcanic activity) and through human activity (Rand et al. 1985). Human activity accounts for most of the PAH contamination, as PAH emissions occur as a result of the burning of fossil fuels and municipal waste, petroleum spills and industrial discharges. PAHs are also listed in the priority pollutants in the EU's WFD. In a water body, pyrene associates with suspended particles and is sequestered in the sediments. Pyrene has a narcotic mode of toxic action (Di Toro et al. 2000) (Figure 1., Table 3).

3.4. Bioavailability experiments

The bioavailabilities of the test compounds were measured by exposing *L. variegatus* to a trace amount of sediment-spiked compound. Thus, each of the tested chemicals was typically spiked into three or four different sediments in order to evaluate the effects of the sediment characteristics on chemical bioavailability. The static exposures lasted from 10 (I, III) to 28 (IV) days, and the samples were taken during the whole exposure period in order to obtain the kinetic bioaccumulation values by measuring the whole body concentration of the worm sample. PCP bioaccumulation in salmon eggs was tested in a static water exposure system lasting three days (II).

3.5. Toxicity experiments

Acute and chronic toxicity experiments lasted from 2 to 28 days and consisted typically of at least 5 different exposure concentrations. The toxicity was evaluated by surveying both lethal and sublethal endpoints. In the acute tests, the lethal toxicity endpoint was used and the tests were conducted as water-only exposures (I, III). Both, *C. riparius* and *L. variegatus* were used in the acute experiments. In the chronic experiments, lethal and sublethal endpoints were measured for *L. variegatus* and *C. riparius* (I, III, IV, V).

The sublethal endpoints for *L. variegatus* consisted of 1) growth as a measure of the wet weight of the organisms, 2) reproduction as a measure of the number of the organisms 3) faecal pellet production, and 4) blood vessel pulsing rate. *C. riparius* growth was measured by measuring the wet weight and the head capsule size of the larvae. *Salmo salar* embryo heat production was measured when the eggs were exposed to PCP in the water-only exposure.

The toxicity endpoints were proportioned to the chemical concentrations in the exposure media and in the organisms' tissues, in order to evaluate the data and test the hypotheses by means of critical body residue.

3.6. Biotransformation

The biotransformation of the test chemical was studied for *L. variegatus* exposed to PCP (IV) and pyrene (V). The chemical residues in the whole body extracts of *L. variegatus* were analyzed by high-performance liquid chromatography (HPLC) in order to measure the amount of the parent chemicals and the metabolites. By using radio-labelled compounds, the tissue pellet was analyzed after extraction in order to evaluate extraction efficiency.

The PCP-exposed worms were extracted first in acetone, then acidified with phosphorus acid (H_3PO_4) and extracted with hexane:acetone. The pyrene-exposed worms were extracted in acetone twice. The extracts were dissolved in acetonitrile:MilliQ-grade water for HPLC analysis.

The extracts were analyzed using an Agilent Zorbax SB-C18 column (3.0 x 100 mm, 3.5 μm) and ODS-C18 pre-column (4.0 x 2.0 mm). A mobile phase for PCP samples consisted of 0.01 M phosphorus acid (A) and acetonitrile (B). The gradient programme was: 0-9 min: 95% of A → 10% of A, 9-12 min 10% of A → 0 % of A, 12-17 min 100% of B. A diode array detector (DAD) was used to detect the compounds at 245 nm. Three fractions were collected with an automatic fraction collector: 0-10.2, 10.2-10.5 and 10.5-17.0 min fractions containing a more water soluble than PCP fraction, a PCP fraction and a less water

soluble than PCP chemical fraction, respectively.

A mobile phase for pyrene samples consisted of MilliQ grade water (A) and acetonitrile (B). The gradient programme was 0-3 min 50% of B, 3-4 min 50 → 100% of B, 4-10 min 100% of B. A fluorometric detector (FLD) was used to detect the compounds at 260 nm. In addition to pyrene, the run also identified 1-hydroxypyrene (1-HP) and 1-hydroxypyrene glucuronide (1-HPG).

3.7. Statistical analyses

The bioaccumulation data were used for estimating the uptake clearance coefficient (k_s), the elimination rate coefficient (k_e) and the bioaccumulation factors (BAF). The toxicokinetic parameters were estimated by plotting the chemical tissue concentration against time and fitting the data to the one-compartment, first order kinetic models (Landrum 1989).

$$C_a = \left[k_s C_s \left(1 - e^{-k_e t}\right)\right] / k_d$$

$$C_a = \left[k_s C_s{}^0 \left(e^{-\lambda t} - e^{-k_e t}\right)\right] / \left[k_e - \lambda\right]$$

Where C_a is the concentration of the compound in the organism, $C_s{}^0$ is the initial contaminant concentration in sediment, k_s is the uptake clearance of the compound from the sediment, k_d is the elimination rate constant, λ is the rate constant for the chemical to become biologically unavailable, and t is time.

The BAF values were obtained from the kinetic estimates (k_s/k_e), and from the ratio of the organism tissue concentration divided by the chemical concentration of the exposure media.

The statistical differences in endpoints between the different exposure treatments were tested by one-way analysis of variance (ANOVA) following by the appropriate post-hoc test. The normality of the data (Kolmogorov-Smirnov test) and the homogeneity of the variances (Levene's test) were checked, and if the assumptions of the parametric test were not fulfilled, the Kruskall-Wallis non-parametric test was used.

The lethal water and sediment concentrations (LC), and lethal body residues (LBR) were estimated by probit analysis (SPSS 11.0, SPSS corp., Chicago, IL, USA), or by fitting the data

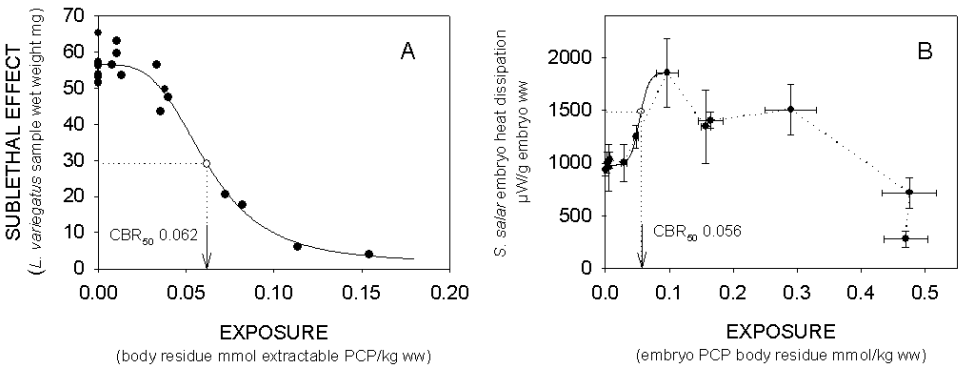

Figure 2. Example of the estimation of CBR_{50} values from the toxicity data for PCP exposed *L. variegatus* (A) (IV) and *Salmo salar* m. *sebago* (B) (II).

to the nonlinear regression model (sigmoidal dose-response) using the constraints 0 for the bottom plateau and 100 for the top plateau (GraphPad Prism 4.0, GraphPad Software, Inc., San Diego, CA, USA). The effective water and sediment concentrations (EC) and the critical body residues (CBR) were estimated by fitting the data to the sigmoidal dose-response (SigmaPlot 8.0, SPSS corp. Chicago, IL, USA, or GraphPad Prism 4.0).

$$y = \min + (\max - \min)/1 + (x/EC_{50})^{Hillslope}$$

Figure 2. shows an example of CBR_{50} estimation. The control group responds to the experimental conditions at the no-effect level of toxicity. The full-effect level for sublethal toxicity can be considered to be in the zone where the exposure is high enough to create partial mortality (Figure 2A.), or when there is a clear change in the expression of sublethal toxicity (Figure 2B.). To obtain reliable estimates for lethal and sublethal toxicity, the maximum and minimum responses are required (Kerr and Meador 1996).

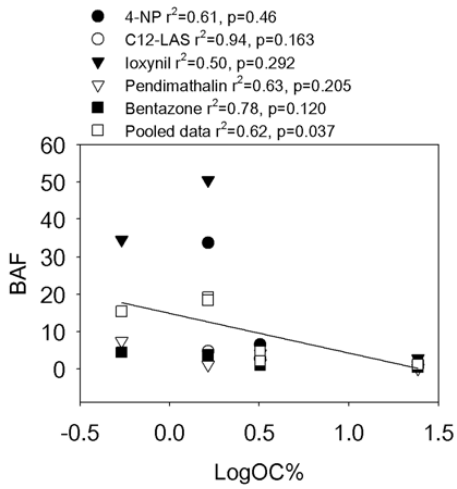

Figure 3. Bioaccumulation factors (BAF) of the test compounds plotted against the log transformed sediment organic carbon content (LogOC%). Coefficient of determination (r^2) and significance of the slope (p) from zero are given for linear regression for pooled data and each compound separately. The linear regression line is shown only for pooled data.

4. Results and discussion

4.1. Bioavailability

4.1.1. Sediment characteristics

The bioavailability of the test compounds clearly varied in the different sediments. The compound bioavailability was modified by the amount of the sediment organic carbon content (OC%) (Figure 3, I, III), which has been supposed to play an especially important role in sequestering pollutants in the non-bioavailable pool in the sediments (e.g. Landrum and Robbins 1990, Burton 1992). The bioavailability of the tested chemicals was systematically lowered when the organic carbon content of the sediment increased. The average values of the BAFs of the model compounds had a significant linear relationship with the log OC% (Figure 3.). The individual chemicals did not, however, show significant relationships. The small particle fraction did not correlate significantly with the BAFs, even for the pooled data.

The EqP theory predicts that the freely dissolved, and hence bioavailable, contaminant in the sediment pore water determines the toxic effects (Lee II 1992, Ingersol 1995, Kane Driscoll and Landrum 1997). The physical and chemical factors that control the partitioning of the chemical between the bioavailable and the non-bioavailable

pools are various. The EqP approach is often applied by normalizing the chemical concentrations based on the lipid content of the organism and the organic carbon content of the sediment (Di Toro 1991, Lee II 1992, Kane Driscoll and Landrum 1997). The EqP approach is valid for non-ionic hydrophobic organic chemicals that are not metabolized, and should theoretically predict the body residue of the exposed organism.

The normalized BSAF values are considered independent of particular sediment or species, and the equilibrium partitioning models, such as BSAF, assume that hydrophobic organic chemicals (HOC) are in equilibrium between sediment, pore water, and the organism (Di Toro 1991).

The BSAF values calculated for pendimethalin (I) clearly evened out the differences in bioavailability among the four different sediments, but generally for all the other test compounds (I, III), normalization to the organism lipid and sediment OC were not the explanatory variables for bioavailability. The BSAF values were generally higher than the theoretical value of 1-2 (Di Toro 1991), which indicates that other factors than passive diffusion through body wall were involved in the bioaccumulation process.

According to EqP theory, the acute LC_{50} estimate from water exposure should theoretically agree with the LC_{50} estimates based on the distribution of the chemical between pore water and sediment organic carbon. Lethal sediment concentrations should thus be derivable from the acute LC_{50} values using the chemical partition coefficient between water and sediment organic carbon (K_{oc}).

The measured LC_{50} values from the toxicity tests normalized to sediment organic carbon content corresponded fairly well with the calculated values for ioxynil and 4-NP (Table 4.). For bentazone the measured values are somewhat higher than calculated, and for C12-LAS the measured values are clearly lower than the calculated values. The explanation for this may be that, in the steady state, the concentration of the parent chemical is achieved, but the total chemical fraction including the metabolites and the bound chemical fraction may increase beyond the duration of the usual bioaccumulation test. Moreover, organism behaviour, such as feeding habits, is not taken into account in the EqP approach (Landrum and Robbins 1990, Granberg and Selck 2007). The sediment organic matter quality has also been proposed as a modifying factor for bioavailability (Kukkonen and Landrum 1994, Kukkonen et al. 2003).

4.1.2. Chemical characteristics

Based on the bioaccumulation factors (BAF) (I, III), the bioavailability of the tested chemicals from the sediment decreased in the order: pendimethalin < C12-LAS < bentazone < 4-NP < ioxynil. A weak relationship was found between the chemical log K_{ow} value and the uptake clearance coefficients (log k_s) (Figure 4.) as shown earlier for different PAHs (Landrum 1989). However, the chemicals used in this study belonged to different chemical classes, which may indicate that there is a general relationship between chemical uptake clearance rate and K_{ow} values. For chemical water solubility, a trend was apparent that with increasing water solubility the bioavailability of the chemical decreased, even though the relationship was not statistically significant.

Table 4. Calculated and measured sediment LC_{50} values normalized to sediment organic carbon (OC) content in *C. riparius* for bentazone, ioxynil, 4-NP and C12-LAS.

Compound	K_{oc}	$LC_{50}^{sed.\ OC}$ calc.	$LC_{50}^{sed.\ OC}$ meas.	Sediment (OC%)
Bentazone	263	68.12	880.9	3.2
			196.1	1.6
Ioxynil	339	2.71	4.44	3.2
4-NP	93325	373.3	293.2	3.2
			139.3	1.6
C12-LAS	67165	738.8	56.4	3.2
			112.8	1.6

$LC_{50}^{sed.\ OC}$ = Lethal sediment concentration for 50% mortality (mmol/kg OC dw).
$LC_{50}^{sed.\ OC}$ calc. = acute LC_{50}^{water} (table 5.) * K_{oc}
$LC_{50}^{sed.\ OC}$ meas. = $LC_{50}^{sediment}$ (Table 5.) / (sediment OC%/100)
OC% = Organic carbon content of the sediment.
K_{oc} = Chemical partition coefficient between water and sediment organic carbon (bentazone: Donigian and Carsel 1987, ioxynil: EC 2004, 4-NP: Düring et al. 2002, C12-LAS: Traina et al. 1996).

It has been reported that the log K_{ow} value plotted against the BAF values of the model compounds has shown a pattern where a chemical with intermediate hydrophobicity has the highest potential to bioaccumulate (Landrum 1989). Chemical hydrophobicity has traditionally been described by the log K_{ow} value, which has been used to predict chemical sorption to the sediment and bioavailability to organisms (Spacie et al. 1995). It is argued that the biological membranes act as a barrier to the hydrophilic chemicals, lowering the bioavailability (Gobas and Mackay 1987). High sorption affinity, e.g. to the sediment, lowers the bioavailability for compounds with a high log K_{ow} value, but their elimination from the organism may be limited due to hydrophobicity (Gobas et al. 1986).

4.1.3. Test organism behaviour

The results implied that sediment characteristics affected the uptake route of PCP (IV). The comparison of chemical residue in sediments ingested and egested was based on analyses of the radiolabel in the defecated pellets and *L. variegatus* tissues. This approach gave circumstantial evidence that sediment characteristics affected the route of exposure between the two test sediments.

Besides the exposure media and the properties of chemicals, the behaviour and other characteristics of an organism may affect bioavailability. The organisms that feed on sediment, such as *L. variegatus*, are exposed to the chemicals also through ingested sediment via the alimentary canal (Loonen et al. 1997, Leppänen and Kukkonen 1998, Conrad et al. 2002). Sediment ingestion is suggested as an important accumulation route for highly hydrophobic contaminants, while for the more water-soluble contaminants sediment ingestion is less important.

Uptake from ingested sediment appeared to be significant in the sediment (S2) with a poor nutritional value for *L.*

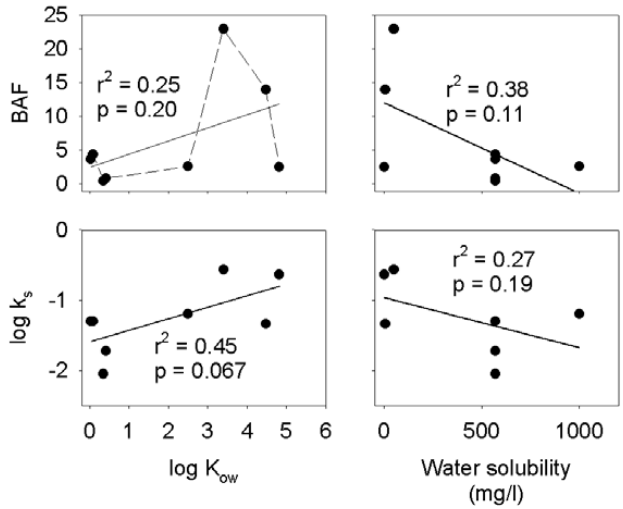

Figure 4. The measured bioaccumulation factors (BAF) and kinetic uptake rate coefficients (log k_s) plotted against model compounds' log K_{ow} and water solubility (mg/l). Coefficient of determination (r^2) and significance of the slope (p) from zero are given for linear regression slope of the pooled data.

variegatus, while in the sediment (S1) with good nutritional value, the uptake of PCP equivalents from ingested sediment in the alimentary canal was not as important a route for bioaccumulation (IV). PCP probably accumulated through the outer cuticle of *L. variegatus*.

The high pH of sediment S1 may have altered the bioaccumulation route directly, since more PCP was presumably dissolved in the pore water, thus amplifying uptake through the outer cuticle. On the other hand, PCP uptake from ingested sediment may have increased simply due to the increased ingestion rate needed to compensate for the poor nutritional value of S2.

4.2. Toxicity

Lethal body residues (LBR_{50}) for bentazone were similar, despite the different sediments in both test species, *L. variegatus* and *C. riparius* (I, Table 5.). On the other hand, the LC_{50} estimates showed great variation among exposure conditions. Similar results were obtained from the sublethal toxicity tests: CBR_{50} estimates were equal despite differences in the EC_{50} values between the different sediments (Table 6.). The results of the toxicity tests with bentazone are thus in accordance with the hypotheses of the CBR approach in that the lethal and sublethal critical body residues were constant despite differences in the exposure conditions or species (McCarty and Mackay 1993).

The results for PCP showed different effective concentrations (EC_{50}) for *L. variegatus* and *S. salar*. On the other hand, the CBR estimates for sublethal effects were similar for the two species (II, IV, Table 6.). The CBRs in *L. variegatus* were based on the measures of the extractable parent PCP. The extractable chemical fraction is in accordance with the proposed sublethal toxic residue for PCP and was assumed to be responsible for the manifestation of toxicity.

Table 5. Lethal exposure concentrations (LC_{50}) and lethal body residues (LBR_{50}) for 50% mortality in C. riparius and L. variegatus for bentazone, ioxynil, 4-NP and C12-LAS.

Organism	Compoud	LC_{50}	95% CL	LBR_{50}	95% CL	Exposure media (sediment OC%)	Exposure time (days)
L. variegatus	bentazone	0.329	0.304-0.349	2.849	2.351-3.551	water	2
C. riparius	bentazone	0.259	0.226-0.295	3.676	2.653-6.991	water	2
C. riparius	bentazone	28.19	20.22-39.66	4.189	3.622-5.498	sediment (3.2)	10
C. riparius	bentazone	3.137	1.029-4.310	2.876	2.375-4167	sediment (1.6)	10
L. variegatus	ioxynil	0.005	0.005-0.005	0.267	-	water	2
C. riparius	ioxynil	0.008	0.007-0.01	0.249	0.17-3.418	water	2
C. riparius	ioxynil	0.142	0.100-162	0.058	0.040-0.441	sediment (3.2)	10
L. variegatus	4-NP	0.006	0.006-0.007	11.56	9.89-15.28	water	2
C. riparius	4-NP	0.004	0.003-0.005	3.742	3.102-4.442	water	2
C. riparius	4-NP	9.383*	8.623-10.14*	-	-	water	10
C. riparius	4-NP	2.228*	1.723-2.732*	-	-	sediment (3.2)	10
L. variegatus	C12-LAS	0.006	0.005-0.006	14.26	6.877-20.5	water	2
C. riparius	C12-LAS	0.011	0.01-0.014	0.816	0.68-1.124	water	2
C. riparius	C12-LAS	1.976*	1.788-2.163*	0.287	0.160-3.087	sediment (3.2)	10
C. riparius	C12-LAS	1.633*	1.278-1.988*	-	-	sediment (3.2)	10
C. riparius	C12-LAS	1.804*	1.636-1.972*	-	-	sediment (1.6)	10

LC_{50} = Lethal sediment (mmol/kg dry weight) or water concentration (mmol/L) for 50% mortality.
LBR_{50} = Lethal body residue (mmol/kg wet weight) for 50% mortality.
95% CL = 95% confidence limits of estimate.
OC% = Organic carbon content of sediment.
*Estimated by fitting to sigmoidal dose-response regression curve using constraints 0 for bottom plateau and 100 for top plateau.

The *S. salar* embryo body residue is assumed to concern parent pentachlorophenol due to the short exposure duration in the toxicity experiment and the early life stage of the organism. Based on these assumptions, the results for PCP are in accordance with the hypotheses of the CBR approach in that the CBR_{50}-values were close to identical, despite the differences in species (*S. salar*/*L. variegatus*), exposure time (2/28 days), exposure media (water/sediment) or the endpoint measured (growth/reproduction/heat production).

The CBR_{50} estimate in *L. variegatus* exposed to water-borne pyrene is in agreement with the values proposed for non-polar narcotic compounds (McCarty and Mackay 1993, Connell et al. 1999). However, there is great variability in the estimates proposed for narcotic compounds, and whether or not the CBR_{50} estimates based on parent pyrene or pyrene equivalent are concerned, both fit into the scale proposed for non-polar narcotic compounds. Nevertheless, the current estimates remain below the concentrations proposed for lethal concentration in this mode-of-toxic-action category.

The toxicity test results for ioxynil, 4-NP and LAS show that the exposure conditions influenced the LBR and CBR values, i.e. the species reacted with different body residues to exposure to these chemicals (I, III, Table 5., Table 6.). Thus the toxicity estimates for these chemicals were not in accordance with the hypotheses of the CBR approach.

4.2.1. CBR and exposure time

In pyrene-exposed *L. variegatus* CBR values decreased, except at the last time point (15 days), when a higher CBR value was recorded. On the last day of the experiment, the organisms in the highest exposure concentration did, however, show the strongest narcotic sublethal effect (decreased pulse rate). In general, the study showed that susceptibility to pyrene increased over time. Similar results have been published for fluoranthene-exposed *Hyalella azteca*, *Chironomus tentans* and *Diporeia spp.*, (Schuler et al. 2004), and for pentachlorobenzene-exposed *H. azteca* (Landrum et al. 2004), in which lethal residues (LR_{50}) decreased over time. Although the opposite results have also been obtained (Landrum et al. 2003), it is probably simply accepted that the toxicity may change over time (Lee et al. 2002, Lee and Landrum 2006).

It has been suggested that the decrease in the dorsal blood vessel pulse rate is a response to an increase of pyrene in the lipid membranes. Lipid membrane has been proposed as a site of toxic action for narcotic substances (van Wezel and Opperhuizen 1995). The decrease in CBR values in pyrene-exposed *L. variegatus* may indicate that a steady state of pyrene was not reached at the site of toxic action, i.e. in the lipid membranes, even though an apparent steady state was reached when total body residue was measured. Thus it seems that pyrene concentration in the *L. variegatus* lipid membranes was probably increasing during the test, this being which was measured as lowering pulse rate over time (V). The chemicals that have a log K_{ow} above 3.3, such as pyrene, have been shown to partition preferentially into triglyceride (non-membrane) lipids (Sandermann Jr. 2003). This may indicate that bioaccumulated pyrene may diffuse slowly from storage lipids to target lipids, increasing the chemical load at the site of toxic action.

It has been suggested that the temporal variability in lethal body residues for PAH exposed aquatic invertebrates is

Table 6. Effective exposure concentration (EC_{50}) and critical body residues (CBR_{50}) for 50% inhibition in sublethal toxic effect in C. riparius, L. variegatus and S. salar for bentazone, ioxynil, C12-LAS, 4-NP, PCP and pyrene. The values are based on chemical-equivalents unless otherwise stated.

Organism	Compound	Endpoint	EC_{50}	95% CL or SE	CBR_{50}	95% CL or SE	Exposure media (sediment OC%)	Exposure time (days)
C. riparius	bentazone	growth	3.195	-14.13-20.52	1.291	-0.553-3.136	sediment (3.2)	10
		head capsule length	2.288	0.418-4.158	0.616	-0.348-1.579		
C. riparius	bentazone	growth	0.045	-1.018-1.108	1.821	-3.446-7.088	sediment (1.6)	10
		head capsule length	0.487	-0.369-1.342	2.435	-1.252-6.123		
C. riparius	ioxynil	growth	0.043	0.029-0.057	0.046	-0.263-0.356	sediment (1.6)	10
C. riparius	C12-LAS	growth	1.488	0.442-2.534	0.172	SE=0.045	sediment (3.2)	10
		head capsule length	1.621	0.442-2.801	0.256	SE=0.065		
C. riparius	C12-LAS	growth	1.562	-0.199-3.323	-		sediment (3.2)	10
		head capsule length	2.293	0.038-4.548	-			
C. riparius	C12-LAS	growth	2.158	1.852-2.464	-		sediment (1.6)	10
		head capsule length	2.786	0.196-5.377	-			
L. variegatus	PCP	growth	0.006	SE=0.002	0.062* 3.42	SE=0.006 SE=0.33	sediment (1.8)	28
		reproduction	0.026	SE=0.066	0.057* 3.15	SE=0.010 SE=0.52		
S. salar	PCP	heat output	0.769	0.326-1.212	0.056	0.047-0.065	water	2
L. variegatus	Pyrene	blood vessel pulse	0.000076	-0.000042- 0.00019	0.118* 0.869	SE=0.349 -0.224-1.963	water	15

EC_{50} = Effective sediment (mmol/kg dry weight) or water (mmol/L) concentration for 50% effect at the endpoint.
CBR_{50} = Critical body residue (mmol/kg organism wet weight) for 50% effect at the endpoint.
95% CL = 95% confidence limits for estimate.
SE = standard error.
OC% = Organic carbon content of sediment.
Sublethal endpoints: growth = wet weight at the end of the experiment.
head capsule length = head capsule length from back part to mandibles at the end of the experiment.
reproduction = number of organisms at the end of the experiment
heat output = organism heat output at the end of the experiment.
* The estimate is based on the extractable parent compound.

controlled not only by toxicokinetic processes but also in association with toxicodynamic processes (Lee et al 2002a, Lee et al. 2002b, Schuler et al. 2004). In other words, toxicity is dependent on the chemical concentration at the site of toxic action, which in turn is dependent on exposure concentration and time (Rozman and Doull 2000). Death therefore occurs when the chemical reaches critical concentration at the site of toxic action, whether or not the steady state whole body concentration is reached in the dose-response experiment. Thus it takes a longer time period before death occurs in the chronic (lower exposure concentration) test compared to the acute test (higher exposure concentration). Moreover, elimination of the chemical from the organism takes place especially in the chronic tests, which may further prolong the manifestation of the lethal effect.

This phenomenon is probably seen in the pyrene-exposed *L. variegatus* (IV). The blood vessel pulse rate decreased over time even though an apparent steady state of pyrene in the organism had been reached. Thus, the manifestation of the toxic effects was determined by the balance between the exposure concentration, the chemical concentration at the site of toxic action and time.

The CBR approach assumes that the effective body residue is constant and independent of exposure time. It has been proposed, however, that temporal variability in toxic body residues is accounted for in the CBR approach in that the body residues for acute and chronic toxicity are different (Lee et al. 2002b). The acute to chronic ratio has been estimated at about 10-12 for lethal endpoint (Kenaga 1982, McCarty 1986, Rand et al. 1995).

There was no clear difference between acute and chronic LBRs in bentazone-exposed *C. riparius*, which may indicate that the mode of toxic action remained the same over time and that bentazone was not biotransformed (Table 5.). For ioxynil- and C12-LAS-exposed *C. riparius*, the acute to chronic toxicity ratio was 4.3 and 2.8, respectively (Table 5.), which may in turn indicate that the chemical mode of toxic action changes, or the chemical is biotransformed and consequently toxico-kinetic and toxico-dynamic processes affect toxicity and LBRs. The low body residue of PCP increased the heat production in *S. salar* embryos, but at higher body residues heat production decreased (II, Figure 2B). This clearly indicates that a certain threshold concentration was crossed, and the expression of toxicity changed. In this case the CBR value is meaningful as a means of evaluating the sensitive toxic effect, as it probably already has deleterious effects on organism survival.

4.2.2. CBR in different species

The LBR_{50} and CBR_{50} values were consistent in different species for bentazone and PCP (Tables 4. and 5., I, IV). For ioxynil, the LBRs were consistent in the acute test (I). According to LBR_{50} estimates, *C. riparius* was more sensitive to both of the surfactants, 4-NP and C12-LAS, than *L. variegatus* (III). The results in this case both agree and disagree with the hypotheses of the CBR approach that the body residues should be constant in different species. It has been stated, however, that differing composition and function among species may cause variability in the CBRs even though the chemical concentration at its site of toxic action was constant (Rand et al. 1995).

In the other researches, *L. variegatus* and *C. riparius* exposed acutely to PCP had different LBR_{50} values, *C. riparius* being more sensitive to toxic stress (Kukkonen 2002). Similarly, *C. tentans*

appeared more sensitive according to lethal body residues compared to two amphipod species, *H. azteca* and *Diporeia spp*. when exposed to fluoranthene (Schuler et al 2004). The amphipod species did, however, have equal lethal body residues. It was concluded that a specific mode of toxic action through toxic metabolites was involved in the manifestation of toxicity, which accounts for the differences in LBRs between the chironomids and the amphipods.

This may explain the differences in LBR and CBR between *L. variegatus* and *C. riparius* for 4-NP and C12-LAS. The chironomids are assumed to have a more developed biotransformation system for metabolizing xenobiotics than the oligochaetes (Verrengia-Guerrero 2002, Schuler et al. 2003), which in turn may indicate that *C. riparius* produced toxic metabolites and was therefore more susceptible to the model compounds. The better biotransformation capability usually appears, however, as faster elimination and lower toxicity (Timbrell 2000).

It has been argued that the whole body residue is already a sufficiently good descriptor of exposure to narcotic compounds (Landrum et al. 1992), being more valuable when normalized to lipid concentration of the organism (Escher and Hermens 2004). Thus, another possible explanation may be offered by the target lipid model, which proposes that variations in toxicity are due to differing species sensitivities and chemical differences (Di Toro et al. 2000). The target lipid model is based on the idea that a target lipid is the site of action in the organism, and that mortality occurs when the chemical concentration in the target lipid reaches a threshold concentration. The threshold concentration is assumed to be species-specific rather than a constant that is applicable to all organisms. For example, rainbow trout (*Oncorhynchus myciss*) was shown to be more susceptible to dihalogenated benzenes (narcotics) compared to guppy (*Poecilia reticulata*) and fathead minnow (*Pimephales promelas*) as regards lethal body residues (LBB_{50}) (van Wezel et al. 1995a). The inter-species differences were associated with differences in the composition of the storage lipids and membrane lipids.

It has been suggested that the total lipid content of an organism alters critical or lethal body residue (Kane Driscoll and Landrum 1997). On the other hand, different LBRs were measured for two benthic copepods, *Schizopera knabeni* and *Coullana* sp., exposed to fluoranthene, and the differences could not be explained by differences in organism lipid contents (Lotufo 1998). The total lipid content explained 50% of the variation in LBRs in fathead minnows exposed to the narcotic substances, di- and trichlorobenzenes, tetrachloroethane and polychlorinated biphenyls (PCB) (van Wezel et al. 1995b).

The lipid partitioning of the narcotic compounds has been shown to be temperature-dependent (van Wezel and Opperhuizen 1995), which consequently makes it more difficult to compare the experimental data. In the current research, the experimental temperature was the same (+ 20ºC) in all the experiments, and therefore the partitioning of the chemical was not affected by temperature. The total lipid concentration was assumed to be approximately the same in *L. variegatus* and *C. riparius* (0.6-1.53% of fresh weight) (III). Egg lipid content of 13% is measured for Atlantic salmon (*Salmo salar*) (Hamor and Garside 1977), which is closely related to Lake salmon. Lake salmon probably has similar egg lipid content. The embryo lipid content is assumed much lower since in the egg the

lipids are mostly found from the yolksac (Kamler 1992). Consequently, due to similar CBRs in the sublethal toxicity to PCP, the lipid contents of *L. variegatus* and *S. salar* embryo were expected to be in the same range.

4.2.3. CBR and environmental and chemical characteristics

The exposure route, whether sediment or water, was not assumed to affect critical body residues of PCP when *L. variegatus* and *S. salar* were compared, the CBRs being similar even though the exposure media were different. This is supported by previous studies, which have shown that lethal body residues were consistent when *L. variegatus* were exposed to PCP in the water-only medium and in two different sediments (Nikkilä et al. 2003). Further, the different exposure concentrations were found not to affect LBRs in the earthworm *Eisenia fetida* when exposed to PCP (Fitzgerald et al. 1997). In addition, LBR_{50} values for PCP in mussel *Dreissena polymorpha* were consistent, even though exposure temperature and pH varied (Fisher et al. 1999).

The differences between the acute and the chronic tests in *C. riparius* as regards lethal body residues of ioxynil indicate that the exposure media had an effect on the LBRs. Ioxynil is a weak acid and therefore pH affects its bioavailability and toxicity. As bioavailability is explicitly related to the measure of LBRs, the intra-cellular pH may have affected the differing LBRs in *C. riparius*. The intra-cellular pH has been shown to decline in zebra mussels *Dreissena polymorpha* exposed to the molluscicide Bayluscicide compared to unexposed organisms (O'Donnell et al. 1996). It has been proposed that the decline of intra-cellular pH alters PCP toxicity and CBRs in zebra mussels (Fisher et al. 1999). It is possible that the 10-day exposure period to ioxynil caused more damage in *C. riparius* and caused the decline in intra-cellular pH compared to the 2-day exposure period. Consequently, more ioxynil was in the hydrophobic form in the chronic test, causing the increased toxic potential of the bioaccumulated chemical. This assumption should not be considered without qualification, since the intra-cellular pH of *C. riparius* was not measured.

4.3. Biotransformation

4.3.1. Pyrene and PCP

The results are in accordance with suggestions that the oligochaetes are able to biotransform pyrene (Leppänen and Kukkonen 2000) and PCP (IV, Knuutinen et al. 1990, Livingstone 1998). The pyrene and PCP-exposed *L. variegatus* tissue extracts were analyzed by HPLC in this study, giving as results the fractions of parent chemicals and metabolites. The amount of the unextractable fraction was substantial, especially in the case of PCP.

For the pyrene-exposed *L. variegatus*, 1-hydroxypyrene (1-HP), which was assumed to be a phase-I metabolite, was found in the tissue extracts. Similar results were reported for the *L. variegatus* exposed to pyrene-spiked sediment (Lyytikäinen et al. manuscript). This finding was assumed as proof that a phase-I like oxidizing enzyme system is present and responsible for metabolizing the pyrene in *L. variegatus*. The oligochaetes contain cytochrome p-450 (CYP-450) enzyme, which metabolizes endo- and exogenous compounds in the organism (Di Giulio et al. 1995, Livingstone 1998, Lee 1998). It is then suggested that the P-450 enzymes are responsible for the biotransformation of pyrene.

There are other findings that support this proposition; *Eisenia andrei* (oligochaeta), have also been shown to be able to metabolize pyrene to 1-HP (Stroomberg et al. 2004). 1-hydroxypyrene glucuronide (1-HPG) was not present, which is consistent with the results of the current study showing that 1-HPG was not present in the pyrene-exposed *L. variegatus* tissue extracts. The marine polychaete *Nereis virens* has been shown to have a high capability for metabolizing pyrene to 1-HP, glucuronidation in the phase II being the most prominent pathway for biotransformation (Giessing et al. 2003, Jørgensen et al. 2005). Springtail *Orchetella cincta* also metabolized pyrene to 1-HP and further to glucoside conjugates (Howsam and Straalen 2003).

The *E. andrei* biotransformation products consisted of three conjugates of 1-HP (Stroomberg et al. 2004). Thus the unknown peaks detected in pyrene-exposed *L. variegatus* tissue in this study may have consisted of 1-HP conjugates similar to *E. andrei*. On the other hand, the developed HPLC method was employed in a recent analysis of pyrene-exposed *Salmo salar* m. *sebago* fries. In this study, a similar peak was identified in a sulphate conjugate of pyrene (Honkanen et al. manuscript). Thus, the unknown peaks may have included the sulphate conjugate of pyrene in the *L. variegatus* tissue extracts.

The results for PCP-exposed *L. variegatus* showed that most of the extractable compounds were hydrophilic biotransformation products, but a fraction of more lipophilic biotransformation products were also found. PCP contains the –OH group and therefore bypasses phase-I reactions and goes straight to phase-II reactions. Pentachloroanisole and 2,3,4,6-tetrachloroanisole were proposed as the lipophilic biotransformation products, and it was suggested that a series of different hydrophilic biotransformation products (pentachlorophenylsulfate, pentachloro-beta-D-glucoside and tetrachloro-p-hydroquinone) found in other organisms exposed to PCP accounted for the water-soluble metabolite products. It has been also shown that reactive oxygen species are generated in the PCP metabolism (Dahlbaus et al. 1996, Po-Hsiung et al. 2002). These intermediates have been shown to be carcinogenic.

Even though contradictory results have also been published on the biotransformation capability of pyrene and PCP in *L. variegatus* (Verrengia-Guerrero 2002), it appears to be certain that at least a moderate biotransformation capability exists.

4.3.2. Tissue-bound residue

The chemical fractions that were not extractable were high; 77-86% for PCP (IV) and 7-56% (average 29.4% SD 11.8, n=70) for pyrene. The radiolabel that remained bound in the tissue may either be the parent chemical or the metabolite. Thus the biotransformation studies raise questions, including the identity of the compounds that remained bound in the tissue and the role of this fraction in toxicity.

In another study where *L. variegatus* was exposed to pyrene, the unextractable radiolabel was assumed to be a product of biotransformation. In this study, parent pyrene was observed to depurate rapidly from the tissues (Leppänen and Kukkonen 2000). In invertebrates in general PAH metabolites have been found to be eliminated more slowly than the parent chemical (James 1989).

Solvent extraction should remove all lipids, and thus it is suggested that the unextractable chemical fraction is covalently bound to proteins or other macromolecules rather than to lipid

material (Belden et al. 2005). A significant proportion of benz(a)anthracene (33-51%) was found to be unextractable from polychaeta *Nereis virens* tissues, and it was suggested that the bioaccumulated chemical was incorporated into the macromolecular pool in the organism (Belden et al 2005).

It is proposed that some oxidized products of PAHs from phase-I, such as diols and phenols, may be further oxidized to diol-epoxides and phenol-oxides (Meador et al. 1995, Livingstone 1998). Some of these transformation products are carcinogenic and bind covalently with DNA, RNA and proteins. It may be thus possible that the tissue-bound fraction in pyrene-exposed *L. variegatus* is composed of those metabolite products of pyrene that are bound covalently to macromolecules and not to lipids.

PCP has actually been shown to bind proteins forcefully (Gülden et al., 2002). This information, together with the finding that the considerable proportion of PCP was in the unextractable fraction, indicates that parent PCP may have been bound covalently to the protein fraction in *L. variegatus*.

After all, it still remains unclear whether the unextractable chemical fraction was the parent chemical or some product of biotransformation, and it remains unsolved whether the bound chemical contributes to the measured toxic effects. The chemical extraction methods should therefore be developed in order to allow characterization of the poorly extractable chemical fractions. Knowledge of the full composition of the chemical fractions could reveal the fraction causing the toxic effects.

4.3.3. The other model compounds

The chemical residues for bentazone, pendimethalin, ioxynil, 4-NP and C12-LAS were based on total radiolabel concentration in the organism tissues (I, III). The radiolabelled model compounds are used collectively in the bioavailability and toxicity studies in ecotoxicological research. For the compounds that are not easily metabolized by aquatic invertebrates, as suggested for C12-LAS (Hwang et al. 2003), the evaluation of the body residue response relationship is straightforward using the method based on the radiolabel. For the compounds that are metabolized, analysis of the radiolabel by liquid scintillation counting introduces uncertainties into the results, as the amount of metabolites is not known.

Studies that quantify chemical body residues reporting only the parent compound may underestimate total bioaccumulation of the parent compound and metabolites (Kane Driscoll and McElroy 1996). On the other hand, LSC analysis may overestimate the body residue by assuming that all of the chemical is parent. Biotransformation is found to be probable extensively in the animal kingdom, even in the most primitive test animals (Snyder 2000), should therefore be considered.

Knowledge of biotransformation, i.e. concentration of the parent chemical(s) and the metabolite product(s) in the tissues of the organism, enhances the accuracy of estimating body residue concentrations. On the other hand, it raises the complication of deciding which pool of the chemical fraction contributes to the toxic effects (Lee and Landrum 2006).

4.4. Mode of toxic action

4.4.1. Bentazone, 4-NP, C12-LAS and pyrene

According to the CBR approach, the LBR_{50} and CBR_{50} values for bentazone

indicate that it acts as a narcotic chemical (McCarty and Mackay 1993). Similarly, the LBR_{50} estimates for 4-NP indicate that it acts acutely through narcosis, which has also been proposed earlier as its mode of toxic action (Matozzo et al. 2004). 4-NP is also an endocrine disruptor having long-term adverse effects (White et al. 1994), but probably at lower concentrations.

The high toxicity values for C12-LAS show that it also has a narcotic mode of toxic action, which has also been suggested previously (Hwang et al. 2003). The variability between the organisms in acute LBR_{50} may, however, signify that the organisms' characteristics (structure and function) have an effect on C12-LAS toxicity (Rand et al. 1995).

It has been shown that differences exist among species as regards their sensitivity to chemicals (Vaal et al. 1997). Moreover, for those compounds that exert their toxicity through a specific receptor, it is suggested that different species respond at different body residues of a particular toxicant, depending on the specific characteristics of the receptor and the toxicant. Thus, 4-NP and C12-LAS may have different modes of toxic action or they may act through different specific receptors. The chemical characteristics of 12C-LAS may lead to binding of the chemical on the organism surface. The surface-bound chemical fraction has therefore been considered as bioaccumulated, even though it may not have significant toxic effects.

CBR_{50} for pyrene in *L. variegatus* fall into the category proposed for non-polar narcotic compounds (McCarty and Mackay 1993). Besides acting through non-polar narcosis, pyrene has been shown to act also through activation of the aryl hydrocarbon receptor, inducing P-450 enzymes expression in vertebrates (Hahn 1998, Incardona et al. 2006).

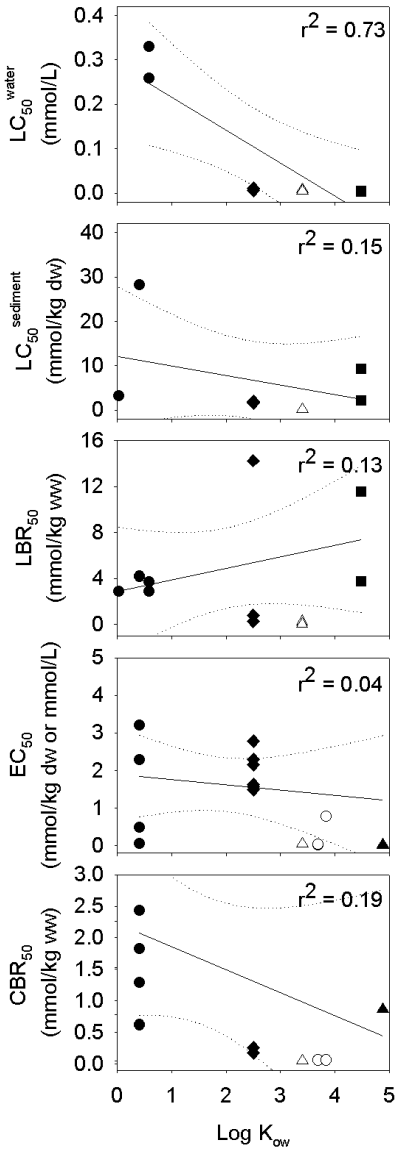

Figure 5. The chemical log K_{ow} plotted against estimated toxicity values. The solid and dotted lines show the linear regression with 95% confidence intervals (•bentazone, Δioxynil, ■4-NP, ♦C12-LAS, ○PCP and ▲pyrene). The regression is drawn for narcotic chemicals (black dots).

The results show that log K_{ow} values for narcotic chemicals correlate negatively, although not statistically significantly, with LC_{50} estimates (Figure 5.). It is suggested that, after being taken up by the organism, the more lipophilic chemicals are more effectively distributed to the site of toxic action in the organisms compared to water-soluble chemicals, which may remain in the water phase in the tissues. The narcotic chemicals' log K_{ow} values do not correlate significantly with the LBR_{50} or CBR_{50} estimates as expected (Figure 5.).

The baseline toxicants have reported to result the lowest toxicity and highest effective body residues (McCarty and Mackay 1993, Connell et al. 1999). Further, the baseline toxicity or narcosis has commonly been divided into different subcategories based on the chemical structure and degree of toxicity of the xenobiotic, namely into polar and non-polar narcosis (Russom et al. 1997). Recent research proposes, however, that there is no difference in lipid membrane concentrations of the polar or non-polar narcotics, and thus equal intrinsic toxic potency is encountered for these two subcategories (Escher and Schwarzenbach 2002).

4.4.2. Ioxynil and PCP

Ioxynil and PCP have a specific mode of toxic action, in that they act as uncouplers of oxidative phosphorylation. The acute LBR_{50} estimates and CBR_{50} estimate for ioxynil are in accordance with the proposed values for respiratory uncouplers. *C. riparius* that had been exposed to sediment-spiked ioxynil for 10 days, had an LBR_{50} estimate that agrees with the chronic values proposed for respiratory uncouplers (0.00015-0.094 mmol/kg ww). Thus, it is suggested that the 10-day exposure period may have been long enough for ioxynil to elicit chronic toxic effects by uncoupling oxidative phosphorylation.

The slight difference between sublethal response (CBR_{50} 0.046 mmol/kg ww) and lethal response (LBR_{50} 0.058 mmol/kg ww) in the case of ioxynil may be linked with the suggestion that only a slight increase in toxic stress caused by a respiratory uncoupler may have significant effects (Willows 1994). In the case of ioxynil toxicity to *C. riparius*, sublethal effects are encountered with a slight increase in body residue.

The CBR_{50} estimates for PCP in *L. variegatus* and *S. salar* are in accordance with the estimates for respiratory uncouplers in chronic exposures. It is supposed, however, that the body residue based on extractable PCP in *L. variegatus* accounts for the toxic effects. Further, it was presumed that due to the short exposure period, no significant biotransformation of PCP occurred in *S. salar* embryo, although the fish embryo has some biotransformation capability at the early stage (Goksøyr and Husøy 1998). Therefore the majority of the radiolabels measured in the fish embryo tissues were presumed to be parent PCP. It is suggested that the specificity of the toxic mode may have had the effect that the CBRs were equal despite the differences in the organisms, exposure media, time and the toxicity endpoint measured.

In the environment, organisms are mostly exposed chronically to low concentrations of xenobiotics. The modes of toxic action should therefore be studied more carefully using low exposure concentrations and long exposure periods, and by investigating the sensitive sublethal endpoints of toxicity.

5. Applications of critical body residues and future prospects

The important goal of the ecotoxicological research is to yield information on the fate and effects of chemicals in the environment for the needs of chemical risk evaluation and assessment. A feasible way to use the CBR data in practice is to improve the ecological risk assessment (ERA) of contaminated sites by comparison of laboratory established CBRs with the tissue residue measures from the field. This study showed that the critical body residues of ecologically relevant sublethal toxicity endpoints could be generated, which therefore enables the direct comparison of laboratory and field data. ERA based on sublethal CBRs would enhance the quality of risk estimates and lower the uncertainty of the assessment, attenuating the importance of the safety factors.

As the CBR approach has not yet been fully tested and validated for most of the chemicals, do not exclude the usefulness of the CBRs in the ERA process. The determinations of the CBRs for more chemicals belonging to different mode-of-action groups are needed, with subsequent evaluation of the CBR approach. More work is also needed to establish the sensitive toxicity endpoints fundamentally important to a population's survival. In this respect, the genotoxic approaches are promising (Snell et al. 2003).

6. Concluding remarks

Due to pollution of the environment, there is a need for prediction of the hazard that pollutants may pose to organisms. Environmental risk assessment is commonly based on the data of toxicity tests conducted with standard species and taking only a few environmental characteristics into account. However, predicting the toxicity of a chemical is difficult because of the differing characteristics of the environments, the chemical and the organisms. This comprises a very complex puzzle to solve.

This study focused on utilization of chemical body residue as the dose-metric for adverse effects, as a means to enhance chemical risk assessment. This study first approved observations for the model compounds used in this study showing that the evaluation of toxicity based on knowledge of the chemical concentrations in the exposure media is misleading, because the characteristics of the ambient medium affect chemical bioavailability. The results indicated that the body residue of the model compounds is a more precise metric for exposure than the concentration of the ambient medium when chemical toxicity is evaluated, as it reduces the variability in toxicity caused by environmental factors.

The main findings of this thesis were that body residue estimates for bentazone, and PCP were consistent, even though the estimates relating to the exposure media varied and the bioavailability of the chemical was variable among the different sediments. For ioxynil, 4-NP and C12-LAS, factors relating to the characteristics of the exposure media, the chemical or the organisms confounded the results in such a way that the body residues also varied. Some of the results are then in accordance with the hypotheses of the CBR approach. The factors that may have affected body residues were related to the exposure conditions and/or species characteristics.

The biotransformation studies showed that *L. variegatus* has a moderate capability to biotransform PCP and pyrene. The measure of the parent chemical and the metabolites enabled a more accurate body residue estimate for

these chemicals. However, it was stated that the biotransformation of the chemical by the organism complicates the evaluation of toxicity due to possible toxic effects of the metabolites.

Acknowledgements

I wish to express my gratitude to my supervisors, Academy Professor Jussi Kukkonen, Professor Matti Leppänen and Professor Ismo Holopainen, for their help and patience during this work. Jussi kindly offered me the opportunity to gain insight into ecotoxicology, guiding me through many different projects during these years. Matti has put large amount of effort helping me in my research. Ismo has improved the language of my papers with great exactitude.

The staff of the Faculty of Biosciences, University of Joensuu, is acknowledged for providing facilities and friendly atmosphere to carry out my research during these years. Prof. Albert A. Koelmans and Docent Matti Viluksela are acknowledged for pre-examination of this thesis, and Rosemary McKenzie kindly checked the language.

My acknowledgements are due to the Finnish Graduate School in Environmental Science and Technology (EnSTe) for support. Funding was also provided by NordTest (projects 1522-01 and 1570-02) and the Academy of Finland (projects 206071, 73166 and 214545).

I owe humble gratitude to all members of Toxiboxi for help and adviced during these years. Certainly, I wouldn't have finished this thesis ever without help I got from many people. Many ecotoxicological and not as ecotoxicological issues have been talked through with my long-term roommate Arto, accompanied with Jaska, Jani and Pete who have also helped and shared many quality conversations with me, pushing me towards the goal. Merja has also given much help and advices. Marja and Julia are greatly obliged, as they have offered invaluable help in the laboratory whenever needed. I thank Olli-Pekka for being advisor in the very beginning of my scientific career. Jussi, Matti, Arto, Merja, Olli-Pekka and Karoliina earn word of thanks for competent co-authoring of the articles.

My parents, Ulla and Tapsa, earn my warm gratitude for creating the foundation for my interest in biology. I also thank my brothers, Lasse and Pete, and my sister, Tuire, for support.

I am deeply indebted to my beloved wife Maarit who has encouraged me in this project with firm faith, being also a critical reviewer of my papers. My dear son Jaakko has helped me to relax by diverting my mind off the thesis regularly. I also appreciate the help of my unborn baby who has set the ultimate deadline for this thesis.

References

Ahel M, Schaffner C and Giger W 1996. Behaviour of alkylphenol polyethoxylate surfactants in the aquatic environment – III. Occurrence and elimination of their persistent metabolites during infiltration of river water to groundwater. Wat Res 30: 37-46.

Arcand Y, Hawari J and Guiot SR 1995. Solubility of pentachlorophenol in aqueous solutions: the pH effect. Wat Res 29: 131-136.

ASTM 2006a. E1688-00a Standard guide for determination of the bioaccumulation of sediment-associated contaminants by benthic invertebrates. Book of standards vol. 11.05. Philadelphia, USA.

ASTM 2006b. E1706-05 Test method for measuring the toxicity of sediment -associated contaminants with freshwater invertebrates. Book of standards vol. 11.05. Philadelphia, USA.

ATSDR 1995. Toxicological profile for polycyclic aromatic hydrocarbons. US Department of Health and Human Services, Agency for Toxic Substances and Disease Registry. Atlanta, Georgia, USA, pp. 458.

Barron MG, Hansen JA and Lipton J 2002. Association between contaminant tissue residues and effects in aquatic organisms. Rev. Environ Contam Toxicol 173: 1-37.

Belden JB, Ownby DR, Lotufo GR and Lydy MJ 2005. Accumulation of trinitrotoluene (TNT) in aquatic organisms: Part 2-Bioconcentration in aquatic invertebrates and potential for trophic transfer to channel catfish (*Ictalurus punctatus*). Chemosphere 58: 1161-1168.

Brinkhurst RO and Jamieson GM 1971. Aquatic oligochaeta of the world. Oliver & Boyd, Edinburgh, UK.

Brown PJ, Long SM, Spurgeon DJ, Svendsen C and Hankard PK 2004. Toxicological and biochemical responses of the earthworm *Lumbricus rubellus* to pyrene, a non-carsinogenic polycyclic aromatic hydrocarbon. Chemosphere 57: 1675-1681.

Burton GA Jr 1992. Sediment toxicity assessment. Lewis Publishers, Chelsea, USA.

Canadian Council of the ministers of the environment 2002. Canadian water quality guidelines for the protection of aquatic life – nonylphenol and its ethoxylates. In: Canadian environmental quality guidelines 1999. Canadian council of the ministers of the environment, Winnipeg, Canada.

Chapman P 2001. Utility and relevance of aquatic oligochaetes in ecological risk assessment. Hydrobiologia 463: 149-169.

Connell DW, Chaisuksant Y and Yu J 1999. Importance of internal biotic concentrations in risk evaluations with aquatic systems. Mar Poll Bull 39: 54-61.

Conrad AU, Comber SD and Simkiss K 2002. Pyrene bioavailability; effect of sediment-chemical contact time on routes of uptake in an oligochaeta worm. Chemosphere 49: 447-454.

Cooney JD 1995. Freshwater tests. In: Fundamentals of aquatic toxicology, effects, environmental fate and risk assessment (Ed. Rand GM). Taylor & Francis, Washington D.C., USA.

Cornelissen G, van Noort PCM and Govers HAJ 1997. Desorption kinetics of chlorobenzenes, polycyclic aromatic hydrocarbons, and polychlorinated biphenyls: sediment extraction with Tenax® and effects of contact time and solute hydrophobicity. Environ. Toxicol. Chem. 16: 1351-1357.

Cornelissen G and Gustafsson Ö 2005. Prediction of large variation in biota to sediment accumulation factors due to concentration-dependent black carbon adsorption of planar hydrophobic organic compounds. Env. Toxicol. Chem. 24: 495-498.

Crosby DG, Beynon KI, Greve PA, Korte F, Still GG and Vouk JW 1981. Environmental chemistry of pentachlorophenol. Pure Appl Chem 53: 1051-1080.3.

Czaplicka M 2004. Sources and transformations of chlorophenols in the natural environment. Sci Tot Environ 322: 21-39.

Dahlbaus, M., Almstadt, E., Henschke, P., Lüttgert, S., Appel, K.E., 1996. Oxidative DNA lesions in V79 cells mediated by pentachlorophenol metabolites. Arch. Toxicol. 70, 457-460.

Di Giulio RT, Benson WH, Sanders BM and van Veld PA 1995, Biochemical mechanisms: metabolism, adaptation, and toxicity. In: Fundamentals of aquatic toxicology (Ed. Rand GM). Taylor & Francis, Washington D.C., USA.

Di Toro DM, Zarba CS, Hansen DJ, Berry WJ, Swartz RC, Cowan CE, Pavlou SP, Allen HE, Thomas NA and Paquin PR 1991. Technical basis for establishing sediment quality criteria for nonionic organic chemicals by using equilibrium partitioning. Environ. Toxicol. Chem. 10:1541–1583.

Di Toro DM and McGrath JA 2000. Technical basis for narcotic chemicals and polycyclic aromatic hydrocarbon citeria. II. Mixtures and sediments. Environ Toxicol Chem 19: 1971-1982.

Di Toro DM, McGrath JA, Hansen DJ 2000. Technical basis for narcotic chemicals and polycyclic aromatic hydrocarbon criteria: I. Water and tissue. Environ. Toxicol. Chem. 19: 1951–1970.

Directive 2006/11/EC of the European Parliamnet and of the Council on pollution caused by certain dangerous substances discharged into the aquatic environment of the Community. February 15th 2006.

Donigian AS and Carsel RF 1987. Modelling the impact of conservation tillage on pesticide concentration in ground and surface waters. Environ Toxicol Chem 6: 241-250.

Düring R-A, Krahe S and Gäth S 2002. Sorption behavior of nonylphenol in terrestrial soils. Environ Sci Technol 36: 4052-4057.

EC 2004. Review report for the active substance ioxynil. European Commission, Health & Consumer Protection Directorate-General. SANCO/4349/2000 final.

Egeler P, Meller M, Schallnaß HJ and Gilberg D 2005. Validation of a sediment toxicity test with the endobenthic aquatic oligochaete *Lumbriculus variegatus* by an international ring test. In co-operation with R. Nagel and B. Karaoglan. Report to the Federal Environmental Agency (Umweltbundesamt Berlin), R&D No.: 202 67 429.

Egeler P, Meller M, Schallnaß HJ and Gilberg D 2006. Validation of a sediment bioaccumulation test with endobenthic aquatic oligochaetes by an international ring test. Report to the Federal Environmental Agency (Umweltbundesamt Berlin), R&D No.: 202 67 437. In press.

Escher BI and Hermens JLM 2002. Internal exposure: linking bioavailability to effects. Environ. Sci. Technol. 36: 455A-462A.

Escher BI and Schwarzenbach RP 2002. Mechanistic studies on baseline toxicity and uncoupling of organic compounds as a basis for modeling

effective membrane concentrations in aquatic organisms. Aquat. Sci. 64: 20-35.

FAO, 1991. Pesticide residues in food - 1991. Evaluations 1991. Part I - Residues. FAO Plant Production and Protection Paper, 113/1, 83-125.

FAO/WHO 1999. Pesticide residues in food – 1998. Evaluations – 1998. Part II – Toxicology. Geneva, World Health Organization, Joint FAO/WHO Meeting on Pesticide Residues (WHO/PCS/01.12).

Fisher SW, Hwang H, Atanasoff M and Landrum PF 1999. Lethal body residues for pentachlorophenol in zebra mussels (*Dreissena polymorpha*) under varying conditions of temperature and pH. Ecotoxicol Environ Saf 43: 274-283.

Fitzgerald DG, Lanno RP, Klee U, Farwell A and Dixon DG 1997. Critical body residues (CBRs): applications in the assessment of pentachlorophenol toxicity to Eisenia fetida in artificial soil. Soil Biol Biochem 29: 685-688.

Franks NP and Lieb NR 1994. Molecular and cellular mechanisms of general anaesthesia. Nature 367: 607-614.

Freire PF, Labrador V, Perez Martin JM and Hazen MMJ 2005. Cytotoxic effects in mammalian Vero cells exposed to pentachlorophenol. Toxicology 210: 37-44.

Gobas FAPC, Opperhuizen A and Hutzinger O 1986. Bioconcentration of hydrophobic chemicals in fish: relationship with membrane permeation. Environ Toxicol Chem 5: 637-646.

Gobas FAPC and Mackay D 1987. Dynamics of hydrophobic organic chemical bioconcentration in fish. Environ Toxicol Chem 6: 495-504.

Goksøyr A and Husøy A-M 1998. Immunochemical approaches to studies of CYP1A localization and induction by xenobiotics in fish. In: Fish Ecotoxicology (Eds. Braunbeck T, Hinton DE and Streit B). Birkhäuser Verlag, Basel, Switzerland.

Granberg ME and Selck H 2007. Effects of sediment organic matter quality on bioaccumulation, degradation, and distribution of pyrene in two macrofaunal species and their surrounding sediment. Mar Environ Res (in press), doi: 10.1016/j.marenvres.2007.02.005.

Gülden, M., Mörchel, S., Tahan, S., Seibert, H. 2002. Impact of protein binding on the availability and cytotoxic potency of organochlorine pesticides and chlorophenols in vitro. Toxicology 175: 201-213.

Hahn ME 1998. The aryl hydrocarbon receptor: A comparative perspective. Comp Biochem Physiol Part C 121: 23-53.

Hamor T and Garside ET 1977. Quantitative composition of the fertilized ovum and constituents parts in the Atlantic salmon *Salmo salar* L. Can J Zool 55: 1650-1655.

Holmstrup M and Krogh PH 1996. Effects of an anionic surfactant, linear alkylbenzene sulfonate, on survival, reproduction and growth of the soil-living collembolan *Folsomia fimetaria*. Environ Toxicol Chem 15: 1745-1748.

Honkanen JO, Heinonen J and Kukkonen JVK 2001. Toxicokinetics of waterborne bisphenol A in landlocked salmon (*Salmo salar* m. *sebago*) eggs at various temperatures. Env Toxicol Chem 20: 2296-2302.

Honkanen JO, Holopainen IJ and Kukkonen JVK 2004. Bisphenol A induces yolk-sac oedema and other adverse effects in landlocked salmon (*Salmo salar* m. *sebago*) yolk-sac fry. Chemosphere 55: 187-196.

Honkanen JO, Wiegand C, Kukkonen JVK. Humic substances modify accumulation but not biotransformation of pyrene in salmon yolk-sac fry. Manuscript.

Hwang H, Fisher SW and Landrum PF 2001. Identifying body residues of HCBP associated with 10-d mortality and partial life cycle effects in the midge, *Chironomus riparius*. Aquat Toxicol 52: 251-267.

Hwang H, Fisher SW, Kim K, Landrum PF, Larson RJ and Versteeg DJ 2003. Assessing the toxicity of dodecylbenzene sulfonate to the midge *Chironomus riparius* using body residues as the dose metric. Environ Toxicol Chem 22: 302-312.

Incardona JP, Day HL, Collier TK and Scholz NL 2006. Developmental toxicity of 4-ring polycyclic aromatic hydrocarbons in zebrafish is differentially dependent on AH receptor isoforms and hepatic cytochrome P4501A metabolism. Toxicol Appl Pharmacol 217: 308-321.

Ingersol CG 1995. Sediment tests. In: Fundamentals of aquatic toxicology, effects, environmental fate and risk assessment (Ed. Rand GM). Taylor & Francis, Washington D.C., USA.

IPCS 1990. Bentazone health and safety guide**,** Health and Safety Guide No. 48. United Nations environment programme, international labour organization, world health organization, Geneva, Switzerland.

James MO 1989. Biotransformation and disposition of PAH in aquatic invertebrates. In: Varanasi, U. (Ed.), Metabolism of Polycyclic Aromatic Hydrocarbons in the Aquatic Environment. CRC Press, Boca Raton, pp. 69–91.

Junghans M, Backhaus T, Faust M, Scholze M and Grimme LH 2006. Application and validation of approaches for the predictive hazard assessment of realistic pesticide mixtures. Aquat Toxicol 76: 93-110.

Kaiser KLE and Valdmanis I 1982. Apparent octanol/water partition coefficients of pentachlorophenol as a function of pH**.** Can J Chem 60: 2104-2106.

Kamler E 1992. Early life history of fish - An energetics approach. Chapman & Hall, London.

Kane Driscoll S and McElroy AE 1996. Bioaccumulation and metabolism of benzo[a]pyrene in three species of polychaete worms. Environ Toxicol Chem 15: 1401-1410.

Kane Driscoll S and Landrum PF 1997. A comparison of equilibrium partitioning and critical body residue approaches for predicting toxicity of sediment-associated fluoranthene to freshwater amphipods. Environ Toxicol Chem 16: 2179-2186.

Kenaga EE 1982. Predictability of chronic toxicity of from acute toxicity of chemicals in fish and aquatic invertebrates. Environ Toxicol Chem 1: 347-358.

Kerr DR and Meador JP 1996. Modeling dose response using generalized linear models. Environ Toxicol Chem 15: 395-401.

Knuutinen J, Palm H, Hakala H, Haimi J, Huhta V and Salminen J 1990. Polychlorinated phenols and their metabolites in soil and earthworms of sawmill environment. Chemosphere 20: 609-623.

Kukkonen JVK and Landrum PF 1996. Distribution of organic carbon and organic xenobiotics among different particle-size fractions in sediments. Chemosphere 32: 1063-1076.

Kukkonen JVK 2002. Lethal body residues of chlorophenol and mixtures of chlorophenols in benthic organisms. Arch Environ Contam Toxicol 43: 214-220.

Kukkonen JVK, Landrum PF, Mitra S, Gossiaux DC, Gunnarsson J and Weston D 2003. Sediment characteristics affecting desorption kinetics of select PAH and PCB congeners for seven laboratory spiked sediments. Environ. Sci. Technol. 37: 4656-4663.

Landrum PF 1988. Toxicokinetics of organic xenobiotics in the amphipod, *Pontoporeia hoyi*: role of physiological and environmental variables. Aquat Toxicol 12: 245-271.

Landrum PF and Robbins JA 1990. Bioavailability of sediment-associated contaminants to benthic invertebrates. In: Sediments: chemistry and toxicity of in-place pollutants (Eds. Baudo R, Giesy JP and Muntau H). Lewis Publishers Inc., Chelsea, MI, USA.

Landrum PF, Lee II H and Lydy MJ 1992. Toxicokinetics in aquatic systems: model comparisons and use in hazard assessment. Environ Toxicol Chem 11: 1709-1725.

Landrum PF and Meador JP 2002. Is the body residue a useful dose metric for assessing toxicity? SETAC Globe, May – June 3: 32-34.

Landrum PF, Steevens JA, Gossiaux DC, McElroy M, Robinson S, Begnoche L, Chernyak S and Hickey J 2004. Time-dependent lethal body residues for the toxicity of pentachlorobenzene to *Hyalella azteca*. Environ Toxicol Chem 23: 1335-1343.

Larson RJ and Woltering DM 1995. Linear alkylbenzene sulfonate (LAS). In: Fundamentals of aquatic toxicology, effects, environmental fate and risk assessment (Ed. Rand GM). Taylor & Francis, Washington D.C., USA.

Lee II H 1992. Models, muddles, and mud: predicting bioaccumulation of sediment-associated pollutants. In: Sediment toxicity assessment (Ed. Burton Jr. GA). Chelsea, MI, USA.

Lee RF 1998. Annelid cytochrome P-450. Comp. Biochem. Physiol., Part C 121: 173-179.

Lee J-H, Landrum PF and Koh C-H 2002a. Toxicokinetics and time-dependent PAH toxicity in the amphipod *Hyalella azteca*. Environ Sci Technol 36: 3124-3130.

Lee J-H, Landrum PF and Koh C-H 2002b. Prediction of time-dependent PAH toxicity in *Hyalella azteca* using a damage assessment model. Environ Sci Technol 36: 3131-3138.

Lee S-J, Kim J-H, Chang Y-S and Moon MH 2006. Characterization of polychlorinated dibenzo-p-dioxins and dibenzofurans in different particle size fractions of marine sediments. Environ Pollut (in press).

Leppänen MT and Kukkonen JVK 1998. Relative importance of ingested sediment and pore water as bioaccumulation routes for pyrene to Oligochaete (*Lumbriculus variegatus*, Müller). Environ Sci Technol: 1503-1508.

Leppänen MT and Kukkonen JVK 2000. Fate of sediment-associated pyrene and benzo[a]pyrene in the freshwater oligochaeta *Lumbriculus variegatus* Müller. Aquat. Toxicol. 49: 199-212.

Li K, Liu W, Xu D, Lee S 2003. Influence of organic matter and pH on bentazone sorption in soils. J Agric Food Chem 51: 5362-5366.

Linders JBHJ, Jansma JW, Mensink BJWG and Otermann K 1994. Pesticides: Benefaction or Pandora's box? A synopsis of the environmental aspects of 243 pesticides, Report, No 679101014, National Institute of Public Health and the Environment (RIVM), The Netherlands, pp.204.

Livingstone DR 1998. The fate of organic xenobiotics in aquatic ecosystems: Quantitative and qualitative differences in biotransfomation by

invertebrates and fish. Comp. Biochem. Physiol. A 120: 43-49.

Loonen H, Muir DCG, Parsons JR and Govers HAJ 1997. Bioacumulation of polychlorinated dibenzo-p-dioxins in sediment by oligochaetes: influence of exposure pathway and contact time. Environ Toxicol Chem 16: 1518-1525.

Lotufo GR 1998. Lethal and sublethal toxicity of sediment-associated fluoranthene to benthic copepods: application of the critical-body-residue approach. Aquat Toxicol 44: 17-30.

Lyytikäinen M, Pehkonen S, Akkanen J, Leppänen M, Kukkonen JVK. Bioaccumulation and biotransformation of polycyclic aromatic hydrocarbons during sediment tests with oligochaetes (*Lumbriculus variegatus*). Manuscript.

Matozzo V, Ballarin L and Marin MG 2004. Exposure of the clam Tapes philippinarum to 4-nonulphenol: changes in anti-oxidant enzyme activities and re-burrowing capability. Mar Pollut Bull 48: 563-571.

Mackay D and Fraser A 2000. Bioaccumulation of persistent organic chemicals: mechanisms and models. Environ Pollut 110: 375-391.

Mackay D, Shiv WY and Ma KC 1995. Illustrated Handbook of Physical-Chemical Properties and Environmental Fate for Organic Chemicals. Volume 4. CRC Press Inc. Boca Raton, FL, USA.

McCarty LS 1986. The relationship between aquatic toxicity QSARS and bioconcentration for some organic chemicals. Environ Toxicol Chem 5: 1071-1080.

McCarty LS, Mackay D, Smith AD, Ozburn GW and Dixon DG 1992. Residue-based interpretation of toxicity and bioconcentration QSARs from aquatic bioassays: neutral narcotic organics. Environ Toxicol Chem 11: 917-930.

McCarty LS and Mackay D 1993. Enhancing ecotoxicological modelling and assessment. Environ Sci Technol 27: 1719-1728.

Meador JP, Stein JE, Reichert WL and Varanasi U 1995. Bioaccumulation of polycyclic aromatic hydrocarbons by marine organisms. Rev. Environ. Contam. Toxicol. 143: 79-165.

Motulsky HJ and Christopoulos A 2003. Fitting models to biological data using linear and nonlinear regression. A practical guide to curve fitting. GraphPad Software Inc., San Diego, CA, USA.

Muir J and Eduljee G 1999. PCP in the freshwater and marine environment of the European Union. Sci Tot Environ 236: 41-56.

Newman MC 1998. Fundamentals of ecotoxicology. Sleeping Bear/Ann Arbor Press, Chelsea, MI, USA.

Nicholls PH 1994. "Physicochemical Evaluation: The Environment", an expert system for pesticides preregistration assessment. BCPC Brighton Conference – Pests and Diseases, Vol. 3, British Crop Protection Council, Farnham, UK, pp. 1337-1342.

Nikkilä A, Halme A and Kukkonen JVK 2003. Toxicokinetics, toxicity and lethal body residues of two chlorophenols in the oligochaete worm, *Lumbriculus variegatus*, in different sediment. Chemosphere 51: 35-46.

Nowosielski BE and Fein JB 1998. Experimental study of octanol-water partition coefficients for 2,4,6-trichlorophenol and pentachlorophenol: derivation of an empirical model of chlorophenol partitioning behaviour. Appl Geochem 13: 893-904.

O'Donnell MJ, Durand ME, Robitaille P-ML, Fisher SW and Stromberg PC 1996. 31P-NMRA analysis of lethal and sublethal lesions produced by KCl-intoxication in the zebra mussel, *Dreissena polymorpha*. J Exp Zool 276: 53-62.

Penttinen O-P, Kukkonen JVK and Pellinen J 1996. Preliminary study to compare body residues and sublethal energetic responses in benthic invertebrates exposed to sediment-bound 2,4,5-trichlorophenol. Environ Toxicol Chem 15: 160-166.

Penttinen O-P and Kukkonen JVK 1998. Chemical stress and metabolic rate in aquatic invertebrates: threshold, dose-response relationship, and mode of toxic action. Environ Toxicol Chem 17: 883-890.

Penttinen O-P and Kukkonen JVK 2006. Body residues as dose for sublethal responses in alevins of landlocked salmon (*Salmo salar* m. *sebago*): a direct calorimetry study. Environ Toxicol Chem 25: 1088-1093.

Petejä-Ronkainen A and Suokas T 2000. Maaperälle ja pohjavedelle riskiä aiheuttavien kohteiden kartoitus Kaakkois-Suomessa. Kaakkois-Suomen ympäristökeskuksen monistesarja 16/2000. 50 p.

Po-Siungh, L., La, D.L., Upton, P.B., Swenberg, J.A., 2002. Analysis of DNA adducts in rats exposed to pentachlorophenol. Carsinogenesis 23, 365-369.

Puolanne J, Pyy O and Jeltsch U 1994. Saastuneet maa-alueet ja niiden käsittely Suomessa. Saastuneiden maa-alueiden selvitys- ja kunnostusprojekti; loppuraportti. Ympäristöministeriö. Muistio 5 1994. 218 p.

Rand GM 1995. Fundamentals of aquatic toxicology. Taylor & Francis, Washington D.C., USA.

Rand GM, Wells PG and McCarty LS 1995. Introduction to aquatic toxicology. In: Fundamentals of aquatic toxicology (Ed. Rand GM). Taylor & Francis, Washington D.C., USA.

Rozman KK and Doull J 2000. Dose and time as variables of toxicity. Ecotoxicology 144:169-178.

Russom CL, Bradbury SP, Broderius SJ, Hammermeister DE and Drummond RA 1997. Predicting modes of toxic action from chemical structure: acute toxicity in the fathead minnow (*Pimephales promelas*). Environ Toxicol Chem 16: 948-967.

Saarikoski J and Viluksela M 1981. Influence of pH on the toxicity of substituted phenols to fish. Arch. Environ Contam Toxicol 10: 747-753.

Sáez M, Gómez-Parra A and Gonzáles-Mazo E 2001. Bioconcentration of linear alkylbenzene sulfonates and their degradation intermediates in marine alga. Fresenius J Anal Chem 371: 486-490.

Sandermann Jr. H 2003. Differential lipid affinity of xenobiotics and natural compounds. FEBS Letters 554: 165-168.

Schuler LJ, Wheeler M, Bailer AJ and Lydy MJ 2003. Toxicokinetics of sediment-sorbed benzo[a]pyrene and hexachlorobiphenyl using the freshwater invertebrates *Hyalella azteca*, *Chirinomus tentans*, and *Lumbriculus variegatus*. Environ Toxicol Chem 22: 439-449.

Schuler LJ, Landrum PF and Lydy MJ 2004. Time-dependent toxicity of fluoranthene to freshwater invertebrates and the role of biotransformation on lethal body residues. Environ Sci Technol 38: 6247-6255.

Simkiss K 1996. Ecotoxicants at the cell membrane barrier. In: Ecotoxicology:

a hierarchical treatment (Eds. Newman MC and Jagoe CH). CRC press, Boca Raton, FL, USA.

Snell TW, Brogdon SE and Morgan MB 2003. Gene expression profiling in ecotoxicology. Ecotoxicology 12: 475-483.

Snyder MJ 2000. Cytochrome P450 enzymes in aquatic invertebrates: recent advances and future directions. Aquat. Toxicol. 48: 529-547.

Soto AM, Justicia H, Wray JW and Sonnenschein C 1991. p-nonyl-phenol: an estrogenic xenobiotic released from "modified" polystyrene. Environ Health Pers 92: 167-173.

Spacie A, McCarty LS and Rand GM 1995. Bioaccumulation and bioavailability in multiphase systems. In: Fundamentals of aquatic toxicology, effects, environmental fate and risk assessment (Ed. Rand GM). Taylor & Francis, Washington D.C., USA.

Strandberg M and Scott-Fordsmand JJ 2004. Effects of pendimethalin at lower trophic levels – a review. Ecotoxicol Environ Saf 57: 190-201.

Streit B 1992. Bioaccumulation processes in ecosystems. Experientia 48: 955-970.

Stroomberg GJ, Zappey H, Steen RJCA, van Gestel CAM, Ariese F, Velthorst NH and van Straalen NM 2004. PAH biotransformation in terrestrial invertebrates – a new phase II metabolite in isopods and springtails. Comp Biochem Physiol, Part C 138: 129-137.

Suomen säädöskokoelma 1022/2006. Valtioneuvoston asetus vesiympäristölle vaarallisista ja haitallisista aineista. Oikeusministeriö, Helsinki.

Suomen säädöskokoelma 214/2007. Valtioneuvoston asetus maaperän pilaantuneisuuden ja puhdistustarpeen arvioinnista. Oikeusministeriö, Helsinki.

Suter II GW 1995. Introduction to ecological risk assessment for aquatic toxic effects. In: Fundamentals of aquatic toxicology, effects, environmental fate and risk assessment (Ed. Rand GM). Taylor & Francis, Washington D.C., USA.

Ten Hulscher TEM, Postma J, den Besten PJ, Stroomberg GJ, Belfroid A, Wegener JW, Faber JH, van der Pol JJC, Hendriks AJ and van Noort PCM 2003. Tenax extraction mimics benthic and terrestrial bioavailability of organic compounds. Environ Toxicol Chem 22: 2258-2265.

Thompson RS and Stewart KM 2003. Critical body residue: a review of the literature and identification of experimental data requirements. Brixham Environemntal Laboratory, Brixham Devon, UK, Project BL7549/B.

Timbrell J 2000. Principles of biochemical toxicology, 3rd ed. Taylor and Francis, UK.

Tomlin C 1994. The pesticide manual, tenth edition. The Bath Press, Bath, UK.

Traina SJ, McAvoy DC and Versteeg DJ 1996. Association of linear alkylbenzenesulfonates with dissolved humic substances and its effect on bioavailability. Environ Sci Technol 30: 1300-1309.

U.S.EPA 1998. Guidelines for ecological risk assessment. EPA/630/R-95/002F. U.S. Environmental Protection Agency, Washington D.C., USA.

U.S.EPA 2000. Methods for Measuring the Toxicity and Bioaccumulation of Sediment-Associated Contaminants with Freshwater Invertebrates, second edition. U.S.EPA 600/R-99/064, Duluth, MN, USA.

Vaal M, Van Der Wal JT, Hermens J and Hoekstra J 1997a. Pattern analysis of

the variation in the sensitivity of aquatic species to toxicants. Chemosphere 35: 1291-1309.

Vaal M, Van Der Wal JT, Hoekstra J and Hermens J 1997b.Variation in the sensitivity of the aquatic species in relation to the classification of environmental pollutants. Chemosphere 35: 1291-1309.

Veith GD, Call DJ and Brooke LT 1983. Structure-activity relationship for the fathead minnow, *Pimephales promelas*: narcotic industrial chemicals. Can. J. Fish. Aquat. Sci. 40: 743-748.

van der Oost R, Beyer J and Vermeulen NPE 2003. Fish bioaccumulation and biomarkers in environmental risk assessment: a review. Environ Toxicol Pharmacol 13: 57-149.

van Wezel AP and Opperhuizen A 1995. Narcosis due to environmental pollutants in aquatic organisms: residue-base toxicity, mechanisms and membrane burdens. Crit. Rev. Toxicol. 25: 255-279.

van Wezel AP, Sijm DTHM, Seinen W and Opperhuizen A 1995a. Use of lethal body burdens to indicate species differences in susceptibility to narcotic toxicants. Chemosphere 31: 3201-3209.

van Wezel Ap, de Vries DAM, Kostense S, Sijm DTHM and Opperhuizen A 1995b. Intraspecies variation in lethal body burdens of narcotic compounds. Aquat Toxicol 33: 325-342.

van Wezel AP, de Vries DAM, Sijm DTHM and Opperhuizen A 1996. Use of lethal body burden in the evaluation of mixture toxicity. Ecotoxicol. Environ. Saf. 35: 236-241.

Water Framework Directive 2000. Directive 2000/60/EC of the European Parliament and of the Council of 23 October 2000 establishing a framework for Community action in the field of water policy. Official Journal L 327, 22/12/2000 P. 1-73.

Verrengia Guerrero NR, taylor MG, Davies NA, Lawrence MAM, Edwards PA, Simkiss K and Wider EA 2002. Evidence of differences in the biotransformation of organic contaminants in three species of freshwater invertebrates. Environ. Pollut. 117: 523-530.

White R, Jobling S, Hoare SA, Sumpter JP and Parker MG 1994. Environmentally persistent alkylphenolic compounds are estrogenic. Endocrinology 135: 175-182.

WHO 2004. Bentazone in Drinking-water Background document for development of WHO Guidelines for Drinking-water Quality WHO/SDE/WSH/03.04/77.

WHO 2005. The WHO recommended classification of pesticides by hazard and guidelines to classification 2004, World Health Organization, Geneva, Switzerland.

Willows RI 1994. The ecological impact of different mechanisms of chronic and sublethal toxicity on feeding and respiratory physiology. In: Water Quality and Stress Indicators in Marine and Freshwater Systems: Linking levels of Organisations. Freshwater Biological Association, Ampleside, UK.

Ying G-G, Williams B and Kookana R 2002. Environmental fate of alkylphenols and alkylphenol ethoxylates – a review. Environ. Int. 28: 215-226.

Erratum

Article I, page 402 and Table 4.: units for k_s should be kg sediment dry weight/kg organism wet weight/hour.