KARI OLAVI KOPONEN

Biotransformation and Histopathological Responses in Chemically Stressed Freshwater Fish

Doctoral dissertation

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ABSTRACT

The usefulness of biotransformation enzyme measurements, both monoxygenase and conjugation enzymes, in conjunction with tissue histopathology as a set of biomarker tools in monitoring chemically affected aquatic environment was tested. The objective was to study the effects of chemical stress on the biotransformation enzyme system of different life-stages of fish. Also, histological cellular studies were conducted in order to be able to link the signs of environmental stress of fish to concrete structural effects caused by chemicals. In this cascade of studies, polychlorinated biphenyls (PCBs) were chosen as xenobiotic model substances. Uptake, effective concentrations, and biological effects of PCBs on fish were followed. Furthermore, the influence of biotic and abiotic factors on the biotransformation and histopathological parameters was investigated. Rainbow trout (Oncorhynchus mykiss) was used as an experimental species in our laboratory studies. In the field studies, brook (Allopetrus brama) and asp (Aspius aspius) were the representative fish species.

In experimental early life stage exposure, PCB 77 penetrated easily through the egg chorion and accumulated into fish embryo. In the laboratory exposure study with juvenile fish, both PCB congeners 77 and 126 readily accumulated into liver tissue of fish. Our field studies indicated that PCBs are still present in feral fish tissues even 20 years after the discharge of PCBs into the studied freshwater lake was ceased. In feral fish, the PCB accumulation was connected to the tissue lipid concentration.

The hepatic monoxygenase system, especially CYP1A enzymes, measured as 7-ethoxyresorufin O-deethylase activity (EROD), reacted rapidly to acute PCB insult both in embryonic and juvenile developmental stages of fish, but also to prolonged environmental chemical exposure to adult feral fish. Our studies also revealed that the EROD system was activated at PCB concentrations, which produced no observed adverse effects on fish. Thus, EROD was considered an effective biomarker for application both in acute and chronic exposure monitoring studies. No clear trend of induction or inhibition of the conjugation system, measured as glutathione S-transferase activity (GST), caused by chemical insult was observed either in experimental PCB exposure studies or in field monitoring. According to our experience, the GST system was not very informative for detecting physiological signs of PCB exposure in fish.

Biotic and abiotic factors were shown to have an effect on biotransformation enzyme activities of fish. Both intra- and interstrain variation in the basal biotransformation enzyme activities of adult fish, but also in the chemically exposed juvenile fish was demonstrated. Also, differences between sexes, as well as seasonal differences in biotransformation of feral fish were apparent.

Histopathological investigation of feral fish living in PCB-polluted environment revealed specific glomerular alterations in the kidney. Of those renal lesions, especially the dilation of glomerular capillaries and mesangial edema could be linked to PCB exposure. The observed cellular changes in other organs (liver, spleen, gonads) were all linked to PCB exposure. Higher prevalence of parasitic infections and rodlet cells in inner tissues of PCB-exposed feral fish may indicate weakened immune defense of those fish.

In conclusion, if abiotic and biotic factors causing variation are minimized, biotransformation enzyme measurements, especially CYP1A enzymes, and histopathological techniques together may compose a set of biomarker tools, which can be utilized for environmental biomonitoring studies.

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To my brother

Ilkka Tapio Koponen
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Vantaa, 25.5.2002

Kari Koponen
ABBREVIATIONS

ABC  adhesion between visceral and parietal layers of Bowman’s capsule
AH  aryl hydrocarbon hydroxylase
AhR  aromatic (aryl) hydrocarbon receptor
BNF  β-naphthoflavone
cytochrome P450
CYP  cytochrome P450 subfamily
DGC  dilation of glomerular capillaries
DMSO  dimethyl sulfoxide
DNA  deoxyribonucleic acid
EC50  effective concentration for 50% of maximal effect
ED50  effective dose for 50% of maximal effect
EROD  7-ethoxyresorufin O-deethylase
FBS  filling of Bowman’s space
GST  glutathione S-transferase
HAH  halogenated aromatic hydrocarbon
ip  intraperitoneal
IUPAC  International Union of Pure and Applied Chemistry
LSI  liver-somatic index
ME  mesangial edema
MO  monoxygenase
mRNA  messenger ribonucleic acid
NADPH  nicotinamide adenine dinucleotide phosphate
PAH  polycyclic aromatic hydrocarbon
PCB  polychlorinated biphenyls
PCDD  polychlorinated dibenz-p-dioxin
PCDF  polychlorinated dibenzofuran
RNA  ribonucleic acid
TEF  toxic equivalency factor
UDPGT  uridine-5’-diphosphoglucuronosyltransferase
ww  wet weight
LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following original articles, referred to by Roman numerals I-VI in the text.


This thesis also contains unpublished data.
CONTENTS

1 INTRODUCTION 15

2 LITERATURE REVIEW 17
   2.1 Biotransformation in fish 17
      2.1.1 Cytochrome P450 monoxygenases (CYP) 18
         2.1.1.1 Induction of fish CYP1A 19
      2.1.2 Conjugation enzymes 22
   2.2 Histopathologic biomarkers 23
   2.3 Polychlorinated biphenyls (PCBs) 25
      2.3.1 Bioaccumulation into fish 27
      2.3.2 Structure-activity 28
      2.3.3 Biological effects and toxicity 29

3 OBJECTIVES 31

4 MATERIALS AND METHODS 32
   4.1 Cellular responses 34
      4.1.1 CYP1A monoxygenases 34
         4.1.1.1 Catalytic activity 34
      4.1.2 Immunoblotting 34
         4.1.1.3 Immunohistochemistry 34
      4.1.2 Conjugation 35
   4.2 Chemical responses 35
      4.2.1 Liquid scintillation counting 35
      4.2.2 Chemical residue analysis 35
   4.3 Histopathology 36
   4.4 Early life stage NOEC-screening 36
   4.5 Statistical analysis 36
5 RESULTS
5.1 Preliminary studies 37
5.2 Bioaccumulation studies 38
5.3 Biotransformation enzyme activities 40
  5.3.1 PCB-exposed fish 40
  5.3.2 Field studies 42
5.4 Histopathology of feral fish 43
  5.4.1 Cellular changes 44
  5.4.2 Parasite infections 45

6 DISCUSSION 46
6.1 Intra- and interstrain variation of biotransformation enzyme biomarkers 46
6.2 PCB uptake 47
6.3 Biological effects of PCBs in fish 49
  6.3.1 Early life stage effects 49
  6.3.2 CYP1A enzyme activities 50
  6.3.3 Conjugation enzyme activities 52
  6.3.4 Histopathological alterations 54
    6.3.4.1 Liver 54
    6.3.4.2 Kidney 56
    6.3.4.3 Reproductive organs 56
    6.3.4.4 Non-specific immunological markers 57
      6.3.4.4.1 Rodlet cells 57
      6.3.4.4.2 Parasitic infections 58
      6.3.4.4.3 Macrophage aggregates 59
  6.4 Difficulties in assessing environmental effects of xenobiotics 59

7 CONCLUSIONS 61

8 REFERENCES 63
1 INTRODUCTION

The modern world is a world of use and abuse of chemicals. Over 100 000 man-made chemicals exist, of which amount tens of thousands eventually enter the aquatic compartment. As a consequence, aquatic organisms readily absorb several of these compounds. If not acutely lethal to an organism the chemical, or proportion of it, will be either excreted, stored, or/and metabolised. The metabolism of xenobiotics is commonly called biotransformation. Biotransformation influences the fate of the chemical by enzyme-catalysed conversion of the parent compound into its metabolites (van Leeuwen and Hermens 1995). Induction of biotransformation enzymes has been widely used to indicate chemical exposure in aquatic organisms. Several PCB congeners are potent inducers of biotransformation enzyme systems in fish (Janz and Metcalfe 1991).

PCBs are one of the most notorious groups of environmental chemicals affecting aquatic life. These chemicals can be substituted with up to ten chlorine atoms, theoretically comprising a total of 209 individual congeners (Safe 1990). PCBs were recognized as significant environmental contaminants in 1960’s, and since then their presence in biota has been established (Mullin et al. 1984). PCBs are persistent environmental contaminants and are causing a great deal of concern globally (Tyler et al. 1998), being identified in practically every environmental matrix of the ecosystem (Rappe and Buser 1989). In the past, a significant portion of the environmental burden of these compounds has resulted from careless disposal practices, accidents and leakage from industrial facilities (Safe 1994). Today, ongoing heavy usage of PCBs in developing countries (Sumpter and Jobling 1995) as well as the recent wars and military conflicts in industrialized regions do have significant contribution to the overall PCB load into the environment.

Animals that live in aquatic environments polluted by lipophilic chemicals, such as PCBs, do accumulate the chemical into their bodies (Connor 1984; Cook et al. 1991). A wide range of toxic effects have been associated with exposure to PCBs in wildlife, including mass die-offs of seal and dolphins, large population declines of European otters, and adverse effects on reproduction and development in many species (UNEP/UNCHS 1999), including fish. The biological effects and toxicity on fish caused by chemical stress occurs at different levels of biological organization. Often changes at biochemical and cellular levels can be detected first, well before the changes at individual, population, or even higher (ecosystem) biological levels occur. In physiology of chemically exposed
fish, changes in biotransformation enzyme activities reflect the metabolic response to chemical stress at molecular and subcellular levels. Those changes can be biochemically detected. As a consequence, xenobiotic metabolism of chemically stressed fish may cause reversible and/or irreversible alterations at cellular and tissue levels, which can be histologically verified.

In this thesis, the effects of pollutants on biotransformation enzyme systems of adult, juvenile, and early life stages of fish were evaluated. Using fish as a model aquatic species, and polychlorinated biphenyls as a model compounds of lipophilic, bioactive aquatic pollutants, the role of both monoxygenase (CYP1A: EROD, AHH) and conjugation (GST, UDPGT) enzyme systems as an early warning biomarkers of sublethal aquatic pollution was evaluated. Since induction/inhibition of biotransformation enzyme system may not be a hazard in itself, a histopathologic tissue analysis of fish was conducted to reveal evidence of possibly increased lesion prevalence in target tissues of fish under sublethal chemical stress.
2 LITERATURE REVIEW

2.1 Biotransformation in fish

In aquatic compartment, organisms have to deal with a myriad of chemicals. The hydrophilic chemicals that are rapidly degraded through abiotic processes like photolysis and hydrolysis usually do not require xenobiotic metabolism. Many of the hydrophobic and not readily biodegraded pollutants, however, will be taken up and accumulated by aquatic organisms, including fish. Now, if the chemical concentration, or the toxic threshold limit of individual specimen towards that foreign substance is exceeded, vital biochemical and/or physiological functions may be disturbed, possibly hindering organisms overall survival or reproductive success. Fish has two major ways to eliminate that chemical: it is either excreted as is, or it may be converted into metabolite through biotransformation (van Leeuwen and Hermens 1995). Sometimes the metabolite turns out to be more toxic than the biotransformed parent compound.

Biotransformation system, which involves the conversion of lipophilic xenobiotics to more water-soluble and more easily excreted compounds, is one of the prime biochemical factors determining the distribution and retention of toxic chemicals in fish (Kleinow et al. 1987). The biochemistry and molecular biology of biotransformation enzyme systems has been the subject of frequent reviews (Goksøyr and Förlin 1992; Stegeman et al. 1992; Stegeman and Hahn 1994; Goksøyr and Husey 1998). The major pathways of biotransformation and elimination are phase I (oxidation, reduction and hydrolysis) and phase II (conjugation, acetylation) metabolism. In phase I reactions, a functional group, e.g., hydroxy (-OH), amino (-NH₂), or carboxyl (-COOH) is introduced into the parent compound. Quantitatively, the most important processes are oxidative, and they are catalysed mainly by microsomal cytochrome P450 monooxygenases, or CYPs (Buehler and Williams 1988). The product of the first biotransformation step, but also the parent compound, can also be metabolised through the phase II reactions, involving glucuronide, sulphate, acetyl and glutathione conjugation (van Leeuwen and Hermens 1995).
2.1.1 Cytochrome P450 monooxygenase (CYP)

The cytochrome P450 enzymes are part of an enzyme system commonly named as mixed function oxidase (MFO) for there major property to build one atom of oxygen into a substrate and reduce the other oxygen atom to water. The cytochrome P450 monooxygenases (CYPs) belong to a large superfamily of membrane bound hemoproteins that are capable of catalysing monooxygenation reactions both in prokaryotes and eukaryotes. In eukaryotes, CYPs are bound to either the microsomal membrane or to the inner mitochondrial membrane. Mitochondrial CYPs are involved in steroid biosynthetic reactions, are found mainly in steroidogenic organs, and generally do not metabolise xenobiotics (Oinonen 1996). The majority of microsomal CYPs transform both endogenous (e.g., steroids, bile acids, prostaglandins, fatty acids) and foreign compounds (Stegeman et al. 1992). Most importantly, only the microsomal CYPs appear to be induced by xenobiotic chemical substrates, involving transcriptional and/or translational activation (Stegeman and Hahn 1994). This system is located primarily in the smooth endoplasmic reticulum of the cells of many organs and tissue types. The distinguishing feature of microsomal reactions is their dependence on the electron transport enzymes (nicotinamide adenine dinucleotide phosphate, NADPH) and the requirement for molecular oxygen (Jimenez and Stegeman 1990). The general scheme for monooxygenase enzyme reactions is

\[ RH + NADPH + O_2 + H^+ \rightarrow ROH + NADP^+ + H_2O \]

where RH is the substrate (e.g., coplanar PCB) and ROH is the hydroxylated product.

The classification of CYPs is based on the degree of amino acid sequence identity between enzymes. Members within the same family share \( \geq 40 \% \) amino acid similarity. Accordingly, the families are further divided to subfamilies (over 55 \% similarity). The naming of a CYP gene is recommended to go along the following rule:
Various CYP forms have been purified from both freshwater as well as from marine fish species (Stegeman and Hahn 1994). Fish CYPs have been shown to belong at least to the subfamilies CYP1A, CYP2B/E/K, CYP3A, CYP4A, CYP11A, CYP17 and CYP19. Polychlorinated biphenyls are the common inducers of the CYP genes 1A1, 1A2, 2B1, and 4A1 (Stegeman and Hahn 1994).

2.1.1.1 Induction of fish CYP1A

The schematic mechanism of xenobiotic-triggered CYP1A induction is presented in fig. 2. In the inductive process, a planar ligand (e.g., PCB) first enters the cell, supposedly by passive diffusion. Once inside the cell, it is readily welcomed by an intracellular aryl hydrocarbon receptor (AhR), a multimeric complex consisting of at least two proteins, a heat shock protein (hsp90) and the Ah-receptor protein. Ah-receptor is a ligand-activated transcription factor that mediates many of the biological effects of halogenated aromatic compounds (Elonen et al. 1998). Consequently, a ligand binds to the Ah-receptor, which transforms to its transcriptionally active form. Transformation involves the dissociation of a hsp90. In order to be translocated into the nucleus, the inducer-receptor complex dimerises with a nuclear translocation factor (ARNT), a competent DNA-binding protein.
Figure 1. Schematic CYP1A production induced by xenobiotic chemical. RO-G = glucuronides (Modified from Leaver 1996).

Ones inside the nucleus the dimerised complex binds to xenobiotic responsive elements of DNA near the promoter region of specific genes. DNA binding induces the Ah gene battery, which consists of at least six genes, two phase I and four phase II genes (Nebert et al. 1990). Chemical stimulates the rate of CYP1A1 gene transcription, resulting in increased levels of messenger RNA (mRNA). The mRNA delivers the information into the cytosol where the synthesis of CYP1A protein takes place in the ribosomal system of the cell. Newly synthesized CYP1A protein binds heme, followed by insertion into the membrane of the endoplasmic reticulum. The CYP1A hemoprotein is the terminal oxidative component of an electron transfer system being responsible for metabolism of various xenobiotics (Andersson and Förlin 1992). Electrons from NADPH are transferred through a flavoprotein (NADPH-cytochrome P450 reductase), CYP1A which then inserts one atom of oxygen into the substrate and reduces the second atom to form water (Gokeşeyr and Förlin 1992).
The induction or inhibition of CYP1A indicates that the chemical affects the physiological equilibrium of the fish at the molecular level. Elevated CYP1A activities increase the potency of the biotransformation system to metabolise the chemical intruder into more polar and excretable products (Golseyr et al. 1994). In some occasions, however, this may lead to the formation of even more toxic intermediates, which can damage the DNA or other cellular macromolecules (Stegeman and Klopper-Sams 1987). During biotransformation processes, chemical carcinogenesis may be contributed by oxygen reactive species and free radicals derived from the xenobiotic (Goepert et al. 1995).

The induction itself can be detected at the levels of mRNA, protein or enzyme activity (Bucheli and Fent 1995). At the enzyme level, the activity of biotransformation enzymes has been studied with prototype substrates that undergo specific functional group transformations (Burke and Mayer 1974). Measurement of two particular catalytic activities, 7-ethoxyresorufin O-deethylase (EROD) and aryl hydrocarbon hydroxylase (AHH), are sensitive means of determining the inductive response of halogenated hydrocarbons in fish. Coplanar PCBs are potent inducers of these enzyme activities (Safe 1994). Although most studies on the CYP1A induction involve liver as a target organ, induction of this enzyme has also been detected in extrahepatic tissues such as kidney, intestine, spleen, gill, and gonad of fish exposed to environmental contaminants (Payne 1976; Lindström-Seppä et al. 1981; Pesonen et al. 1987; Van Veld 1990; Stegeman and Hahn 1994).

Complementary to catalytic EROD and AHH activity measurements, immunohistochemical and immunochemical analysis of affected tissues have been used in identifying specific cell types involved in the CYP1A induction response, as well as their intratissue localization (Stegeman et al. 1991; Smolowitz et al. 1991). Compared with immunochemical methods (Western blots, ELISA) in which tissue homogenisation is required prior to analysis, immunohistochemistry may be more informative technique, because it preserves the cellular structure of a tissue sample (Husey et al. 1996).
2.1.2 Conjugation enzymes

In comparison to Phase I enzyme system, relatively little is known about the enzymology of conjugating enzyme system of fish. In phase II reactions, water-soluble endogenous molecules are conjugated to the parent compound or oxygenated intermediates of phase I, thus making the initial compound water-soluble end-product, which can be easily excreted through bile or urine or over the gill (Goksøyr and Förlin 1992; Roy and Hänninen 1993). Perhaps the most important of these enzymes are glutathione transferases (GST), UDP-glucuronosyltransferases (UDPGT), and sulfotransferases (ST), which, accordingly, link metabolites to glutathione, glucuronic acids, and sulphate (Stegeman et al. 1992; Buhler and Williams 1988). In general, the major pathway for electrophilic compounds is conjugation with glutathione, while for nucleophilic compounds conjugation with glucuronic acid is the major route (George 1994). PCBs are capable of inducing both glutathione transferase (GST) and glucuronosyltransferase (UDPGT) enzyme activities (Safe 1994). In contrast, there is no proof of xenobiotic-induced sulfotransferase (STs) enzyme activities in aquatic species (Fourreman 1989).

GSTs are a multigene superfamily of dimeric, multifunctional, primarily cytosolic enzymes (George 1994). Hepatic glutathione S-transferase (GST) activity has been purified and partially characterized from four teleost species, rainbow trout (Oncorhynchus mykiss), carp (Cyprinus carpio), plaice (Pleuronectes platessa), and salmon (Salmo salar). Most of the GST isoforms are proven active towards the artificial substrate 1-chloro-2,4-dinitrobenzene (CDNB) (George 1994). GST is an antioxidative enzyme, and essentially all of the glutathione-linked enzymes can be considered to be part of a general defence system against oxidation products in biological systems (Mannervik 1990). In addition to their role in protecting an organism from peroxidative cellular damage, GSTs participate in cellular transport of endogenous compounds (heme, bilirubin, steroids) and detoxification of both endogenous and reactive xenobiotics (George 1994; Smith et al. 1989). In the GST catalysed reactions, a glutathione reacts with electrophilic compounds and is capable of replacing hydrogen, chlorine or nitrogens. Glutathione is readily conjugated in the first step of the mercapturic acid formation as shown below:
In this general scheme RX is aromatic ring or halide compound

Glucuronidation is another important pathway for detoxification and excretion of xenobiotic compounds in fish (George 1994). Uridine diphosphoglucuronosyltransferase (UDPGT) enzymes are membrane-bound proteins residing primarily on the luminal side of the endoplasmic reticulum (Fourman 1989). The UDPGT enzyme catalyses the conjugation of glucuronic acid from the high-energy nucleotide, UDP-glucuronic acid (UDPGA), to an appropriate substrate. The general scheme for this conjugation is:

\[
\text{UDPGA} + R-XH \rightarrow R-X-GA + UDP
\]

In this reaction X is either O, COO, or NH, and GT is glucuronosyltransferase.

Both UDPGA and the substrate may be transported from cytoplasm by membrane translocases (Iyanagi et al. 1989). The substrate may also be reactive intermediate resulting from monooxygenase activity (Jimenez and Stegeman 1990). Produced glucuronide conjugates, O-, N-, S-, or C-glucuronides, are then excreted through the bile, urine or blood. According to George (1994), neither S- nor C-glucuronide formation has been studied in fish. This group of isoenzymes accepts broad range of substrates. Induction of UDPGT system has been reported in fish exposed to PCBs (Andersson et al. 1985; Monod et al. 1988).

### 2.2 Histopathologic biomarkers

Fish living under constant and diverse chemical stress often display high prevalence of pathological lesions (Malins et al. 1988). Therefore, histopathological tissue changes can be utilised as indicators of prior exposure to environmental stressors. Histopathologic biomarkers are higher-level responses, reflecting previous change in physiological and/or biochemical function (Hinton et al. 1992), and thus can be utilised in order to link these changes to actual damage or alteration at cell and higher
levels of biological organisation. The biology of fish tumors can be related to the tumor studies in mammals because fish anatomy, physiology, toxicology, and pathology often parallel those of mammals (Moore and Myers 1994). Tumor epizootics and neoplastic pathobiology of fish, as well as the use of histopathologic biomarkers have been a subject of several reviews (Mix 1986; Harshburger and Clark 1990; Hinton et al. 1992; Moore and Myers 1994). These lesions - or any contaminant-induced physiological or biochemical changes in an organism - signal effects resulting from prior or recent exposure to toxic substances (Hinton and Lauren 1990).

The induction or inhibition of enzymes metabolising xenobiotics indicate changes in the toxicokinetic phase, which are not immediately related to the mechanism of toxicity (Walker 1998). Hypothetically, an exposure to hazardous pollutant can induce biotransformation enzyme system, leading to accelerated metabolism and increased amount of toxic intermediate metabolites, which in turn may lead to cellular toxicity, subsequently detected by histopathologic biomarkers (Hinton et al. 1992). Importantly, morphological markers of sublethal tumorigenic effects may be predictive of more severe biological changes (Moore and Myers 1994). Proliferative lesions in several organs have been characterized from feral fish residing in an aquatic environment polluted by PCBs and other organic compounds (Gardner and Pruell 1989).

The liver is the major site of the drug-metabolising system mediated by cytochrome P-450. Activation of potentially harmful chemicals to their ultimate metabolites may result in neoplastic changes within the liver (Hinton and Laurén 1990). Therefore, liver is probably the most utilized target organ for effects of xenobiotic chemicals. Teleostean kidney receives the vast majority of postbranchial blood, so the renal lesions might be expected to be also good indicators of environmental pollution (Hinton and Laurén 1990). The characteristic feature of teleostean spleen as a hematopoietic organ and temporary reservoir for blood (Takashima 1982) also gives it histopathological biomarker value. Also, reproductive organs of fish are important indicators of aquatic pollution, especially since PCBs are known for their ability to disrupt the endocrine systems of animals. Furthermore, in fish and in mammals, the skin is very susceptible to chemical and environmental stress (Hinton and Lauren 1990).

The immune system has the potential for assessing the toxic effects of chemicals. Several immunologic responses at the cellular level, both specific and non-specific, can be detected parallel
to histopathological evaluation of fish tissues (Weeks et al. 1992). Probably the most typical defence mechanism in fish is a nonspecific, granulomatous inflammatory reaction (Haaparanta et al. 1996). Phagocytic cells, including granulocytes and macrophages, are drawn to an inflamed area by both endo- and exogenous chemoattractants (Secombes and Fletcher 1992). Macrophages are oftentimes organized into melano-macrophage centers (MMCs), which are thought to have a role in detoxification and recycling of foreign material (Wolke 1992). Also, the rodlet cells (RCs), which have been hypothesised to be, e.g., migrating secretory cells or granular leukocytes (Leino 1974; Cenini 1984), have been thought to form a part of the nonspecific immune defence against infectious agents, parasitic infections, or toxicant-induced stress (Iger and Abraham 1997; Dezfuli et al. 1998).

2.3. Polychlorinated biphenyls (PCBs)

Polychlorinated biphenyls (PCBs) are organochlorines that are produced by the chlorination of biphenyl (Table 1). These chemicals can be substituted with up to ten chlorine atoms, theoretically comprising a total of 209 individual congeners (Safe 1990). They have characteristically low water solubility and high lipophilicity. Furthermore, their thermal conductivity is high and electric conductivity is low. Depending on the degree of chlorination, these physico-chemical properties brought about a wide range of different applications. Thus, PCBs have been used as electric fluids in transformers and capacitors, and in hydraulic and heat exchange fluids, but also in adhesives, dedusting agents, pesticide extenders, paints, flame retardants, and in carbonless copying paper (Safe 1992; Fiedler et al. 1994), to name but a few. Commercial PCBs contain a mixture of isomers: the product may comprise from 20 % up to 60 % of chlorine.

Because PCBs are man-made substances they do not occur naturally in environment. Production of PCBs started in 1930s, declined rapidly during the 1970s, and it is believed to have ceased in mid-1980s. The total amount of PCB produced during that time is estimated at 1.5 million tons (Rantanen 1992), about 70 % of which is still in use or in stock (Tyler et al. 1998). A significant portion of the environmental burden of these compounds has resulted from careless disposal practices, accidents and leakage from industrial facilities (Safe 1994). Furthermore, ongoing heavy usage of PCBs in developing countries is leading to increasing concentrations in the global environment as a whole (Sumpter and Jobling 1995). Recently, wars and military conflicts in various
industrialized regions in Europe have undoubtedly caused serious environmental problems, most of which are yet to be monitored. As an example, after the Kosovo conflict, environmental inspection of UNEP/UNCHS Balkan Task Force reported several tons of PCBs to be released into the atmosphere, soil, rivers and groundwater by military activities (UNEP/UNCHS 1999).

Table 1. Characterization of polychlorinated biphenyls by their name, formula, chlorine content and lipophilicity.

<table>
<thead>
<tr>
<th>IUPAC number</th>
<th>Name (…chlorobiphenyl)</th>
<th>Molecular formula</th>
<th>Number of isomers</th>
<th>Chlorine percentage</th>
<th>Log K&lt;sub&gt;ow&lt;/sub&gt;*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 – 3</td>
<td>mono…</td>
<td>C&lt;sub&gt;12&lt;/sub&gt;H&lt;sub&gt;6&lt;/sub&gt;Cl</td>
<td>3</td>
<td>18,8</td>
<td>4,09 – 4,69</td>
</tr>
<tr>
<td>4 – 15</td>
<td>di…</td>
<td>C&lt;sub&gt;12&lt;/sub&gt;H&lt;sub&gt;6&lt;/sub&gt;Cl&lt;sub&gt;2&lt;/sub&gt;</td>
<td>12</td>
<td>31,8</td>
<td>4,65 – 5,30</td>
</tr>
<tr>
<td>16 – 39</td>
<td>tri…</td>
<td>C&lt;sub&gt;12&lt;/sub&gt;H&lt;sub&gt;6&lt;/sub&gt;Cl&lt;sub&gt;3&lt;/sub&gt;</td>
<td>24</td>
<td>41,3</td>
<td>5,16 – 5,89</td>
</tr>
<tr>
<td>40 – 81</td>
<td>tetra…</td>
<td>C&lt;sub&gt;12&lt;/sub&gt;H&lt;sub&gt;6&lt;/sub&gt;Cl&lt;sub&gt;4&lt;/sub&gt;</td>
<td>42</td>
<td>48,7</td>
<td>5,66 – 6,36</td>
</tr>
<tr>
<td>82 – 127</td>
<td>penta…</td>
<td>C&lt;sub&gt;12&lt;/sub&gt;H&lt;sub&gt;6&lt;/sub&gt;Cl&lt;sub&gt;5&lt;/sub&gt;</td>
<td>46</td>
<td>54,3</td>
<td>6,20 – 6,95</td>
</tr>
<tr>
<td>128 – 169</td>
<td>hexa…</td>
<td>C&lt;sub&gt;12&lt;/sub&gt;H&lt;sub&gt;6&lt;/sub&gt;Cl&lt;sub&gt;6&lt;/sub&gt;</td>
<td>42</td>
<td>58,9</td>
<td>6,74 – 7,42</td>
</tr>
<tr>
<td>170 – 193</td>
<td>hepta…</td>
<td>C&lt;sub&gt;12&lt;/sub&gt;H&lt;sub&gt;6&lt;/sub&gt;Cl&lt;sub&gt;7&lt;/sub&gt;</td>
<td>24</td>
<td>62,8</td>
<td>7,24 – 7,52</td>
</tr>
<tr>
<td>194 – 205</td>
<td>octa…</td>
<td>C&lt;sub&gt;12&lt;/sub&gt;H&lt;sub&gt;6&lt;/sub&gt;Cl&lt;sub&gt;8&lt;/sub&gt;</td>
<td>12</td>
<td>66,0</td>
<td>7,80 – 8,0</td>
</tr>
<tr>
<td>206 – 208</td>
<td>nona…</td>
<td>C&lt;sub&gt;12&lt;/sub&gt;HCl&lt;sub&gt;9&lt;/sub&gt;</td>
<td>3</td>
<td>68,7</td>
<td>7,71 - 8,09</td>
</tr>
<tr>
<td>209</td>
<td>deca…</td>
<td>C&lt;sub&gt;12&lt;/sub&gt;Cl&lt;sub&gt;10&lt;/sub&gt;</td>
<td>1</td>
<td>71,1</td>
<td>8,18</td>
</tr>
</tbody>
</table>

* Log K<sub>ow</sub> = Octanol-water partition coefficient.

PCBs were recognized as significant environmental contaminants in 1960’s, and since then their presence in biota has been established (Mullin et al. 1984). Less than a half of the theoretical total of 209 PCB congeners account for nearly all of the environmental PCB contamination, and fewer than 25 of these congeners account for most of the total PCB burden in animal tissues (McFarland and Clarke 1989). Once introduced into the environment, the stable PCBs degrade relatively slowly and undergo cycling and transport within the various components of the global ecosystem (Baker and Eisenreich 1990; Safe 1994). Due to an atmospheric transport alone, it has been estimated that between 11 to 17 tonnes of total PCB enters the North Sea every year (Kramer et al. 1991).
PCBs are acknowledged to be among the most widespread and persistent chemical contaminants in coastal aquatic environments (Varanasi et al. 1992). Recent lake studies indicate that PCBs may be recycled from sediments back into the overlying water (Sanders et al. 1997). Instead of being free in the water column, PCBs tend to absorb onto particles and microorganisms. Ultimately, PCBs accumulate in aqueous sediments, from where they can be picked up and accumulated or metabolised by various bottom-feeding and other aquatic species. Environmental processes (partitioning, transformation, bioaccumulation) alter the composition of PCB mixtures in aquatic biota: evaporated or dissolved congeners are usually lower in chlorine content, and therefore more inclined to metabolism and elimination. Thus, congeners absorbed to sediment tend to be higher in chlorine content and persistence than the original mixtures (Hutzinger et al. 1974).

2.3.1 Bioaccumulation into fish

PCBs do accumulate in fish (Cook et al. 1991). Water serves as a route of exposure for chemicals that are water-soluble or are adsorbed to fine particles suspended in the water column (Macuubbin et al. 1990). In fish, lipophilic chemicals are absorbed through ingestion, dermal exposure, and through gills (Randall et al. 1996). Generally, the major transport mechanism for lipophilic xenobiotics is believed to be passive diffusion (James and Kleinow 1994). A key parameter in assessing the potential environmental behaviour of PCBs and other lipophilic chemicals is a relative solubility of the chemical in water and lipids, often measured as the octanol-water partition coefficient ($K_{ow}$) (Table 1). Characteristic lipophilicity ($\log K_{ow}$ from 4.5 to over 8) and hydrophobicity of chlorinated hydrocarbons makes them soluble in the fatty tissues of aquatic biota. However, decreasing bioaccumulation factors for PCBs with $\log K_{ow} > 6$ have been described in field studies with fish (Bremle et al. 1995).

The chemical concentration in the animal itself is one of the most important factors when biological effects of pollution to aquatic organisms are evaluated. The physicochemical properties of the xenobiotic pollutant, the route of exposure, as well as organism’s capacity to metabolise these compounds all have significant influence to the extent of accumulation (James and Kleinow 1994; Varanasi et al. 1992). The body burden of an individual animal depends on numerous factors, including an organism’s age/size, its lipid content, and its trophic position (Larsson et al. 1996; Kucklick and Baker 1998; Datta et al. 1998). Bioaccumulation of PCBs does not necessarily mean
that they are in the active role in organism. Because of their lipophilic nature, PCBs are usually stored in the lipid-rich tissues of the body. These chemical stores may become temporarily inactive, becoming biologically available only when lipid reserves are mobilised (Tyler et al. 1998). During the spawning event, energy reserves are mobilized into gonad production and/or spent in migration. In spawning, a portion of the chemical load is transferred to eggs (Miller 1993).

A trophic transfer could be another important factor influencing accumulation of lipophilic compounds into aquatic species (Opperhuizen and Jongeneel 1986). Longer food web of predator species usually results in higher levels of accumulated chemical in an organism (Cabana and Rasmussen 1994). Aquatic organisms tend to selectively bioaccumulate the most persistent congener. As a result, bioaccumulated individual congeners appear to be more toxic and more persistent in the body than parent commercial PCBs (Aulerich et al. 1986; Hovinga et al. 1992). While bioaccumulation is important in terms of the magnification of the chemical within food chains, it is not the sole determinant of the hazard of chemical to aquatic species (Lech and Vodicnik 1982).

2.3.2 Structure activity

The structure-binding relationships for the PCBs have shown that the most active compounds are substituted on both para (4 and 4') and at least two meta (3, 3', 5 and/or 5') positions (Safe 1990). Since PCBs do not have reactive functional groups, they must be first hydroxylated to make them more polar and excretable. Hydroxylation mainly occurs at para or meta positions whenever these sites are unsubstituted. Particularly three of these congeners, namely 3,3',4,4'-tetrachlorobiphenyl (PCB 77), 3,3',4,4',5-pentaclorobiphenyl (PCB 126), and 3,3',4,4',5,5'-hexachlorobiphenyl (PCB 169), possess a planar configuration that allows them to interact with, and bind to the Ah-receptor (Aaliborg et al. 1994). These congeners are also called coplanar PCBs (Fig. 2).
2.3.3 Biological effects and toxicity

Just like bioaccumulation of the chemical, also the biological effects and toxicity of PCBs depend on several factors including the chlorine content and purity of the commercial mixture, the animal species and strain, age, sex and reproductive stage of the animal, as well as the route and duration of exposure to the PCB mixture (Safe 1994). PCBs display biochemical and toxic responses, some of which are similar to polychlorinated dibenzo-\(\mu\)-dioxins and polychlorinated dibenzofurans (Asplund et al. 1994). Various abnormal alterations in cellular morphology of tissues have been observed in environmentally stressed fish. Morphologically, typical signs of Ah receptor mediated toxicity in early life stages of fish include yolk sac and pericardial edema, subcutaneous hemorrhages, foreshortened maxilla and sac fry mortality, the symptoms also resembling the blue-sac disease (Spitsberger et al. 1991; Walker et al. 1994; Kim and Cooper 1998; Elonen et al. 1998).

The worst-case scenarios of chemical-driven adverse biological changes in living tissues are non-reversible cellular alterations leading to malignant tumors, cancer and ultimately to the death of an organism. Epidemiological studies to date have not been able to demonstrate convincing causal relationship between human PCB exposure and increased risk of carcinogenesis. However, carcinogenicity data on rats is sufficient (Norback and Weltman 1985; Brunner et al. 1996). PCBs have not been shown to have direct genetic activity, but in causing rodent liver tumors those PCB congeners with carcinogenic activity are found to be promoters rather than initiators of carcinogenesis (Moore and Myers 1994). Indeed, PCBs are capable of activating genes involved in
generating carcinogenic compounds from polycyclic aromatic hydrocarbons (PAHs) and TCDD (George 1994).

Human are not safe and protected from the environmental exposure of halogenated aromatic hydrocarbons (HAHs). In addition to inhalation and occupational exposure, these chemicals effectively enter the food chain, and eventually end up into human diet. Serum samples collected in 1993-1995 revealed a significant PCB exposure of humans consuming Great Lakes fish (Humphrey et al. 2000). PCBs are able to accumulate into the human fetus by placental transport and continue to do so postnatally if the infants are breast-fed, as the chemical may be present in human milk (Zetterström 1999). Consequently, consumption of PCB-contaminated fish by pregnant women has been associated with decreased birth weight and deficits in cognitive function in their infants and children (Bermis and Seegal 1999). Also, various toxic responses have been described for the planar PCBs, but also for the non-planar PCB congeners. These include body weight loss, dermal toxicity, immunotoxicity, reproductive deficits, hepatotoxicity, teratogenicity, hypo- and hyperplastic tissue responses, promotor activity in carcinogenesis, and the capability to induce the CYP1A (Safe 1990; Ahlborg et al. 1994, Pohjanvirta and Tuomisto 1994).

In recent years, environmental research has also been concerned about the ecological and human health impacts of chemicals possibly disrupting the endocrine system that regulates growth, metabolism, reproduction, and immune functions. Individual congeners and commercial mixtures of PCBs, as well as their hydroxylated metabolites (PCDDs and PCDFs) have been shown to be able to mimic endogenous hormones, thus being capable of modulating and/or interfering the endocrine system of animals (Colborn and Smolen 1996; Tyler et al. 1998). The similarity of toxic effects of halogenated aromatic compounds in different fish species indicates that the Ah-receptor is apparently responsible for the toxicity in these organisms (Elonen et al. 1998).
3 OBJECTIVES

The general objective of this thesis was to demonstrate – using both laboratory experiments and field studies – the use of biotransformation enzyme activity measurements together with histopathological tissue analysis as biomarkers of both acute and sublethal chemical stress on aquatic compartment. Fish were used as a model organism, and polychlorinated biphenyls as model compounds of lipophilic, persistent and bioactive aquatic pollutants.

To select the most suitable fish species and strain for the laboratory studies the intra- and interstrain variation of both basal and chemically (BNF) challenged biotransformation enzyme system (EROD, GST, UDPGT) in adult and juvenile fish (I) was determined. Also, a preliminary study on the acute toxic behaviour of the chemical on fish was tested (II).

Enzymes from both phase I and phase II systems (AHH, EROD, GST, UDPGT) where tested to find the ones suited for the selected fish species and selected chemical exposure (PCB 77 and 126). In this laboratory study, the biotransformation enzyme system in juveniles of three representative trout strains under PCB-exposure was tested (III).

To follow the accumulative behaviour of the model chemical (PCBs), the uptake of both PCB 77 and PCB 126 in the liver tissue of i.p. injected juvenile trout were measured (III). Also, the transport of the chemical from water compartment into living tissue was tested. Moreover, the accumulation pattern of PCB 77 as well as the exposure time needed to activate the biotransformation (EROD, GST) enzyme system of embryonic fish (II) was determined.

The biomarker value of biotransformation enzyme system (EROD, GST) in monitoring chronic, sublethal PCB exposure of both adult and reproductively immature feral fish was tested in field. The small freshwater lake (Lake Kermala) with known and measured PCB pollution was monitored in comparison to reference locations (IV).

Since an induction/inhibition of biotransformation enzyme system may not be a hazard in itself, the potential toxic effects of chronic PCB exposure on feral fish using histopathology as an endpoint was studied (V and VI).
4 MATERIALS AND METHODS

Rainbow trout (*Oncorhynchus mykiss*), one of the most widely used fish species in both aquaculture and laboratory studies all over the world, was also the species of choice for our laboratory experiments (I, II, III). All the fish material for our exposure studies was obtained from a Finnish fish farm Savon taimen Oy. In the field studies, two behaviourally different Cyprinid-species, bream (*Abramis brama*) (V, VI) and asp (*Aspius aspius*) (IV, VI) were caught at the study sites as representatives for the feral freshwater fish species. Descriptions of the analysis used in this thesis are listed in table 2. Details about sample collection and preparation as well as any analytical method employed in this series of studies are described in the each original article.
Table 2. Analysis performed in this thesis.

<table>
<thead>
<tr>
<th>Analysis</th>
<th>Detection method</th>
<th>Dimensions</th>
<th>Study</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cellular responses:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CYP1A</td>
<td>Immunoplotting of CYP1A</td>
<td>pmol/mg protein</td>
<td>III</td>
<td>Kloepper-Sams et al. 1987</td>
</tr>
<tr>
<td>Western plot</td>
<td>Gel electrophoresis, densitometer</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Immunohistochemistry</td>
<td>Light microscopy</td>
<td>Localization of</td>
<td>III</td>
<td>Smolowicz et al. 1991</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CYP1A protein</td>
<td></td>
<td></td>
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<tr>
<td><strong>Catalytic activity</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EROD</td>
<td>7-ethoxyresorufin O-deethylase</td>
<td>pmol/min/mg</td>
<td>I-IV</td>
<td>Burke and Mayer 1974</td>
</tr>
<tr>
<td></td>
<td>Spectrophotometer</td>
<td>protein</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AHH</td>
<td>Aryl hydrocarbon hydroxylase</td>
<td>pmol/min/mg</td>
<td>III</td>
<td>Nebert and Gelboin 1968</td>
</tr>
<tr>
<td></td>
<td>Spectrophotometer</td>
<td>protein</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Conjugation</strong></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GST</td>
<td>Glutathione S-transferase</td>
<td>nmol/min/mg</td>
<td>I-IV</td>
<td>Habig et al. 1974</td>
</tr>
<tr>
<td></td>
<td>Spectrophotometer</td>
<td>protein</td>
<td></td>
<td></td>
</tr>
<tr>
<td>UDPGT</td>
<td>UDP-glucuronosyltransferase</td>
<td>pmol/min/mg</td>
<td>I, III</td>
<td>Hänninen 1968</td>
</tr>
<tr>
<td></td>
<td>Spectrophotometer</td>
<td>protein</td>
<td></td>
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<tr>
<td><strong>Others</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Histopathology</td>
<td>Light microscopy</td>
<td>Cellular alterations</td>
<td>V, VI</td>
<td>Myers et al. 1994, 1998</td>
</tr>
<tr>
<td>BIORAD</td>
<td>Total protein content</td>
<td>mg protein</td>
<td>I-IV</td>
<td>Bradford 1976</td>
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<td></td>
<td>Spectrophotometer</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LSI</td>
<td>Liver-somatic index ( Liver weight/body weight) x 100</td>
<td>dimensionless</td>
<td>III</td>
<td>Adams and McLean 1985</td>
</tr>
<tr>
<td></td>
<td>(Liver weight/body weight) x 100</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Chemical responses:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LSC</td>
<td>Liquid scintillation counting</td>
<td>C^{14} counts/minute</td>
<td>II</td>
<td>Aalto et al. 1994</td>
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<tr>
<td></td>
<td>Liquid scintillation counter</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>iPCB</td>
<td>Chemical residues of total PCB</td>
<td>µg/kg wet weight</td>
<td>III, IV</td>
<td>Tarhanen et al. 1999</td>
</tr>
<tr>
<td></td>
<td>Gas chromatograph, mass spectrometer</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
4.1 Cellular responses

4.1.1 CYP1A monooxygenases

4.1.1.1 Catalytic activity (Studies I, II, III and IV)

The hepatic and renal monooxygenase activities of the fish were measured from the microsomal fraction using 7-ethoxyresorufin (final concentration 2 µM) as a substrate. The deethylation of 7-ethoxyresorufin (EROD) was measured with a Shimadzu fluorescence spectrophotometer (RF-5001PC) in a kinetic reaction with resorufin as reference (Burke and Mayer 1974). Aryl hydrocarbon hydroxylase (AHH) activity was determined using benzo(a)pyrene (85 µM) as substrate: the hydroxylated benzo(a)pyrene was measured spectrofluorometrically (Perkin-Elmer MPF-43A) using 3-hydroxybenzo(a)pyrene as reference (Nebert and Gelboin 1968).

4.1.1.2 Immunoplotting (Study III)

Fish cytochrome P4501A equivalents were measured from microsomal protein samples using SDS polyacrylamide gel electrophoresis in a 12% acrylamide gel (Kloepfer-Sams et al. 1987) with monoclonal antibody (Mab 1-12-3) to scup P450E and with secondary antibody [goat anti-mouse IgG linked to alkaline phosphatase (Bio-Rad)]. The results were quantified densitometrically (Shimadzu Dual-Wavelength Flying-Spot Scanner CS-9000).

4.1.1.3 Immunohistochemistry (Study III)

The immunohistochemical (IHC) detection of P4501A in fish tissue sections (liver, kidney, intestine) exposed to PCB 77 and PCB 126 was done by an indirect peroxidase labeling method (Smolowitz et al. 1991) using monoclonal antibody Mab 1-12-3 to scup P4501A1. BNF-treated fish were used as positive controls. Color development was achieved by incubation in 3-amino-9-ethylcarbazole. Sections were counterstained with Mayer's hematoxylin and mounted in glycerol. IHC detection of CYP1A was scored both for the occurrence of the stain and for the intensity of staining for each tissue.
4.1.2 Conjugation (Studies I, II, III and IV)

Glutathione S-transferase (GST) activity in the cytosolic fraction was analysed by a Perkin-Elmer Lambda 2 UV/VIS spectrophotometer with 1-chloro-2,4-dinitrobenzene (CDNB) as a substrate (Habig et al. 1974). Microsomal UDP-glucuronosyltransferase (UDPGT) activity was measured spectrophotometrically (Shimadzu UV-240) using p-nitrophenol as the aglycone (Hänninen 1968). Total protein contents in microsomal as well as in cytosolic fractions of kidney and liver were measured by the protein-dye binding method (BIORAD) of Bradford (1976).

4.2 Chemical responses

4.2.1 Liquid scintillation counting (Study II)

A Wallac 1214 Rackbeta scintillation counter was used to measure weak beta emission produced by \(^{14}C\)-labelled PCB 77 (specific activity 37.1 mCi/mmol). The prepared solutions were placed in capped vials into a lightproof chamber where two photomultiplier tubes detected the scintillations and converted them into electrical pulses for recording (Aalto et al. 1994).

4.2.2 Chemical residue analysis (Studies III and IV)

In study III, total content of PCB-congeners 77 and 126 (purchased from Cambridge Isotope Laboratories (CIL.) MA, USA) in trout liver tissue was done by soxhlet extraction with a solvent mixture hexane-acetone (1:1). The organic phase was analysed for PCB content with a gas chromatograph (GC) coupled to a series mass spectrometry (MS). In study IV, the data for muscle chemistry is combined from three separate samplings during 1996-1998. In 1996 analysis (Lake Kernaala asp), muscle tissue samples were extracted with the solvent mixture of acetone-hexane-petrolhexane-ether (5:5:2:5:9:1) in a Soxhlet apparatus. 2,4,6-TCB was used as an internal standard. The total PCB content of the organic phase was analysed with the dual GC with an electron-capture detection (ECD). In chemical analysis during 1997-1998 (bream from Lake Kernaala and Lake Alasjärvi; asp from River Kokemäenjoki) the same internal standard was used. However, the soxhlet extraction was done with toluene. The organic phase was analysed for PCB content with a GC coupled to a series MS.
4.3 Histopathology (Studies V and VI)

Tissue samples for histopathology were preserved in 10% neutral buffered formalin. Preserved tissues were processed routinely and embedded in paraffin, sectioned (5 μm) and stained with Gill’s hematoxylin and eosin for light microscopy. For histological examination slides were evaluated randomly three times and the results of each evaluation combined.

4.4 Early Life Stage NOEC-screening (Study II)

The No Observed Effect Concentration (NOEC) -screening of PCB 77 was conducted by placing eyed embryos (50 embryos/vial) and newly hatched yolk sac larvae of the same strain (25 larvae/vial) into duplicate vials each containing 200 ml of reconstituted water and various concentrations of PCB 77 (1, 10, 100, 500 μg/l, respectively) (PCB 77 purchased from Cambridge Isotope Laboratories (CIL) MA, USA). Test solutions were prepared by dilution of a stock solution. Duplicate control vials, as well as controls containing dimethyl sulfoxide (DMSO), the carrier agent for PCB, were run in addition to the test series. During the test water quality characteristics and dissolved oxygen concentration were measured daily. All the embryos and larvae were inspected twice a day for grossly visible abnormalities and mortality.

4.5 Statistical analysis

Statistical analysis was run using SPSS/PC+ (Release 6.0 and 7.5) program. Normality test of the variables was made with the Kolmogorov-Smirnov test. The assumption of equal variances was tested with the analysis of variance (Cochran’s C test or Bartlett Box-F-test). In each case the degree of heterogeneity was significant (p < 0.05) in every variable tested. So, further analysis was made using non-parametric methods (Kruskal-Wallis oneway analysis of variances, p < 0.05; Mann-Whitney’s U-test with Bonferroni’s correction, p < 0.05) to detect statistical significance.
5 RESULTS

5.1 Preliminary studies

Study (I) was designed to assess the intra- and interstrain variability of CYP1A enzyme activities and conjugation enzyme reactions of different rainbow trout strains. To find the most suitable strain for our purposes, the most common trout strains were tested for their biotransformation characteristics. In two separate experiments, monooxygenase (EROD) and conjugation (GST and UDPGT) enzyme activities were measured from liver and kidney samples of commonly utilized trout breeds in Finland. In the preliminary study, the differences in the basal biotransformation enzyme activities were detected in two separately cultivated rainbow trout strains. Results denoted statistically significant intra- (UDPGT, GST) and interstrain (EROD) differences in biotransformation enzyme activities. In the laboratory experiment, the corresponding enzyme activities were measured from three different rainbow trout strains exposed to β-naphthoflavone (BNF), a model xenobiotic compound. The results revealed significant interstrain differences especially in hepatic EROD and GST activities. UDPGT activities in the liver, as well as the measured monooxygenase and conjugation activities in the kidney showed no notable variance between strains.

In the search of sublethal PCB concentrations for accumulation and biotransformation studies on embryonic fish, a NOEC test was conducted (II). The 12-day PCB 77 NOEC screening with rainbow trout embryos showed no mortality in any of the chemical concentrations. When the same NOEC-test was conducted with yolk sac larvae, no mortality in any of the test concentrations was observed during the first 7 exposure days. However, the scanning electron micrographs (SEM) of the lateral skin of the larvae at the 96-hour time point showed a pronounced increase in mucus production in comparison to controls (Fig. 3) (Koponen, unpublished).
Figure 3. Scanning electron micrographs (2000-x magnification) of rainbow trout larvae after 96-hour exposure to waterborne PCB 77: (A) Lateral skin of control larvae. Normal appearing microridge pattern with mucus producing cells (mu) and emerging chloride cells (ch) is evident. (B) Lateral skin of PCB 77-exposed larvae (500 μg/l) with an extensive increase in mucus secretion (Koponen, unpublished).

At the end of the NOEC exposure (12 days) there was no mortality in DMSO control groups and only 2.5 % mortality in water control groups. At the highest PCB 77-concentration (500 μg/l), dead larvae were first observed at day 9 (10 %), with the final mortality percentage of 55 at the end of the study. The final percent mortalities after 12 days exposure in the lower test concentrations (100, 10, and 1 μg/l) were 10, 7.5, and 2.5, respectively.

5.2 Bioaccumulation studies

The accumulation rate and possible accumulation threshold limits of PCB 77 into embryonic stage of fish were determined (II). The advanced rainbow trout embryos were exposed up to 7 days to waterborne sublethal concentrations of PCB 77 (1, 10 and 100 μg/l). Results indicated direct accumulation of the chemical into the eggs, but the exposure time was not long enough for PCB 77 to reach the steady state. However, at the two lowest test concentrations a temporary plateau at chemical accumulation was reached at the third exposure day. The chemical uptake increased again at day 7 in all the exposure groups.
Table 3. PCB uptake in study fish at different life stages and through different exposure routes.

<table>
<thead>
<tr>
<th>Fish</th>
<th>Exposure</th>
<th>PCB in tissue</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>O. mykiss</td>
<td>embryo</td>
<td>whole PCB 77</td>
<td>1 µg/l water 3</td>
</tr>
<tr>
<td>O. mykiss</td>
<td>embryo</td>
<td>whole PCB 77</td>
<td>10 µg/l water 3</td>
</tr>
<tr>
<td>O. mykiss</td>
<td>embryo</td>
<td>whole PCB 77</td>
<td>100 µg/l water 3</td>
</tr>
<tr>
<td>O. mykiss</td>
<td>embryo</td>
<td>whole PCB 77</td>
<td>1 µg/l water 7</td>
</tr>
<tr>
<td>O. mykiss</td>
<td>embryo</td>
<td>whole PCB 77</td>
<td>10 µg/l water 7</td>
</tr>
<tr>
<td>O. mykiss</td>
<td>embryo</td>
<td>whole PCB 77</td>
<td>100 µg/l water 7</td>
</tr>
<tr>
<td>O. mykiss</td>
<td>juvenile</td>
<td>liver PCB 77</td>
<td>100 µg/kg ip 6</td>
</tr>
<tr>
<td>O. mykiss</td>
<td>juvenile</td>
<td>liver PCB 77</td>
<td>1000 µg/kg ip 6</td>
</tr>
<tr>
<td>O. mykiss</td>
<td>juvenile</td>
<td>liver PCB 77</td>
<td>5000 µg/kg ip 6</td>
</tr>
<tr>
<td>O. mykiss</td>
<td>juvenile</td>
<td>liver PCB 126</td>
<td>100 µg/kg ip 6</td>
</tr>
<tr>
<td>O. mykiss</td>
<td>juvenile</td>
<td>liver PCB 126</td>
<td>1000 µg/kg ip 6</td>
</tr>
<tr>
<td>O. mykiss</td>
<td>juvenile</td>
<td>liver PCB 126</td>
<td>5000 µg/kg ip 6</td>
</tr>
</tbody>
</table>

A. aspius subadult muscle reference background environment chronic 47 IV
A. aspius subadult muscle tPCB sublethal environment chronic 2764b IV
A. brama adult muscle reference background environment chronic 9 IV
A. brama adult muscle tPCB sublethal environment chronic 59c IV
A. brama adult muscle tPCB sublethal environment chronic 159d IV

*The analysis data is presented as mean values without standard deviation. For detailed results see corresponding articles I-IV. b Piironen 1996, c Kernaala north, d Kernaala south.

In the study III the accumulation potency of two coplanar PCB congeners, PCB 77 and PCB 126 were determined. Juvenile rainbow trout were intraperitoneally injected with both congeners, and chemical analysis of liver tissue was performed 6 days after the injection. Results showed PCB 77 to be more readily accumulated into liver tissue: the contents of congener 126 in liver were about three times lower than the contents of congener 77.
PCB accumulation of environmentally exposed fish was also studied. Chemical analysis for total PCB in muscle tissue of two feral fish species, bream and asp, was determined in Lake Kernaala and reference locations. The PCB accumulation into fish in reference waters was extremely low in comparison to Lake Kernaala fish. Both bream and asp from Lake Kernaala showed clear PCB accumulation profile. In comparison to Lake Alasjärvi bream, the muscle and lipid PCB content was 18 and 13 times higher in Kernaala South and 7 and 7 times higher in Kernaala North. In asp the difference was even more pronounced: the PCB content in muscle and in lipid in Lake Kernaala asp was 59 and 26 times higher than in reference samples. In Lake Kernaala, a significant intra-lake variation in PCB accumulation profile was also apparent: bream caught from the northern end of the lake revealed 3 times lower PCB content in muscle and 2 times lower amount of PCB in lipid proportion than bream caught from southern sampling point.

5.3 Biotransformation enzyme activities

5.3.1 PCB-exposed fish

Biotransformation enzyme activities were measured both in laboratory and in field studies. In laboratory studies, embryonic (II) and juvenile (III) stages of rainbow trout were experimentally exposed to PCBs. First, the exposure time needed to activate the monooxygenase (EROD) and conjugation (GST) enzyme systems of fish at the advanced embryonic stage was determined. Rainbow trout embryos were exposed to sublethal waterborne concentrations of PCB 77 (1, 10 and 100 mg/l) and sampled at days 3 and 7. The CYP1A system, measured as EROD enzyme activity, was shown to be a sensitive indicator of embryonic chemical exposure, being induced at the low PCB concentrations and after a short exposure time. When compared to control values at the third exposure day, dose-related 5.5- (1 μg/l), 6.4- (10 μg/l), and 8.8- (100 μg/l) fold increases in EROD induction were detected. Similarly, corresponding increases in EROD induction after seven exposure days were 9.0-, 6.9-, 8.0- fold in comparison to controls. The lack of dose-response at the latter sampling point might have occurred because the embryonic monooxygenase activities already had attained their maximal induction capacity with the lower doses. The conjugation enzyme system (GST) appeared also to be functioning, although it did not respond to the PCB exposure. GST enzyme activities at the third exposure day seemed to be slightly induced at the lowest test concentration. However, induction diminished at the middle concentration, and GST activity in the
highest dose group was essentially the same as that in controls. At day 7, GST enzyme activities were slightly under control values in all three PCB dose groups.

Table 4. Biotransformation enzyme activities of reference/control fish as well as environmentally and artificially exposed fish in wild, in cultured and in laboratory conditions.

<table>
<thead>
<tr>
<th>Study</th>
<th>Fish origin/ study site</th>
<th>Devel. stage</th>
<th>Sex</th>
<th>Inducing chemical</th>
<th>Dose or conc.</th>
<th>Exp. route</th>
<th>Exp. time</th>
<th>EROD</th>
<th>AHF</th>
<th>GST</th>
<th>UDPGT</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>RT</td>
<td>cultured, F</td>
<td>adult</td>
<td>m/f 15</td>
<td>reference</td>
<td>environmental</td>
<td>chronic</td>
<td>19</td>
<td>nm</td>
<td>299</td>
<td>754</td>
</tr>
<tr>
<td></td>
<td>RT</td>
<td>cultured, F</td>
<td>adult</td>
<td>m/f 15</td>
<td>reference</td>
<td>environmental</td>
<td>chronic</td>
<td>12</td>
<td>nm</td>
<td>323</td>
<td>457</td>
</tr>
<tr>
<td></td>
<td>RT</td>
<td>cultured, F</td>
<td>adult</td>
<td>m/f 15</td>
<td>reference</td>
<td>environmental</td>
<td>chronic</td>
<td>10</td>
<td>nm</td>
<td>272</td>
<td>649</td>
</tr>
<tr>
<td></td>
<td>NL</td>
<td>laboratory</td>
<td>juvenile</td>
<td>f 15</td>
<td>control</td>
<td>ip olive oil</td>
<td>7 days</td>
<td>51</td>
<td>nm</td>
<td>574</td>
<td>111</td>
</tr>
<tr>
<td></td>
<td>NL</td>
<td>laboratory</td>
<td>juvenile</td>
<td>f 15</td>
<td>BNF</td>
<td>ip 20 mg/kg</td>
<td>7 days</td>
<td>625</td>
<td>nm</td>
<td>356</td>
<td>131</td>
</tr>
<tr>
<td></td>
<td>T</td>
<td>laboratory</td>
<td>juvenile</td>
<td>f 15</td>
<td>control</td>
<td>ip olive oil</td>
<td>7 days</td>
<td>50</td>
<td>nm</td>
<td>513</td>
<td>122</td>
</tr>
<tr>
<td></td>
<td>T</td>
<td>laboratory</td>
<td>juvenile</td>
<td>f 15</td>
<td>BNF</td>
<td>ip 20 mg/kg</td>
<td>7 days</td>
<td>535</td>
<td>nm</td>
<td>237</td>
<td>139</td>
</tr>
<tr>
<td>II</td>
<td>RT</td>
<td>laboratory</td>
<td>embryo</td>
<td>?/20</td>
<td>control</td>
<td>water 0</td>
<td>3 days</td>
<td>0.3</td>
<td>nm</td>
<td>160</td>
<td>nm</td>
</tr>
<tr>
<td></td>
<td>RT</td>
<td>laboratory</td>
<td>embryo</td>
<td>?/20</td>
<td>PCB 77</td>
<td>water 1 µg/l</td>
<td>3 days</td>
<td>1.4</td>
<td>nm</td>
<td>190</td>
<td>nm</td>
</tr>
<tr>
<td></td>
<td>RT</td>
<td>laboratory</td>
<td>embryo</td>
<td>?/20</td>
<td>PCB 77</td>
<td>water 10 µg/l</td>
<td>3 days</td>
<td>1.6</td>
<td>nm</td>
<td>177</td>
<td>nm</td>
</tr>
<tr>
<td></td>
<td>RT</td>
<td>laboratory</td>
<td>embryo</td>
<td>?/20</td>
<td>PCB 77</td>
<td>water 100 µg/l</td>
<td>3 days</td>
<td>2.2</td>
<td>nm</td>
<td>158</td>
<td>nm</td>
</tr>
<tr>
<td></td>
<td>RT</td>
<td>laboratory</td>
<td>embryo</td>
<td>?/20</td>
<td>PCB 77</td>
<td>water 1 µg/l</td>
<td>7 days</td>
<td>0.7</td>
<td>nm</td>
<td>179</td>
<td>nm</td>
</tr>
<tr>
<td></td>
<td>RT</td>
<td>laboratory</td>
<td>embryo</td>
<td>?/20</td>
<td>PCB 77</td>
<td>water 10 µg/l</td>
<td>7 days</td>
<td>6.3</td>
<td>nm</td>
<td>159</td>
<td>nm</td>
</tr>
<tr>
<td></td>
<td>RT</td>
<td>laboratory</td>
<td>embryo</td>
<td>?/20</td>
<td>PCB 77</td>
<td>water 100 µg/l</td>
<td>7 days</td>
<td>4.8</td>
<td>nm</td>
<td>164</td>
<td>nm</td>
</tr>
<tr>
<td></td>
<td>RT</td>
<td>laboratory</td>
<td>embryo</td>
<td>?/20</td>
<td>PCB 77</td>
<td>water 100 µg/l</td>
<td>7 days</td>
<td>5.6</td>
<td>nm</td>
<td>169</td>
<td>nm</td>
</tr>
</tbody>
</table>

The analysis data are presented as mean values without standard deviations. For detailed results see corresponding articles.

nm = not measured, RT = rainbow trout, ST = Savon ranta, NL = Nižné laka, T = Tiere, FE = female, f = female, m = male, n = not performed injection.

LA = Lake Alašjärvi, K = Lake Kerimäki, RK = River Kolensjoki, m = male, f = female, p = not performed injection.
The biotransformation potency of the two most toxic PCB congeners, PCB 77 and PCB 126 was studied with immature fish. Juvenile rainbow trout received i.p. injection (0.1, 1 and 5 mg/kg) of either PCB 77 or PCB 126. After 6 exposure days, biotransformation enzyme activities (EROD, AHH, UDPGT and GST) in liver and kidney, and hepatic CYP1A protein content were analysed. Both congeners strongly induced EROD and AHH activities in liver and kidney. In the liver the highest dose of both congeners and in kidney the highest dose of PCB 126 did not increase the CYP activities as much as would have been expected. The immunochemical CYP1A protein analysis showed the induction to proceed in a dose-related manner even at the highest doses, strengthening the hypothesis of the suppression of the catalytic enzyme system in overdose exposure situations. With respect to conjugation reactions, both hepatic and renal UDPGT activities were induced with both of the congeners tested. Instead, the GST activities did not show any clear response to PCB exposure.

5.3.2 Field studies

The biotransformation enzyme activities were also detected in the field (IV). In the PCB polluted lake Kernaala, the enzyme activities (EROD; GST) of feral fish (bream and asp) were detected both in spring and fall, and compared to the corresponding enzyme values of fish caught from the reference locations (Lake Alasjärvi/bream, River Kokemäenjoki/asp). In Kernaala spring sampling (before the spawning time of bream), male bream showed significantly higher hepatic EROD activities than female bream. In fall sampling, after the spawning time of bream, EROD activities were remarkably diminished both in male and in female fish, respectively. In Alasjärvi fall sampling, both male and female bream showed notably lower EROD activities when compared to Kernaala fall sampling. Also Lake Kernaala asp showed clearly induced hepatic EROD activities when compared to the reference fish caught from River Kokemäenjoki. Female asp displayed significantly higher EROD activities than male asp in Lake Kernaala. The hepatic EROD activities in reference asp were hardly measurable, being at the same basal level in both sexes.

Differences between sexes, as well as seasonal differences were apparent in conjugation (GST) enzyme activities in Lake Kernaala bream. Prespawning male fish showed significantly higher GST activities than female fish. Also, there was notable increase in conjugation enzyme activities from spring to fall in both sexes. However, GST values of both genders from Kernaala fall sampling were
essentially at the same level as the reference values from Lake Alasjärvi, respectively. Male asp showed somewhat higher hepatic GST values than female fish. However, no significant gender-specific differences in hepatic GST activities were found inside either sampling. GST activities were higher in both male and female asp in Lake Kernaala than in asp caught from reference location.

5.4 Histopathology of feral fish

An overall histopathological evaluation of feral fish was conducted to find evidence of abnormal cellular alterations that could reflect the prolonged chemical stress (Table 5). The histopathological analysis of feral fish (V, VI) consisted of comprehensive morphological tissue examination of gill, liver, kidney, spleen, gonad and intestine. Table 4 also contains some unpublished data.

Table 5. Histopathological alterations observed in Lake Kernaala bream and asp.

<table>
<thead>
<tr>
<th>Target organ</th>
<th>...linked to season, age, or reproductive stage of fish</th>
<th>...indirectly linked to chemical stress</th>
<th>...directly linked to chemical stress</th>
<th>Study</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gill</td>
<td>lamellar edema, hyperplasia of respiratory epithelium, increased RC prevalence</td>
<td>increased RC prevalence</td>
<td>none observed</td>
<td>unpubl.</td>
</tr>
<tr>
<td>Skin</td>
<td>increased mucus formation</td>
<td>increased mucus formation</td>
<td>increased mucus formation</td>
<td>unpubl.</td>
</tr>
<tr>
<td>Liver</td>
<td>fibrosis surrounding blood vessels and bile ducts, fatty vacuolation of hepatocytes, increased MMC, parasite and RC prevalence</td>
<td>increased parasite and RC prevalence</td>
<td>none observed</td>
<td>V, VI</td>
</tr>
<tr>
<td>Kidney</td>
<td>hyaline droplets, tubular dilution, newly formed nephrons, increased MMC, DGC, ME, FBS and ABC prevalence</td>
<td>increased RC, DGC, ME, FBS and ABC prevalence</td>
<td>DGC, ME</td>
<td>V, VI</td>
</tr>
<tr>
<td>Spleen</td>
<td>MMC, inflammation</td>
<td>increased RC and parasite prevalence</td>
<td>none observed</td>
<td>V, VI</td>
</tr>
<tr>
<td>Gonad</td>
<td>MMC</td>
<td>none observed</td>
<td>none observed</td>
<td>VI</td>
</tr>
<tr>
<td>Intestine</td>
<td>inflammation</td>
<td>none observed</td>
<td>none observed</td>
<td>V, VI</td>
</tr>
</tbody>
</table>

RC = rosette cell, MMC = melanomacrophage centers, DGC = dilation of glomerular capillaries, ME = mesangial edema, ABC = adhesion of Bowman’s capsule, FBS = filling of Bowman’s space
5.4.1 Cellular changes

In the posterior kidney of both bream and asp caught from Lake Kernaala, several pathological changes were diagnosed. In the renal corpuscle, high prevalence of dilution of glomerular capillaries (DGC), and mesangial edema (ME) were detected in both bream and asp, while the occurrence of these lesions was low or totally absent in reference locations. In both fish species, yet another two renal lesions, the filling of Bowman’s space (FBS) and an adhesion between parietal and visceral layers of Bowman’s capsule (ABS), were apparent in higher prevalence than in corresponding reference fish. Oftentimes all these lesions were co-occurring in Lake Kernaala fish. Other changes in kidney included increased tubular dilation, hyaline droplets, protein resorption, and increased numbers of developing nephrons. However, there were no statistically significant differences in either of the prevalence or tissue distribution of these changes between Lake Kernaala and reference locations.

All the cellular alterations observed in liver of either bream or asp were linked to season or current stage of parasitic infections. A fibrosis surrounding hepatic arteries, veins and bile ducts was clearly evident in Lake Kernaala bream sampled in spring. At fall samplings, however, the prevalence of this condition was significantly lower both in Kernaala and in Alasjärvi bream. Apart from pathogen-induced inflammation, a focal increase in inflammatory cells co-occurred with the fibrotic lesions in liver. This type of perivascular/peribiliary fibrosis was not found in asp. A moderate prevalence of focal fatty vacuolation of hepatocytes was observed in bream in Kernaala spring sampling. In fall sampling, corresponding prevalence for focal fatty vacuolation was significantly lower in Lake Kernaala, while no such hepatic alteration was found from Lake Alasjärvi bream. In asp, a high occurrence of multifocal to diffuse fatty change in liver parenchyma was evident in both sampling locations. Of the other tissues studied, gill, gonad and intestine revealed no lesions or abnormal cellular alteration that could have been directly linked to prolonged chemical stress.

RCs were evident in every freshwater bream caught from a PCB-polluted lake, Lake Kernaala. The frequency of RCs corresponded strongly with the current phase of parasitic infections in liver, kidney, and spleen. Also, RCs were evident multifocally in every gill and intestine sample studied. However, no statistical differences in RC number or intratissue location between samplings were detected in these organs. Interestingly, no RCs were detected in gonads.
5.4.2 Parasite infections

In Kernaal samplings, a variable proportion of bream were infected by the larval nematode, *Raphidascaris acus* (liver, kidney, and spleen), an unspecified species of *Myxidium* (liver), and/or an unspecified myxosporean (kidney) parasite. There was a highly significant seasonal difference in occurrence of nematode-associated hepatic granulomas in Lake Kernaala. Interestingly, hepatic nematode infection in bream caught from reference location was 3-times lower than in fish captured simultaneously from Lake Kernaala. An unspecified *Myxidium* infection in bream liver in Lake Kernaala showed seasonal variation. No hepatic parasites were found from any of the asp examined in this study. In kidney, the prevalence of unspecified myxosporean infection in bream showed significant seasonal variation. The prevalence of myxosporean infection in kidney of Lake Kernaala asp was significantly higher than in reference asp. It is important to note that no myxosporeans were found in glomeruli, or proximal or distal convoluted segments. Larval nematode infection in bream kidney was low in all samplings. Neither nematode infection nor parasite-associated granulomas were found from any of the asp kidney studied.
6 DISCUSSION

6.1 Intra- and interstrain variation of biotransformation enzyme biomarkers

For the reliable and repeatable study results both in the field and in the laboratory, it is important to minimise the number of factors causing variation by standardising the study conditions as homogenous as possible. According to recent findings, equally important is to minimise the variability caused by the selected fish breed. In the search for the best possible test species of fish, several rainbow trout strains were selected. The basal activities of biotransformation enzymes were shown to have interstrain, but also significant intrastain variation in the field sampling of adult rainbow trout strains. In the laboratory study, three strains tested differed moderately from each other in their responses to a model xenobiotic compound, BNF. Eventually, the Suvon taimen strain was selected for further studies for its higher induction capacity both in hepatic and renal EROD activities in comparison to other strains tested.

In this study, EROD enzyme system displayed a clear induction, being sufficiently sensitive to indicate significant interstrain differences. Among monoxygenase activities in the liver of fish, EROD has been induced most strongly by PAH-type xenobiotics (Bucheli and Fent 1995). On the contrary, BNF-exposure did not produce any significant effect on hepatic or renal UDPGT activities. In the present study the exposure response was quite similar in all three strains showing no notable interstrain variation. In several fish species, glucuronidation has been found to be one of the most important pathways for the conjugation of numerous xenobiotics. It has been shown previously that BNF-treatment moderately induces UDPGT activities in fish liver (Zhang et al. 1990; Clarke et al. 1991). Interestingly, in our BNF exposure, cytosolic GST was showing inhibition instead of induction. In agreement, Zhang and co-workers (1990) discovered that although a single dose of BNF induced GST activity, a second treatment 3 days later repressed the activity. This has been thought to be due to inhibition by a metabolite of BNF (George 1994). However, when rainbow trout were intraperitoneally injected with BNF, a 2 fold (Andersson et al. 1985) and 2.5 fold (Goksoyr et al. 1987) induction of hepatic GST activities were found.

This study highlights the need for intercalibration of fish strains used in the experimental studies in aquatic toxicology. Particularly in exposure studies, the true effects of toxic compounds can easily
be disguised behind the variability caused by the strain itself. This can be taken into account by ensuring that the same fish population is consistently used in such studies.

6.2 PCB uptake

Experimental early life stage exposure showed how easily PCB 77 (the model PCB congener used in this laboratory study) moved through the egg chorion and accumulated into fish embryos. No threshold for chemical uptake was evident, and the accumulation continued throughout the test. PCB 77 was also rapidly uptaken by larval fathead minnow (Lindström-Seppä et al. 1994). This particular PCB congener is one of the most potent accumulator chemicals into living organisms, although similarly significant accumulation into lake trout embryo and larvae has been observed with less toxic PCB congener as well (Broyles and Noveck 1979). In the present study, no clear steady state in the accumulation pattern was seen, only a temporary plateaus in the middle of the exposure. These results are in accordance with Feijtel et al. (1997), who approximated that for the most PCBs the time to reach 95% steady state would be over 15 days.

According to Walker and Peterson (1994) the main route of accumulation of lipophilic chemicals in feral fish eggs is through the transfer from maternal tissue rather than through water or sediments. That hypothesis is agreed by Miller (1993), who demonstrated that the total PCB concentrations in lake trout eggs from Lake Michigan and Lake Superior were 66 and 78% of the total PCB contents in maternal muscle tissue on a lipid-normalized basis. Consequently, it is obvious that these highly lipophilic, persistent chemicals are capable of accumulating into the fish embryo throughout their early development. Ones inside the egg chorion, the majority of PCBs are stored in embryonic yolk (Broyles and Noveck 1979), until redistributed when sac fry start to utilise its yolk reserves. Interestingly, when larvae of striped bass were exposed to Aroclor 1254 through water and food, the PCB accumulated from water was retained for longer periods, and PCB body burdens obtained from food were eliminated faster than by fish that accumulated PCB from water (Wang 1998).

In our laboratory exposure study with juvenile fish, both PCB congeners 126 and 77 were shown to be readily accumulating into liver tissue of juvenile rainbow trout. The hepatic contents of congener 126 were about three times lower than the contents of congener 77. The fact that PCB 126 contains more chlorine than PCB 77 could explain the difference in their accumulation potential in our
experiment. A rapid uptake and distribution of the chemical was also seen in fathead minnow (Pimephales promelas) exposed to PCB 77 (Lindström-Seppälä et al. 1994). According to Safe (1990), highly chlorinated compounds are not as effectively accumulated as the less chlorinated counterparts.

Results of the field studies in Lake Kernaala and reference locations indicated that, regardless of rather low discharge rate over the time (about 900 litres during the period of 1956-1983), PCBs are still present and accumulating into feral fish. Also, especially in asp, the muscle PCB content was apparently connected to the tissue lipid concentration, which was nine times greater than in bream. Therefore, the fact that subadult asp accumulate more lipophilic PCBs than adult bream could be more dependent on the muscle lipid content rather than the age and size of the fish. The amounts of accumulated PCBs analysed from tissues of freshwater fish silverside (Odontesthes bonariensis) also reflected the differences in tissue lipid content (Menone et al. 2000). Due to the high lipid content of the liver it usually accumulates chemicals from blood stream very rapidly. In the experimental exposure of adult fathead minnow, PCB 77 concentrations in muscle were measured to be lower than those in ovary or liver (Lindström-Seppälä et al. 1994). However, the lipid-normalized amounts of accumulated PCBs in feral bream from the River Elbe were similar both in muscle and in liver (Marth et al. 1997). In the study of Kucklick and Baker (1998), the lipid content of the aquatic organisms studied explained 81% of the variability in wet weight tPCB concentrations.

Some decrease in total PCBs in fish after the termination of the accumulation phase has been recorded (Bruggeman et al. 1984). The role of biotransformation in the depuration of PCBs has been discussed in detail elsewhere (de Boer et al. 1994). In general, fish is apparently able to metabolise certain PCB congeners, but metabolism may not play a significant role in determining the body burden of these chemicals (Varnas et al. 1992). If metabolism/depuration of PCBs take place in an environment where chemically steady state prevails, the body burden analysis would underestimate the total amount of the chemical processed by an organism under prolonged environmental exposure. In an experimental elimination study, a large percentage of two non-ortho substituted PCB 77 and PCB 126 as well as the most of the two mono-ortho substituted PCBs 105 and 118, remained in rainbow trout early life stages through the sac fry stage (Zabel et al. 1995). When Hajslovia et al. (1997) studied the depuration of PCBs from heavily contaminated carp
(Cyprinus carpio) transferred to clean water for 25 months, an apparent removal of PCBs from the muscle tissue was detected, but no decrease of total fish body burden for highly chlorinated PCBs was recorded. In the same study, starvation caused rapid increase of PCBs in fat occurred due to the significant decrease of lipid content in muscle tissue of carp (Hajalovia et al. 1997). Temporal variations in the nutritional status of animal can be considerable, and these variations may influence toxicokinetic processes and biomarker responses as well (Joergensen et al. 1999).

6.3 Biological effects of PCBs in fish
6.3.1 Early life stage effects

Early life stages of fish are known to be the most sensitive developmental stages of fish to the lethal effects of HAlHs (Prince and Cooper 1989; Weis and Weis 1989; Spitsbergen et al. 1991; Walker et al. 1992). In our studies, however, regardless of the immediate uptake of the chemical, the model congener (PCB 77) was not acutely toxic neither to embryos nor larvae rainbow trout. Even in the highest, environmentally non-realistic waterborne concentration (100 µg/l), both embryos and larvae survived showing no or little signs of acute toxicity. Walker and Peterson (1991) also reported no increase in mortality during the egg stage of rainbow trout exposed to injected doses of PCB 77.

Interestingly, our studies indicated notable increase in mucus production in the skin of PCB-exposed trout larvae (see Fig. 3), supposing that the symptoms of stress were already apparent before any concrete evidence of toxicity was monitored. Dermal toxicity of PCBs has been observed both in human and in laboratory animals (Hara 1985; Allen et al. 1974). Fish skin forms an important interface between internal tissues and the external environment. Exposure to toxic substances causes accelerated mucus production in fish skin (Varanasi et al. 1975). The gas exchange in teleostean embryos and larvae is primarily cutaneous (Rombough 1988). Therefore, especially when the early life stages of fish are concerned, the extensive mucus secretion, while protecting fish against environmental trauma, can also alter important physiological functions of embryonic and larval fish. Several other studies have also found fish skin to be very susceptible to environmental stressors (Pickering et al. 1982; Varanasi et al. 1975; Miller and Mackay 1982).

The xenobiotic metabolism of embryonic and larval fish is not yet fully functioning, which is one of the reasons for their high sensitivity to chemical stress at these early stages. Morphologically, typical
signs of Ah receptor mediated toxicity in early life stages of fish include yolk sac and pericardial edema, subcutaneous hemorrhages, foreshortened maxilla and sac fry mortality, the symptoms also resembling the blue-sac disease (Spitsbergen et al. 1991; Walker et al. 1994; Kim and Cooper 1997; Elonen et al. 1998). In TCDD-induced blue-sac disease, it has been hypothesized that the induction of CYP1A1 in vascular endothelium may cause changes in hemodynamic and/or vascular permeability that leads to edema and early life stage mortality of fish (Guiney et al. 1990). Non-ortho PCBs (IUPAC numbers 81, 169, 77, and 126) have been shown to cause signs of toxicity identical to those of TCDD in early life stages of rainbow trout, while several mono-ortho substituted PCBs (IUPAC numbers 4, 28, 52, 105, 118, 128, 138, 156, and 170) did not cause any symptoms of blue-sac disease or sac fry mortality (Zabel et al. 1995; Walker and Peterson 1991).

6.3.2 CYP1A enzyme activities

A cytochrome P450 1A-like protein has been found in all fish species so far investigated. To date, the fish CYP1A seems to be the most utilized of phase I biotransformation enzymes in aquatic biomonitoring studies. Numerous laboratory and field studies have provided evidence for induction of CYP1A in fish exposed to PAHs, PCBs, dioxins and related compounds (Payne et al. 1987; Kleinow et al. 1987; Elskus and Stegeman 1989; Van Veld et al. 1990; Stein et al. 1992; Stegeman et al. 1992; Goksøyr et al. 1994). The induction of CYP1A is rapid and can be monitored within hours after exposure (Haasch et al. 1989; Klopper-Sams and Stegeman 1989).

In our early life stage exposure study, every waterborne PCB 77 concentration (1-100 ug/l) caused induction in embryonic EROD enzyme system shortly after the exposure, clearly before the first adverse effects or mortality occurred. The hepatic monoxygenase system of the fetal lake trout during embryonic and larvae stages was shown to be induced by exposure to Aroclor 1254 (Binder and Lech 1984). The sensitivity of the CYP1A system to PCBs in killifish embryos increased nine-fold within 24 h of hatching (Binder and Stegeman 1984).

In the juvenile-exposure study, the effects of PCB 126 and PCB 77 on CYP1A and conjugation activities in immature rainbow trout were determined. After six exposure days, both hepatic and renal EROD and AHH activities were increased in a dose-dependent manner. Congener 126 exposed trout generally attained higher EROD and AHH activities in comparison to fish exposed to
congener 77. Also, both hepatic and renal EROD enzyme activities were more potently induced than AHH enzyme activities. In comparison to the dose needed to cause EROD induction in juvenile catfish (*Ictalus punctatus*), more than ten times higher dose of Aroclor 1254 was required to induce AHH enzyme system (Ankley et al. 1986). These results are in consensus with several other studies (Janz and Metcalfe 1991; Safe 1990; Walker and Peterson 1991; Safe 1994). CYP1A expression is highest in the liver, and it is localized in hepatocytes and endothelial cells (Smolowitz et al. 1991). Extrahepatic activity is also present and is usually most pronounced in kidney (Melancon et al. 1988; Husey et al. 1994). Our findings of higher EROD activities in liver than in kidney are parallel to the more recent study, in which juvenile rainbow trout injected with various doses of Clophen A50 and TCB expressed similar patterns of time-dependent increase in EROD activity in the liver and kidney, but renal EROD activities were 5- to 10-fold lower (Blom & Förlin 1997). CYP1A is not normally constitutively expressed in significant levels (Stegeman and Hahn 1994). However, such constitutive expression of CYP1A in kidney has recently been observed in adult *F. heteroclitus* (Elskus et al. 1999). Also, EROD activities in kidney higher than those in liver in lake trout caught from Lake Ontario in 1994 has been reported (Palace et al. 1994). CYP1A induction may occur in extrahepatic tissues even when elevated activity cannot be detected in the liver (Payne 1984).

In our study, an inhibition of hepatic catalytic activities with higher dose (5 mg/kg) was apparent. The induction of CYP1A protein showed that the *de novo* synthesis of cytochrome P4501A did continue even when catalytic activities did not show any further increase. PCB 77 induced microsomal CYP1A and hepatic EROD activities of juvenile rainbow trout, with maximum induction detected not earlier than 13 days after the injection (Tyle et al. 1991). Thus, measuring only catalytic activities can give a misleading picture of current PCB induction in fish. Instead of induction, several studies have demonstrated that high doses of PAHs and PCBs can lead to inhibition of catalytic activity (Gooch et al. 1989; Haasch et al. 1993; Lindström-Seppä et al. 1994).

In some experimental studies a nonresponsiveness of CYP1A enzymes to PCBs has been documented. In the study of Stahl et al. (1984) redfish receiving a single ip injection of Aroclor 1254 did not show any increase in microsomal monooxygenases. More recently, both dietary and intraperitoneal exposure of adult zebra fish to Aroclor 1254 failed to increase CYP1A protein and did not induce hepatic EROD activity (Troxel et al. 1997). It is also possible that chronic exposure to
PCB may impair the induction potency of the CYP1A enzyme system in fish. Feral perch caught from a highly PCB-polluted lake in Sweden had exceptionally low levels of hepatic CYP1A expression, and they did not respond to treatment with PCB 77 in the laboratory either, implying that the prior exposure profile of fish could have impaired their responsiveness to PCB (Förlin and Celander 1995). Recent study of Elksus and co-workers (1999) demonstrated reduced CYP1A expression in a population of Fundulus heteroclitus from highly contaminated Newark Bay, NJ.

Most of the laboratory experiments where xenobiotic metabolism of fish has been evaluated have been conducted in water temperatures higher than the prevailing environmental water temperatures. In our study, where juvenile rainbow trout were exposed to PCBs, the water temperature was set to 4 - 6 °C to better resemble the North-European winter conditions. Despite of the rather low water temperature in the experiment, the induction levels of PCB-exposed fish were notably elevated, but the magnitude of induction was probably lower than it would have been in warmer water. Teleostean biotransformation system functions more efficiently in high rather than in low water temperatures, although fish have been shown to be physiologically adaptive towards changes in water temperature (Koivusaari et al. 1981; Förlin et al. 1984). Andersson and Koivusaari (1985) showed that the induction in warm-acclimated rainbow trout juveniles was about two times higher and the inductive response occurred faster than in cold-acclimated fish. In general, contrary to the warm-blooded organisms, biotransformation of PCBs in fish is slow (Boon et al. 1992).

Even though certain PCBs may resemble hormones for their mode of action, biotransformation enzyme activities are not necessarily suitable indicators of antiestrogenic effects of PCBs in fish. When the anti-estrogenicity of PCB 126 and other Ah-receptor agonists in carp (Cyprinus carpio) hepatocytes was evaluated, the suppression of the secretion of the yolk protein vitellogenin was not directly correlated with EROD activity, and the anti-estrogenic effects occurred at higher concentrations than the induction of CYP1A (Smeets et al. 1999).

6.3.3 Conjugation enzyme activities

In our early life stage biotransformation experiment, the conjugation GST enzyme system was shown to be functioning already at the advanced embryonic stage, although no response to the studied chemical stress to waterborne PCB 77 was detected. Overall, there was no clear dose-
response of GST to PCB 77 in any of the concentrations at either post-exposure time point (3rd and 7th exposure days) examined in this study. When juvenile rainbow trout were ip-exposed to coplanar PCBs 126 and 77, increased GST activities were detected at the low dose (0.1 mg/kg) at day 6 post-injection. However, GST induction was not evident with higher PCB doses.

In the same experiment, a slight, but not significant increase in UDPGT activity following PCB exposure was measured. These results are consistent with the recent study of Blom & Förlin (1997), who found the UDPGT to be markedly increased 4 weeks after a single injection of 0.1 mg TCB/kg body weight. Further in that study, the induction with 100 mg Clophen A50/ kg body weight did not occur until 15 weeks after injection. When five times higher dose of the same commercial PCB mixture was administered, a significant UDPGT induction in rainbow trout liver was achieved in 4 weeks (Andersson et al. 1985). It can be deduced that coplanar PCBs did not affect conjugation activities as effectively as monooxygenase activities. However, too short exposure period in our laboratory experiment could be the main reason for the lack of significant conjugation response.

UDP GT enzyme system has been induced by exposure to organic contaminants (Clarke et al. 1991). From the biomonitoring point of view, the feature of hepatic UDPGT enzyme system of not being affected by spawning or gonadal development (Clarke et al. 1991) should make it a potential tool for biomarker studies.

In our field study, hepatic cytosolic GST enzyme activity measurements of both bream and asp failed to show any clear indication of chemical stress in Lake Kernaala fish. Significant gender and seasonal differences of conjugation GST enzyme activities in Lake Kernaala bream were found. These results are in accordance with the study of George and Buchanan (1990), in which cytosolic GST was shown to display a marked seasonal variation in both male and female plaice, Pleuronectes platessa. The studies of GST activities in PCB-exposed fish, both in laboratory and in the field, have yielded confusing results. High levels of GST activities have been measured in the livers of fish caught downstream from a PCB incineration plant along the River Rhone (Monod et al. 1988). In contrast, no significant changes was seen in hepatic GST enzyme activities of three cyprinid species collected from the PCB- and PAH-polluted part of the river Po, Italy (Vigano et al. 1998). GST activities have been reported to be induced, inhibited, or to be unaffected by PCB exposure (Andersson et al. 1985; Monod et al. 1988; Vigano et al. 1998).
Interesting approach to a problem of inconsistent results gained from GST studies in fish was recently put forward by Petrivalsky and coworkers (1997). They demonstrated that the effects of xenobiotics on cytosolic GST would be substrate dependent. In that case, it is possible that the substrate used in our GST protocol (1-chloro-2,4-dinitrobenzene, CDNB) would probably not be the best biochemical marker in ecotoxicological biomarker studies. In fact, when Ankley et al. (1986) intraperitoneally exposed immature catfish with Aroclor 1254, no significant changes in cytosolic GST activity toward CDNB, NBC (p-nitrobenzyl chloride), EA (ethacrynic acid), or DCNB (1,2-dichloro-4-nitrobenzene) were detected. However, they found notable increase in GST activity with ENPP (1,2-epoxy-3-(p-nitrophenoxyl)propane) as a substrate. Both mammals and fishes possess multiple forms of hepatic cytosolic GST. While these GSTs have different specifications for substrate, they all exhibit some degree of activity toward CDNB (Sijm and Opperhuizen 1989). In that respect, the use of that particular substrate in this thesis study, including both laboratory and field experiments, as well as different species and life stages of fish, was justified.

Without a doubt, conjugation enzymes have a role in biotransformation of xenobiotics. In some cases, however, exposure of fish to chemicals that increase monooxygenase activities does not activate GST or UDPGT systems (James and Bend 1980; Balk et al. 1980). On the other hand, compounds that also increase monooxygenases have increased hepatic conjugation enzyme activities (Andersson et al. 1985). Also, phase II enzymes are often not so responsive to contaminant induction as CYP enzymes ( Ankley et al. 1986; Jimenez and Stegeman 1990). Eventually, the data about the effects of PCBs on teleostean phase II enzymes is still rather limited and confusing.

6.3.4 Histopathological alterations

6.3.4.1 Liver

For various laboratory animal species liver is a common target organ of PCB-mediated toxicity (Kimbrough et al. 1978; Safe 1989). In our studies, despite of notably induced hepatic biotransformation enzyme activities of Lake Kernaala fish in comparison to reference locations, histopathological evaluation of liver tissue of feral fish revealed no reliable evidence of toxicant-induced hepatic neoplastic lesions or hepatotoxicity. The observed cellular changes in liver tissue were all linked to season: diffuse fatty change, found from both bream and asp in Lake Kernaala,
but also in reference locations, has been influenced by nutritional state of fish and as an response to ageing (Segner and Braunbeck 1988; Hinton et al. 1992). However, this condition has been also associated with exposure of fish to variety of different carcinogenic agents and after a variety of hepatotoxic insults (Hendricks et al. 1984; Schwaiger et al. 1997). Excessive fat accumulation was reported in the liver of lake sturgeon caught from PCB-polluted sites of St. Lawrence River, Montreal (Rousseaux et al. 1995). Furthermore, diffuse cellular vacuolation of hepatocytes has been reported in white suckers caught from PCB- and PAH-polluted Sheboygan River, Wisconsin (Schrank et al. 1997).

In Lake Kernaala fish, the fibrosis surrounding hepatic blood vessels and bile ducts of bream often co-occurred with locally increased number of inflammatory cells. The prevalence of fibrosis decreased from spring to fall, with no significant differences to fish caught from the reference location. While the mean age of the bream was the same in all samplings, the observed fibrosis was most likely due to seasonal variation rather than chemical stress of fish. An inflammatory fibrotic liver changes due to a manifestation of a hepatic nematode parasite have been observed in fish (Rousseaux et al. 1995). However, similar fibrotic changes have been reported in livers of St. Lawrence lake sturgeon (Acipenser fulvescens) taken from study sites polluted with PCBs and PAHs (35). Hyperplasia, inflammation and fibrosis of the intrahepatic bile ducts in the livers of mirror carp (Cyprinus carpio) have been observed after administration of a low dose of TCDD (van der Weiden et al. 1994).

The utility of toxicopathologic hepatic lesions in wild fish as biomarkers of contaminant effect has been shown previously (Myers et al. 1990, 1994; Malins et al. 1984, 1985). Hepatic PCB concentrations are significant risk factors for early toxicopathologic lesions in juvenile English sole (Pleuronectes vetulus) (Myers et al. 1998). Dietary exposure to and bioaccumulation of total PCBs have been demonstrated to be significant risk factors for hepatic lesion occurrence in marine estuarine fish (Collier et al. 1998). Furthermore, in a comprehensive histopathological study of three species of bottom fish sampled at Pacific Coast of United States, a prevalence of hepatic lesions was most commonly associated with exposure to PCBs and PAHs (Myers et al. 1994). PCB congeners with carcinogenic activity are found to be promoters rather than initiators of carcinogenesis. Indeed, PCBs have been shown to be capable of activating genes involved in generating carcinogenic compounds from PAHs (George 1994). Concentrations of those carcinogenic chemicals in Lake
Kernaala sediment and fish tissue are low, which could partly explain the absence of neoplastic liver lesions in Lake Kernaala fish. Thus, PCB may serve either as a marker for other contaminants that are responsible for observed effects, or that other contaminants present in fish may interact synergistically with the PCBs to produce the observed toxicity (Seegal 1999).

6.3.4.2 Kidney

Inner organs with high blood flow like liver, but also kidney, effectively bioaccumulate lipophilic chemicals. Kidney consists of head kidney, which serves as the primary hemopoietic organ, and the trunk kidney, which is composed of nephrons and interstitial lymphoid tissue, being an important excretory organ of teleostean fishes (Hibiya 1982; Gronman 1982). Therefore, also teleostean kidney has a potential for future histopathological marker organ.

In the Lake Kernaala field study, the fish kidney was the only organ showing specific cellular alterations that could have been linked to PCB exposure. For the most part the tissue lesions were located in renal glomeruli of both feral fish species studied. The glomerulus is the main component of the renal corpuscle and is mainly composed of blood capillary loops. The capillaries and the mesangial region play an important role in the normal filtration of blood (Takashima 1982). Dilation of glomerular capillaries (DGC), as well as mesangial edema (ME) was characterized from the majority of feral fish caught from Lake Kernaala. Since no or insignificant prevalence of such alterations were observed in fish caught from the reference locations, it was concluded that chemical stress might have been the primary or the secondary factor finally culminating to glomerular lesions in trunk kidney.

In the study of Fischer-Scherl et al. (1991), fish free of any parasites and bacterial or viral diseases showed obliterated Bowman’s spaces of renal corpuscles after chronic Atrazine exposure. Kidney cells of three different cyprinid species have been shown to be vulnerable to the clastogenic effects of sublethal exposure to commercial PCB mixture Aroclor 1254 (Al-Sabti 1985). Also, interrenal stress responsiveness of tilapia (Oreochromis mossambicus) was impaired by dietary exposure to PCB 126 (Quabius et al. 1997). In our knowledge, there are very few studies dealing with the issue of renal, and more specifically, glomerular biomarker studies on aquatic organisms.
6.3.4.3 Reproductive organs

Our field studies revealed no histopathologic evidence of reproductive dysfunction of feral fish caused by prolonged PCB exposure. However, tolerance to stress in fish is likely to be the lowest in the reproductive system (Gerking 1980). Wildlife from ecosystems exposed to PCBs and other endocrine disrupting chemicals display a variety of reproductive alterations (Gray and Metcalfe 1998). Reduced fecundity, disorganisation of lobules and spermatogenic elements, fibrosis of lobule walls, and fatty necrosis has been demonstrated in fish exposed to PCBs (Sangalang et al. 1981; Johnson et al. 1988; Safe 1990; Casillas et al. 1991). In contrast, Suedel et al. (1997) exposed fathead minnow (Pimephales promelas) to five different di-ortho PCB congeners for a total of 13 weeks, but found no adverse effects on reproductive parameters. Estrogenic responses of PCBs that are able to mimic the natural hormone estrogen in fish have received great attention. The fact remains, however, that all the xenoestrogens discovered to date are comparatively weak estrogens at environmentally realistic concentrations (Sumpter and Jobling 1995; Arukwe et al. 1997).

6.3.4.4 Non-specific immunological markers

Higher prevalence of certain parasitic infections in Lake Kernaala fish than in fish caught from reference locations makes one suspect the lowered immune defence ability of the infected specimen. Indeed, abnormally high overall occurrence of rodlet cells in Lake Kernaala bream also talks strongly for the theory of immune dysfunction of those fish. In the current thesis study, however, no direct measure of teleostean immunotoxicity was employed. Instead, morphological parameter such as the occurrence of rodlet cells and macrophage aggregates, which both are supposed to have an role in non-specific defence mechanism of fish, were studied in inner organs of feral fish. In addition, increased parasitism as an indirect response of chemical stress was also thought to be a sign of weakened immune defence of fish.

6.3.4.4.1 Rodlet cells

In the histopathological study of Lake Kernaala bream population the higher than normal presence of rodlet cells in inner tissues of fish was noted. The rodlet cells, also considered to be parasitic protozoan, are nowadays morphologically agreed to be normal endogenous components of fish
tissue (Iger and Abraham 1997). Rodlet cells have been found in dozens of marine and freshwater fish species at a variety of tissues (Leino 1974; Barber et al. 1979; Smith et al. 1995). Although widely distributed, these cells seem to be frequently associated with tissue epithelium. The development and ultra structure of rodlet cells have been extensively studied, while the definitive function of these cells still remains to be solved. The most recent studies have supposed these cells to form a part of the non-specific defence mechanism of epithelial tissues of fish, being stress- or toxicant-induced type of fish cells produced in response to sublethal environmental conditions. Chemical stress has been shown to increase the occurrence of rodlet cells in fish (Smith et al. 1995).

In our study, significant seasonal fluctuation in both number and location of rodlet cells, together with the evident co-occurrence of these cells with the current parasitic infections in target organs suggests that the long-term sublethal PCB pollution was not the primary factor effecting rodlet cell occurrence in Lake Kernaala fish. However, it is possible that chronic exposure to PCBs may have weakened the immune defence of fish and made them more vulnerable to parasitic infections, thus indirectly having an influence also on rodlet cell proliferation.

6.3.4.4.2 Parasitic infections

In this thesis study, a 3-fold higher prevalence of hepatic nematodes in Lake Kernaala bream in comparison to reference location, Lake Alajärvi, was observed. These small, adjacent lakes belong to the same watershed, and the bream caught simultaneously from both lakes had the same age and maturational stage. Therefore, notably higher prevalence of hepatic nematodes in Lake Kernaala bream could be partly explained by a chronic sublethal chemical exposure: it may have suppressed the immune response of fish and thus made them more vulnerable to secondary parasitic infections.

Chemical exposure may reduce immune competence, leading to infectious disease, possibly neoplasia or death (Hinton et al. 1992). Pollution may also promote an increase in parasite prevalence by favouring the survival and reproduction of the intermediate hosts (Haaparanta 1997). Juvenile salmon from an urban estuary polluted by PCBs and PAHs have been shown to exhibit an impaired immune system and to be more susceptible to the marine pathogen *Vibrio anguillarum* than were salmon from reference estuaries or hatcheries (Arkoosh et al. 1994). Increased susceptibility to pathogens is supposed to be associated with immune dysfunction and exposure to chemical contaminants present in polluted aquatic environment. In recent years, mass mortalities
among seals and dolphins have been connected to co-occurring virus infections and high levels of persistent lipophilic pollutants in their bodies, supporting the hypothesis that a contaminant-related suppression of the immune system might have contributed to the severity of the virus outbreaks (de Swart et al. 1996).

6.3.4.3 Macrophage aggregates

Our findings of non-specific appearance of macrophage aggregates in liver, kidney and spleen of both asp and bream (Koponen, unpublished) are in accordance with the studies of Haaparanta et al. (1996) and Husjy et al. (1996), which concluded that MAs have limited and controversial value as indicators of environmental pollution. It has been suggested that melanin in macrophages of fish may play a role in neutralising potentially toxic free radicals (Agius 1985). The prevalence of MAs is known to vary among individual fish, species, and organs. The frequency of MAs in liver has also been correlated to exposure to environmental pollution (Wester et al. 1994).

6.4 Variation causing factors in assessing environmental effects of PCBs in fish

Establishing of biological effects and toxicity of a certain pollutant or chemical group is a multidimensional problem, in which both laboratory and field studies are obligatory in order to gain reliable data for environmental risk assessment. Individual PCB congeners are known to exhibit different physicochemical properties and biological effects. Also, the composition of PCB residues in environmental matrices is highly variable and their mechanisms of toxicity may differ significantly (Giesy and Kannan 1998). Because the commercial PCBs and PCBs in the environmental extracts are merely complex mixtures of congeners, the ultimate impact of PCBs on the environment may be due to the individual components of these mixtures (Safe 1994). On the other hand, studies with chemical mixtures could also provide valuable information about the possible interactive effects between these compounds (Li et al. 1999). The synergistic and/or antagonistic interactions between PCBs themselves, as well as with other chemicals determine the net toxicological potency of chemical stress on aquatic biota. In biological monitoring the total PCB approach is oftentimes used when the potential of this chemical group in causing toxic effects in wildlife is surveyed.
In addition to thousands of existing xenobiotics and their possible antagonistic/synergistic relationships, several other biotic and abiotic factors may influence the biotransformation enzyme activities of fish (Fig. 4). These include species differences and intraspecies variation, developmental and reproductive stage of fish, gender, hormonal factors, season, temperature, diet and nutritional stress (for review see Payne et al. 1987; Jimenez and Stegeman 1990). Deeper insight into species differences in toxic potency of biphenyl congeners has been recently provided by Abnet et al. (1999). In their study, comparisons between aryl hydrocarbon receptors from human, rainbow trout, and zebra fish showed that while mono-ortho PCBs were able to activate the human Ah receptor, they were generally ineffective in activating the corresponding fish AhR2s.

**Figure 4.** Example of the myriad of variation causing factors affecting the biomonitoring of environmentally stressed fish.

In search for biomarkers in wild fish populations, it is important to bear in mind that not all the teleosts respond similarly to exposure to the same classes of toxicants (Collier et al. 1992). The occurrence and current stage of infectious diseases in fish must also be considered (Hinton et al. 1992). In addition, fish age, rather than the gender, must be accounted for in any analysis comparing lesion risk to site of capture or chemical risk factors (Myers et al. 1994). In some fish species it has been shown that in certain pollution associated liver lesion, both EROD and GST activities have
been inhibited in comparison to normal liver tissue (Stalker et al. 1991). In pathological investigations it should be noted that the structures of different organs can undergo highly significant changes according to the animal’s state of development, the composition of its aquatic environment and endocrine factors (Roberts 1978). It would be beneficial if biochemical and cellular changes caused by certain chemical could also be verified in strictly controlled laboratory studies before concrete conclusions are to be drawn. It is obvious that in the field, and even in the laboratory, it is difficult to take into account and/or eliminate all the disturbing factors that may affect the objective and error-free interpretation of the study results.

7 CONCLUSIONS

In this thesis the influence of biotic and abiotic factors (species, strain, age, sex and season) on the biotransformation parameters were shown. We demonstrated intra- and interstrain variation already in the basal activities of biotransformation enzymes in adult fish. The same trend was also observed in chemically exposed juvenile fish. In laboratory testing, as well as in environmental monitoring studies, the elimination of factors causing errors is essential in order to gain reliable and repeatable results for risk assessment purposes.

The behaviour of a xenobiotic model substance (uptake, effective concentrations, and biological effects) PCB was followed. Under experimental conditions, PCB readily penetrated through the embryonic chorion of fish eggs. While the chemical to some extent was adsorbed into the eggshell and into the interstitial fluid, the embryo most likely took up most of the PCB. In juvenile fish, the chemical effectively concentrated into the main metabolic organ, the liver. PCBs were also found in muscle tissue of the feral fish, underlining the fact that even though the influx of the chemical into Lake Kernaala ceased nearly 20 years ago, these highly persistent chemicals are still actively cycling in the ecosystem of the lake.

The hepatic EROD enzymes reacted rapidly to acute PCB insult both in embryonic and juvenile developmental stages of fish, but also to environmental chemical exposure to adult fish. Our studies also revealed that the EROD system was activated at PCB concentrations, which produced no observed adverse effects on fish. Thus EROD was considered an effective biomarker for application
both in acute and chronic exposure monitoring studies. It must be noted, however, that prolonged PCB exposure has been demonstrated also to inhibit the EROD system in feral fish.

In our studies, we showed that the GST system is already functioning at the embryonic stage of fish. However, no clear trend of induction or inhibition of the GST system caused by chemical insult was observed either in experimental PCB exposure studies or in field monitoring. Furthermore, this enzyme system reflected the seasonal changes in field studies. According to our experience, the GST system was not very informative for detecting physiological signs of PCB exposure in fish.

Histological cellular studies were included into this thesis in order to be able to link the signs of environmental stress of fish to concrete biological effects caused by chemicals. Induction of the EROD enzyme system was a sign of activated xenobiotic metabolism of feral fish living in a PCB polluted lake. Indeed, in the kidney of those feral fish we were able to detect specific glomerular alterations, which could be, either directly or indirectly, linked to PCB pollution. Liver, an important target organ of chemical insult, showed no clear signs of prolonged chemical stress.

It can be concluded that biotransformation enzyme activity measurements in fish, especially the EROD activity, are informative in the same way as temperature measurements in the human body, or pH measurements in the aquatic environment: abnormal readings demonstrate a deviation from the so called steady state, or normal conditions. Additional analyses are needed, however, before we can pinpoint the factors causing these changes. For more reliable interpretation, biotransformation enzyme analyses in fish could be supported by histological observations. Histopathologic analysis offers an informative but labour-intensive way to interpret data from several organ systems and permits localisation of lesions within specific tissues and cell types. If applied together, these techniques compose a set of biomarker tools, which can be successfully utilized for biomonitoring studies.
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