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Review article

Fish bioaccumulation and biomarkers in environmental risk assessment: a review

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Abstract

In this review, a wide array of bioaccumulation markers and biomarkers, used to demonstrate exposure to and effects of environmental contaminants, has been discussed in relation to their feasibility in environmental risk assessment (ERA). *Fish bioaccumulation markers* may be applied in order to elucidate the aquatic behavior of environmental contaminants, as bioconcentrators to identify certain substances with low water levels and to assess exposure of aquatic organisms. Since it is virtually impossible to predict the fate of xenobiotic substances with simple partitioning models, the complexity of bioaccumulation should be considered, including toxicokinetics, metabolism, biota-sediment accumulation factors (BSAFs), organ-specific bioaccumulation and bound residues. Since it remains hard to accurately predict bioaccumulation in fish, even with highly sophisticated models, analyses of tissue levels are required. The most promising fish bioaccumulation markers are body burdens of persistent organic pollutants, like PCBs and DDTs. Since PCDD and PCDF levels in fish tissues are very low as compared with the sediment levels, their value as bioaccumulation markers remains questionable. Easily biodegradable compounds, such as PAHs and chlorinated phenols, do not tend to accumulate in fish tissues in quantities that reflect the exposure. Semipermeable membrane devices (SPMDs) have been successfully used to mimic bioaccumulation of hydrophobic organic substances in aquatic organisms. In order to assess exposure to or effects of environmental pollutants on aquatic ecosystems, the following suite of *fish biomarkers* may be examined: biotransformation enzymes (phase I and II), oxidative stress parameters, biotransformation products, stress proteins, metallothioneins (MTs), MXR proteins, hematological parameters, immunological parameters, reproductive and endocrine parameters, genotoxic parameters, neuromuscular parameters, physiological, histological and morphological parameters. All fish biomarkers are evaluated for their potential use in ERA programs, based upon six criteria that have been proposed in the present paper. This evaluation demonstrates that phase I enzymes (e.g. hepatic EROD and CYP1A), biotransformation products (e.g. biliary PAH metabolites), reproductive parameters (e.g. plasma VTG) and genotoxic parameters (e.g. hepatic DNA adducts) are currently the most valuable fish biomarkers for ERA. The use of biomonitoring methods in the control strategies for chemical pollution has several advantages over chemical monitoring. Many of the biological measurements form the only way of integrating effects on a large number of individual and interactive processes in aquatic organisms. Moreover, biological and biochemical effects may link the bioavailability of the compounds of interest with their concentration at target organs and intrinsic toxicity. The limitations of biomonitoring, such as confounding factors that are not related to pollution, should be carefully considered when interpreting biomarker data. Based upon this overview there is little doubt that measurements of bioaccumulation and biomarker responses in fish from contaminated sites offer great promises for providing information that can contribute to environmental monitoring programs designed for various aspects of ERA.

Keywords: Fish; Environmental risk assessment (ERA); Bioaccumulation; Biomarkers

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1. Introduction

The environment is continuously loaded with foreign organic chemicals (xenobiotics) released by urban communities and industries. In the 20th century, many thousands of organic trace pollutants, such as polychlorinated biphenyls (PCBs), organochlorine pesticides (OCPs), polycyclic aromatic hydrocarbons (PAHs), polychlorinated dibenzofurans (PCDFs) and dibenzo-*p*-dioxins (PCDDs) have been produced and, in part, released into the environment. Since the early sixties mankind has become aware of the potential long-term adverse effects of these chemicals in general and their potential risks for aquatic and terrestrial ecosystems in particular. The ultimate sink for many of these contaminants is the aquatic environment, either due to direct discharges or to hydrologic and atmospheric processes (Stegeman and Hahn, 1994). The presence of a xenobiotic compound in a segment of an aquatic ecosystem does not, by itself, indicate injurious effects. Connections must be established between external levels of exposure, internal levels of tissue contamination and early adverse effects. Many of the hydrophobic organic compounds and their metabolites, which contaminate aquatic ecosystems, have yet to be identified and their impact on aquatic life has yet to be determined. Therefore, the exposure, fate and effects of chemical contaminants or pollutants in the aquatic ecosystem have been extensively studied by environmental toxicologists.

Ecological or environmental risk assessment (ERA) is defined as the procedure by which the likely or actual adverse effects of pollutants and other anthropogenic activities on ecosystems and their components are estimated with a known degree of certainty using scientific methodologies (Depledge and Fossi, 1994). ERA has become increasingly important since environmental scientists as well as the general public have learned that chemicals which are not toxic to humans can have deleterious effects on natural resources which are generally valued, e.g. DDTs jeopardizing predatory birds and fish, death of fish and other aquatic organisms due to acid deposition in poorly buffered lakes which also contributes to the die-back of forests (Bascietto et al., 1990). The risk assessment process can be divided into a scientifically oriented *risk analysis* and a more politically oriented *risk management*. Risk analysis is a process, which comprises some or all of the following elements: hazard identification, effect assessment, exposure assessment and risk characterization (Van Leeuwen and Hermens, 1995). Environmental risk management deals with regulatory measures based on risk assessment (Van Leeuwen and Hermens, 1995). Risk management and risk analysis, are closely related but different processes: in risk analysis the risk of a certain situation is determined, whereas risk management examines solutions to the problem. Although ERA is generally performed by predictive methods, the interest in the assessment of pollution that began in the past and may have ongoing consequences in the future is increasing. These so-called *retrospective ERAs* are primarily concerned with establishing the potential relationship between a pollutant source and an ecological effect caused by exposure of organisms to the pollutant (Suter, 1993).

The ability of various pollutants (and their derivatives) to mutually affect their toxic actions complicates the risk assessment based solely on environmental levels (Calabrese, 1991). Deleterious effects on populations are often difficult to detect in feral organisms since many of these effects tend to manifest only after longer periods of time. When the effect finally becomes clear, the destructive process may have gone beyond the point where it can be reversed by remedial actions or risk reduction. The sequential order of responses to pollutant stress within a biological system is visualized in Fig. 1 (modified from Bayne et al., 1985). Such scenarios have triggered the research to establish early-warning signals, or **biomarkers**, reflecting the adverse biological responses towards anthropogenic environmental toxins (Bucheli and Fent, 1995). Biomarkers are measurements in body fluids, cells or tissues indicating biochemical or cellular modifications due to the presence and magnitude of toxicants, or of host response (NRC, 1987). Effects at higher hierarchical levels are always preceded by earlier changes in biological processes, allowing the development of early-warning biomarker signals of effects at later response levels (Bayne et al., 1985). In an environmental context, biomarkers offer promise as sensitive indicators demonstrating that toxicants have entered organisms, have been distributed between tissues, and are eliciting a toxic effect at critical targets (McCarthy and Shugart, 1990). In this respect, it is also interesting to study the development and application of sensitive laboratory **bioassays**, based upon the responses of biomarkers, such as the CYP1A responses in EROD or CALUX assays with hepatoma cell lines (Sawyer and

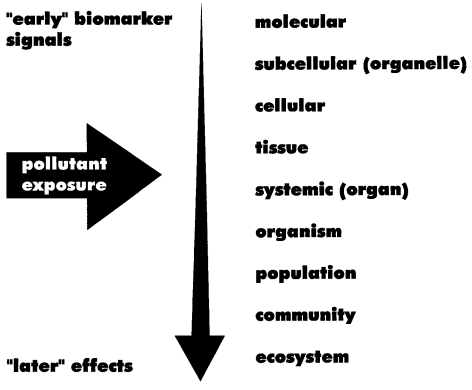

Fig. 1. Schematic representation of the sequential order of responses to pollutant stress within a biological system. Modified from Bayne et al. (1985).

Safe, 1982; Murk et al., 1996). Bioassays offer many advantages for comparing the relative toxicity of specific chemicals or specific effluents. However, toxicity tests also have serious limitations for biological monitoring (BM) because most do not account for the effect of chemical specification in the environment, kinetics and sorption of chemicals to sediment, accumulation through food chains and modes of toxic action which are not readily measured as short-term effects (McCarthy and Shugart, 1990). Depledge and Fossi (1994) suggested the use of biomarkers in toxicity tests as an attempt to link biomarker responses to effects on life-history characteristics (e.g. survival and reproduction), which will provide a further foundation for the use of biomarkers in environmental assessment.

For several reasons, fish species have attracted considerable interest in studies assessing biological and biochemical responses to environmental contaminants (Powers, 1989). Monitoring species should be selected from an exposed community on the basis of their relationship to the assessment endpoint as well as by following some practical considerations (Suter, 1993). For the assessment of the quality of aquatic ecosystems, both criteria are met for numerous species of fish. Fish can be found virtually everywhere in the aquatic environment and they play a major ecological role in the aquatic food-webs because of their function as a carrier of energy from lower to higher trophic levels (Beyer, 1996). The understanding of toxicant uptake, behavior and responses in fish may, therefore, have a high ecological relevance. Most of the general biomarker criteria appear to be directly transferable to certain fish biomarkers (Stegeman et al., 1992). Between different fish species, however, considerable variation in both the basic physiological features and the responsiveness of certain biomarkers towards environmental pollution may become apparent. Despite their limitations, such as a relatively high mobility, fish are generally considered to be the most feasible organisms for pollution monitoring in aquatic systems.

In Section 2, general information on different types of **biomarkers** will be discussed, together with criteria and properties for valid biomarkers in environmental field research. The process of ERA will be elucidated in Section 3. The monitoring of aquatic pollution, which is the most important aspect of the ERA process with respect to biomarkers, will be discussed in Section 4. The processes governing the bioaccumulation of organic trace pollutants in fish, as well as the use of fish as a bioconcentrator to identify certain substances with low water levels will be reviewed in Section 5. Biota-sediment accumulation data will be presented and discussed with regard to the potential use of fish body burdens in the assessment of exposure to various groups of organic pollutants. In Section 6 an overview will be presented of virtually all **fish biomarkers** (biological and biochemical parameters) that have been used in order to assess exposure to and effects of environmental contaminants. Emphasis will be placed on the biochemical responses to organic trace pollutants, and the feasibility of these markers as early-warning signals for environmental hazards. An extensive summary with the overall conclusions of this review will be presented in Section 7, and the perspectives will be given in the final Section 8.

The scope of this review will be to give an overview of fish bioaccumulation and biomarker studies and to discuss the advantages and limitations of applying these parameters in the assessment of environmental risks in aquatic ecosystems. At present, ERA processes are mainly based upon the determination and prediction of contaminant levels in the various ecosystem compartments, and upon comparison of these concentrations with legislative threshold values or environmental safety standards. However, there is a growing awareness that focusing on chemical data alone is insufficient to reliably assess the potential risks of the complex mixture of contaminants in the aquatic environment. There is an increasing trend to use the behavior of pollutants (bioavailability, bioaccumulation, and biotransformation) as well as pollution-induced biological and biochemical effects on aquatic organisms to evaluate or predict the impact of chemicals on aquatic ecosystems. Emphasis in this review will, therefore, be placed on the use of bioaccumulation and biomarker responses in fish as monitoring tools for the assessment of the risks and hazards of environmental pollutants for the aquatic ecosystem, as well as on its limitations.

2. Biomarkers

Several definitions have been given for the term '**biomarker**', which is generally used in a broad sense to include almost any measurement reflecting an interaction between a biological system and a potential hazard, which may be chemical, physical or biological (WHO, 1993). A biomarker is defined as a change in a biological response (ranging from molecular through cellular and physiological responses to behavioral changes) which can be related to exposure to or toxic effects of environmental chemicals (Peakall, 1994). Van Gastel and Van Brummelen (1994) redefined the terms 'biomarker', 'bioindicator' and 'ecological indicator', linking them to different levels of biological organization. They considered a biomarker as any biological response to an environmental chemical at the sub-individual level, measured inside an organism or in its products (urine, faeces, hair, feathers, etc.), indicating a deviation from the normal status that cannot be detected in the intact organism. A bioindicator is defined as an organism giving information on the environmental conditions of its habitat by its presence

or absence or by its behavior, and an ecological indicator is an ecosystem parameter, describing the structure and functioning of ecosystems.

According to the NRC (1987), WHO (1993), biomarkers can be subdivided into three classes:

- biomarkers of **exposure**: covering the detection and measurement of an exogenous substance or its metabolite or the product of an interaction between a xenobiotic agent and some target molecule or cell that is measured in a compartment within an organism;
- biomarkers of **effect**: including measurable biochemical, physiological or other alterations within tissues or body fluids of an organism that can be recognized as associated with an established or possible health impairment or disease;
- biomarkers of **susceptibility**: indicating the inherent or acquired ability of an organism to respond to the challenge of exposure to a specific xenobiotic substance, including genetic factors and changes in receptors which alter the susceptibility of an organism to that exposure.

The subdivision of biomarkers in the literature is rather diffuse since biomarkers of exposure and those of effect are distinguished by the way they are used, not by an inherent dichotomy (Suter, 1993). The responses of biomarkers can be regarded as biological or biochemical *effects* after a certain toxicant *exposure*, which makes them theoretically useful as indicators of both *exposure* and *effects*. Biomarkers of exposure can be used to confirm and assess the exposure of individuals or populations to a particular substance (group), providing a link between external exposure and internal dosimetry. Biomarkers of effect can be used to document either preclinical alterations or adverse health effects due to external exposure and absorption of a chemical. Biomarkers of susceptibility help to elucidate variations in the degree of responses to toxicant exposure observed between different individuals. The bioaccumulation of certain persistent environmental contaminants in animal tissues may be considered to be a biomarker of exposure to these chemicals (NRC, 1987; WHO, 1993). According to the definitions given by Van Gastel and Van Brummelen (1994), however, body burdens are not considered to be biomarkers or bioindicators since they do not provide information on deviations related to 'health'. In order to avoid confusion, in this review the analytical–chemical indicators (body burdens) will be referred to as **bioaccumulation markers**, while all biological (biochemical, physiological, histological and morphological) indicators measured inside an organism or its products will be referred to as **biomarkers**.

In general, phenomena are more universal on a cellular level than at higher levels of biological organization, so biochemical responses may be similar in a large variety of organisms. Good biomarkers are sensitive indices of both pollutant bioavailability and early biological responses. Biomarkers may be used after exposure to dietary, environmental or occupational sources, to elucidate cause–effect and dose–effect relationships in health risk assessment, in clinical diagnoses and for monitoring purposes. Generally, biomarker responses are considered to be intermediates between pollutant sources and higher-level effects (Suter, 1990). When these compensatory responses are activated, the survival potential of the organism may already have begun to decline because the ability of the organism to mount compensatory responses to new environmental challenges may have been compromised (Depledge and Fossi, 1994). The most compelling reason for using biomarkers is that they can give information on the biological effects of pollutants rather than a mere quantification of their environmental levels. Biomarkers may provide insight into the potential mechanisms of contaminant effects. By screening multiple biomarker responses, important information will be obtained about organism toxicant exposure and stress. A pollutant stress situation normally triggers a cascade of biological responses, each of which may, in theory, serve as a biomarker (McCarthy et al., 1991). Above a certain threshold (in pollutant dose or exposure time) the pollutant-responsive biomarker signals deviate from the normal range in an unstressed situation, finally leading to the manifestation of a multiple effect situation at higher hierarchical levels of biological organization (Fig. 2, modified from McCarthy et al., 1991). Improper application or interpretation of biomarker responses, however, may lead to false conclusions as to pollutant stress or environmental quality. Certain responses established for one species are not necessarily valid for other species. Moreover, ecotoxicological data obtained in laboratory studies can be difficult to translate into accurate predictions of effects that may occur in the field (ECETOC, 1993). Since both overestimation and underestimation of effects may occur, laboratory observations on biomarkers must always be validated with field research. Biomarkers applied in both the laboratory and the field, can provide an important linkage between laboratory toxicity and field assessment. For field samples, biomarker data may provide an important index of the total external load that is biologically available in the 'real world' exposure.

In order to evaluate the strength and weaknesses of fish biomarkers objectively, we propose six criteria comprising the most important information that should be available or has to be established for each candidate biomarker (based upon the criteria formulated by Stegeman et al., 1992):

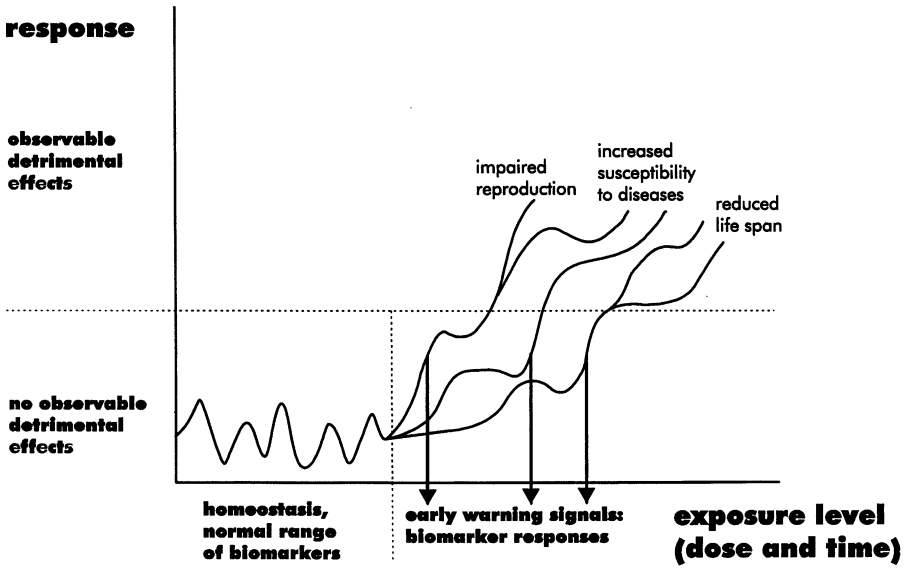

Fig. 2. The principal scheme of responses in organisms to the detrimental effects of pollutant exposure. Modified from McCarthy et al. (1991).

- the assay to quantify the biomarker should be reliable (with quality assurance (QA)), relatively cheap and easy to perform;
- the biomarker response should be sensitive to pollutant exposure and/or effects in order to serve as an early warning parameter;
- baseline data of the biomarker should be well defined in order to distinguish between natural variability (noise) and contaminant-induced stress (signal);
- the impacts of confounding factors to the biomarker response should be well established;
- the underlying mechanism of the relationships between biomarker response and pollutant exposure (dosage and time) should be established;
- the toxicological significance of the biomarker, e.g. the relationships between its response and the (long-term) impact to the organism, should be established.

In addition to these criteria it has been suggested that biomarkers should preferentially be non-invasive or non-destructive, to allow or facilitate environmental monitoring of pollution effects in protected or endangered species (Fossi and Marsili, 1997). Responses of 'ideal' biomarkers correlate with the health or fitness of the organism. With regard to the test organism, its basic biology and physiology should be known so that sources of uncontrolled variation (growth and development, reproduction, food sources) can be minimized (Stegeman et al., 1992).

Fish biomarkers may be useful tools in several steps of the risk assessment process: effect, exposure and hazard assessment, risk characterization or classification, and monitoring the environmental quality of aquatic ecosystems. The authors believe that phased increases in the extent and complexity of environmental monitoring scenarios will test and confirm previous understanding of biomarker responses and gradually expand that understanding. In time the biomarkers will thus become a routine, well-characterized and scientifically and legally defensible tool for monitoring and assessing environmental pollution. Based on the magnitude and pattern of the biomarker responses, the environmental species offer the potential of serving as *sentinels* demonstrating the presence of bioavailable contaminants and the extent of exposure, *surrogates* indicating potential human exposure and effects and *predictors* of long-term effects on the health of populations or the integrity of the ecosystem (McCarthy and Shugart, 1990).

3. Environmental or ecological risk assessment (ERA)

Risk assessment can be defined as the process of assessing magnitudes and probabilities to the adverse effects of human activities or natural catastrophes (Suter, 1993). The development of risk assessment has been driven by the need to allocate scarce resources to estimate human-related risks (Power and McCarty, 1997). Risk assessment clearly separates the scientific process of estimating the magnitude and probability of effects (**risk analysis**) from the process of choosing among alternatives and determining acceptability of risks (**risk management**). The entire risk assessment process consists of eight steps, which are defined as follows (Van Leeuwen and Hermens, 1995):

- *Hazard identification* is the identification of the adverse effects, which may be caused by chemicals. The likelihood of harm due to exposure distinguishes risk from hazard.

- *Effect assessment* is the estimation of the relationship between dose or level of exposure to chemicals and the incidence and severity of an effect. Most of the experiments are carried out in order to determine a *no effect level* (NEL), which can be converted to a *predicted no effect level* (PNEL) or a *predicted no effect concentration* (PNEC) for other species.
- *Exposure assessment* is the estimation of concentrations or doses to which human populations or environmental compartments are or may be exposed. For existing chemicals exposure can be assessed by measuring concentrations, while for new chemicals a *predicted environmental concentration* (PEC) can be estimated.
- *Risk characterization* is an integration of the first three steps of the risk assessment process in order to estimate the incidence and severity of the deleterious effects likely to occur due to actual or predicted exposure to chemicals. For newly developed chemicals the PEC/PNEC ratio, i.e. the *risk quotient*, can be determined. The risk quotient, combined with uncertainty factors, links the risk analysis to the risk management by quantifying the hazards and risks for specific situations (Duke and Taggart, 2000; Jager et al., 2001).
- *Risk classification* is the evaluation of risks in order to decide if risk reduction is required. Generally, risk classification is performed using two risk levels, in which the upper limit is the *maximum permissible level* (MPL) and the lower limit is the *negligible level* (NL).
- *Risk–benefit analysis* is the drawing up of a balance sheet of the respective risks and benefits of a proposed risk-reduction action.
- *Risk reduction* is taking measures to protect man and/or the environment from the risks identified. A risk reduction may be achieved by defining *safety standards*, such as the *acceptable daily intake* (ADI).
- *Monitoring* is a repetitive observation for defined purposes of one or more chemical or biological elements according to a prearranged schedule over time and space, using comparable and standardized methods (see Section 4). Monitoring is essential in several stages of the **ERA** process (De Zwart, 1995). During a *problem formulation* chemical and BM of ambient waters may indicate deviations from the normal (alarm and trend function), thus triggering problem recognition. During the *risk analysis stage* chemical monitoring (CM) of receiving waters as well as selected effluents can help in exposure characterization, while BM may be used to predict ecological effects (instrument function and early-warning). During the *risk management stage* monitoring will help by verifying control strategy results and in checking compliance (control function).

ERA has been employed primarily to deal with chemicals. In the last decade much research was devoted to ERA, mainly by international bodies like the World Health Organization (WHO), the Organization for Economic Co-operation and Development (OECD) and the European Centre for Ecotoxicology and Toxicology of Chemicals (ECETOC). The objective of risk-based environmental regulation is to balance the degree of permitted risk against the cost of risk reduction and against competing risks. Ecological risk assessment has several advantageous properties in environmental decision-making (Suter, 1993). It provides a quantitative basis for comparing and prioritizing risks as well as a systematic means of improving the understanding of risks. In addition, it estimates clear consistent endpoints. Whilst Suter (1990) criticized the ambiguous endpoints of other approaches (such as 'ecosystem integrity') as being too vague to be subject to formal quantitative analysis, Cairns and McCormick (1992) successfully refuted the accusation by pointing out that most of the components that are encompassed by the term (such as species diversity, population dynamics, nutrient cycling rates, etc.) are indeed quantifiable and can be used to measure ecosystem well-being. Ecological risk assessors analyze the effects of human actions on the natural environment. Although risk assessment is widely used, consensus on an acceptable, comprehensive decision-making framework that clearly establishes the role of policy and science in formulating environmental management principles has not emerged, according to Power and McCarty (1997). Power and McCarty (1998) carried out a comparative analysis of seven representative frameworks for ERA/risk management.

Like other risk assessment processes, **ERA** is primarily concerned with *predictive assessments* (e.g. PEC and PNEC) that estimate the nature, probability and magnitude of effects of proposed actions (Suter, 1990). However, emphasis has been shifting to *retrospective assessments*, i.e. assessments of human actions that were initiated in the past and may have ongoing consequences in the future, such as waste sites, acid rain and existing pesticides (Suter, 1993). Retrospective assessment falls into three categories with respect to the direction of inference (Fig. 3). Source-driven assessments begin with an existing pollution source, such as a spill or an effluent, and attempt to determine the nature of the effects. Exposure-driven assessments are prompted by evidence of exposure without prior evidence of a source or effects. Effects-driven assessments begin with an observed effect such as a declining animal population or a fishless lake, and attempt to determine a cause. In all cases, the logical link between sources and effects is exposure (Suter, 1993). The retrospective assessment of hazards and risks of existing chemicals may be established by actual measurements of concentrations and

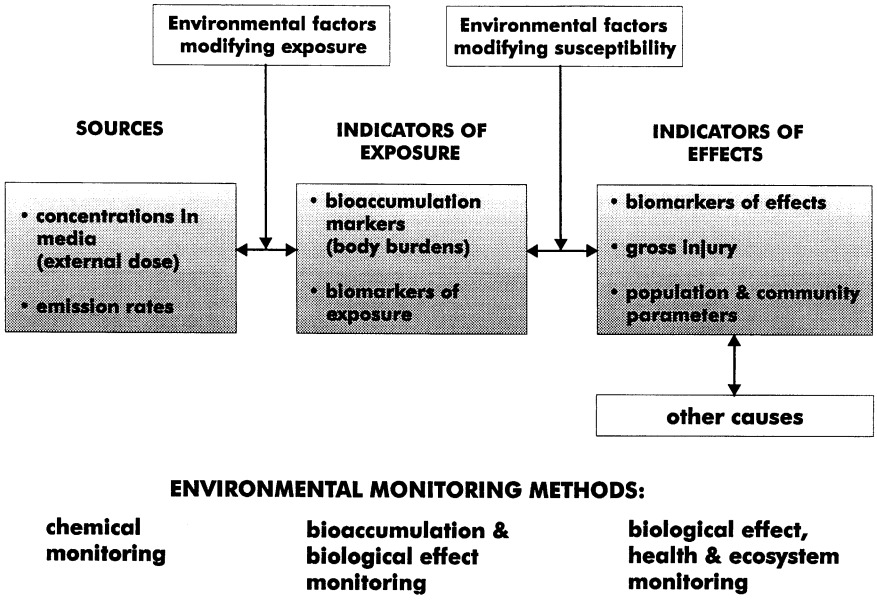

Fig. 3. The relationship among the components of the risk characterization stage of retrospective assessments based on the process of ecological epidemiology, including their respective environmental monitoring methods. Based on Suter (1993), Henderson et al. (1989), De Zwart (1995).

effects in the field, using bioaccumulation and biomarkers.

Any risk assessment must have defined **endpoints**. An assessment endpoint is a formal expression of the environmental values to be protected (Suter, 1993). Defining an assessment endpoint involves two steps: (1) identifying the valued attributes of the environment that are considered to be at risk, and (2) defining these attributes in operational terms. Suter (1993) proposed five criteria that any endpoint should satisfy:

1) societal relevance (understood and valued by public and decision-makers);
2) biological relevance (important to a higher level of the biological hierarchy);
3) unambiguous operational definition;
4) accessibility to prediction and measurement;
5) susceptibility to the hazardous agent(s).

Since assessment endpoints are often vaguely defined or undefined, it has not always been clear what should be measured in retrospective ERAs (Suter, 1990). Population- and ecosystem-level measures are relevant to assessment endpoints but, due to compensatory and adaptive mechanisms, they are often resistant to effects and are not diagnostic for pollutants impacts. Sub organism-level measures are potentially much more diagnostic and sensitive to pollutants, but the relevance of a biochemical or histological response to population and community level assessment endpoints is poorly defined. Organism-level measures are intermediate in relevance, sensitivity and diagnostic utility. The parameters that are measured in a monitoring study or toxicity test are generally referred to as indicators. The formal, usually quantitative, expression of the results of toxicity testing or monitoring of an indicator is a measurement endpoint, which is the toxicological or biological input to risk assessment. The most useful measurement endpoints for risk assessments are multidimensional descriptive models such as concentration–response functions, rather than a single number, such as provided by 50% lethal concentration (LC_{50}) tests.

4. Monitoring aquatic pollution

Monitoring is a repetitive observation for defined purposes of one or more chemical or biological elements according to a prearranged schedule over time and space, using comparable and standardized methods (according to the definition of the United Nations Environmental Program (UNEP)). This last step in the risk management process, which is most relevant with respect to biomarkers, may serve a number of purposes: the *control function* to verify the effectiveness of risk reduction or to ensure that previously formulated standards are being met, the *signal or alarm function* to detect sudden adverse changes in the environment, the *trend function* to enable the prediction of future developments and the *instrument function* for the recognition and clarification of underlying processes. It is important that environmental monitoring programs should only be undertaken if the objectives clearly state what the data are going to be used for (Peakall and Walker, 1994). The five **environmental monitoring** methods which may be performed in order to assess risks of contaminants for organisms and to classify the environmental quality of ecosystems are listed below (see also Fig. 3):

- **chemical monitoring (CM)**: exposure assessment by measuring levels of a selected set of well-known contaminants in abiotic environmental compartments;
- **bioaccumulation monitoring (BAM)**: exposure assessment by measuring contaminant levels in biota or determining the critical dose at a critical site (bioaccumulation);
- **biological effect monitoring (BEM)**: exposure and effect assessment by determining the early adverse alterations that are partly or fully reversible (biomarkers);
- **health monitoring (HM)**: effect assessment by examining the occurrence of irreversible diseases or tissue damage in organisms;
- **ecosystem monitoring (EM)**: assessment of the integrity of an ecosystem by making an inventory of, for instance, species composition, density and diversity.

A regular, systematic use of living organisms to evaluate changes in environmental or water quality, as in BAM, BEM, HM and EM, is called **biological monitoring (BM)** or **biomonitoring** (De Zwart, 1995). The term '**toxicity monitoring**' is used to describe measurements on the direct biomolecular and physiological responses of individual organisms towards toxicants in an experimental setup, including bioassays and biological early-warning systems, e.g. in BEM and HM. A so-called **integrated monitoring** program is a study consisting of coordinated monitoring activities comprising both chemical and biological measurements in a variety of environmental media or compartments. An example of an effect-based integrated monitoring program is the so-called **Triad** approach, consisting of analyses of chemical contamination (CM), assessment of toxicity using bioassays (BEM) and determination of the in faunal community structure (EM), which is described in detail by Chapman (1990). Analyses of Triad data in order to determine the pollution status can involve comparisons of Ratio-to-Reference (RTR) values, ranking, multivariate analyses and Mantel's test on disease clustering (Mantel, 1967). Van Gastel and Van Brummelen (1994) proposed a stepwise integrated **ERA** of chemicals, consisting of four different biomonitoring levels using biomarkers, bioassays, bioindicators and ecological indicators:

I) **sub-organismal (biomarkers):** At the level of biochemical and physiological processes, deviations from the normal situation ('health') can be measured using biochemical techniques.
II) **Organisms (bioassays):** Survival, growth and reproduction of individuals are chosen as endpoints of the classic laboratory ecotoxicity tests.
III) **Populations (bioindicators):** At this level, effects are manifested as changes in the genetic structure, the age structure or the abundance of a population.
IV) **Ecosystems (ecological indicators):** At this level, changes in species composition, abundance and diversity may be indicative of the effects of pollution on communities.

A proper understanding of the relationships between biomarker responses and survival, growth or reproduction is generally considered to be a prerequisite for the use of biomarkers in **ERA** (Van Gastel and Van Brummelen, 1994). It is more and more acknowledged that biomonitoring (in addition to CM) is necessary for a reliable **ERA**. In the following sections a wide range of bioaccumulation markers (Section 5) and biomarkers (Section 6) will be discussed with regard to their feasibility in **ERA** processes.

5. Fish bioaccumulation markers and ERA

Exposure assessment has to provide information on steady-state concentrations of potentially toxic xenobiotics in a selected environmental compartment. Methods for assessing exposure to a chemical fall into two categories (WHO, 1993):

- measurement of levels of chemical agents and their metabolites and/or derivatives in cells, tissues, body fluids or excreta, i.e. BAM;
- measurement of biological responses such as cytogenetic and reversible physiological changes in the exposed individuals, i.e. BEM, which will be discussed in Section 6.

Measurement of covalent adducts formed between chemical agents and cellular macromolecules (protein, DNA) or their excretion products have characteristics of both categories. In evaluating exposure, a distinction is made between the external dose, defined as the amount of a chemical agent in environmental contact with the organism as determined by CM, and the internal dose, which is the total amount of a chemical agent absorbed by the organism over a period of time as determined by BAM. Bioaccumulation markers and biomarkers of exposure will reflect the distribution of the chemical or its metabolites, respectively, throughout the organism (Fig. 3). Theoretically, this distribution can be tracked through various biological levels (e.g. tissue, cell, etc.) to the ultimate target (WHO, 1993).

When released into the environment substances will be subject to transport and transformation processes. These processes together with emission patterns, environmental parameters and physicochemical properties of the substances, will govern their distribution and con-

centration in environmental compartments such as water, air, soil, sediment and biota (ECETOC, 1993). Contaminant distributions over different trophic levels in the food web may provide a means to determine structure in aquatic communities (Russell et al., 1999). Organic trace contaminants, such as PCBs, OCPs, PAHs, PCDFs and polychlorinated dibenzo-*p*-dioxins (PCDDs) are ubiquitously present in the aquatic environment. The partition behavior of these hydrophobic chemicals in sediment, water and biota is mainly determined by lipid and organic carbon contents; the more hydrophobic a compound, the greater the partitioning to these phases (Meador et al., 1995).

5.1. Bioaccumulation

Persistent hydrophobic chemicals may accumulate in aquatic organisms through different mechanisms: via the direct uptake from water by gills or skin (bioconcentration), via uptake of suspended particles (ingestion) and via the consumption of contaminated food (biomagnification). Even without detectable acute or chronic effects in standard ecotoxicity tests, bioaccumulation should be regarded as a hazard criterion in itself, since some effects may only be recognized in a later phase of life, are multi-generation effects or manifest only in higher members of a food-web, e.g. impact of PCBs on the hatching success of eggs (Tillitt et al., 1992). Bioaccumulation of chemicals in biota may be a prerequisite for adverse effects on ecosystems (Franke et al., 1994).

Contaminant levels in biota are determined primarily by the uptake and elimination kinetics, which are typical for both chemicals and organisms (Gobas et al., 1988). A model of the processes governing bioaccumulation (uptake and clearance) in aquatic organisms is presented in Fig. 4. According to this model, the concentration of a chemical in biota (C_B) over time (t) can be expressed by:

$$\frac{dC_B}{dt} = [k_w C_w + k_F C_F] - k_B C_B$$

$$= [k_w C_w + k_F C_F] - [k_{EXC} + k_{MET}] C_B \quad (1)$$

Here, C refers to a concentration; k to a rate constant; and the subscripts W, F, B, EXC and MET to water, food, biota, excretion and metabolism, respectively. Uptake of organic pollutants in fish may be direct via exchange with the water phase ($k_W C_W$) or indirect via the consumption of contaminated food ($k_F C_F$) (Thomann, 1989). Although biotransformation of organic trace pollutants ($k_{MET} C_B$) has been reported for fish, clearance mainly occurs by simple release from the (lipoid) gill membranes and via faecal excretion into the surrounding water ($k_{EXC} C_B$) (Brown, 1994).

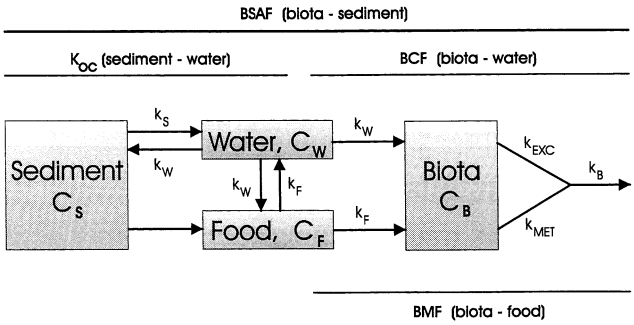

Fig. 4. Bioaccumulation model for aquatic organisms. K_{OC}: sorption coefficient; BCF: bioconcentration factor; BSAF: biota-sediment accumulation factor; BMF: biomagnification factor. C refers to a concentration and k to a rate constant. The subscripts S, W, F, B, EXC and MET refer to sediment, water, food, biota, excretion and metabolism, respectively. The digestible sediment fraction is considered to be part of the food. Adapted from Van der Oost et al. (1996a).

5.2. Bioconcentration

The bioconcentration factor (BCF) of a chemical is the ratio of its concentrations in the organism and in water during steady state or equilibrium (Opperhuizen, 1991). For the partitioning of chemicals between water and the lipid phases of organisms, the steady state BCF is defined as:

$$\mathrm{BCF} = \frac{k_W}{k_B} = \frac{C_B}{C_W} \quad (2)$$

Uptake of chemicals in organisms from water probably follows a passive diffusion mechanism analogous to that of oxygen uptake (Thomann and Connolly, 1984). The linear relationship demonstrated between HCB exposure and tissue residues in clams supported a bioenergetics-based model which indicated that decreased oxygen levels in water result in a more rapid uptake and an increased body burden of hydrophobic chemicals (Boese et al., 1988). Yang et al. (2000) demonstrated a significant correlation between the uptake constant (k_W) of organochlorine compounds and fish oxygen consumption, regardless of fish size and species. Moreover, they demonstrated a significant relationship between the depuration rate constant (K_B) and fish oxygen uptake. The uptake rate depends on the water concentrations, which will generally be higher for less hydrophobic compounds (Gobas et al., 1993). It has been suggested that the rate of uptake of hydrophobic chemicals in fish increase with a higher lipid content of the biological membranes (Spacie and Hamelink, 1982). The time required reaching a steady state between the water and fish concentrations can be determined by caging uncontaminated fish in polluted areas and measuring pollutant tissue levels after different exposure times. For rainbow trout, the estimated equilibrium times ranged between 15 and 256 days for various PCB congeners (Vigano et al., 1994) and between 56 and 275

days for persistent OCPs (Galassi et al., 1996). The BCF is usually derived from parameters assessed in uptake and elimination experiments, and is presented as a single value without statistical analyses. Bailer et al. (2000) presented a strategy for obtaining standard errors, a measure of precision, confidence intervals and a statistical test for the BCF, which they considered to be a biological endpoint of great ecotoxicological value.

The fate of chemicals is largely determined by sorption to suspended particulates and sediments (Loonen et al., 1994a). Sorption depends on the characteristics of both the sediments and the chemicals involved (see Section 5.4). If sorption of hydrophobic chemicals is considered as a partitioning between water and the organic fraction of sediment, then the equilibrium sorption coefficient (K_{OC}) can be expressed as:

$$K_{OC} = \frac{k_W}{k_S} = \frac{C_S}{C_W} \quad (3)$$

Here the subscripts S and W refer to sediment and water, respectively. If the processes of bioconcentration and sorption on sediments (i.e. the upper part of the model in Fig. 4) have both reached equilibrium, then Eqs. (2) and (3) can be combined to define the biota-sediment accumulation factor (BSAF):

$$\mathrm{BSAF} = \frac{C_B}{C_S} = \frac{\mathrm{BCF}}{K_{OC}} \quad (4)$$

Both the BCF for the partitioning of chemicals between water and the lipid phases of organisms and the K_{OC} for the partitioning between water and the organic fraction of the sediment depend upon the hydrophobicity of the chemicals (Connor, 1984). Various relationships have been derived between the log-transformed values of the 1-octanol/water partition coefficient (log K_{OW}) and the BCF of organic chemicals. Devillers et al. (1996) compared seven linear and non-linear BCF models with a dataset of 436 experimental BCF values recorded for 227 chemicals, in order to estimate their accuracy. For chemicals with a log $K_{OW} < 6$ all models yielded equivalent results, but for the highly hydrophobic chemicals (log $K_{OW} > 6$) a bilinear model proved to be superior to the other models considered. Hawker and Connel (1985) derived an equation that related the K_{OW} to the time needed to approach equilibrium between water and biota levels. This allowed them to predict that only compounds with log $K_{OW} < 6$ attain equilibrium within 1 year. Furthermore, Thomann (1989) demonstrated that the excretion rate of hydrophobic chemicals from aquatic organisms was inversely related to the log K_{OW} of the compounds. These studies are indicative of the relevance of the K_{OW} to assess the bioaccumulation potential. Among others, Connor (1984) demonstrated a tendency toward higher BSAF values for chlorinated aromatic hydrocarbons with higher K_{OW} values. The shallow slopes of the log BSAF/log K_{OW} regression lines in the study of Connor indicated a high affinity of both fish tissue and sediment organic matter (OM) for organic compounds. According to the equation of Connor (1984) the BSAF increases from 0.05 to 1.80 for chemicals with log K_{OW} values between 4 and 8. In response to Breck (1985), Connor combined the non-linear relationships between BCF and K_{OW} (Mackay, 1982) and between K_{OC} and K_{OW} (Karickhoff, 1984) and postulated that the BSAF was 0.077, independent of the K_{OW}. Van der Kooij et al. (1991), however, assumed the BSAF (i.e. the BCF/K_{OC} ratio) of chlorinated hydrocarbons to be approximately 2 (independent of K_{OW}) and postulated that steady-state BSAF values varied between 1 and 4. In various field studies, however, higher BSAF values were observed, especially for compounds with log K_{OW} values higher than 6 (e.g. Van der Oost et al., 1996a; Leadly et al., 1998). Tracey and Hansen (1996) reviewed data on PCB, PAH and OCP BSAFs from various laboratory and field experiments with fish and invertebrates. The analyses revealed similar BSAF values for various benthically-coupled species, both within and among different habitat groups, and indicated that the sum of total exposures from all routes is similar across species. Belfroid et al. (1996) assumed that a competitive process between sediment and biomass for the hydrophobic compound would result in an optimum accumulation at a certain point, but that in most cases bioaccumulation based on bulk soil and sediment concentrations is independent of the hydrophobicity (K_{OW}) of the compound.

5.3. Biomagnification

Biomagnification is the ratio between the uptake of the chemicals from food and their clearance (Sijm et al., 1992). During steady state, the biomagnification factor (BMF) can be defined as:

$$\mathrm{BMF} = \frac{k_F}{k_B} = F_F \frac{E_F}{k_B} \quad (5)$$

Here, F_F refers to the amount of food transported through the intestines per gram of fish per day and E_F to the efficiency of uptake of the chemical from food. Since bioaccumulation of persistent and extremely hydrophobic compounds cannot always be explained satisfactorily by simple partitioning processes between sediment, water and fish (Van der Oost et al., 1988; Thomann, 1989), it is likely that the uptake via contaminated food (biomagnification) contributes significantly to the bioaccumulation of these contaminants in fish. If a primary source of chemical input to an aquatic ecosystem is slow release from polluted sediments then it is plausible that uptake by benthic organisms followed by predation by

larger organisms such as fish may be a significant source of bioaccumulation (Farrington, 1991). Thomann (1989) demonstrated, by comparing predicted levels of a food chain bioaccumulation model with field observations, that biomagnification was not significant for chemicals with log K_{OW} values up to approximately 5. For chemicals with log K_{OW} values between 5 and 7, the observed bioaccumulation factors (BAFs) in feral fish indicated significant elevations (up to two orders of magnitude) above the calculated BCF values. LeBlanc (1995), however, demonstrated that significant biomagnification is only observed for chemicals with log $K_{OW} > 6.3$. He suggested that trophic-level differences in bioaccumulation might be due higher BCFs as a consequence of decreased chemical elimination efficiencies of organisms occupying increasing trophic levels. Russell et al. (1999) provided clear evidence for biomagnification of chemicals with log K_{OW} values greater than 6.3, some evidence for biomagnification of chemicals with log K_{OW} values between 5.5 and 6.3, and no evidence for biomagnification for chemicals with log K_{OW} values less than 5.5. Fisk et al. (1998) demonstrated that the persistent organochlorines with a log K_{OW} of approximately 7 have the greatest potential for food chain accumulation in fish.

The mechanism of biomagnification and food chain accumulation of organic chemicals can be explained with a fugacity-based hypothesis (Gobas et al., 1988), which has been validated by experimental findings (Gobas et al., 1993). Fugacity is equivalent to chemical activity or chemical potential as it pertains to the tendency of a chemical to escape from a phase, such as water or food (Clark et al., 1988). A difference in fugacity provides a driving force for net passive chemical transport from high to low fugacity phases. Food digestion in the gastrointestinal tract was found to increase the chemical fugacity in the food 4–5-fold by altering the fugacity capacity, while an additional 2–3-fold increase in the chemical concentration and fugacity is caused by a reduction of the food volume due to absorption from the gastrointestinal tract (Gobas et al., 1993). Food digestibility and absorption were found to be critical factors controlling BMFs and dietary uptake efficiencies under laboratory and field conditions (Gobas et al., 1999). Based upon diet information, bioenergetics modeling and determinations of PCBs in both predator and prey fish, Madenjian et al. (1998a,b) estimated that the lake trout and the coho salmon in Lake Michigan, USA, retained respectively, 80 and 50% of the PCBs that are contained within their food.

As a result of biomagnification, experimentally derived BCF or BSAF values of very hydrophobic substances may deviate significantly from those predicted with partitioning models (Thomann, 1989; Van der Oost et al., 1996a). Biomagnification may be more important for larger fish than for smaller fish, since relative gill ventilation volumes decrease with size while relative feeding rates are almost equal (Opperhuizen, 1991). When biomagnification is an important uptake route, individual and site-specific variations in bioaccumulation patterns may, at least partly, be due to differences in the fish diet (Van der Oost et al., 1996a). This hypothesis may be investigated by determining the levels of $d^{15}N$ in the contents of the gastrointestinal tract of fish, since this stable isotope is reported to be an integrative measure of trophic position, increasing with higher trophic levels (Cabana and Rasmussen, 1994).

5.4. Bioavailability

When measuring bioaccumulation behavior the bioavailability of the substance considered is a crucial parameter for valid results (Franke et al., 1994). In a review by Belfroid et al. (1996), the bioavailability was defined as the fraction of the bulk amount of the chemical present in soil/sediment and (interstitial) water that can potentially be taken up during the organism's lifetime into the organism's tissues (excluding the digestive tract). When the concentration in fish is not related to the real bioavailable concentration in the water, this might result in underestimation of the bioconcentration potential (Kristensen and Tyle, 1991). Deviations in BSAF values predicted with the partitioning models may thus partly be due to differences in bioavailability of the chemicals, possibly resulting in a pronounced site-specific variation in bioaccumulation profiles of certain contaminants (Van der Oost et al., 1996a).

Sediment characteristics such as particle size and OM content may be important factors in determining the bioavailability of hydrophobic chemicals. Conflicting results have been reported on the influence of particle size on bioavailability (Belfroid et al., 1996). Usually, organisms preferentially ingest the smaller sediment particles (enriched in OM), which results in an increased contaminant uptake. Normalization of the contaminant concentrations to OM or OC only partially accounts for this increased uptake. OM is the main determinant of sorption of hydrophobic compounds to soils and sediments (Belfroid et al., 1996). Therefore, the bioavailability of these chemicals generally decreases with increasing soil or sediment OM contents (Landrum and Faust, 1991). These differences are taken into consideration when bioaccumulation is studied with the OM normalized BSAF value, which is also referred to as the bioavailability index (Rifkin and LaKind, 1991). However, since OM cannot be considered constant, normalizing sorption constants to OM content may be less accurate than generally assumed. The OM composition may have an additional influence on sorption characteristics (Belfroid et al., 1996). It was suggested that not only the sediment OM content should

be taken into account, but that the sedimentary soot phase also has a significant impact on bioavailability of organic trace pollutants like PAHs (Gustafsson et al., 1997). Environmental behavior of PAHs could be quantitatively explained using the soot–carbon-normalized partition coefficient. Belfroid et al. (1995) indicated that the sorption and bioavailability of a chemical were also affected by the residence time in soil and sediments, also referred to as aging. This generally means that with a longer residence time in sediments the bioavailability of certain compounds, e.g. PAHs, decreases due to a decreased desorption rate (White et al., 1999). For several other factors, such as clay content, moisture content and presence of oil or metals, influences on sorption and bioavailability are known to be expected (Belfroid et al., 1996).

It has been demonstrated that the bioaccumulation of PCBs and chlorobenzenes (Schrap and Opperhuizen, 1990), pesticides (Muir et al., 1994), polychlorinated dioxins and dibenzofurans (Loonen et al., 1994a) and PAHs (Haitzer et al., 1999) can be affected by the presence of particles in the aquatic phase, such as sediment, humic acids and other dissolved organic matter (DOM). In these studies it was suggested that a reduction of the uptake of hydrophobic chemicals was caused by a reduced bioavailability of the compounds due to sorption on particles. On the one hand, it was demonstrated that distinct decreases in bioaccumulation of very hydrophobic contaminants due to DOM with a high binding capacity occurred at environmentally representative DOM concentrations (Haitzer et al., 1999). Sediment-bound chlorobenzenes, on the other hand, were bioavailable to benthic deposit feeders, indicating that ingestion of sediment particles was a significant uptake route for these hydrophobic pollutants (Boese et al., 1990). Pollutant uptake via sediments will only contribute significantly to the body burden of organisms which are able to digest (parts of) the sediment (Opperhuizen, 1991). This uptake route, which is probably of minor importance in fish, is represented in the bioaccumulation model of Fig. 4 as a dotted arrow between sediment and food. Digestible sediment is thus considered as part of the food in this model.

5.5. Biotransformation

An organism has two major ways of eliminating a chemical: it is either excreted in its original form (the parent compound) or it is biotransformed by the organism. Biotransformation generally leads to the formation of a more hydrophilic compound which is more easily excreted than the parent compound (Vermeulen, 1996). The organ most commonly involved in the biotransformation of foreign compounds is the liver, because of its function, position and blood supply. Biotransformation may also alter the toxicity of a compound, which may be either beneficial or harmful to the organism. In case of a detoxication reaction the toxicity of the compound is reduced while the excretion is generally elevated. In case of bioactivation, however, the compound is transformed into a reactive metabolite, which is more toxic than the parent compound. The biotransformation process may thus be important in determining the activity of a compound, the duration of that activity and the half-life of the compound in the body (Vermeulen, 1996).

An increased clearance of contaminants through effective biotransformation (metabolism) may cause severe deviations in BSAF values as predicted with partitioning models. However, when uptake rates are significantly higher than metabolic clearance rates bioaccumulation can still occur even though the substance is readily biodegradable (Franke et al., 1994). Pollutant concentrations in tissues and differences in excretion of metabolites can be a function of tissues and conditions controlling the activity of biotransformation enzymes (Farrington, 1991). These conditions include spawning, nutritional status, conditions and duration of exposure to organic pollutants and life cycle stage of the animal. Another important example involves the interactive effects of one chemical pollutant on another (Farrington, 1991). Simultaneous exposure of fish to PCBs and PAHs, for instance, significantly influences the extent of uptake and metabolism of each (Stein et al., 1984). Biotransformation reactions of xenobiotic organic chemicals in fish have been extensively reviewed by Sijm and Opperhuizen (1989). Fish tissue levels of chemicals that are easily biotransformed (e.g. low chlorinated PCBs, PAHs, non-2,3,7,8-substituted PCDD/Fs) are most likely not suitable as bioaccumulation markers for exposure assessment, since their tissue levels do not reflect levels in the surrounding environment (Van der Oost et al., 1996a).

5.6. Bioaccumulation models

One of the most important steps in the risk assessment process is the determination of potential exposure. Typical exposure estimation involves combining predicted concentrations for target chemicals with certain assumptions about the environmental fate of these chemicals and the activity patterns of the receptors (Valberg et al., 1996). Subsequently, the results of the exposure assessment are combined with toxicity information to provide a quantitative estimate of risk. Although some linear correlations have been demonstrated between the octanol/water partition coefficient (log K_{OW}) and the BCF (e.g. Mackay, 1982), their relationship has been proved to be very poor for many types of chemicals. It cannot be expected that the K_{OW} will be a good model of the bioaccumulation behavior of all organic chemicals in fish, because many factors

influencing accumulation are not taken into consideration (Franke et al., 1994), including:

- phenomena of active transport;
- the influence on the diffusion behavior through cell membranes;
- different rates of metabolism in various organisms and the accumulation behavior of the metabolites;
- accumulation in specific organs and tissues (also by adsorption to biological surfaces like gills and skin);
- special structural properties (e.g. amphiphilic substances, dissociating substances leading to multiple equilibrium processes);
- uptake and depuration kinetics, remaining level of the substances or of metabolites after depuration.

In addition, it is important to consider the interactive effects of one chemical pollutant on the accumulation behavior of another (Farrington, 1991; Van der Oost et al., 1991a). Despite these limitations, it is generally accepted that substances with log K_{OW} values higher than or equal to 3 have the potential to bioaccumulate (Franke et al., 1994). The log K_{OW} may be more suitable for priority ranking for testing and assessing dangerous substances than for predicting reliable BCFs for unknown new substances (Franke, 1996).

The best-known and most applied model to predict body burdens of contaminants in aquatic organisms is the equilibrium partitioning theory (EPT). In the EPT it is assumed that the concentration of a chemical in the organism is solely determined by the concentration in the water phase and the lipid content of the species (e.g. Van der Kooij et al., 1991). In the past decades there has been substantial progress in all aspects of biogeochemical research related to the issues of bioavailability and disposition of toxic organic chemicals: solubility, sorption, uptake, metabolism, retention, release and excretion. Predictive equations have been derived or have evolved empirically that relate molecular structural characteristics or properties to biogeochemical behavior. Some predictive models for the bioaccumulation of xenobiotic organic chemicals in fish are listed below:

- Thomann and Connolly (1984) constructed a model for PCBs in the Lake Michigan food chain, using growth, respiration or metabolic rate, assimilation efficiency of food (biomagnification), and bioenergetics. The model was validated in the field with alewife and lake trout.
- McCarty (1987) used the interrelationship between K_{OW}, BCF and toxicity, in combination with first-order, one-compartment assumptions, to estimate toxicant kinetic parameters. The proportional relationship could be used to convert kinetics data from a bioconcentration basis to a toxicity basis and vice versa.
- Van der Kooij et al. (1991) designed a partitioning model for toxic chemicals between water, organic matter (OM) of sediments and particulate matter and biota lipids. The BSAF was estimated to be approximately 2 for organic chemicals, independent of their log K_{OW} value.
- Sijm et al. (1992) proposed a life-cycle biomagnification model for hydrophobic organic chemicals, using lipid content, growth, uptake and elimination kinetics, as well as reproduction and biotransformation. Most parameters were derived from long-term laboratory experiments with guppies.
- Thomann et al. (1992), Gobas (1993) both developed rather similar models for predicting chemical residues in aquatic food webs, which have gained general scientific acceptance and are being used for both scientific and regulatory applications. Both models incorporate the cumulative results of research originating from the early 1980s on bioaccumulation processes in aquatic food webs. Both models contain rate equations for estimation of steady-state conditions but also treat some chemical distributions as equilibrium partitioning. Burkhard (1998) compared both models and observed that the BAFs of the Gobas model were slightly better in agreement with measured BAFs (determined from Lake Ontario data). The K_{OW} and the sediment–water column chemical concentration quotient were the dominant sources of uncertainties for predicted BAFs by both models. Both models can be used in **ERA** studies for assessing the risks of bioaccumulative chemicals to aquatic organisms and to organisms that consume them, including wildlife and humans.
- Hendriks (1995b) developed a model to predict non-equilibrium concentrations of trace contaminants in biota by relating the main non-steady state parameter, the outflow rate, to the K_{OW} and the size of the species. This intermediately complex model allowed estimations for fairly unknown substances and species.
- Park and Erstfeld (1997) developed a kinetic model to simulate bioaccumulation of toxic chemicals under natural conditions, using sediment sorption/desorption, bioavailability, uptake and elimination kinetics. Biomagnification was not taken into account. The model was validated in the laboratory with chlordane in goldfish.
- Luk and Brockway (1997) designed a bioenergetics-based pollutant accumulation model, including age-dependencies for diet composition and energy densities of prey and consumer. Sensitivity analysis indicated metabolic and growth-related parameters to be most critical. The model was fitted to the Lake Ontario water and fish PCB concentrations.
- Morrison et al. (1997) developed a mathematical model that estimates chemical concentrations in

phytoplankton, zooplankton, filter-feeding and detrivorous benthic invertebrates and fish. A validation experiment illustrated that 95% of observed concentrations in invertebrates and fish were within a factor 2 of model-predicted concentrations.

5.7. Bioaccumulation assessment using SPMDs

Passive sampling via SPMDs, introduced by Huckins et al. (1990), is a so-called biomimetic technique used to simulate body residues and target concentrations of complex organic mixtures. SPMDs usually consist of low-density polyethylene (LDPE) lay-flat tubing, filled with either natural lipids or the model lipid triolein (1,2,3-tri[*cis*-9-octadecenoyl]glycerol). Thus far, SPMDs have been used for sampling organic contaminants in water, sediments, air and soil. A wide range of nonpolar to moderately polar chemicals, including PAHs, PCBs, OCPs, PCDDs, PCDFs, and chlorophenols–anisoles–veratroles has been sampled successfully with SPMDs (Booij et al., 1998). Other recently developed passive samplers are diffusive gradients in thin films (DGTs) for metal sampling (Zhang et al., 1998) and the solid phase micro-extraction (SPME) fibres to estimate the bioaccumulation potential in effluents and surface waters (De Maagd, 2000; Verbruggen et al., 2000). All these methods are designed to mimic aquatic animals as bioconcentrators of low level environmental pollutants.

In order to predict water concentrations of contaminants by levels accumulated in the SPMDs, more has to be known about the uptake kinetics (Rantalainen et al., 2000). Uptake of chemicals depends upon their chemical and physical properties (notably K_{OW}) but also upon temperature, turbulence, flow rate of the water column and biofouling of the membrane surface. Booij et al. (1998) described a method to estimate the uptake kinetics in both laboratory and in field situations by spiking the SPMDs, prior to exposure, with a number of 'performance reference compounds' that do not occur in the environment. The release rate of these compounds is a measure for the exchange kinetics between the SPMD and water. A fairly good agreement was found between SPMD-derived water concentrations of organochlorine compounds and measured analyte values of tangential-flow ultrafilter permeates (Ellis et al., 1995) values obtained by using a resin column water sampler (Rantalainen et al., 1998). In several studies the uptake rates in SPMDs were compared with those of aquatic invertebrates and fish. Meadows et al. (1998) demonstrated that the uptake rate constants (k_U) of PCBs estimated in SPMDs were similar to those in brown trout, with k_U values for SPMDs ranging from one to two times those of the fish. The pattern of congener uptake in the fish and SPMDs was also similar. PCB and PCDD/F congener profiles obtained in SPMDs reflected those in feral longnose suckers in a Canadian river (Rantalainen et al., 1998). A poor comparability, on the other hand, was observed between organochlorine levels in SPMDs and those in feral carp and sauger or feral and caged channel catfish in the Mississippi river (USA), which was most probably due to the metabolism and depuration of these compounds by fish (Ellis et al., 1995).

Although SPMDs do not fully fall within the scope of this review, they are briefly described here since they may be used as an alternative for fish in order to investigate certain aspects in **ERA** studies. SPMDs can be applied to investigate bioavailability of nonpolar compounds (Huckins et al., 1990; Petty et al., 1995; Sabuliunas et al., 1998) and temporal trends in the levels of waterborne contaminants (Bergqvist et al., 1998; McCarthy et al., 2000) and to evaluate the significance of point and non-point contaminant sources (McCarthy et al., 2000). Integrated research with SPMD sampling and bioassay testing of the extracts may be a valuable approach for the assessment of levels and effects of bioavailable hydrophobic pollutants.

5.8. Exposure assessment using contaminant bioaccumulation in fish

Although many studies have been carried out to investigate the accumulation of organic trace pollutants in aquatic organisms, generally, no standardized methods were used. Since the levels of hydrophobic contaminants in the water phase are usually too low for reliable quantification, it is difficult to study bioconcentration in the field. Compared with the water column, sediment is a more appropriate environmental compartment to be related to levels of pollutants in biota (Connor, 1984; Rifkin and LaKind, 1991). Sediment characteristics change slowly, which makes it easier to collect representative samples, which, for example, are less dependent on seasonal variations. In addition, sediments may serve as a storage compartment for long-term release, reflecting the history of discharges to an area (Connor, 1984). There are, however, some factors, such as sediment composition, bioturbation, sediment erosion, patchiness, degradation processes, etc. which have to be taken into account in designing the proper sampling strategy (Kelly et al., 1994). The affinity of a non-polar compound for sediments depends primarily on its hydrophobicity. Since the sorption of hydrophobic contaminants is, among other factors, determined by the sediments' OM content (Landrum and Faust, 1991), sediment pollutant levels normalized on an OM basis provide more information on the bioavailable fraction. Since the capacity of an organism to accumulate hydrophobic contaminants is highly dependent upon their tissue lipid weight (LW), biota levels should be expressed on a LW basis. For comparative research it is, therefore, important to study bioaccumulation using the LW/OM-based BSAFs. Despite

the standardized BSAF method, one should realize that bioaccumulation in field experiments will always be affected by site-specific differences in bioavailability (Van der Oost et al., 1996a). Sometimes the biota suspended solid accumulation factor (BSSAF) is also used to quantify bioaccumulation (e.g. Hendriks et al., 1998; Burkhard and Lukasewycz, 2000).

Laboratory derived BCFs of 227 organic chemicals have recently been reviewed by Devillers et al. (1996). Laboratory testing of bioaccumulation with high water concentrations may lead to low BCF values, consequently signaling low risk. Since the BCF is being used for classifying and labeling dangerous substances and in ERA, low BCFs due to high test concentrations may be misleading and underestimate the risk (Franke, 1996). To explore the validity of laboratory derived bioaccumulation ratios to field conditions, Hendriks (1995a) collected values from experiments and compared these with ratios observed in field surveys. LW-normalized concentrations of persistent organics in fish were about twice as high as those expected from laboratory studies, while LW-normalized concentrations of less persistent organics in fish were more than 1000 times lower than expected (Hendriks, 1995a).

An extensive monitoring program in the Dutch rivers revealed that the largest contribution to the overall organic microcontaminant burden in aquatic organisms came from traditionally monitored chemicals, such as PCBs, PAHs and OCPs (Hendriks et al., 1998). An overview of bioaccumulation studies using sediment and fish levels of PCBs, OCPs, PAHs, PCDDs and PCDFs is presented in Tables 1–4. Comparison between different studies is difficult because both fish and sediment levels are expressed on the basis of various dimensions (dry weight [DW], fresh weight [FW], lipid weight [LW] or organic matter or carbon [OM or OC]). Only a few studies reported both LW-based fish tissue levels and OM-based sediment levels. Bioaccumulation characteristics of different analyte groups are discussed in the following paragraphs, emphasis being placed on the use of pollutant tissue levels as bioaccumulation markers for exposure assessment. It will be discussed below whether or not the BSAFs of the compounds are consistent with the partitioning model of Van der Kooij et al. (1991), which predicted LW:OM-normalized BSAF values for organic chemicals ranging from 1 to 4, independent of their log K_{OW} values.

5.8.1. *Polychlorinated biphenyls (PCBs)*

Biota-sediment accumulation ratios (BASFs) of different PCB congeners in fish are listed in Table 1. The BSAF values range from 0.1 for CB 77 in channel catfish (Gale et al., 1997) to 2286 for total PCB in lake trout (Veith et al., 1977). The results of Veith et al. (1977), as well as other results of PCBs analyses reported before 1985, however, should be regarded as unreliable due to the primitive analytical methods which were used at that time. For completeness, however, these values are still listed in the Table 1. If bioaccumulation is determined using the same dimensions, less variation is observed in BSAF values. When the LW:OM-normalized data are considered, BSAF values are generally higher than those predicted by EPT (Van der Kooij et al., 1991), indicating that bioaccumulation cannot be explained solely by partitioning. Since log K_{OW} values of most PCB congeners are higher that 5. It is most likely that biomagnification through trophic transfer is the primary mechanism governing the accumulation of these compounds in fish, as was confirmed by numerous field surveys (e.g. Van der Oost et al., 1988, 1996a; Zaranko et al., 1997; Metcalfe and Metcalfe, 1997). There is a trend towards higher BSAF values for higher chlorinated PCB congeners, which might be due to biotransformation of lower chlorinated congeners, an elevated biomagnification of the higher chlorinated congeners, or both (Lake et al., 1995). It was demonstrated in three-spined sticklebacks that higher chlorinated PCB congeners generally showed higher BMFs than the lower chlorinated congeners (Vanbavel et al., 1996). Despite the fact that BSAF values are reported to be independent of the K_{OW} (Ankley et al., 1992; Belfroid et al., 1996), a significant negative correlation between the BSAFs of extremely hydrophobic PCBs (seven or more Cl-substituted) and the log K_{OW} was observed in striped mullet and spotted sea trout (Maruya and Lee, 1998), which supports the hypothesis that the highly chlorinated congeners are less efficiently transferred in the food web due to restricted membrane permeability (Kannan et al., 1998).

Indications for biotransformation of lower chlorinated PCB congeners in fish have been demonstrated in several studies (De Boer et al., 1993; Elskus et al., 1994; Sijm et al., 1992; Brown, 1992; Sijm and Opperhuizen, 1989). In field research, a comparison between PCB levels in fish and sediments may be used to assess selective depletion of certain PCB congeners, indicating a selective metabolic clearance of those compounds by fish (De Boer et al., 1993). Strong indications for a metabolic degradation of the non-ortho-substituted congeners CB 77 and 126 were accordingly observed in yellow eel, as opposed to observations in other fish species, e.g. pike-perch, cod, sole, dab and flounder (De Boer et al., 1993). In a long-term elimination study in which PCB-exposed eel were transferred to a relatively clean lake, it was demonstrated that elimination half-lives of tetra- and penta-CBs ranged from 340 to 1450 days, while for most hexa-, hepta- and octa-CBs no measurable elimination was observed at all (De Boer et al., 1994).

Although large variations in PCB BSAF values were observed between different species of fish and different sampling sites (Table 1), PCB tissue levels seem to be

Table 1
Biota-sediment accumulation factors (BSAFs) of polychlorinated biphenyls (PCBs) in fish

Species	Mean fish sediment concentration ratios of congeners											Dimensions	Reference
	CB 28	CB 52	CB 77	CB 101	CB 105	CB 118	CB 126	CB 138	CB 153	CB 180	Sum PCB		
Atlantic cod *Gadus morhua*						119		72	106		72	LW:DW	Beyer et al., 1996
Barbel *Barbus barbus*				61				163	172	156	123	DW:DW	Galassi et al., 1994
Bleak *Alburnus alburnus*				83				187	214	232	156	DW:DW	Galassi et al., 1994
Brown bullhead *Ameiurus nebulosus*	< 1	2–4		2–15	3–27	4–24		2–38	2–53	4–66		DW:DW	Leadly et al., 1998
Brown trout *Salmo trutta*											1000	LW:DW	Mowrer et al., 1982
											4	FW:FW	Niimi and Oliver, 1989
Burbot *Lota lota*											958	LW:DW	Mowrer et al., 1982
											1243	LW:DW	Veith et al., 1977
Channel catfish *Ictalurus punctatus*			0.1–0.6		0.9–3.6	1.0	0.1–0.4					LW:OM	Gale et al., 1997
											2	LW:OM	Tracey and Hansen, 1996
Chub *Leuciscus cephalus*				49				128	135	154	105	DW:DW	Galassi et al., 1994
Coho salmon *Oncorhynchus kisutch*											3–8	FW:FW	Niimi and Oliver, 1989
Croacker *Micropogonias undulatus*											1	LW:OM	Tracey and Hansen, 1996
Cyprinid *Chondrostoma soetta*				38				85	96	106	77	DW:DW	Galassi et al., 1994
Eel *Anguilla anguilla*	2	3		3				4	8	7		LW:OM	Van der Oost et al., 1988
											13–16	LW:OM	Van der Oost et al., 1991a
	3–45	4–18		5–17		7–28		16–43	8–40	6–42		LW:OM	Van der Oost et al., 1996a
	1	7		7		8		12	22	9		LW:DM**	Hendriks, 1995a
	2	14		12		8		18	32	19		LW:OM**	Hendriks et al., 1998
English sole *Parophrys vetulus*											7–14	FW:FW	Stein et al., 1992
Fathead minnow *Pimephales promelas*											2	LW:OM	Tracey and Hansen, 1996
Flounder *Platichthys flesus*									4–11			LW:OM	Vethaak et al., 1996
Gudgeon *Gobio gobio*											292	LW:DW	Mowrer et al., 1982
Killifish *Fundulus heteroclitus*											1–275	DW:DW	Elskus and Stegeman, 1989
											4–33	DW:DW	Lake et al., 1995
Lake trout *Salvelinus namaycush*											2286	LW:DW	Veith et al., 1977
											16	FW:FW	Niimi and Oliver, 1989
Medaka *Oryzias latipes*											4	LW:OM	Tracey and Hansen, 1996
Perch *Perca fluviatilis*				65				170	182	175	132	DW:DW	Galassi et al., 1994
											55	LW:DW	Brevik et al., 1996
											542	LW:DW	Mowrer et al., 1982
Pike *Esox lucius*											18–35	LW:OM	Van der Oost et al., 1991a
			6				33					LW:OM	Järnberg et al., 1993
Rainbow trout *Salmo gairdneri* or *Oncorhynchus mykiss*											2–9	FW:FW	Niimi and Oliver, 1989
Roach											6–12	LW:OM	Van der Oost et al., 1991a
Rutilus rutilus											5–37	LW:OM	Van der Oost et al., 1994b
	4	12		12		10		10	17	7		LW:OM**	Hendriks, 1995a
Rutilus pigus				34				72	78	72	61	DW:DW	Galassi et al., 1994
Rutilus rubilio				42				96	110	97	79	DW:DW	Galassi et al., 1994
Rock sole *Lepidopsetta bilineata*											5–12	FW:FW	Stein et al., 1992
Rudd *Scardinius erythrophthalmus*				57				150	172	168	124	DW:DW	Galassi et al., 1994
Scup *Stenotomus chrysops*											2	LW:OM	Tracey and Hansen, 1996

Table 1 (*Continued*)

Species	Mean fish sediment concentration ratios of congeners										Dimensions	Reference
	CB 28	CB 52	CB 77	CB 101	CB 105	CB 118	CB 126	CB 138	CB 153	CB 180	Sum PCB	
Sea trout (spotted) *Cynoscion nebulosus*										1	1	LW:OM	Maruya and Lee, 1998
Sheatfish *Silurus glanis*				36				70	82	79	61	DW:DW	Galassi et al., 1994
Spot *Leiostomus xanthurus*											1	LW:OM	Tracey and Hansen, 1996
Starry flounder *Platichthys stellatus*											3–10	FW:FW	Stein et al., 1992
Stripet mullet *Mugil cephalus*										3	3	LW:OM	Maruya and Lee, 1998
Summer flounder *Paralichthys dentatus*											3	LW:OM	Tracey and Hansen, 1996
Weakfish *Cynoscion regalis*											4	LW:OM	Tracey and Hansen, 1996
White perch *Morone americana*											1	LW:OM	Tracey and Hansen, 1996
Winter flounder											8–40	DW:DW	Elskus et al., 1994
Pleuronectes americanus											1	LW:OM	Tracey and Hansen, 1996
						2–7		1–3	1–3			FW:FW	Hellou et al., 1999

Symbols and abbreviations *. DW, dry weight; FW, fresh weight; LW, lipid weight; OM, organic matter or organic carbon. **, suspended solids instead of sediments.

indicative of the exposure of aquatic animals to these chemicals and may thus be used as bioaccumulation markers for exposure assessment in **ERA** studies. Devault et al. (1996) observed that trends in lake trout PCB concentrations in Lakes Michigan and Superior reasonably mimic those in the water column over the long term.

5.8.2. *Organochlorine pesticides (OCPs)*

BSAFs of different OCP compounds in fish are listed in Table 2. The BSAF values range from 0.02 for chlordane in goldfish (Park and Erstfeld, 1997) to 88 for HCB and *p*,*p*′-DDT in eel (Van der Oost et al., 1996a) and *p*,*p*′-DDE in spotted gar (Ford and Hill, 1991). These differences, which are less dramatic than those found for PCBs but still cover more than two orders of magnitude, may be due partly to differences in the dimensions on the basis of which biota and sediment levels where expressed.

LW:OM-based BSAF values of HCHs, drins and DDD in eel (Van der Oost et al., 1996a) and drins in roach (Van der Oost et al., 1994b) were within the ranges of the partitioning model of Van der Kooij et al. (1991), indicating a distribution mainly governed by a bioconcentration mechanism. The higher BSAFs found for DDE, DDT and HCB in eel (Van der Oost et al., 1996a) and for HCB, HCHs and DDTs in roach (Van der Oost et al., 1994b) indicated that both bioconcentration and biomagnification mechanisms were involved in the accumulation of these compounds. Indications for a biomagnification of toxaphene and DDT congeners were also found in various fish species from different trophic levels (Ford and Hill, 1991), while laboratory studies revealed that dry weight-based BSAF values for HCB in fathead minnows ranged from 12 to 15 (Schuytema et al., 1990).

Biotransformation has been reported to influence the accumulation of several OCP compounds in fish (Sijm and Opperhuizen, 1989). DDT can be partly metabolized to DDE (Sijm and Opperhuizen, 1989; Streit, 1992), which is in line with the high DDE BSAFs found in most species of fish (Table 2). High metabolic clearance rates have been reported for HCH congeners in fish (Butte et al., 1991), although BSAF values found for summed HCHs are generally rather high (Table 2). Epoxidation to heptachlor epoxides is found to be the major biotransformation route of heptachlor (Fendick et al., 1990).

Although large variations in BSAF values were observed for OCPs in different species of fish and between different sampling sites (Table 2), most OCP tissue levels seem to be indicative of the exposure of aquatic animals to these chemicals and may thus be used as bioaccumulation markers for exposure assessment.

Table 2
Biota-sediment accumulation factors (BSAFs) of organochlorine pesticldes (OCPs) in fish

Species	Mean fish-sediment concentration ratios (range) of compounds:													Dimensions*	Reference
	HCB	Lindane	Sum HCH	Sum drins	Sum hepta-chlor	Chlordane	Toxaphene	Mirex	pp-DDE	pp-DDD	pp-DDT	sum-DDT	Sum-OCP		
Barbel *Barbus barbus*	1								73					DW:DW	Galassi et al., 1994
Bleak *Alburnus alburnus*	0.3								78					DW:DW	Galassi et al., 1994
Bowfin *Amia calva*				0.7			23		26	27	18	25		FW:FW	Ford and Hill, 1991
Brown bullhead *Ameiurus nebulosus*								2	6–26					DW:DW	Leadly et al., 1998
Brown trout *Salmo trutta*	0.2							2				4		FW:FW	Niimi and Oliver, 1989
Carp *cyprinus carpio*				0.5			26		60	38	9	49		FW:FW	Ford and Hill, 1991
Channel catfish *Ictalurus punctatus*			0.1–0.6		0.9–3.6								4	LW:OM	Tracey and Hansen, 1996
Chub *Leuciscus cephalus*	0.4								49					DW:DW	Galassi et al., 1994
Coho salmon *Oncorhynchus kisutch*	0.2							1–6				3–7		FW:FW	Niimi and Oliver, 1989
Cottonmouth *Agkistrodon piscivorus*				0.3			0.3		18	0.3	< 1	11		FW:FW	Ford and Hill, 1991
Croacker *Micropogonias undulatus*													3	LW:OM	Tracey and Hansen, 1996
Cyprinid *Chondrostoma soetta*	0.2								38					DW:DW	Galassi et al., 1994
Eel *Anguilla anguilla*													28–42	LW:OM	Van der Oost et al., 1991a
	3–88		1–9	1–5	2–23				12–66	1–5	7–88	6–23		LW:OM	Van der Oost et al., 1991a
	3	20	5–20	10					20	10	1			LW:OM**	Hendriks, 1995a
	7	19	8	6	1				15	12	3			LW:OM**	Hendriks et al., 1998
Fathead minnow *Pimephales promelas*	12–15													DW:DW	Schuytema et al., 1990
													3	LW:OM	Tracey and Hansen, 1996
Goldfish *Carassius auratus*						0.02								FW:FW	Park and Erstfeld, 1997
Killifish *Fundulus heteroclitus*										1–140					Elskus and Stegeman, 1989

Table 2 (*Continued*)

Species	Mean fish-sediment concentration ratios (range) of compounds:													Dimensions*	Reference
	HCB	Lindane	Sum HCH	Sum drins	Sum hepta-chlor	Chlordane	Toxaphene	Mirex	pp-DDE	pp-DDD	pp-DDT	sum-DDT	Sum-OCP		
Lake trout *Salvelinus namaycush*	0.8							13				15		FW:FW	Burkhard and Lukasewycz, 2000
Mosquitofish *Gambusia affinis*				0.17			2		5	4	1	5		FW:FW	Ford and Hill, 1991
Perch *Perca fluviatilis*	0.1								66					DW:DW	Galassi et al., 1994
		1.5							1	0.1	0.1	0.2		LW:DW	Brevik et al., 1996
Pike *Esox lucius*													32–35	LW:OM	Van der Oost et al., 1991a
Rainbow trout *Salmo gairdneri* or *Oncorhynchus mykiss*	0.2–0.4							2–7				2–6		FW:FW	Niimi and Oliver, 1989
Roach *Rutilus rutilus*													14–19	LW:OM	Van der Oost et al., 1991a
	15–22		16–18	1–2								18–32		LW:OM	Van der Oost et al., 1991b
	3	5	3–7	10					20	9	1			LW:DW**	Hendriks, 1995a
Rutius pigus	0.3								31					DW:DW	Galassi et al., 1994
Rutilus rubilio	0.1								43					DW:DW	Galassi et al., 1994
Rudd *Scardinius erythrophthalmus*	0.1								75					DW:DW	Galassi et al., 1994
Scup *Stenotomus chrysops*													3	LW:OC	Tracey and Hansen, 1996
Sheatfish *Silurus glanis*	0.2								29					DW:DW	Galassi et al., 1994
Smallmouth buffalo *Ictiobus bubalus*				0.7			46		65	46	36	59			Ford and Hill, 1991
Spot *Leiostomus xanthurus*													3	LW:OM	Tracey and Hansen, 1996
Spotted gar *Lepisosteus oculatus*				0.7			23		88	79	21	80		FW:FW	Ford and Hill, 1991
Water snakes *Nerodia spp*.				0.3			3		10	1	< 1	7		FW:FW	Ford and Hill, 1991
Weakfish *Cynoscion regalis*													5	LW:OM	Tracey and Hansen, 1996
White perch *Morone americana*													3	LW:OM	Tracey and Hansen, 1996

Symbols and abbreviations *, DW, dry weight; FW, fresh weight; LW, lipid weight; OM, organic matter or organic carbon; **, suspended solids instead of sediments.

Table 3
Biota-sediment accumulation factors (BSAFs) of polycyclic aromatic hydrocarbons (PAHs) in fish

Species	Mean fish-sediment concentration ratios (range) of compounds										Dimensions*	Reference
	Fluorene	Phenanthrene	Fluoranthene	Pyrene	Chrysene	Sum PAH 2**	Sum PAH 3**	Sum PAH 4**	Sum PAH 5**	Sum PAH 6**	Sum PAH	
Antarctic fish *Notothenia gibberifrons*											0.24–1.25	DW:DW	McDonald et al., 1995
Brown bullhead *Ictalurus nebulosus*		2–10	2–6	2–5	1–2						1–6	FW:DW	Baumann and Harshbarger, 1995
											0.01–0.10	LW:OM	Van der Oost et al., 1991a
Eel *Anguilla anguilla*						0.24–2.9	0.09–1.6	0.01–0.4	0.003–0.06	0.02–0.20	0.04–0.56	LW:OM	Van der Oost et al., 1994a
Killifish *Fundulus heteroclitus*	0.05–0.36	0.004–0.05	0.0005–0.001	0.0003–0.001	0.0001–0.001						0.001–0.012	DW:DW	Elskus and Stegeman, 1989
Lake trout *Salvelinus namaycush*		0.00011	0.00016	0.00710	0.00033							LW:OM	Burkhard and Lukasewycz, 2000
Pike *Esox lucius*											0.02–0.09	LW:OM	Van der Oost et al., 1991a
Roach *Rutilus rutilus*											0.02–0.13	LW:OM	Van der Oost et al., 1991a
						0.5–2.3	0.1–0.6	0.02–0.14	0.01–0.06	0.01–0.13		LW:OM	Van der Oost et al., 1994a
Sunfish *Lepomis macrochirus*											0.00001–0.8	LW:OM	Thomann and Komlos, 1999

Symbols and abbreviations *; DW, dry weight; FW, fresh weight; LW, lipid weight; OM, organic matter or organic carbon; **, number of aromatic rings.

Table 4
Biota-sediment accumulation factors (BSAFs) of polychlorinated dibenzodioxins and dibenzofurans (PCDD/Fs) in fish

Species	Mean fish-sediment concentration ratios (range) of congeners												Dimensions*	Reference
	2378-TCDD	12378-PCDD	123678-HxCDD	1234678-HpCDD	OCDD	Sum PCDD	2378-TCDF	12378-PCDF	123678-HxCDF	1234678-HpCDF	OCDF	Sum PCDF		
Carp *cyprinus carpio*	0.270	0.060	0.035	0.005			0.060		0.037	0.003			LW:OW	Kuehl et al., 1987
Channel catfish *Ictalurus punctatus*	0.15–0.48	0.19–0.31	0.06–0.28	0.01–0.71	0.01–0.86	0.01–0.72	0.01–0.19	0.004–0.21	0.01–0.04	0.001–0.07	0.001–0.07	0.003–0.17	FW:OM	Gale et al., 1997
Eel *Anguilla anguilla*	0.220	0.002	0.020	0.001	0.001	0.001–0.13	0.020	0.001		0.005	0.002	0.001–0.13	LW:OM	Van der Oost et al., 1996a
Guppy *Poecilia reticulata*	0.155	0.080	0.024	0.014	0.003		0.014	0.002	0.021	0.016			LW:OM	Loonen et al., 1994a
Lake trout *Salvelinus namaycush*	0.41	0.33	0.06	0.002	0.0004		0.43	0.65	0.07	0.006			FW:FW	Endicott and Cook, 1994
White sucker *Catostomus commersoni*					0.49								LW:OM	Muir et al., 1992a,b
Winter flounder *Pleuronectes americanus*							1–1.5						FW:FW	Hellou et al., 1999

Symbols and abbreviations *; DW, dry weight; FW, fresh weight; LW, lipid weight; OM, organic matter or organic carbon.

5.8.3. Polycyclic aromatic hydrocarbons (PAHs)

The bioaccumulation of PAHs by various marine organisms has been extensively reviewed by Meador et al. (1995). BSAFs of different PAH compounds in fish are listed in Table 3. The BSAF values range from 0.01 for total PAH in eel (Van der Oost et al., 1991a) to 10 for phenanthrene in brown bullhead (Baumann and Harshbarger, 1995). All LW:OM-based BSAF values were much lower than the values predicted with the EPT model of Van der Kooij et al. (1991), indicating a reduced uptake or an increased clearance of these compounds. BSAF values of PAHs in sunfish declined with increasing K_{OW}, probably due to low gut assimilation efficiency and increased metabolism (Thomann and Komlos, 1999). Partitioning of combustion-derived PAHs between water and sediment may be much less than predicted, possibly because associations with particles are much stronger than expected (Meador et al., 1995; Lamoureux and Brownawell, 1999). De Maagd (1996) demonstrated site-specific PAH sorption coefficients, even after normalization on organic carbon content.

PAH congeners in the aquatic environment can be transformed by chemical (photo) oxidation or biological transformations (Neff, 1985). PAH biotransformation occurs in many aquatic organisms, but it is most effective in the liver of fish. PAHs are easily metabolized by the phase I enzymes of the mixed function oxygenase system (MFO) to more hydrophilic products like phenols, dihydrodiols, quinones and epoxides (Sijm and Opperhuizen, 1989; Lech and Vodicnik, 1985). Reported half-lifes of parental PAHs in rainbow trout range from 1 day for acenaphtylene to 9 days for phenanthrene (Meador et al., 1995). Some of the PAHs can be excreted directly as unconjugated polar metabolites in bile (via the gallbladder), but most PAH will be excreted after conjugation by phase II enzymes (Vermeulen et al., 1992).

Since the elimination of PAHs is generally very efficient in fish, no bioaccumulation of these compounds has generally been demonstrated. PAH fish tissue levels are, therefore, not indicative of the levels to which the animals were exposed and cannot be used as bioaccumulation markers for exposure assessment. In order to assess the exposure of fish to PAHs it is more appropriate to determine PAH metabolite levels in bile or tissue DNA adduct levels (Tuvikene, 1995; Meador et al. 1995). Metabolite levels in an organism or in its excreta are the result of a clear interaction of a chemical with the biological matrix and often reflect the induction of enzymatic reactions. Metabolite levels could, therefore, be considered to be biomarkers. Similarly, the formation of DNA adducts after exposure to mutagenic and carcinogenic chemicals in the field must be considered as a biomarker (Van Gastel and Van Brummelen, 1994). PAH metabolites and DNA adducts will be discussed in more detail in Section 6.4 (biotransformation products) and Section 6.10 (genotoxic parameters), respectively.

5.8.4. Polychlorinated dibenzo-p-dioxins and dibenzofurans (PCDD/Fs)

BSAFs of different PCDD and PCDF compounds in fish are listed in Table 4. The BSAF values range from 0.0004 for OCDD in lake trout (Endicott and Cook, 1994) to 0.86 for OCDD in channel catfish (Gale et al., 1997). Generally, BSAF values of PCDFs and PCDDs are about three orders of magnitude lower than those of PCBs and OCPs (Rifkin and LaKind, 1991; Loonen et al., 1994a; Van der Oost et al., 1996a), and generally lower than those predicted with the partitioning model of Van der Kooij et al. (1991). In view of the log K_{OW} values of the PCDF/Ds, which range from 6 to 8 (Sijm et al., 1989b; Loonen et al., 1994b), it was anticipated that their partitioning between different phases of the aquatic environment would be similar to that of the highly chlorinated PCB congeners. Several reasons have been discussed in the literature to explain this apparent lack of bioaccumulation, namely biotransformation, reduced lipid solubility, reduced membrane transport and reduced bioavailability (Opperhuizen and Sijm, 1990; Loonen et al., 1994a,b). In the bioaccumulation model of Fig. 4, this may be demonstrated by a reduced uptake or an increased metabolic clearance.

Uptake rate constants of PCDFs and PCDDs from water to fish are comparable to those of other chlorinated hydrocarbons (Opperhuizen and Sijm, 1990; Loonen et al., 1994b). The lower uptake rates of the hepta- and octachlorinated congeners may result from reduced membrane permeability due to a larger effective cross-sectional diameter of the molecules (Opperhuizen and Sijm, 1990). Rifkin and LaKind (1991) reported that fish accumulate dioxins by ingestion (biomagnification), rather than by bioconcentration. In a study with guppies it was determined that both the BMF and the uptake efficiencies of the PCDFs and PCDDs were low compared with those found for other hydrophobic compounds (Loonen et al., 1991). Muir et al. (1992a,b) demonstrated that PCDF/Ds in the water phase were not readily bioavailable, since they were almost entirely associated with small-sized particles or with dissolved organic carbon. A congener-specific reduction in bioavailability (Loonen et al., 1994a) might explain the decreasing BSAF values with an increasing number of chlorine substituents in both dibenzofurans and dioxins found in most studies (Table 4).

The lack of accumulation of non-2,3,7,8-substituted PCDF/D congeners has been attributed to selective biotransformation (Sijm et al., 1989a, 1993). Endicott and Cook (1994) suggested that the low fresh weight-based biota-sediment ratios of PCDFs and PCDDs in feral lake trout were due to extensive metabolism of the compounds. However, since the biological half-lives of

the PCDF/Ds in trout range from 46 to 105 days (Van den Berg et al., 1994), this explanation does not seem satisfactory. The toxicokinetics and metabolism of PCDDs and PCDFs, and their relevance for toxicity, have been extensively reviewed by Van den Berg et al. (1994). Despite the low BSAF values of PCDD and PCDF congeners, fish tissue levels seem to be exposure-related (Van der Oost et al., 1996a). However, since their extremely low BSAF values cannot be explained satisfactorily, the PCDD/F fish tissue levels should not be regarded as valid bioaccumulation markers for exposure assessment.

6. Fish biomarkers and ERA

It is virtually impossible to monitor all contaminants of anthropogenic (predominantly halogenated hydrocarbons) and natural origin (heavy metals and most PAHs) which form a potential threat to the environment. In order to assess the overall quality of the aquatic environment, however, a more promising approach is to examine biochemical responses reflecting the potential of contaminants to impair physiological processes in the exposed organisms (McCarthy and Shugart, 1990). BEM by determining early adverse alterations (partially or fully reversible) is often necessary for a reliable study of bioavailable aquatic pollution. A biomarker can be defined as a biological response, which can be related to exposure to or toxic effects of environmental chemicals (Section 3). Biomarkers can be used to assess the health status of organisms and to obtain early-warning signals of environmental risks (Payne et al., 1987). Since many of the biomarkers are short-term indicators of long-term adverse effects, these data may permit intervention before irreversible detrimental effects become inevitable (McCarthy and Shugart, 1990). Various biochemical parameters in fish have been tested for their responses to toxic substances and their potential use as biomarkers of exposure or effect. Biomarkers, which have been investigated most extensively, are enzymes involved in the detoxication of xenobiotics and their metabolites (biotransformation enzymes, antioxidant enzymes). In fish, the liver is the organ most commonly involved in the detoxication of foreign compounds. This fact makes it the target organ upon which this Section of the review will be mainly focused.

Biotransformation or metabolism can be defined as an enzyme-catalyzed conversion of a xenobiotic compound into a more water-soluble form, which can be excreted from the body more easily than the parent compound (Lech and Vodicnik, 1985). Biotransformation of xenobiotic chemicals often involves enzymes that have a relatively low degree of substrate specificity when compared with enzymes involved in the metabolism of constitutive compounds (Melancon et al., 1992; Vermeulen, 1996). The toxicity of a foreign compound may be affected by metabolism, which can be either beneficial (detoxication) or harmful (bioactivation) to an organism. The various pathways of toxication and detoxification and the possible consequences of biotransformation of xenobiotics are illustrated in Fig. 5. Toxic effects may manifest themselves when the parent compound or its metabolites bind to cellular macromolecules, which may ultimately lead to membrane disruption, cell damage and or genotoxic effects that subsequently can lead to development and progression of diseases (e.g. cancer). Metabolism is, therefore, an important determinant of the activity of a compound, the duration of that activity and the half-life of the compound in the body (Timbrell, 1991).

Xenobiotic chemicals may be biotransformed in the liver according to the simplified mechanism of route I in Fig. 6, which can be subdivided into phases I, II and III. Phase I is a non-synthetic alteration (oxidation, reduction or hydrolysis) of the original foreign molecule, which can then be conjugated in phase II and catabolized in phase III (Commandeur et al., 1995). The phase III type enzymes (e.g. peptidases, hydrolases and β-lyase) that catalyze the catabolism of conjugated metabolites to form easily excretable products fall beyond the scope of this review. Many environmental contaminants (or their metabolites) have been shown to exert toxic effects related to oxidative stress (Winston and Di Giulio, 1991). Defence systems have evolved to combat oxyradical formation, using antioxidant enzymes. Studies on the induction of these antioxidant enzymes by contaminant-generated increases in oxyradicals have often been inconclusive (Winston and Di Giulio, 1991).

It is possible to analyze the impact of toxic xenobiotics on fish with various types of exposure and effect biomarkers, most of which are included in the overview below. The respective sections in which the different biomarker groups are discussed in more detail are given in parenthesis.

- **Biotransformation enzymes**: Generally, the most sensitive effect biomarkers are alterations in levels and activities of biotransformation enzymes. In fish, the activity of these enzymes may be *induced* or *inhibited* upon exposure to xenobiotics (Bucheli and Fent, 1995). Enzyme induction is an increase in the amount or activity of these enzymes, or both. A two-phase cyt P450 induction was, for instance, observed in PCB-exposed rainbow trout: the first phase induction consisted of activation of existing enzymes, while the second phase included de novo enzyme synthesis (Sijm and Opperhuizen, 1989). It is generally assumed that de novo protein synthesis is the most important enzyme induction process (Stegeman and Hahn, 1994). Inhibition is the opposite of induction. In this case, enzymatic activity is blocked, possibly due

to a strong binding or complex formation between the enzyme and the inhibitors. Two major types of enzymes involved in xenobiotic biotransformation are distinguished:
  - **Phase I enzymes** (Section 6.1);
  - **Phase II enzymes and cofactors** (Section 6.2);
- **Oxidative stress parameters** (Section 6.3): Many environmental contaminants (or their metabolites) have been shown to exert toxic effects related to oxidative stress (Winston and Di Giulio, 1991). Oxygen toxicity is defined as injurious effects due to cytotoxic reactive oxygen species (ROS), also referred to as reactive oxygen intermediates (ROIs), oxygen free radicals or oxyradicals (Di Giulio et al., 1989a). Of particular interest are the reduction products of molecular oxygen which may react with critical cellular macromolecules, possibly leading to enzyme inactivation, lipid peroxidation (LPO), DNA damage and, ultimately, cell death (Winston and Di Giulio, 1991). The activities of the **antioxidant enzymes**, which defend the organisms against ROS, are critically important in the detoxification of radicals to non-reactive molecules.
- **Biotransformation products** (Section 6.4): Another type of biomarker is the elevation in levels of biotransformation products, such as metabolite levels in body fluids or the amount of covalent adducts formed between metabolites of biodegradable chemicals and cellular macromolecules (proteins, RNA, DNA) (Melancon et al., 1992). These biomarkers may have the characteristics of both exposure and effect assessment categories.

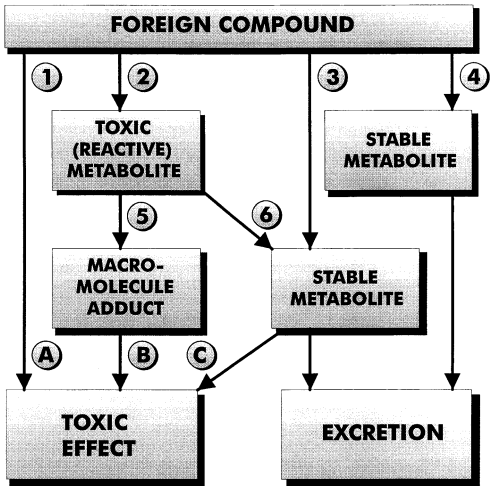

Fig. 5. Possible toxication and detoxification pathways of xenobiotic compounds: (1) direct toxic effect (A); (2) metabolic activation; (3) formation of a stable metabolite which may cause a toxic effect (C); (4) detoxification. The reactive metabolite formed by bioactivation (2) may cause a toxic effect (B) through reaction with critical targets (5) or be detoxified through reaction with a protective agent (6). Adapted from Timbrell (1991), slightly modified.

- **Stress proteins, metallothioneins and multixenobiotic resistance** (Section 6.5): The stress proteins (also called *heat-shock proteins*, HSP) comprise a set of abundant and inducible proteins involved in the protection and repair of the cell against stress and harmful conditions (Sanders, 1993). Special groups of stress proteins are the *metallothioneins* (MTs), which are inducible by both essential and toxic heavy metals (Stegeman et al., 1992; Viarengo et al., 2000), and the *P*-glycoproteins of the multixenobiotic resistance (MXR) mechanism, which may be induced or inhibited by a wide variety of chemicals (Bard, 2000).
- **Haematological parameters** (Section 6.6): Several haematological parameters in fish are potential effect biomarkers. The leakage of specific enzymes (e.g. transaminases) into the blood may be indicative of the disruption of cellular membranes in certain organs (Moss et al., 1986). Although less specific, other haematological parameters, like hematocrit, hemoglobin, protein and glucose, may be sensitive to certain types of pollutants as well. In addition, the blood levels of specific steroid hormones or proteins normally induced by these hormones may be indicative for certain reproductive effects due to endocrine disruption (see Section 6.8).
- **Immunological parameters** (Section 6.7): A large number of environmental chemicals have the potential to impair components of the immune system. Both antibody- and cell-mediated immunity may be depressed by certain pollutants, as reviewed by Vos et al. (1989). Although most research on this system has been performed on mammalian species, it may be considered a promising field to search for new fish biomarkers (Wester et al., 1994).
- **Reproductive and endocrine parameters** (Section 6.8): The impact of xenobiotic compounds on reproductive and endocrine effects has attracted growing interest in recent years. Since a decreased reproductive capability in feral fish may in the long run threaten the survival of a large number of susceptible species, these parameters certainly deserve thorough examination. Hormone regulation may be impaired as a consequence of exposure to environmental pollutants (Spies et al., 1990).
- **Neuromuscular parameters** (Section 6.9): With respect to neuromuscular functions, recent studies indicated that the 'old' biomarker acetylcholinesterase (ACHE), which is sensitive to organophosphate (OP) and carbamate pesticides, may be responding to low levels of contaminants in the environment (Payne et al., 1996).
- **Genotoxic parameters** (Section 6.10): The exposure of an organism to genotoxic chemicals may induce a cascade of events (Shugart et al., 1992): formation of structural alterations in DNA, procession of DNA damage and subsequent expression in mutant gene

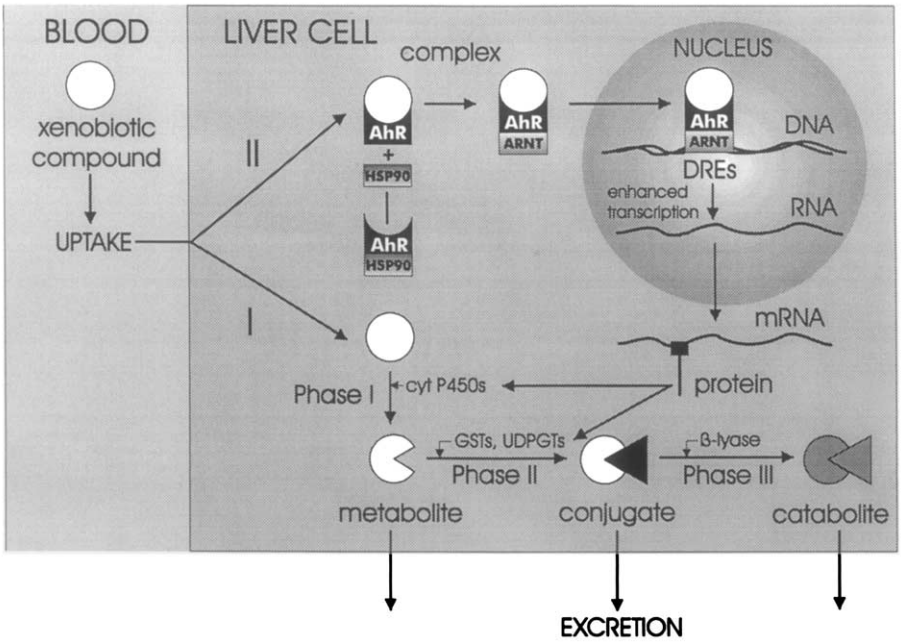

Fig. 6. Simplified presentation of the fate of xenobiotic compounds in the liver cell. Route I, a possible mechanism for detoxification or toxication, and route II, a possible mechanism for enzyme induction. AhR, aryl hydrocarbon receptor; HSP90, 90 kDa heat shock protein; ARNT, Ah receptor nuclear translocator; DREs, dioxin responsive elements; cyt P450s, cytochrome P450 isozymes; GSTs, glutathione *S*-transferases; UDPGTs, UDP-glucuronyl transferases.

products, and diseases (e.g. cancer) resulting from the genetic damage. The detection and quantification of various events in this sequence may be employed as biomarkers of exposure and effects in organisms exposed to genotoxic substances in the environment.

- **Physiological and morphological parameters** (Section 6.11): The actual measurement of adverse effects or of the consequences of those effects may also be used as biomarkers. Determination of adverse effects can be performed histopathologically, by investigating lesions, alterations or tumour formation (neoplasms) in fish tissues.

Since the main objective of this review is to examine the use of fish biomarkers in classifying the water quality for ERA, emphasis will be placed on the most susceptible parameters, which may be used as early-warning systems for pollutant stress. Some of the most commonly measured biomarker responses in fish, i.e. the biotransformation enzymes and the products of biotransformation, will therefore, be discussed in more detail, while other potential biomarker responses will only be discussed briefly in the following sections. The discussion will focus on the feasibility of various parameters as biomarkers for ERA. An overview of the literature on pollutant-induced responses in fish is presented in Tables 5–12. In these tables, a separation is made between four groups of biomarker responses, each group being divided into laboratory and field studies: phase I-related enzymes (Tables 5 and 6), phase II enzymes and cofactors (Tables 7 and 8), antioxidant enzymes (Tables 9 and 10) and various other types of commonly used biomarkers (Tables 11 and 12).

When laboratory experiments were carried out with different pollutant doses, or after different exposure times, the most prominent of the reported effects are presented (Tables 5, 7, 9 and 11). The strongest observed effects are also presented for multiple-site field studies or field caging studies with different exposure times, i.e. generally those at the most polluted site and after the longest exposure time, respectively (Tables 6, 8, 10 and 12). The relative frequencies of the responses in various biological and biochemical parameters, as presented in Tables 5–12, are illustrated in the graphs of Figs. 7–10. In these graphs, the percentages of negative or positive pollutant-induced biomarker responses for all fish species, as reported in the literature considered for this review, are visualized for both laboratory and field studies. In the following paragraphs, the function and responses of the parameters presented in the Tables and Figures will be discussed, together with those of other biological parameters (see overview).

6.1. Phase I enzymes

The first phase of metabolism, unmasking or adding reactive functional groups, involves oxidation, reduction or hydrolysis (Goeptar et al., 1995). For the majority of xenobiotic compounds the phase I reactions are catalyzed by microsomal monooxygenase (MO) enzymes,

also known as the mixed-function oxidase (MFO) system (i.e. cytochrome P450 [cyt P450], cytochrome b_5 [cyt b_5], and NADPH cytochrome P450 reductase [P450 RED]). Most oxidative phase I biotransformations in fish are catalyzed by these cytochrome P450-dependent MOs. Cytochromes P450, comprising a large and still expanding family of heme proteins, are membrane-bound proteins which predominantly are located in the endoplasmic reticulum of the liver (Stegeman et al., 1992; Bucheli and Fent, 1995). To a lesser extent, they have also been found in various other fish organelles and tissues (Celander, 1993). The cyt P450 reactions can be grouped according to the type of substrate and can be divided largely into the synthesis and degradation of endogenous substrates and the metabolism of xenobiotic substrates (Stegeman et al., 1992; Goksøyr and Förlin, 1992). The biochemistry and molecular biology of MOs, including current perspectives on forms, functions and regulation of cytochrome P450 in aquatic species, have been extensively reviewed by Stegeman and Hahn (1994).

The most important feature of the MFO system is its ability to facilitate the excretion of certain compounds by phase I metabolism, as it transforms lipophilic xenobiotics to more water-soluble compounds (Bucheli and Fent, 1995). Xenobiotic phase I biotransformation via the MO system follows a reaction cycle which can be divided into several steps (Stegeman and Hahn, 1994; Bucheli and Fent, 1995; Goeptar et al., 1995). In the first step, the substrate binds to prosthetic heme ferric iron (Fe^{3+}) of the enzyme. Following substrate binding, the iron is reduced by electron transfer from the flavoprotein NADPH cytochrome P450 reductase (P450 RED). Subsequently, O_2 is bound; a critical point at which catalysis may proceed or be interrupted, resulting in release of active oxygen (superoxide). The next steps involve the addition of a second electron, usually via cytochrome b_5 (cyt b_5), and the formation of a peroxide, followed by cleavage of the O–O bond, the formation of a substrate radical, the hydroxylation of that radical and the release of the product.

Hydrophobic organic micropollutants may induce the activity of the xenobiotic metabolizing enzyme systems in many species, including fish (Kleinow et al., 1987). The mechanism of enzyme induction has been studied most extensively for cyt P450 isozymes. Years ago, Knudson and Poland (1982) proposed a model to describe the mechanism by which TCDD and related compounds regulate gene expression through the aromatic hydrocarbon receptor (AhR). An updated rendition of this model (according to Bucheli and Fent, 1995; Safe, 2001) is shown in Fig. 6 (route II). Enzyme induction is initiated by the binding of a specific xenobiotic to a protein complex that comprises the Ah receptor and the heat-shock protein 90 (HSP 90), the latter being subsequently released. The Ah receptor complex then binds to aryl hydrocarbon nuclear transferase (ARNT, a.k.a. Ah receptor nuclear translocator) and migrates to the cell nucleus. In the nucleus of the cell ARNT binds to a DNA recognition sequence upstream of the cyt P450 genes, also known as the xenobiotic regulatory element (XRE) or dioxin responsive element (DRE). Transcription factors now have ready access to the promotor region of the CYP1A gene. Consequently, messenger RNA (mRNA) synthesis is increased, resulting in elevated protein levels (Stegeman and Hahn, 1994).

Since the MFO system is sensitive to certain environmental pollutants, its activity may serve as a biological monitor for exposure to certain classes of xenobiotic chemicals (Sijm and Opperhuizen, 1989; Bucheli and Fent, 1995). Induction of cyt P450 1A (CYP1A), the best studied biomarker for environmental contamination in aquatic ecosystems, has been reviewed by Goksøyr and Förlin (1992), Bucheli and Fent (1995). There is strong evidence that the content and activity of induced CYP1A in fish is related to levels of aromatic and polychlorinated aromatic hydrocarbons in the animals and the environment in a dose-dependent manner (Stegeman and Lech, 1991; Stegeman and Hahn, 1994). In general, the structural features associated with CYP1A induction in fish are similar to those in mammals (Stegeman and Hahn, 1994). Even for the best-known groups of inducers (i.e. PAHs and HAHs), however, our understanding is not sufficient to identify the most important contributors to environmental induction. Presumably, structure/activity relationships (SARs) for induction of P450s are at least partly a reflection of receptor-binding SARs. These relationships have not been directly examined in aquatic species, although it was demonstrated that the correspondence between AhR presence and CYP1A inducibility in fish is nearly perfect (Stegeman and Hahn, 1994).

An overview of the responses of phase I-related enzymes to environmental pollutants in both laboratory and field studies is presented in Tables 5 and 6, respectively. Since phase I responses in laboratory studies were extensively reviewed in 1989 by Sijm and Opperhuizen, this review focused on the most recent studies.

6.1.1. Total cytochrome P450 (cyt P450)

Xenobiotic phase I biotransformation in fish is mainly mediated by the cyt P450-dependent MO or MFO system (Fig. 6, route I). Although most cytochrome P450 proteins do not show any response to pollutants, the strong and selective induction of some P450 isoenzymes may cause a significant elevation in total cyt P450 levels. Generally, this response is less sensitive than that in the levels or activities of selected isoenzymes (Bucheli and Fent, 1995). It was demonstrated that single xenobiotic compounds can act as inducers of

Table 5
Laboratory studies on responses of organic trace pollutants on fish hepatic phase I-related enzymes

Species	Pollutants	cyt P450*	CYP1A*	AHH*	EROD*	cyt b5*	P450 RED*	Others**	Reference
Antarctic fish *Notothenia gibberifrons*	PAH (BaP)				++				McDonald et al., 1995
Arctic charr *Salvelinus alpinus*	PAH (BaP)	=			++			CND: ++ T6βH: =	Wolkers et al., 1996
Atlantic cod *Gadus morhua*	Crude oil (PAHs)				++				Aas et al., 2000
Atlantic salmon	PAH (BNF)		++						Goksøyr et al., 1991a
Salmo salar	PAH (BNF)	+	++		++	=	+	ECOD: ++	Goksøyr and Larsen, 1991
	Crude oil				+				Gagnon and Holdway, 2000
	PAHs		++		++			mRNA: ++	Stagg et al., 2000
Atlantic tomcod *Microgadus tomcod*	PAH (BNF, BaP), PCB 77 or 2378-TCDD							mRNA: ++	Courtenay et al., 1999
Barbel *Barbus plebejus*	PCB (Aroclor 1260)	+			++			ECOD: ++	Hugla and Thome, 1999
Blenny	PAH (BNF, heavy gas oil)	+	+		++			mRNA: +	Celander et al., 1994
Zoarces viviparus	PAH (BNF)		+		++				Förlin and Celander, 1993
Blue-striped grunt *Haemulon sciurus*	PAH (BNF)	+	+		++				Stegeman et al., 1990
Brook trout	PCDD (2,3,7,8,-TCDD)				++				Cormier et al., 2000b
Salvelinus fontinalis	PAH (BaP)				+				Padros et al., 2000
Brown bullhead *Ameiurus nebulosus*	PAH (BNF)	=			++				Hasspieler et al., 1994
Bullhead *Cottus gobio*	Organotins (TBT, TPT)	−−			−−		−		Fent and Bucheli, 1994
California killifish *Fundulus parvipinnis*	PAH (BaP)			+					Von Hofe and Puffer, 1986
Carp	PCDD (2,3,7,8-TCDD)	+			++				Van der Weiden et al., 1994
Cyprinus carpio	PCDD (2,3,7,8-TCDD)		++		++				Van der Weiden et al., 1992
	PAH (BaP, chrysene)		++		++				Van der Weiden et al., 1993
	PCBs (Aroclor 1254)				++				Melancon and Lech, 1983
	PAH (BNF)	+		++	++			PROD: ++	Riviere et al., 1990
	Deltamethrin	=			−			APDM: −	Banca et al., 1997
	PCB (Aroclor 1254)	+		++	++	=	=	ECOD: +	Ueng et al., 1992
	PAH (BNF)		++		++				Agradi et al., 2000
	17a-ethynylestradiol	=	−		−		+	b5RED: =	Sole et al., 2000
Channel catfish	PCB (Aroclor 1254)	=		++	++	=	=	APDM: =	Ankley et al., 1986
Ictalurus punctatus	BKME	=			++				Mather-Mihaich and Di Giulio, 1991
	PAH (BNF)	=			++				Hasspieler et al., 1994
	2,4-D+picloram	=			+				Gallagher and Di Giulio, 1991
	2-aminocanthracene (AA)				−				Watson et al., 1995
	PAH (BaP)		+	+					Ploch et al., 1998
	PCB 126		++		++				Rice and Roszell, 1998
	PCOD/F		+						Fiedler et al., 1998
Chinook salmon *Oncorhynchus tshawytscha*	PAH (BNF)		++					mRNA: ++	Campbell and Devlin, 1996
Cockscomb prickleback *Anoplarchus purpurescens*	BNF or oiled sediment		++						Woodin et al., 1997
Cod	PAH (BNF)		+						Goksøyr, 1991
Gadus morhua	PAH (BNF		+						Goksøyr et al., 1991a
	PCDD (2,3,7,8-TCDD)		+		++				Hektoen et al., 1994

Table 5 (*Continued*)

Species	Pollutants	cyt P450*	CYP1A*	AHH*	EROD*	cyt b5*	P450 RED*	Others**	Reference
Crucian carp *Carassius auratus*	PCDDs and metals				++				Chen et al., 1998
Dab	PCB (CB 77)		++		+				Sleiderink and Boon, 1996
Limanda limanda	PAH (BNF)		+		++				Förlin and Celander, 1993
	PAH (3MC)				++				Lemaire et al., 1996
	PAH (BaP) and PCB (CB 126)				+				Van Schanke et al., 2002
Eel *Anguilla anguilla*	PAH (BaP)	=		++	++		=		Lemaire-Gony and Lemaire, 1992
	PAH (DNOC)	=					++		Braunbeck and Völkl, 1991
	Organotins (TBT, TPT)	−−			−−		−		Fent and Bucheli, 1994
	PAH (BaP, BNF)				++				Pacheco and Santos, 1997
	PAH (BaP, BNF)				+				Pacheco and Santos, 1998
	PAH (BNF)				+				Fenet et al., 1998
	PAH (BaP)				++				Rotchell et al., 1999
	PAH (BaP)				++				Rotchell et al., 2000
	PAH (BNF)		++		++				Agradi et al., 2000
	PAH (BNF or BaP), PCB		++		++				Schlezinger and Stegeman, 2000
English sole	PAH (BaP)		++	++					Varanasi et al., 1986
Parophrys vetulus	PCB (Aroclor 1254), PAH (BaP)			+/++				EH: =	Collier and Varanasi, 1991
Fathead minnow *Pimephales promelas*	PCB (CB 77)		++		++				Lindström-Seppä et al., 1994
Flounder	PCB (Clophen A50)	=			+				Besselink et al., 1998
Platichthys flesus	PCB (CB 156), PAH (BaP)		+		++			AE: =	Beyer et al., 1997
	PCBs				+				Besselink et al., 1998
	PAH (BaP)				++				Rotchell et al., 1999
	PAH (BaP)				+				Rotchell et al., 2000
Fourhorn sculpin *Myoxocephalus quadricornis*	BKME				++				Förlin et al., 1985
Gizzard shad	PAH (BaP)				++				Levine et al., 1994
Dorosoma cepedianum	PAH (BaP)				++			mRNA: ++	Levine and Oris, 1997
Guppy *Poecilia reticulata*	Phenobarbital (PB)	+		−		=			Varanasi et al., 1987
Killifish *Fundulus heteroclitus*	PAH (BNF)	+	++		++		+	mRNA: ++	Kloepper-Sams and Stegeman, 1992
	PAH (BNF) or 3,3,7,8-TCDF		++		++			mRNA: ++	Bello et al., 2001
Lake sturgeon *Acipenser fulvescens*	PCDF (2,3,7,8-TCDF)				+				Palace et al., 1996
Lake trout	PAH (BNF)		++		++				Förlin and Celander, 1993
Salmo trutta	Propiconazole		+		+				Egaas et al., 1999
Mountain whitefish *Prosopium williamsoni*	PAH (BNF)	+	++		++	=			Kloepper-Sams and Benton, 1994
Mudfish *Clarias anguillaris*	PCBs, OCPs		+		++				Gadagbui and Goksøyr, 1996
Mullit *Chelon labrosus*	PCB (Phenoclor DP 6)	+				+		APDM: +	Narbonne and Gallis, 1979
Plke *Esox lucius*	PAH (BNF)		+		++				Förlin and Celander, 1993
Perch *Perca fluviatilis*	PCB (Clophen A50), PAH (BNF)		+		+/++				Förlin and Celander, 1993
Plaice *Pleuronectes platessa*	PCB (Clophen A40)	+	+		=				Boon et al., 1992
Polar cod *Boreogadus saida*	Crude oil		++		++			mRNA: ++	George et al., 1995

Table 5 (*Continued*)

Species	Pollutants	cyt P450*	CYP1A*	AHH*	EROD*	cyt b5*	P450 RED*	Others**	Reference
Rainbow trout *Salmo gairdneri* or *Oncorhynchus mykiss*	PCB (Aroclor 1254), PAH (BNF)				+/++			PROD: =	Addison et al., 1987
	Phenobarbitone				=				Addison et al., 1987
	PAH (BNF)				++			EH: =	Andersson et al., 1985
	PCB (Clophan A50)				++			EH: =	Andersson et al., 1985
	OCP+OPP (endosulfan/disulfoton)				=		=		Arnold et al., 1995
	PAH (BNF)	=	++		++		=	E2H: =	Celander and Förlin, 1991
	PAH (BNF)	+	++	+	++			mRNA: +	Celander et al., 1993
	PCB (CB 153)				++		=		Da Costa and Curtis, 1995
	Atrazine				=		=	AE: =	Egaas et al., 1993
	PCB (CB 126 and sediment extracts)				++				Engwall et al., 1994
	Organotins (TBT, TPT)	−−			− −		=		Fent and Bucheli, 1994
	PCB (Clophen A50), PAH (BNF)		++		++				Förlin and Celander, 1993
	PAH (BNF)		++						Goksøyr et al., 1991a
	PAH (BNF)		++						Goksøyr, 1991
	PAH (BNF)		++		++			mRNA: ++	Haasch et al., 1993a
	PAH (BNF, ISF)		+		++			ECOD: ++	Haasch et al., 1994
	PCDD (2378-TCDD)		+		++			AE: =	Hektoen et al., 1994
	PCB (Clophen A50)				++				Holm et al., 1994
	PCB (CBs 77 and 126)		++	++	++				Huuskonen et al., 1996
	OCP (endosulfan)		+	+	+		=	AE: +	Jensen et al., 1991
	BKME				++				Lehtinen et al., 1990
	OCP (HCB)				=/+				Lindström-Seppä et al., 1996
	BKME				++				Martel et al., 1995
	PCB (Aroclor 1254, CB 77)	+			++			ECOD: ++	Melancon and Lech, 1983
	PCDF (2378-TCDF)				++				Muir et al., 1992a,b
	PCDD (2378-TCDD)				++				Newsted and Giesy, 1993
	PCB (33′44′-TCB)				++				Otto and Moon, 1995
	BKME containing sediments		++		+				Otto et al., 1994
	OCP (HCB)			=	=				Roy et al., 1995
	PCB (CB 118)		+	+	++		=	AE: =	Skaare et al., 1991
	PAH (engine exhaust extract)				++	=	=	EH: =	Tjärnlund et al., 1996
	PCB (CB 77)	+			++			ECOD: ++	Tyle et al., 1991
	PCOD (2378-TCDD)	++			++				Van der Weiden et al., 1992
	PCDD (2378-TCDD)		++		++				Van der Weiden et al., 1992
	PAH, PCB in sediment extr.			+	+			APDM: +	Vigano et al., 1995
	PCB (33′44′-TCB)				++			mRNA: ++	Otto et al., 1996
	PCB (CB 169)		+		+			mRNA: +	Donohoe et al., 1999
	PCDDs				++				Parrott et al., 1995
	PCB (22′44′55′-HCB)		+		++			mRNA: +	Foster et al., 1998
	PAH (BNF)				+				Fenet et al., 1998
	2378-TCDD				++				Machala et al., 1998

Table 5 (*Continued*)

Species	Pollutants	cyt P450*	CYP1A*	AHH*	EROD*	cyt b5*	P450 RED*	Others**	Reference
	creosote containing sediments				++				Hyotylainen and Oikari, 1999
	PAH (BaP)			++	++			mRNA: ++	Levine and Oris, 1999
	PAH (BNF)				++			ADH: =	Lemaire et al., 1996
	PCB (33′44′-TCB)				++				Blom and Förlin, 1997
	PAH (retene)				++				Fragoso et al., 1999
	Propiconazole				++		=	mRNA: ++	Levine et al., 1999
	PCB (33′44′-TCB)				++			mRNA: ++	Otto et al., 1997
Redfish *scianops ocellatus*	Brevetoxin	=			+			PROD: =	Washburn et al., 1994
Roach	PAH (BNF)				++				O'Hare et al., 1995
Rutilus rutilus	BKME				++				Aaltonen et al., 2000b
Safi fish *Siganus canaliculatus*	PAH (BNF)	+		+	+	=	=	ECOD: +	Raza et al., 1995
Sand flathead *Platycephalus bassensis*	Treated waste water	=			=				Mosse et al., 1996
Scup *Stenotomus chrysops*	PCDF (2,3,7,8-TCDF)	+	++		++		=	mRNA: ++	Hahn and Stegeman, 1994
	PBB (33′44′-TCB)		++		++	+	+	mRNA: ++	White et al., 1997
	Triphenyltin	=	−		−	=	−	CYP2/3A: =	Fent et al., 1998
Sea bass *Dicentrarchus labrax*	PAH (3MC)				++			ADH: +	Lemaire et al., 1996
Skate *Raja erinacea*	PCB 126		++		++				Hahn et al., 1998
Southern flounder *Paralichthys lethostigma*	PAH (BaP)			++					Little et al., 1984
Speckled sanddab *Citharichthys stigmaeus*	PAH (BaP)			+					Von Hofe and Puffer, 1986
Squirrelfish *Holocentrus*	PAH (BNF)	=	+		=				Stegeman et al., 1990
Starry flounder	PAH containing sediments			+					Collier et al., 1992
Platichthys stellatus	PAH (BaP)			+					Varanasi et al., 1986
Stickleback *Gasterosteus aculeatus*	PCB (Clophen A50)				++			P6βH: ++	Holm et al., 1994
Striped mullet *Mugil cephalus*	Crude oils	+				+	+	B5RED: =	Chambers, 1979
Sturgeon *Acipenser naccarii*	PAH (BNF)		++		++				Agradi et al., 2000
Sunfish *Lepomis macrochirus*	PAH (BNF, BaP)				++				Oikari and Jimenez, 1992
Tilapia *Oreochromis niloticus*	PCB (Aroclor 1254)	+		++	++	+	=	ECOD: +	
Turbot *Scophthalmus maximus*	PAH (BaP)		+		+				Peters et al., 1997
Whitefish *Coregonus lavaretus*	BKME				++				Soimasuo et al., 1995
	BKME				++			PROD: ++	Soimasuo et al., 1998b
Yellow perch *Perca flavescens*	PBB (CB 126)		−/+		−/+				Dabrowska et al., 2000
Zebrafish	PCDD (2,3,7,8-TCDD)		++		++				Buchmann et al., 1993
Brachydanio rerio	PAH (BNF) or 2,3,7,8-TCDD		+		+				Troxel et al., 1997
	PCBs				+				Orn et al., 1998

Symbols and abbreviations: −−, strong inhibition (< 20% of control); −, inhibition; =, no (significant) response; +, induction; ++, strong induction (> 500% of control); *, cyt P450, cytochrome P450; CYP1A, cytochrome P450 1A isozyme; AHH, aryl hydrocarbon hydroxylase; EROD, ethoxyresorufin *O*-deethylase; cyt b5, cytochrome b5; P450 RED, NAD(P)H cytochrome P450 reductase. **, mRNA, cytochrome P450 1A 'messenger' RNA; CND, caffeine *N*-demethylase; T6βH, testosterone 6β-hydroxlase; P6βH, progesterone 6β-hydroxylase; EH, epoxide hydroxylase; E2H, estradiol 2-hydroxylase; PROD, pentoxyresorufin *O*-dealkylase; APDM, aminopyrine *N*-demethylase; b5RED, cytochrome b5 reductase; AE, aldrin epoxidase; ADH, aldehyde dehydrogenase.

specific isoenzymes, but inhibit others. This may result in a considerable alteration of isoenzyme levels, whereas the amount of total cyt P450 is not always affected (Miranda et al., 1990). The total amount of cyt P450 proteins is generally determined by scanning the difference spectrum between the oxidized and the reduced forms from 400 to 500 nm (Omura and Sato, 1964).

An increase in the total cyt P450 levels was observed in laboratory studies with various species of fish exposed to organic trace pollutants (Table 5). Notably PAHs, PCBs and PCDFs caused a significant increase in total cyt P450 levels, while PCDDs caused a very strong increase ($>500\%$ of control). These results are confirmed by many field studies, which reported significant increases of hepatic cyt P450 levels in fish from polluted environments (Table 6). A strong and significant decrease in cyt P450 levels was observed in bullhead, eel and rainbow trout exposed to organotins (Table 5). In feral brown bullhead and roach from polluted environments cyt 450 was significantly decreased (Table 6). The cyt P450 responses for all fish species from 39 laboratory studies and 35 field studies are summarized in Fig. 7A. A significant increase in cyt P450 levels was observed in 53% of the laboratory studies and 51% of the field studies, while strong increases ($>500\%$ of control) were observed in 3 and 6% of the laboratory and field studies, respectively.

Although a positive response in cyt P450 levels was observed in more than 50% of the reported laboratory and field studies, its value as a biomarker for **ERA** in aquatic ecosystems is limited since the responses of individual isoenzymes (notably CYP1A, Section 6.1.2) are more specific and sensitive. Total cyt P450 determinations may, however, be useful in testing the condition of subcellular microsomal fractions for EROD analyses, since degradation of cyt P450s during microsome isolation or storage may be identified as an increased peak in the difference spectrum at 420 nm. Moreover, levels and activities of CYP1A isoenzymes may be normalized to a cyt P450 basis, in order to obtain so-called 'turnover' values (e.g. EROD/P450).

6.1.2. Cytochrome P450 1A (CYP1A)

In fish, the class of cyt P450 isozymes which is responsible for the biotransformation of a myriad of xenobiotic compounds (PAHs, PCBs, dioxins, etc.) is the CYP1A subfamily, comprising two genes, CYP1A1 and CYP1A2 (Goksøyr and Förlin, 1992; Stegeman and Hahn, 1994). The CYP1A protein levels can be determined immunologically, using mono- or polyclonal antibodies with ELISA, Western-blotting or histochemical techniques (Bucheli and Fent, 1995). Goksøyr and Husøy (1998) reviewed biochemical and toxicological aspects concerning the cytochrome P450 system, with a more detailed description of CYP1A induction responses in fish. They also discussed the ecotoxicological consequences of CYP1A induction and the use of immunochemical techniques for CYP1A detection as a biomarker in environmental monitoring. Although the liver is the most important organ with respect to cytochromes P450, immunohistochemical staining revealed that CYP1A was expressed in other tissues as well. Route-specific cellular expression of CYP1A was observed in mummichog following exposure to the aqueous and dietary BaP (Van Veld et al., 1997). Aqueous BaP exposure resulted in higher CYP1A levels in gills, heart, liver and blood vessels, while dietary BaP exposure resulted in high levels in gut epithelium. Elevation of the CYP1A protein levels due to exposure to environmental pollutants is preceded by an increase in CYP1A mRNA levels (Stegeman and Hahn, 1994). Measurement of CYP1A mRNA by Northern blots is becoming an integral part of investigations on CYP1A regulation, while several field trials have shown its suitability as a biomarker (Bucheli and Fent, 1995). Population differences in CYP1A mRNA inducibility were observed in Atlantic tomcod from polluted and reference sites (Courtenay et al., 1999). Since differences were also observed between the responsiveness to PAHs and halogenated aromatic hydrocarbons (HAHs), the authors suggested that the CYP1A transcription in the tomcod was modulated by more than one molecular mechanism. A similar reduction in the sensitivity to AhR agonists was observed in killifish from the heavily contaminated New Badford Harbor (NBH) in the US (Bello et al., 2001). It was suggested that this reduction was caused by an alteration in the AhR signal transduction pathway in NBH fish, most probably due to chronic exposure to high levels of HAHs. Other CYP isoenzymes are generally less responsive to environmental pollutants (Celander, 1993), although elevated CYP3A levels have been demonstrated in harp seals, most probably due to toxaphene exposure (Wolkers et al., 2000). Since CYP3A is associated with steroid metabolism (notably 6β-testosterone hydroxylation), this may be important with respect to reproductive toxicity (Section 6.8, Celander et al., 1989).

Numerous studies have demonstrated an increase in the hepatic CYP1A protein levels in various species of fish after exposure to organic trace pollutants (Table 5). Notably, PAHs, PCBs, PCDDs and PCDFs caused a significant or a very strong increase ($>500\%$ of control) in CYP1A content. These results are confirmed by many field studies in which a strong and significant increase of hepatic CYP1A protein levels was observed in many species of fish from polluted environments (Table 6). At environmentally relevant doses, organotins such as tributyltin (TBT) intensified the PCB-induced CYP1A induction in channel catfish, while a decrease of induction was observed at higher TBT doses (Rice and Roszell, 1998). A study with liver microsomes from scub indicated that 3,3′,4,4′-TCB inactivated CYP1A by

Table 6
Field studies on responses of organic trace pollutants on fish hepatic phase I-related enzymes

Species	Pollutants	cyt P450*	CYP1A*	AHH*	EROD*	CYT b5*	P450 RED*	Others**	Reference
Atlantic cod *Gadus morhua*	PCBs, PAHs		+		+				Goksøyr et al., 1994
Atlantic/Baltic salmon	BKME			++	++				George et al., 1992b
Salmo salar	Halogenated xenobiotics		+		+		+		Pesonen et al., 1999
	Oil spill (PAHs)		++		++				Stagg et al., 2000
Antarctic rockcod *Notothenia coriiceps*	PAHs				++				McDonald et al., 1995
Barbel	PCBs and PAHs				++			ADPM:+	Vigano et al., 1998
Barbus plebejus	PCBs and PAHs				+				Flammarion and Garric, 1997
Blue-striped grunt *Haemulon sciurus*	PCBs, PAHs	=	+		=				Stegeman et al., 1990
Bream	PCBs				++			ECOD:++	Jedamski-Grymlas et al., 1995
Abramis brama	BKME	+		++	++		+		Lindström-Seppä and Oikari, 1991
	BKME				+				Kantoniemi et al., 1996
Brown bullhead *Ictalurus nebulosus*	Paint, organic solvents etc.	−			=	=			Gallagher and Di Giulio, 1989
	PCBs, DDE				+				Otto and Moon, 1996b
	PAHs				+				Arcand-Hoy and Metcalfe, 1999
Bullhead *Cottus gobio*	BKME			+					Bucher et al., 1993
Burbot *Lota lota*	BKME				+				Kloepper-Sams and Benton, 1994
	PCBs	=		=	=				Lockhart and Metner, 1992
Carp	BKME (PCDD/Fs)				++			ECOD:=	Ahokas et al., 1994
Cyprinus carpio	PHAHs, PAHs	+	++		++		+		Curtis et al., 1993
	PHAHs, PAHs	+			++	+	=		Van der Oost et al., 1998
	PCBs and PAHs				++			ADPM:+	Vigano et al., 1995
Channel catfish	PAHs, PCBs	=	+		++			ECOD:+	Haasch et al., 1993b
Ictalurus punctatus	PCDDs		++		++			PROD:+	Ronis et al., 1992
Chub	Various pesticides				+				Vindimian et al., 1993
Leuciscus cephalus	WWTP effluent				+				Kosmala et al., 1998
	PCBs and PAHs				+				Flammarion and Garric, 1997
Cockscomb prickleback *Anoplarchus purpurescens*	Oil spill		++						Woodin et al., 1997
Cod	Oil spill		++						Goksøyr et al., 1991b
Gadus morhua	PAHs, PCBs, DDTs		+		+				Beyer et al., 1996
Comber	PAHs, PCBs, PCDDs				+				Burgeot et al., 1994
Serranus cabrilla	PAHs				+				Narbonne et al., 1991
	PAHs				+				Burgeot et al., 1996
Serranus hepatus	PAHs				+				Burgeot et al., 1996
Dab	PAHs, PCBs		+		+				Sleiderink, 1995a
Limanda limanda	PAHs, PCBs, PCDDs				+				Burgeot et al., 1994
	PAHs, PCBs, DDTs		=		++				Goksøyr et al., 1991b
	Unknown		+		+				Förlin and Celander, 1993
	PCBs, OCPs				++				Eggens et al., 1992
	PAHs, PCBs	+	+		+				Sleiderink et al., 1995b
	PAHs, PCBs		+		++				Sleiderink and Boon, 1995
	Unknown				++			mRNA:+	Renton and Addison, 1992

Table 6 (*Continued*)

Species	Pollutants	cyt P450*	CYP1A*	AHH*	EROD*	CYT b5*	P450 RED*	Others**	Reference
	Oil spill				+				Kirby et al., 1999a
Dragonet *Callionymus lyra*	PAHs, PCBs, PCDDs				+				Burgeot et al., 1994
Eel *Anguilla anguilla*	PCBs, OCPs, PAHs, PCDF/Ds	=	++		++	+	+	PROD:+	Van der Oost et al., 1996b
	PCBs, OCPs, PAHs, PCDF/Ds	+			+			PROD:+	Van der Oost et al., 1991b
	PAHs				+				Fenet et al., 1998
	BKME				+				Pacheco and Santos, 1999
	Pesticides		+		+				Agradi et al., 2000
	PAHs and PCBs		++		++				Schlezinger and Stegeman, 2000
English sole	PAHs and others			+				EN: =	Collier et al., 1992
Parophrys vetulus	PAHs, PCBs		+	++	++				Collier et al., 1995
	PAHs, PCBs			++	++				Stein et al., 1992
Flounder	PCBs, OCPs, PAHs		+		++			ECOD: =	Goksøyr et al., 1991b
Platichthys flesus	PCBs, PAHs		=		=				Eggens et al., 1995
	PCBs, PAHs, DDTs		+		++				Beyer et al., 1996
	Domestic/industrial waste				+				Weber and Karbe, 1995
	PCBs, PAHs		=		+/=			mRNA:+	Vethaak et al., 1996
	PCBs, HCB	+	++		++				Stegeman et al., 1988
	PCBs, HCB			+	++				Addison and Edwards, 1988
	PAHs and PCBs				+				Kirby et al., 1999b
Golden ide *Leuciscus idus*	PAHs, DDTs	+						ECOD:+	Jedamski-Grymlas et al., 1994
Grayling *Thymallus thymallus*	PCBs	++		+	++		+	ECOD:++	Monod et al., 1988
Grey mullet	OCPs				++				Rodriguez-Ariza et al., 1993
Mugil sp.	PAHs, PCBs, OCPs				++			ECOD:+	Rodriguez-Ariza et al., 1994
Gudgeon	Various pesticides				+				Vindimian et al., 1993
Gobio gobio	PCBs, PAHs				+				Flammarion and Garric, 1997
Killifish	PCBs, OCPs, PAHs	=	+		+	=		AE: =	Elskus and Stegeman, 1989
Fundulus heteroclitus	PCBs		+		+			mRNA:+	Haasch et al., 1993b
	Oil spill, pesticides							AE:+	Burns, 1976
Largemouth bass	Unknown		+		=				Schlenk et al., 1996
Micropterus salmoides	PCBs				+			mRNA:+	Haasch et al., 1993b
Longnose sucker *Catostomus catostomus*	BKME				+				Kloepper-Sams and Benton, 1994
Minnow *Phoxinus phoxinus*	Various pesticides				+				Vindimian et al., 1993
Mountain whitefish *Prosopium williamsoni*	BKME	=	++		++	=			Kloepper-Sams and Benton, 1994
	BKME			++					Kloepper-Sams and Owens, 1993
Nase	PCBs	+		+	++		−	ECOD:+	Monod et al., 1988
Chondrostoma nasus	Unknown	+			++				Masfaraud et al., 1990
	PCBs and PAHs				++			ADPM:+	Vigano et al., 1995
Nile tilapia *Oreochromis niloticus*	PCBs, HCS	++				+	=		Bainy et al., 1996
Northern squawfish *Ptychocheilus oregonensis*	PHAHs, PAHs	=	=		=		=		Curtis et al., 1993

Table 6 (*Continued*)

Species	Pollutants	cyt P450*	CYP1A*	AHH*	EROD*	CYT b5*	P450 RED*	Others**	Reference
Perch *Perca fluviatilis*	BKME		++		+			PROD: +	Huuskonen and Linström-Seppä, 1995
	BKME				+				Lindström-Seppä et al., 1992
	PHAHs, PAHs				+				Balk et al., 1996
	BKME	=		+	+		=		Lindström-Seppä and Oikari, 1991
	BKME		+						Förlin and Celander, 1993
	BKME				++				Förlin et al., 1995
	BKME				++				Kloepper-Sams and Owens, 1993
	BKME				+			PROD: =	Karels et al., 1998
	BKME				+				Kantoniemi et al., 1996
	BKME				+				Förlin et al., 1995
	PAHs and heavy metals		=	−					Tuvikene et al., 1999
Pike	PCBs, OCPs, PAHs	+			+			PROD: +	Van der Oost et al., 1991b
Esox lucius	PCDDs, PCDFs				+				Förlin et al., 1992
Plaice	PCBs, OCPs, PAHs		+		+			ECOD: +	Goksøyr et al., 1991b
Pleuronectes platessa	PCBs, PAHs		=		=				Eggens et al., 1995
	PCBs, OCPs				+				Galgani et al., 1991
	Oil spill				+				Kirby et al., 1999a
Rainbow surfperch *Hypsurus caryi*	Petroleum seep		++						Spies et al., 1996
Rainbow trout *Salmo gairdneri* or	BKME				+				Lindström-Seppä et al., 1992
Oncorhynchus mykiss	PCBs				=				Otto et al., 1996
	BKME	=		+	++		=		Lindström-Seppä and Oikari, 1990
	Petrochemical waste	=						ECOD: +	Nikunen, 1985
	Oil			++					Payne and Penrose, 1975
	PAHs				+				Fent et al., 1998
	PAHs and heavy metals		=	−					Tuvikene et al., 1999
	PAHs			++	++				Senchez-Hemandez et al., 1998
Red mullet	PAHs, PCBs, PCDDs				+				Burgeot et al., 1994
Mullus barbatus	PAHs				+				Burgeot et al., 1996
Redbreast sunfish	PAHs, PCBs, phenols				++				Jimenez et al., 1990
Lepomis auritus	PCBs, Hg	+			+	+	+		Adams et al., 1990
	PCBs				+				Ham et al., 1997
Roach	PCBs, OCPs, PAHs	−	=		=	−	=		Van der Oost et al., 1994a
Rutilus rutilus	PCBs, OCPs, PAHs	+			+				Van der Oost et al., 1991b
	BKME	=		+	++		=		Lindström-Seppä and Oikari, 1991
	PCBs	+		++	++		=	ECOD: +	Monod et al., 1988
	Various pesticides				+				Vindimian et al., 1993
	BKME				+			PROD: +	Karels et al., 1998
	BKME				+				Kantoniemi et al., 1996
	PAHs and heavy metals		=	−					Tuvikene et al., 1999
Rock sole	PAHs, PCBs		+	+	+				Collier et al., 1995
Lepidopsetta bilineata	PAHs, PCBs			+	+				Stein et al., 1992

Table 6 (*Continued*)

Species	Pollutants	cyt P450*	CYP1A*	AHH*	EROD*	CYT b5*	P450 RED*	Others**	Reference
Rubberlip surfperch *Rhacochilus toxotes*	Petroleum seep		+						Spies et al., 1996
Ruffe *Gymnocephalus cernua*	Domestic/industrial waste				+			ECOD: +	Weber and Karbe, 1995
Sand flathead *Platycephalus bassensis*	PAHs				++			ECOD: +	Holdway et al., 1994
Sardine *Sardina pilchardus*	PAHs, PCBs, DDE				=				Peters et al., 1994
Seabream *Diplodus annularis*	Oil, metals and others	=		+					Bagnasco et al., 1991
Shorthorn sculpin *Myoxocephalus scorpius*	PAHs				+				Stephensen et al., 2000
Speckled sanddab *Citharichthys stigmaeus*	PCBs, PAHs, DDTs			++					Rice et al., 1994
Spoonhead sculpin *Cottus ricei*	BKME			++					Gibbons et al., 1998
Spot *Leiostomus xanthurus*	PAHs	+	++		++				Van Veld et al., 1990
Squirrelfish *Holocentrus rufus*	PCBs, PAHs	+	=		=				Stegeman et al., 1990
Starry flounder	PAHs and others			+				EH: =	Collier et al., 1992
Platichthys stellatus	PAHs, PCBs		+	++	+				Collier et al., 1995
	PAHs, PCBs, OCPs			+					Spies et al., 1988
	PAHs, PCBs			+	+				Spies et al., 1996
Stone loach *Noemacheilus barbatulus*	WWTP effluent				+				Kosmala et al., 1998
Tilapia	PCBs, OCPs		+		++				Gadagbui and Goksøyr, 1996
Oreochromis niloticus	unknown	+	++		++	+			Bainy et al., 1999
	Sewage, industrial waste	=		+	++				Ueng et al., 1992
Toadfish *Opsanus tau*	PAHs		=	=	=				Collier et al., 1993
White sucker	BKME		+		+				Van den Heuvel et al., 1995
Catostomus commersoni	BKME			++					Hodson et al., 1992
	BKME			++	++				Munkittrick et al., 1992
Whitefish *Coregonus muksun*	BKME	=		++	++		=		Lindström-Seppä and Oikari, 1989
	BKME				+			mRNA: +	Soimasuo et al., 1998a
Winter flounder	oil spill	=				=	=	b5RED: =	Payne et al., 1984
Pseudopleuronectes americanus	PAHs				++				Payne et al., 1987
	PCBs		+		+			mRNA:+	Elskus et al., 1992

Symbols and abbreviations: −−, strong inhibition (< 20% of control); −, inhibition; =, no (significant) response; +, induction; ++, strong induction (> 500% of control); *, cyt P450, cytochrome P450; CYP1A, cytochrome P450 1A isozyme; AHH, aryl hydrocarbon hydroxylase; EROD, ethoxyresorufin *O*-deethylase; cyt b5, cytochrome b5; P450 RED, NAD(P)H cytochrome P450 reductase. **, mRNA, cytochrome 450 1A 'messenger' RNA; AE, aldrin epoxidase; PROD, pentoxyresorufin *O*-dealkylase; ECOD, ethoxycoumerin *O*-deethylase; b5RED, cytochrome b_5 reductase.

Table 7
Laboratory studies on responses of organic trace pollutants on fish hepatic phase II enzymes and cofactors

Species	Pollutants	GSH*	GSSG*	GST*	UDPGT*	Others**	Reference
Carp	OPP (dichlorvos)	=					Hai et al., 1995
Cyprinus carpio	PAH (BNF)			=	=		Riviere et al., 1990
	17a-ethynylestradiol			=	=		Sole et al., 2000
Catfish	OPP (diclorvos)	=					Hai et al., 1995
Ictalurus nebulosus							
Channel catfish *Ictalurus punctatus*	BKME (short term, 1 day)	−					Mather-Mihaich and Di Giulio, 1991
	BKME (long term, 2 weeks)	+					Mather-Mihaich and Di Giulio, 1991
	PCB (Arochor 1254)			=	=		Ankley et al., 1986
	OCP (picloram and 2,4-D)			=			Gallagher and Di Giulio, 1991
	PAH containing sediments	+	+				Di Giulio et al., 1989a,b
Cod	PCDD (2,3,7,8-TCDD)			=			Hektoen et al., 1994
Gadus morhua							
Crucian carp *Carassius auratus*	PCDDs and metals in sediment	+		+			Chen et al., 1998
Dab	PAH (3MC)			=	=	DTD: =	Lemaire et al., 1996
Limanda limanda	PAH (BaP) and PCB (CB 126)			=			Van Schanke et al., 2002
Eel	PAH (DNOC)				++	ARST: +	Braunbeck and Völkl, 1991
Anguilla anguilla	PAH (BaP)			=			Lemaire-Gony and Lemaire, 1992
	PAH (BNF)			=			Fenet et al., 1998
English sole	PAH (BaP)			=			Collier and Varanasi, 1991
Parophrys vetulus							
Flounder	PCB (CB 156), PAH (BaP)			=	=		Beyer et al., 1997
Platichthys flesus							
Killifish *Fundulus heteroclitus*	PAH (BNF) or 2,3,7,8-TCDF			+	=		Bello et al., 2001
Lake sturgeon	PCDF (2,3,7,8-TCDF)				=		Palace et al., 1996
Acipenser fulvescens							
Lake trout	Propiconazole			+			Egaas et al., 1999
Salmo trutta							
Mudfish	PCBs, OCPs			+	+		Gadagbui and Goksøyr, 1996
Clarias anguillaris							
Plaice	PCB (Aroctor 1254)				+		Clarke et al., 1992
Pleuronectes platessa	PCB (Clophen A40)			+			Boon et al., 1992
Rainbow trout *Salmo gairdneri* or *Oncorhynchus mykiss*	PCB (Clophen A50), PAH (BNF)			+	+		Andersson et al., 1985
	OCP + OPP (endosulfan/difussion)			+			Arnold et al., 1995
	PAH (BNF)			+	+		Celander et al., 1993
	PCB (CB 153)				=		Da Costa and Curtis, 1995
	Atrazine			=			Egaas et al., 1993
	PCB (Clophen A50)			+	+	DTD: =	Förlin et al., 1996
	PAH (3MC)			=	=	DTD: +	Förlin et al., 1996
	PCDD (2,3,7,8-TCDD)			−			Hektoen et al., 1991
	PCB (CBs 77 and 126)			+	+		Huuskonen et al., 1996
	OCP (endosulfan)			=			Jensen et al., 1991
	OCP (HCB)	+		= / +			Lindström-Seppä et al., 1996
	PCB (3,3′,4,4′-TCB)	+	+	+	++	GSH/GSSG: =	Otto and Moon, 1995
	BKME containing sediments	+	=	=	+		Otto et al., 1994
	HCB			=	=		Roy et al., 1995
	PAH (engine exhaust extract)			=		DTD: =	Tjärnlund et al., 1996
	PAH, PCB in sediment extracts			=	+		Vigano et al., 1995
	PCB (33′44′-TCB)	=					Otto et al., 1996

Table 7 (*Continued*)

Species	Pollutants	GSH*	GSSG*	GST*	UDPGT*	Others**	Reference
	PAH (BNF)			=			Fenet et al., 1998
	TCDD, PCB, DDE			+			Machala et al., 1998
	PAH (BNF)			=	+	DTD: =	Lemaire et al., 1996
	PCB (33′44′-TCB)			=	=		Blom and Förlin, 1997
Redfish *Sciaenops ocellatus*	Brevetoxin			=			Washburn et al., 1994
Scup *Stenotomus chrysops*	PBB (33′44′-TCB)				+		White et al., 1997
Sea bass *Dicentrarchus labrax*	PAH (3MC)			−	=	DTD: =	Lemaire et al., 1996
Seabream *Sparus aurata*	PCB (Arocolor 1254)			+			Pedrajas et al., 1995
	OCP (deielderin), OPP (malathion)			−			Pedrajas et al., 1995
Starry flounder *Platichthys stellatus*	PAH containing sediments			=			Collier et al., 1992
Sunfish *Lepomis macrochirus*	PAH (BaP)			−			Oikari and Jimenez, 1992
Viviparous blenny *Zoarces viviparus*	PAH (BNF)			=	+		Celander et al., 1994
Whitefish *Coregonus lavaretus*	BKME				−		Soimasuo et al., 1995
Zebrafish *Brachydanio rerio*	PCDD (2,3,7,8-TCDD)				+		Baumann et al., 1993

Symbols and abbrevations: − −, strong inhibition (< 20% of control); −, inhibition; =, no (significant) response; +, induction; + +, strong induction (> 500% of control) *, GSH, reduced glutathione; GSSG, oxidized glutathione; GST, glutathione-*S*-transferease, UDPGT, UDP

uncoupling the catalytic cycle, resulting in formation of ROS within the active site (Schlezinger et al., 1999). The ROS formed by CYP1A may contribute to the toxicity of planar HAHs. The CYP1A responses for all fish species from 60 laboratory studies and 48 field studies are summarized in Fig. 7B. A significant increase in CYP1A levels was observed in 91% of the laboratory studies and 85% of the field studies, while strong increases (> 500% of control) were observed in 43 and 39% of the laboratory and field studies, respectively.

In all fish species considered, hepatic CYP1A protein levels seem to be a very sensitive biomarker of exposure to PAHs and HAHs, which will certainly be feasible in **ERA** procedures. Levine and Oris (1999) suggested that CYP1A expression due to exposure to rapidly metabolized substances should preferably be measured in tissues that make direct contact with the environment, such as the gill and intestine. CYP1A determinations may be used in various steps of the **ERA** process, such as quantification of impact and exposure of various organic trace pollutants, environmental monitoring of organism and ecosystem 'health', identifying subtle early toxic effects, triggering of regulatory action, identification of exposure to specific compounds, toxicological screening and the research on toxic mechanisms of xenobiotics (Stegeman et al., 1992). The CYP1A response has been validated for use in **ERA** monitoring programs (Bucheli and Fent, 1995), assuming that all potential variables that may affect this parameter are considered in the experimental design.

6.1.3. *Ethoxyresorufin O-deethylase (EROD) and aryl hydrocarbon hydroxylase (AHH)*

Next to CYP1A protein or mRNA levels, a common method to examine the responses of the CYP1A isoenzyme is to determine its catalytic activity. The first CYP1A related activity to be proposed as an indicator of pollutant exposure was the aryl hydrocarbon hydroxylase (AHH) activity (Payne, 1976). This activity is usually measured by determining the hydroxylation of benzo[a]pyrene (Collier et al., 1995). The activity of ethoxyresorufin *O*-deethylase (EROD), however, appeared to be the most sensitive catalytic probe for determining the inductive response of the cyt P450 system in fish (Goksøyr and Förlin, 1992). The EROD activity is measured by following the increase in fluorescence of the reaction product resorufin (Burke and Mayer, 1974). Recently, an extensive review was published, compiling and evaluating existing scientific information on the use, limitations, and procedural considerations for EROD activity in fish as a biomarker of chemical exposure (Whyte et al., 2000).

Increases in both AHH and EROD activities have been observed in many species of fish after exposure to organic trace pollutants (Table 5). Notably, PAHs, PCBs, PCDDs and PCDFs caused very strong increases (> 500% of control) in CYP1A catalytic activities. It was recently observed that substances such as nitrated polycyclic aromatic hydrocarbons (NPAHs) and N-heterocyclic aromatic hydrocarbons (azarenes), which are ubiquitous in the environment, may contribute

Table 8
Field studies on responses of organic trace pollutants on fish hepatic phase II enzymes and cofactors

Species	Pollutants	GSH*	GSSG*	GST*	UDPGT*	Others**	Reference
Atlantic/Baltic salmon	BKME			=	=		George et al., 1992b
Salmo salar	halogenated xenobiotics			+		GSH/GSSG:-	Pesonen et al., 1999
Barbel *Barbus plebejus*	PCBs and PAHs	=		+	+		Vigano et al., 1995
Bream	PCBs			+			Jedamski-Grymlas et al., 1995
Abramis brama	BIKME	+		+	+		Lindström-Seppä and Oikari, 1991
	BIKME		=	=			Kantoniemi et al., 1996
Brown bullhead *Ameiurus nebulosus*	PCBs, DDE	−		+	=		Otto and Moon, 1996b
Bullhead *Cottus gobio*	BKME	−					Bucher et al., 1993
Carp	PHAHs, PAHs	−	+	+	+	GSH/GSSG:-	Van der Oost et al., 1998
Cyprinus carpio	PCBs and PAHs	=		=	+		Vigano et al., 1995
Chub (*Leuciscus cephalus*)	Heavy metals			+			Lenartova et al., 1997
Cod (*Gadus morhua*)	PAHs, PCBs, DDTs			+			Beyer et al., 1996
Eel *Anguilla anguilla*	PCBs, PAHs, OCPs, PCDD/Fs	=	+	+	+		Van der Oost et al., 1996b
	PAHs			=			Fenet et al., 1998
English sole	PAHs and others			=			Collier et al., 1992
Parophrys vetulus	PAHs, PCBs	++					Stein et al., 1992
Flounder	PAHs, PCBs, DDTs				=		Goksøyr et al., 1991b
Platichthys flesus	PCBs, PAHs, DDTs,			=			Beyer et al., 1996
	Domestic/industrial waste			=			Weber and Karbe, 1995
Grey mullet *Mugil* sp.	OCPs			+			Rodriguez-Ariza et al., 1993
Nase *Chondrostoma soetta*	PCBs, PAHs	=		=	+		Vigano et al., 1995
Nile tilapia *Oreochromis niloticus*	PCBs, HCHs	=					Bainy et al., 1996
Perch *Perca fluviatilis*	BKME			=	+		Huuskonen and Linström-Seppä, 1995
	BKME	+		=	=		Lindström-Seppä and Oikari, 1991
	BKME				+		Förlin et al., 1995
	BKME			=	=		Kantoniemi et al., 1996
	BKME			−	=		Tuvikene et al., 1999
	PAHs and heavy metals						
Plaice *Pleuronectes platessa*	PCBs, OCPs				=		Goksøyr et al., 1991b
Rainbow trout	PCBs	=	=	−	=		Otto et al., 1996
Salmo gairdneri or *Oncorhynchus mykiss*	BKME			=	=		Lindström-Seppä and Oikari, 1990
	BKME				+		Oikari and Kunnamo-Ojala, 1987
	BKME				−		Oikari et al., 1985
	Petrochemical waste				+		Nikunen, 1985
	PAHs			=			Fent et al., 1998
	PAHs and heavy metals			−	=		Tuvikene et al., 1999
Red mullet *Mullus barbatus*	PAHs			+		DTD: =	Burgeot et al., 1996
Rock sole *Lepidopsetta bilineata*	PAHs, PCBs	+					Stein et al., 1992
Ruffe *Gymnocephalus cernua*	Domestic/Industrial waste			=			Weber and Karbe, 1995
Roach	PCBs, PAHs, OCPs	=	=	−			Van der Oost et al., 1994a
Rutilus rutilus	BKME	+		=	=		Lindström-Seppä and Oikari, 1991
	BKME			=	=		Kantoniemi et al., 1996
	PAHs and heavy metals			−	=		Tuvikene et al., 1999

Table 8 (*Continued*)

Species	Pollutants	GSH*	GSSG*	GST*	UDPGT*	Others**	Reference
Seabream *Diplodus annularis*	oil, metals and others	=		−		DTD: =	Bagnasco et al., 1991
Shorthorn sculpin *Myoxocephalus scorpius*	PAHs			=		DTD: =	Stephensen et al., 2000
Starry flounder	PAHs and others			=			Collier et al., 1992
Platichthy stellatus	PAHs, PCBs	+					Stein et al., 1992
Tilapia *Oreochromis niloticus*	PCBs, OCPs			+	+		Gadagbui and Goksøyr, 1996
White sucker *Catostomus commersoni*	BKME			−			Hodson et al., 1992
Whitefish *Coregonus muksun*	BKME			=/+			Lindström-Seppä and Oikari, 1989

Symbols and abbrevations: − −, strong inhibition (< 20% of control); −, inhibition; =, no (significant) response; +, induction; + +, strong induction (> 500% of control);*, GSH, reduced glutathione; GSSG, oxidized glutathione; GST, glutathione *S*-transferease, UDPGT, UDP glucuronyl transferase; **, GSH/GSSG, thiol:disulfide ratio; DTD, DT diaphorase.

significantly to fish CYP1A induction in PAH-contaminated environments (Jung et al., 2001). Generally, a good correlation will be observed between CYP1A protein levels and EROD activity (e.g. Van der Oost et al., 1996b). Numerous field studies demonstrated a strong and significant increase of hepatic CYP1A protein levels and activity in many species of fish from polluted environments (Table 6). A strong and significant decrease in EROD activities, however, was observed in bullhead, eel and rainbow trout exposed to organotins, while a decreased AHH activity was observed in phenobarbital-exposed guppies (Table 5). The PCB-induced EROD induction in channel catfish was inhibited by low doses organotins, although EROD activity was still elevated compared with reference fish (Rice and Roszell, 1998). Studies of Fent et al. (1998) with scub exposed to triphenyltin (TPT) indicated a degenerative effect of organotins on the fish microsomal MO system, although some differences are observed between organotins, and between species. EROD activity and CYP 1A protein were also reduced in carp, after i.p. administration of 17α-ethynylestradiol (Sole et al., 2000). AHH responses for all fish species from 23 laboratory studies and 33 field studies are summarized in Fig. 7C. A significant increase in AHH activity was observed in 88% of the laboratory studies and 90% of the field studies, while strong increases (> 500% of control) were observed in 43 and 39% of the laboratory and field studies, respectively. The EROD responses for all fish species from 137 laboratory studies and 127 field studies are summarized in Fig. 7D. A significant increase in EROD activities was observed in 88% of the laboratory studies and 90% of the field studies, while strong increases (> 500% of control) were observed in 69 and 37% of the laboratory and field studies, respectively.

Both AHH and EROD activities in fish liver are very sensitive biomarkers, and may thus be of great value in **ERA** processes. Although certain chemicals may inhibit EROD induction or activity, this interference is generally not a drawback to the use of EROD as a biomarker (Whyte et al., 2000). Together with levels of CYP1A protein and mRNA, the induction of CYP1A catalytic activities may be used both for the assessment of exposure and as early-warning sign for potentially harmful effects of many organic trace pollutants. Research on mechanisms of CYP1A-induced toxicity suggests that EROD activity may not only indicate chemical exposure, but may also precede effects at various levels of biological organization (Whyte et al., 2000). Certain confounding variables, which may affect the enzyme activities, however, will have to be considered when interpreting the responses in these parameters.

6.1.4. *Cytochrome b_5 (cyt b_5)*

Cyt b_5 is involved in the cyt P450-mediated biotransformations through electron donation by NADH via cytochrome b_5 reductase (Timbrell, 1991). It is unknown whether or not the AhR-mediated mechanism of induction is also involved in the induction of cyt b_5 levels in fish inhabiting polluted environments.

Cyt b_5 levels were measured in a limited number of laboratory and field studies only. Increased cyt b_5 levels were observed in laboratory studies with PCB exposed mullet and striped mullet exposed to crude oils (Table 5). Two field studies, with eel and Nile tilapia from polluted environments, demonstrated a significant increase in cyt b_5 levels, while a significant decrease was observed in roach from a polluted site (Table 6). The cyt b_5 responses for all fish species from 12 laboratory studies and ten field studies are summarized in Fig. 7E. A significant increase in cyt b_5 levels was observed in 33% of the laboratory studies and 50% of the field studies, while strong increases (> 500% of control) were

Table 9
Laboratory studies on responses of organic trace pollutants on fish hepatic antioxidant enzymes

Species	Pollutants	SOD*	GPOX*	GRED*	CAT*	Others**	Reference
Carp	OPP (paraquat)	=	+		=		Gabryelak and Klekot, 1985
Cyprinus carpio	OPP (paraquat)	+					Vig and Nemcsok, 1989
	OPP (paraquat)	=	=		=	LPOX: −	Hai et al., 1995
	17a-ethynylestradiol	=	=		=		Sole et al., 2000
Catfish *Ictalurus nebulosus*	OPP(dichlorvos)	=	=		=	LPOX: +	Hai et al., 1995
Channel catfish *Ictalurus punctatus*	BKME	=	=		+	LPOX: =	Mather-Mihaich and Di Giulio, 1991
	PAH containing sediments				+		Di Giulio et al., 1989a,b
	OCP (2,4-D and picloram)				=		Gallagher and Di Giulio, 1991
Dab *Limanda limanda*	PAH (3MC)	=		=	= / −		Lemaire et al., 1996
Eel *Anguilla anguilla*	PAH (DNOC)				−		Braunbeck and Völkl, 1991
Lake sturgeon *Acipenser fulvescens*	PCDF (2,3,7,8-TCDF)	+	=		=	LPOX: + +	Palace et al., 1996
Rainbow trout	BKME containing sediments	−			=		Otto et al., 1994
Salmo gairdneri or *Oncorhynchus mykiss*	PAH + PCB in sediments extract		=	=			Vigano et al., 1995
	OPP (Paraquat)	−				TBARS: + +	Pedrajas et al., 1995
	PCB (Aroclor 1254), OCP (dieldrin)	=				TBARS: +	Pedrajas et al., 1995
	HCB contaminated food	+	+	+			Roy et al., 1995
	PCB (3,3′,4,4′-TCB)	=	+	+ +	−		Otto and Moon, 1995
	OCP + OPP (endosulfan/disulfoton)				=	G6PDH: =	Arnold et al., 1995
	PCB (Clophen A50)			+			Förlin et al., 1996
	PAH (3MC)			=			Förlin et al., 1996
	PAH, PCB (engine exhaust extract)			+	−	G6PDH: =	Tjärnlund et al., 1996
	DDE			+			Machala et al., 1998
	PAH (BNF)	=	=		=		
Red mullet *Mullus barbatus*	PCB (Arcolor 1254)	=	+	−	+	LPOX: +	Rudneva-Titova and Zherko, 1994
Scorpion fish *Scorpoena porcus*	PCB (Arcolor 1254)	=	+	+	+	LPOX: +	Rudneva-Titova and Zherko, 1994
Sea bass	Cd and Cu				−	LPOX: +	Romeo et al., 2000
Dicentrarchus labrax	PAH (3MC)	+ / =	− / +		=		Lemaire et al., 1996
Viviparous blenny *Zoarces viviparus*	PAH (BNF, heavy gas oil)			=			Celander et al., 1994

Symbols and abbrevations: − −, strong inhibition (< 20% of control); −, inhibition; =, no (significant) response; +, induction; + +, strong induction (> 500% of control);*, SOD, superoxide dismutase; GPOX, glutathione peroxidase; GRED, glutathione reductase, CAT, catalase; **, LPOX, lipid peroxidation; G6PDH, glucose-6-phosphate dehydrogenase; TBARS, thiobarbituric acid reactive substances.

observed in none of the reviewed laboratory and field studies.

Although cyt b_5 levels may be elevated in some fish species after exposure to organochlorine compounds, its value as a biomarker in **ERA** procedures remains questionable. Further research is required to elucidate the mechanism of cyt b_5 induction.

6.1.5. NADPH cytochrome P450 reductase (P450 RED)

Cyt P450 activity depends on reduction of the heme iron by electron transfer from the flavoprotein P450 RED, and in some cases from cyt b_5 (Kloepper-Sams and Stegeman, 1992; Goeptar et al., 1995). P450 RED is, therefore, regarded as a typical MO enzyme in fish liver (Braunbeck and Völkl, 1991).

Some studies reported an increased hepatic P450 RED activity in fish after exposure to PAHs and crude oil, but most studies could not demonstrate any significant alterations (Table 5). These results are confirmed by field studies, although significant increases of hepatic P450 RED activity were observed in salmon, bream, carp, eel, sunfish and grayling from polluted environments. In most cases no significant differences could be observed between fish from control and polluted sites (Table 6). A significant decrease in P450 RED activities was observed in bullhead, eel, scup and rainbow trout exposed to organotins (Table 5), and in

Table 10
Field studies on responses of organic trace pollutants on fish hepatic antioxidant enzymes

Species	Pollutants	SOD*	GPOX*	GRED*	CAT*	Others**	Reference
Atlantic/Baltic salmon *Salmo salar*	Halogenated xenobiotics		=	+			Pesonen et al., 1999
Barbel *Barbus plebejus*	PCBs and PAHs		=	=			Vigano et al., 1995
Brown Bullhead *Ameiurus nebulosus*	PCBs, DDE	+	=		=		Otto and Moon, 1996b
Bullhead *Cottus gobio*	BKME	=			=	G6PDH: =	Bucher et al., 1993
Carp *Cyprinus carpio*	PHAHs, PAHs	+	=	=	=		Van der Oost et al., 1998
Channel catfish *Ictalarus punctatus*	BKME				+		Mather-Mihaich and Di Giulio, 1991
Chub	heavy metals	=	+	=		TBARS: =	Lenartova et al., 1997
Leuciscus cephalus	PCBs and PAHs		=	=			Vigano et al., 1995
Dab *Limanda limanda*	PAHs	+			+		Livingstone et al., 1993
Eel *Anguilla anguilla*	PCBs, PAHs, OCPs, PCDD/Fs	=	=		=		Van der Oost et al., 1996b
Grey mullet *Mugil* sp.	OCPs	+	+	=	+	LPOX:-	Rodriguez-Ariza et al., 1993
Nase *Chondrostoma soetta*	PCBs and PAHs		=	=			Vigano et al., 1995
Nile tilapia *Oreochromis niloticus*	PCBs, HCHs	+	=	–	–	G6PDH: +	Bainy et al., 1996
Rainbow trout *Salmo gairdneri*	PCBs		–	=			Otto et al., 1996
Red mullet *Mullus barbatus*	PAHs	+	–		+		Burgeot et al., 1996
Sardine *Sardina pilchardus*	PAHs, PCBs, DDE	+			+		Peters et al., 1994
Seabream *Diplodus annularis*	Oil and others		–	=		G6PDH: +	Bagnasco et al., 1991
Shorthorn sculpin *Myoxocephalus scorpius*	PAHs		=	+	+		Stephensen et al., 2000
Spot *Leiostomus xanthurus*	PAHs	+					Roberts et al., 1987

Symbols and abbrevations: – –, strong inhibition (< 20% of control); –, inhibition; =, no (significant) response; +, induction; + +, strong induction (> 500% of control); *, SOD, superoxide dismutase; GPOX, glutathione peroxidase; GRED, glutathione reductase; CAT, catalase; **, G6PDH, glucose-6-phosphate dehydrogenase; LPOX, lipid peroxidation; TBARS, thiobarbituric acid reactive substances.

nase from a PCB-polluted environment (Table 6). The P450 RED responses for all fish species from 24 laboratory studies and 18 field studies are summarized in Fig. 7F. A significant increase in P450 RED activity was observed in 25% of the laboratory studies and 33% of the field studies, while a strong increase (> 500% of control) was only observed in a laboratory study with eel exposed to PAHs (Braunbeck and Völkl, 1991).

Although the hepatic P450 RED activity in a limited number of fish species appears to be affected by environmental contaminants, it should not be considered as a valid biomarker for **ERA** since most studies could not demonstrate any significant effects (Fig. 7F).

6.2. Phase II enzymes and cofactors

The so-called second phase of metabolism involves a conjugation of the xenobiotic parent compound or its metabolites with an endogenous ligand (Fig. 6). Conjugations are addition reactions in which large and often polar chemical groups or compounds such as sugars and amino acids are covalently added to xenobiotic chemical compounds and drugs (Lech and Vodicnik, 1985). The majority of the phase II type enzymes catalyze these synthetic conjugation reactions, thus facilitating the excretion of chemicals by the addition of more polar groups (e.g. glutathione (GSH) and glucuronic acid (GA)) to the molecule (Commandeur et al., 1995; Mulder et al. 1990). Some xenobiotic compounds possess the requisite functional groups (such as –COOH, –OH or $–NH_2$) for direct metabolism by conjugative phase II enzyme systems, while others are metabolized by an integrated process involving prior action of the phase I enzymes (George, 1994; Lech and Vodicnik, 1985; Sijm and Opperhuizen, 1989). Phase II enzymes can play an important role in homeostasis as well as in detoxification and clearance of many xenobiotic compounds. The major pathway for electrophilic compounds and metabolites is conjugation with GSH, while for nucleophilic compounds conjugation with GA is the major route (George, 1994). Other pathways play a minor role in fish and are the preferred route for only a few compounds. Sulphatation is a competitive pathway to glucuronidation for PAH metabolites, but it is only effective at very low substrate concentrations (George, 1994).

Relatively little is known about the enzymology and molecular biology of piscine phase II systems, the current knowledge being confined to those catalyzing glucuronidation and GSH conjugation (George, 1994). In addition to the phase I CYP1A genes, the Ah gene battery also comprises phase II genes like NADPH menadione oxidoreductase, aldehyde dehydrogenase, UDPGT and GST (Nebert et al., 1990; Celander,

Table 11
Laboratory studies on responses of organic trace pollutants on fish PAH related parameters, serum transaminases and gross indices

Species	Pollutants	Bile MET*	DNA add*	ALT*	AST*	LSI*	CF*	Reference
Antarctic fish, *Notothenia gibberifrons*	PAHs	++						Yu et al., 1995
Atlantic cod, *Gadus morhua*	Curde oil (PAHs)	++	++					Aas et al., 2000
Atlantic salmon	PAH (BNF)					=		Goksøyr and Larsen, 1991
Salmo salar	Crude oil	++						Gagnon and Holdway, 2000
Bluegill sunfish, *Lepomis macrochirus*	PAH (BaP)			+	=			Oikari and Jimenez, 1992
Brown bullhead	PAH (BaP)		++					Sikka et al., 1990
Ictalurus nebulosus	PAHs	++						Leadly et al., 1999
	PAH (BaP)		+					Ploch et al., 1998
Brook trout, *Salvelinus fontinalis*	PAH (BaP)	++						Padros et al., 2000
Brown trout	Domestic waste water: acute			+	+			Bucher and Hofer, 1990
Salmo trutta	Domestic waste water: chronic			=	=			Bucher and Hofer, 1990
California killifish, *Fundulus parvipinnis*	PAH (BaP)		+					Von Hofe and Puffer, 1986
Carp	Atrazine			=	=			Neskovic et al., 1993
Cyprinus carpio	Pyrethroid (deltamethrin)				+			Balint et al., 1995
Channel catfish *Ictalurus punctatus*	OCP (2, 4-D and pidoram)			=		-		Gallagher and Di Giulio, 1991
	PAH (BaP)		+					Ploch et al., 1998
Dab	PCB (CB 77)					=		Sleiderink and Boon, 1996
Limanda limanda	PAH (BaP) & PCB (CB 126)	++	++					Van Schanke et al., 2002
English sole	PAH (BaP)	++	++					Varanasi et al., 1987
Parophrys vetulus	PAH (BaP)		++					Varanasi et al., 1986
	PAH (BaP)		++					Varanasi et al., 1989b
	PAH (BaP)	++						Collier and Varanasi, 1991
			++					Stein et al., 1993
Flounder	PCB (clophen A50)					+	=	Besselink et al., 1998
Platichthys flesus	PCB (CB 156)				=	=	=	Beyer et al., 1997
	PAH (BaP)	++			++	=	=	Beyer et al., 1997
	PAH (BaP)	++						Hylland et al., 1996
	PAH (BaP)		++					Malmström et al., 2000
Killyfish, *Fundulus heteroclitus*	PAH (BNF)					-		Kloepper-Sams and Stegeman, 1992
Mudfish *Clarias anguillaris*	PCBs, OCPs					+	=	Gadagbui and Goksøyr, 1996
Pike, *Esox lucius*	B(a)P		++					Ericson et al., 1999a
Rainbow trout *Salmo gairdneri* or *Oncorhynchus mykiss*	PAH, PCB in sediment extracts	+						Vigano et al., 1995
	BKME containing sediments					=	=	Otto et al., 1994
	PAH (BaP)		+					Potter et al., 1994
	PCDF (2, 3, 7, 8-TCDF)					=		Muir et al., 1992b
	BKME					+		Lehtinen et al., 1990
	PCDD (2, 3, 7, 8-CDD)					+	=	Newsted and Giesy, 1993
	PCDD (2, 3, 7, 8-CDD)					=		Van der Weiden et al., 1992
	PAH (BNF, ISF)					=		Celander and Förlin, 1991
	PCB (3, 3′, 4, 4′-TCB)					+		Otto and Moon, 1995
	PCB (CB 153)					=		Da Costa and Curtis, 1995
	OCP+OPP (endosulfan/disulfoton)					+	=	Arnold et al., 1995
	PAHs (effluent discharges)		+					Sagelsdorff, 1995
	PAH (engine exhaust extract)					+		Tjärnlund et al., 1996

Table 11 (*Continued*)

Species	Pollutants	Bile MET*	DNA add*	ALT*	AST*	LSI*	CF*	Reference
	Creosote containing sediments	++						Hyotylainen and Oikari, 1999
	PAH (retene)	++						Fragoso et al., 1999
Redbreast sunfish, *Lepomis auritus*	PCBs, Hg				=	+		Adams et al., 1990
Sand flathead	Treated waste water	=						Mosse et al., 1996
Platycephalus bassensis	Chlorinated phenols	++						Brumley et al., 1998
Scup, *Stenotomus chrysops*	PCDF (2, 3, 7, 8-TCDF)					=		
Speckled sanddab, *Citharichthys stigmaeus*	PAH (BaP)		+					Von Hofe and Puffer, 1986
Stickleback, *Gasterosteus aculeatus*	PCB (Clophen A50)					=		Holm et al., 1994
Striped mullit, *Mugil cephalus*	Crude oil					=		Chambers, 1979
Turbot, *Scophthalmus maximus*	PAH (BaP)		++					Peters et al., 1997
Viviparous blenny, *Zoarces viviparus*	PAH (heavy gas oil)					+		Celander et al., 1994
Zebrafish	PAH (BaP)		++					Hsu and Deng, 1996
Brachydanio rerio	PCBs					-		Orn et al., 1998

Symbols and abbreviations: − −, strong inhibition (< 20% of control); −, inhibition; =, no (significant) response; +, induction; ++, strong induction (> 500% of control); *, bile MET, metabolites in bile; DNA add, DNA adducts (liver); ALT, alanine transaminase (serum); AST, aspertate transaminase (serum); LSI, liver sometic index (liver); CF, condition factor (whole body).

1993). The mechanism of induction for most forms of phase II enzymes is, therefore, probably regulated via the Ah-receptor as well (Sutter and Greenlee, 1992; Hayes and Pulford, 1995). As compared with phase I systems, the induction responses of phase II enzymes are generally less pronounced (Andersson et al., 1985; George, 1994), so that they may be masked by natural variability factors (such as sex, maturity, nutrition, season, temperature, etc.). Still, even slight alterations of the phase II activity may be harmful to an organism. The levels of phase II cofactors may also be affected after exposure to environmental pollutants (Stegeman et al., 1992; Lindström-Seppä and Oikari, 1991; Van der Oost et al., 1996b). The responses of fish phase II enzymes and cofactors in laboratory and field studies are listed in Tables 7 and 8, respectively.

6.2.1. *Reduced and oxidized glutathione (GSH and GSSG)*

Reduced GSH, a tripeptide consisting of *g*-glutamine, cysteine and glycine, can be conjugated in the initial step of mercapturic acid formation (George, 1994; Commandeur et al., 1995). Among its functions are two contrasting roles in detoxifications, as a key conjugate of electrophilic intermediates, principally via GST activities in phase II metabolism, and as an important antioxidant (Stegeman et al., 1992; Commandeur et al., 1995). GSH reacts with electrophilic compounds and replaces hydrogen, chlorine, nitro-groups and all kinds of other so-called leaving groups. The mercapturic acids are formed after catabolism of the GSH conjugate with peptidases and acetylases (Lech and Vodicnik, 1985; Commandeur et al., 1995). Urinary excretion of mercapturic acids is a useful tool in the assessment of toxicologically relevant internal doses in persons exposed to electrophilic chemicals, and may be applied as a non-invasive biomarker in human occupational and environmental toxicology (De Rooij et al., 1997). A microplate assay for total GHS and GSH disulfide contents, based upon a first order recycling reaction resulting in the formation of 5,5′-dithiobis 2-nitrobenzoic acid (TNB), was described by Vandeputte et al. (1994).

Influxes of oxyradical-generating compounds may alter GSH status and/or metabolism in several ways (Stegeman et al., 1992; Otto and Moon, 1995). Increased fluxes of oxyradicals can impose a drain on intracellular reducing equivalents with potentially profound consequences on a variety of metabolic processes. The consumption of GSH due to the direct scavenging of oxyradicals or as a cofactor for glutathione peroxidase (GPOX) activity may represent such a drain: NADPH must be oxidized to maintain GSH levels via glutathione reductase (GRED) (Di Giulio et al., 1995). More indirectly, oxidative stress can impose a drain on the reductant pool as a consequence of the energetic costs of mounting a defence against an increased flux of

Table 12
Field studies on responses of organic trace pollutants on fish PAH related parameters, serum transaminases and gross indices

Species	Pollutants	bile MET*	DNA add*	ALT*	AST*	LSI*	CF*	Reference
Antarctic fish *Notothenia coriiceps*	PAHs	+						McDonald et al., 1995
Barbel	PAHs		=					Kurelec et al., 1989
Barbus barbus	PCBs and PAHs					=	=	Flammarion and Garric, 1997
Bream	PCBs, PAHs					+		Slooff et al., 1983
Abramis brama	PCBs						=	Jedamski-Grymlas et al., 1995
	PAHs		=					Kurelec et al., 1989
	BKME		+					Kantoniemi et al., 1996
Brook trout *Salvelinus fontinalis*	PAHs		+					Ray et al., 1995
Brown bullhead	PAHs		++					Dunn et al., 1987
Ictalurus nebulosus	PCBs, DDE					=	+	Otto and Moon, 1996b
	PAHs	++						Leadly et al., 1999
	PAHs		++					Dunn et al., 1990
	PAHs	+						Arcand-Hoy and Metcalfe, 1999
	PCBs, OCPs and PAHs					+		Leadly et al., 1998
Brown trout *Salmo trutta*	domestic waste water				=			Bucher and Hofer, 1990
Carp	PAHs		=					Kurelec et al., 1989
Cyprinus carpio	PCBs, OCPs, PCDDs	+		=	+	=	=	Van der Oost et al., 1998
Chub	PHAs		=					Kurelec et al., 1989
Leuciscus cephalus	WWTP effluent					+		Kosmala et al., 1998
	PCBs and PAHs					=	=	Flammarion and Garric, 1997
Cod	PAHs, PHAHs		++					Ericson et al., 1996
Gadus morhua	PAHs, PCBs, DDTs	++			=	+	=	Beyer et al., 1996
Dab *Limanda limanda*	PAHs, PCBs, DDTs					+	+	Goksøyr et al., 1991b
	PAHs, PCBs						=	Sleiderink and Boon, 1995
	PAHs		=					Poginsky et al., 1990
	PAHs		=					Chipman et al., 1992
Eel *Anguilla anguilla*	PCBs, PAHs, OCPs, PDDD/Fs	+	++					Van der Oost et al., 1994a
	PCBs, PAHs, OCPs, PDDD/Fs			=	=	=	=	Van der Oost et al., 1996b
English sole	PAHs, PCBs	++						Varanasi et al., 1987
Parophrys vetulus	PAHs and others	++						Collier et al., 1992
	PAHs	++	++					Varanasi et al., 1989a
	PAHs, PCBs	=	+					Stein et al., 1992
	PAHs		+					Poginsky et al., 1990
	PAHs and PCBs					+	+	Kirby et al., 1999a,b
Flounder	PAHs		++					Baan et al., 1994
Platichthys flesus	PAHs, PCBs, DDTs	++			++	=	=	Beyer et al., 1996
	PAHs, PCBs	++	+			=	=	Vethaak et al., 1996
	PAHs, PCBs, DDTs					=	=	Goksøyr et al., 1991b
Killifish *Fundulus heteroclitus*	PCBs, OCPs, PAHs					=		Elskus and Stegeman, 1989
Mountain whitefish *Prosopium williamsoni*	BKME					=		Kloepper-Sams and Owens, 1993
Mugil *Mugil auratus*	PAHs		=					Kurelec et al., 1989
Oyster toadfish *Opsanus tau*	PAHs	++	++					Collier et al., 1993
Perch *Perca fluviatilis*	BKME					+	+	Huuskonen and Linström-Seppä, 1995

Table 12 (*Continued*)

Species	Pollutants	bile MET*	DNA add*	ALT*	AST*	LSI*	CF*	Reference
	BKME					+		Kloepper-Sams and Owens, 1993
	creosote		++					Ericson et al., 1999b
	BKME (chiorophe-nolics)	++						Karels et al., 1998
	BKME		+					Kantoniemi et al., 1996
Plaice	PAHs, PCBs	=						Eggens et al., 1995
Pleuronectes platessa	PAHs, PCBs, DDTs					+	=	Goksøyr et al., 1991b
Rainbow surfperch *Hypsurus caryi*	Petroleum seep	+						Spies et al., 1996
Rainbow trout	PCBs					=	=	Otto et al., 1996
Salmo gairdneri or *Oncor-hynchus mykiss*	BKME					=	=	Lindström-Seppä and Oikari, 1990
	Petrochemical waste						=	Nikunen, 1985
Red mullet *Mullus barbatus*	PAHs		+					Burgeot et al., 1996
Redbreast sunfish *Lepomis auritus*	BKME					−	=	Adams et al., 1992
Roach	PCBs, PAHs, OCPs		=			=	=	Van der Oost et al., 1994b
Rutilus rutilus	BKME			−	−			Jeney et al., 1996
	BKME (chlorophe-nolics)	+						Karels et al., 1998
	BKME		+					Kantoniemi et al., 1996
Rock sole *Lepidopsetta bilineata*	PAHs, PCBs	=	=					Stein et al., 1992
Rubberlip surfperch *Rhacochilus toxotes*	Petroleum seep	=/+						Spies et al., 1996
Shorthorn sculpin *Myoxocephalus scorpius*	PAHs	++	++			+		Stephensen et al., 2000
Starry flounder	PAHs and others	++						Collier et al., 1992
Platichthys stellatus	PAHs, PCBs	=	+					Stein et al., 1992
Tilapia *Oreochromis niloticus*	PCBs, OCPs					−	=	Gadagbui and Goksøyr, 1996
White sucker	BKME				=			McMaster et al., 1994
Catostomus commersoni	BKME					+	−	Hodson et al., 1992
	PAHs	+						Cormier et al., 2000a
	PAHs		+					El Adlouni et al., 1995
Winter flounder	PAHs		+					Varanasi et al., 1989a
Pseudopleuronectes ameri-canus	PAHs		+					Gronlund et al., 1991
Whitefish *Coregonus muksun*	BKME					=/+	=	Lindström-Seppä and Oikari, 1989

Symbols and abbreviations: −−, strong inhibition (< 20% of control); −, inhibition; =, no (significant) response; +, induction; ++, strong induction (> 500% of control); *, bile MET, metabolites in bile; DNA add,DNA adducts [liver]; ALT, Alanine transaminase [serum]; AST, Aspertate transaminase [serum]; LSI, liver sometic index [liver]; CF, condition factor [whole body].

oxyradicals, i.e. biosynthesis of antioxidants (Winston and Di Giulio, 1991).

Perhaps the most obvious direct effect of certain pollutants is a decrease in thiol status, i.e. the ratio of reduced to oxidized glutathione (GSH:GSSG), due to either direct radical scavenging or increased peroxidase activity (Stegeman et al., 1992; Otto and Moon, 1995). Alternatively, normal GSH:GSSG ratios can be maintained due to increased activities of GRED or increased GSH synthesis. In mammals, GSH synthesis is considered to be tightly regulated via feedback inhibition by GSH on a rate-limiting synthetic enzyme (Lauterburg et al., 1983). In the healthy cell GSH:GSSG ratios are typically very high, greater than 10:1 (Stegeman et al., 1992). In rainbow trout it was observed that EROD induction (due to PCB exposure) was intensified in GSH-supplemented fish tissues, while the reverse was observed in GSH deficient fish (Otto et al., 1996, 1997). Strong indications were found that the tissue thiol status modulates Ah receptor inducible CYP1A gene expression and catalytic activity, indicating a 'cross-talk' between the GSH and cytochrome P450 systems.

Both GSH and GSSG levels have only been measured in a limited number of laboratory and field studies.

Increased GSH levels were observed in laboratory studies with fish exposed to BKME, PAHs, PCBs and HCB (Table 7). Some field studies, e.g. with bream, perch and roach from BKME-polluted environments, demonstrated a significant increase in GSH levels, while significant decreases were observed in bullhead and carp from polluted sites (Table 8). Two laboratory studies, with channel catfish and rainbow trout exposed to PAH-containing sediments and PCBs, respectively, reported elevated hepatic GSSG levels (Table 7). Elevated GSSG levels in the field was only observed in one study with eel from a polluted site (Table 8). The GSH responses for all fish species from ten laboratory studies and 17 field studies are summarized in Fig. 8A. A significant increase in GSH levels was observed in 60% of the laboratory studies and 35% of the field studies. The GSSG responses for all fish species from three laboratory studies and four field studies are summarized in Fig. 8B. A significant increase in GSSG levels was observed in two of the laboratory studies (67%) and two of the field studies (50%). Strong increases ($>500\%$ of control) in hepatic GSH levels were only observed in English sole from a site which was heavily polluted with PAHs and PCBs (Stein et al., 1992), while no strong increases in GSSG levels were observed in any of the reviewed laboratory and field studies.

Due to the limited number of observations on the responses in GSH and GSSG levels, these parameters cannot yet be considered as valid biomarkers for **ERA** purposes. The key role played by GSH in detoxifications and the responsiveness of this system to xenobiotics, however, motivates continued research on its feasibility as a biomarker. Although it seems that pollutant-induced effects on GSH levels are restored by feedback-mechanisms, the hepatic GSH:GSSG ratio may be a potential biomarker for oxidative stress (Van der Oost et al., 1996b).

6.2.2. *Glutathione S-transferases (GSTs)*

The conjugation of electrophilic compounds (or phase I metabolites) with GSH is catalyzed by the glutathione *S*-transferases (GSTs), a multigene superfamily of dimeric, multifunctional, primarily soluble enzymes. Apart from their essential functions in intracellular transport (heme, bilirubin and bile acids) and the biosynthesis of leukotrienes and prostaglandins, a critical role for GSTs is obviously defence against oxidative damage and peroxidative products of DNA and lipids (George, 1994). The susceptibility of different fish species to chemical carcinogenesis may be modulated by the activity of GST (Varanasi et al., 1987). On the basis of substrate specificity, immunological cross-reactivity and protein sequence data, the soluble GSTs have been grouped into four classes: a, m, p and q (George, 1994). These enzymes are mainly located in the cytosolic fraction of the liver (Sijm and Opperhuizen, 1989). Most studies determine the total GST activity using the artificial substrate 1-chloro-2,4-dinitrobenzene (CDNB), which is conjugated by all GST isoforms with the exception of the q-class enzymes (George, 1994; Van der Aar et al., 1996).

The toxicity of many exogenous compounds can be modulated by induction of GSTs. Effects of inducing agents on total hepatic GST activity, measured by CDNB conjugation, have been observed in several fish species (George, 1994). As for CYP1A, the mechanism of induction for most GSTs in mammals is regulated via the Ah-receptor (Pickett and Lu, 1989; George, 1994). An additional form of GST induction which functions independently of the Ah-receptor has been elucidated and requires metabolism of the compound before transcriptional activation of the respective subunit gene can take place (Rushmore and Pickett, 1990). Due to the role that GSTs play in conjugating reactive epoxide species and other electrophiles, induction of these enzymes must be considered to be beneficial, although metabolic activation of halogenated xenobiotics by GST is also well recognized (Armstrong, 1990; Commandeur et al., 1995). There is a limited amount of information regarding sexual, seasonal and developmental differences in GST activity in fish (Stegeman et al., 1992).

An increase in hepatic GST activity has been reported in several studies after exposure of fish to PAHs, PCBs, OCPs and PCDDs, but most studies did not demonstrate any significant alterations (Table 7). Attempts to detect chemically induced activities of GSTs in free-living fish also yielded conflicting results. Several studies reported GST activities to be significantly increased, but in most cases no significant differences were observed between fish from control and polluted sites (Table 8). A significant decrease in GST activities was observed in rainbow trout, sea bass, seabream and sunfish exposed to PCDDs, pesticides or PAHs (Table 7), and in some fish species in polluted environments (Table 8). The GST responses for all fish species from 43 laboratory studies and 39 field studies are summarized in Fig. 8C. A significant increase in GST activity was observed in 33% of the laboratory studies and in 33% of the field studies, while no strong increases ($>500\%$ of control) were reported in any of the laboratory and field studies considered.

Hepatic total GST activity in fish does not seem to be feasible as a biomarker for **ERA**, since increased activities are only observed in a limited number of fish species. In addition, the exposure to pollutants like PCDDs and PAHs may cause both induction and inhibition of the enzyme activity. However, more research on this parameter, which is of paramount importance for major detoxification processes, may elucidate specific isoenzymes that have a more sensitive and selective response to pollutants. Hepatic cytosolic

GST activity toward ethacrynic acid appeared to be induced in rainbow trout exposed to PCB 153 or *p,p′*-DDE, but was not induced after 2,3,7,8-TCDD exposure (Machala et al., 1998). This observation indicates that this parameter may be a suitable biomarker for exposure to nonplanar PCBs and organochlorines that do not induce CYP1A activity.

6.2.3. UDP-glucuronyl transferases (UDPGTs)

The synthesis of glucuronides by microsomal UDP-glucuronyl transferases (UDPGTs) is a major pathway for the inactivation and subsequent excretion of both endogenous and xenobiotic organic compounds (Lech and Vodicnik, 1985; Mulder et al., 1990; George, 1994). GA conjugation first requires synthesis of uridine 5′-diphosphoglucuronic acid (UDPGA). UDPGT catalyzes the transfer of UDPGA to a wide variety of acceptor substrates (aglycones) to form O-, N-,S- and C-glucuronides (Kasper and Henton, 1980; Mulder et al., 1990), the majority being O-glucuronides (George, 1994). As with most enzymes that exhibit a broad specificity for structurally diverse compounds, multiple isoenzymes belonging to a number of multigene families are found (George, 1994). The different UDPGT isoenzymes are generally named after their acceptor substrates, e.g. bilirubin, steroid and phenol UDPGTs. While the liver is quantitatively the most important site for glucuronidation of xenobiotics in fish, significant activities have also been detected in extrahepatic tissues, including kidney, gills and intestine (George, 1994).

In mammals, it was found that the UDPGT isoenzymes display a differential induction by xenobiotic compounds, and they also fall into four developmental clusters, which suggests differential regulation during development (Burchell and Coughtrie, 1989). In a study by Clarke et al. (1992), it was demonstrated that multiple UDPGT isoforms with differing substrate specificities were also present in fish. The UDPGT isoform, which preferentially conjugates planar phenols, is induced by PAHs, most probably via an Ah receptor-dependent mechanism (Nebert et al., 1990; George, 1994). UDPGT activity in fish is reported to be influenced by sex, season, pH and temperature differences (Stegeman et al., 1992). Due to the ease of the assay, most studies have utilized 4-nitrophenol as the acceptor substrate.

Although an increased UDPGT activity normally results in elevated detoxification of the inducing agent, undesirable effects may also occur (Stegeman et al., 1992). As is the case for cyt P450, UDPGT enzymes metabolize endogenous substrates such as steroid hormones. Induced UDPGTs may, therefore, seriously alter steroid metabolism, possibly with profound effects on the reproductive success of an organism (Stegeman et al., 1992). Steroid hormone glucuronides formed in the testis and seminal vesicles of fish appear to be important pheromones, acting as stimulants of ovulation and sperm production and also enhancing vitellogenesis in many species (George, 1994). A decreased UDPGT activity can result in bilirubin accumulation (Oikari and Nakori, 1982).

Hepatic UDPGT activities were reported to be increased in most laboratory studies with fish exposed to PAHs, PCBs, OCPs, PCDDs and BKME-contaminated sediments (Table 7). These results are in line with a number of field experiments in which an increased UDPGT activity was found in fish from polluted sites (Table 8). Significant decreases in UDPGT activities were only observed in BKME exposed whitefish and rainbow trout caged in a BKME-polluted environment (Table 8). The UDPGT responses for all fish species from 27 laboratory studies and 26 field studies are summarized in Fig. 8D. A significant increase in UDPGT activity was observed in 52% of the laboratory studies and in 42% of the field studies, while strong increases ($>500\%$ of control) were only reported in two laboratory studies, with eel exposed to dinitro-*o*-cresol (Braunbeck and Völkl, 1991) and rainbow trout exposed to PCB (Otto and Moon, 1995).

Although not as sensitive as phase I enzymes, the UDPGT activity appears to be the phase II parameter which is most responsive to pollutant exposure. It, therefore, seems to be valid as a biomarker in certain **ERA** monitoring programs, although more research will be required to investigate the potential pollution-induced responses of the various UDPGT isoenzymes.

6.3. Oxidative stress parameters

Many pollutants (or their metabolites) may exert toxicity related to oxidative stress. For instance, Winston and Di Giulio (1991) found elevated rates of idiopathic lesions and neoplasia among fish inhabiting polluted environments to be related to the increased oxidative stress associated with pollutant exposure. Oxygen toxicity is defined as injurious effects due to cytotoxic reactive oxygen species (ROS), also referred to as reactive oxygen intermediates (ROIs), oxygen free radicals or oxyradicals (Di Giulio et al., 1989a; Halliwell and Gutteridge, 1999; Winzer, 2001). These reduction products of molecular oxygen (O_2) are the superoxide anion radical ($O_2^{-\bullet}$), hydrogen peroxide (H_2O_2) and the hydroxyl radical ($OH^{\bullet}$), an extremely potent oxidant capable of reacting with critical cellular macromolecules, possibly leading to enzyme inactivation, lipid peroxidation (LPOX), DNA damage and, ultimately, cell death (Winston and Di Giulio, 1991).

Numerous endogenous sources of oxyradical production exist, but of more immediate interest with respect to environmental biomarkers is the ability of a number of structurally diverse compounds to enhance intracellular oxyradical production through the process of redox

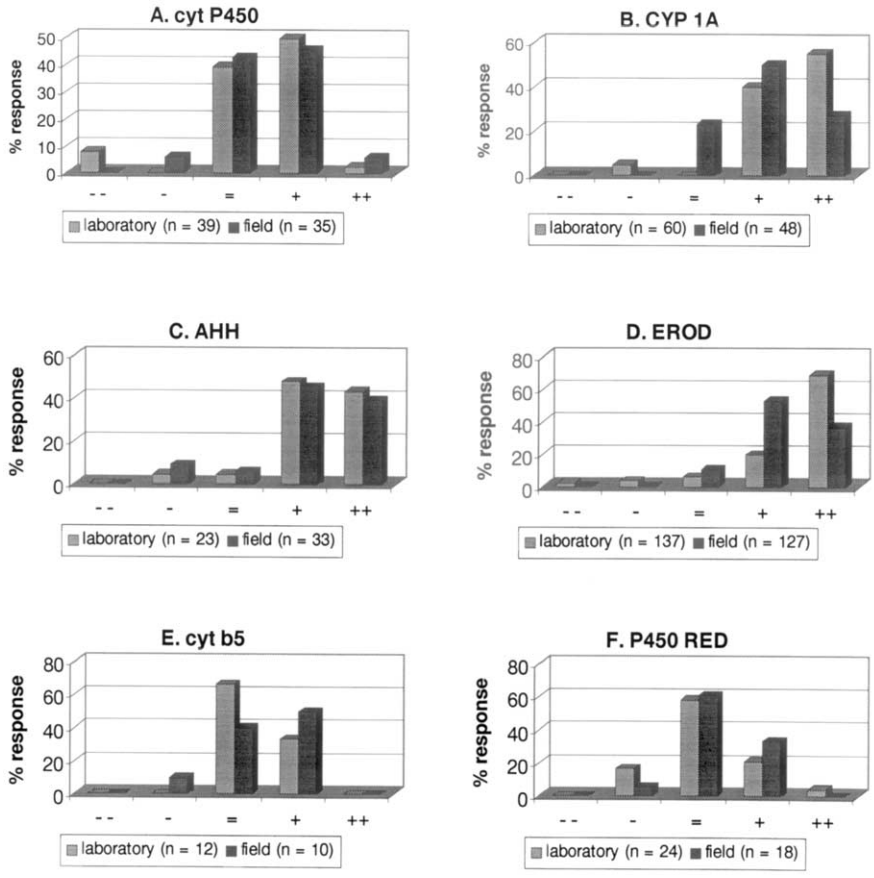

Fig. 7. Frequencies of pollutant-induced responses of phase I-related enzymes in fish: (A) cytochrome P450 (cyt P450); (B) cytochrome P450 1A (CYP1A); (C) aryl hydrocarbon hydroxylase (AHH); (D) ethoxyresorufin *O*-deethylase (EROD); (E) cytochrome b_5 (cyt b_5); (F) cytochrome P450 (c) reductase (P450 RED). $--$, strong decrease ($<20\%$ of control); $-$, decrease; $=$, no (significant) response; $+$, increase; $++$, strong increase ($>$ 500% of control).

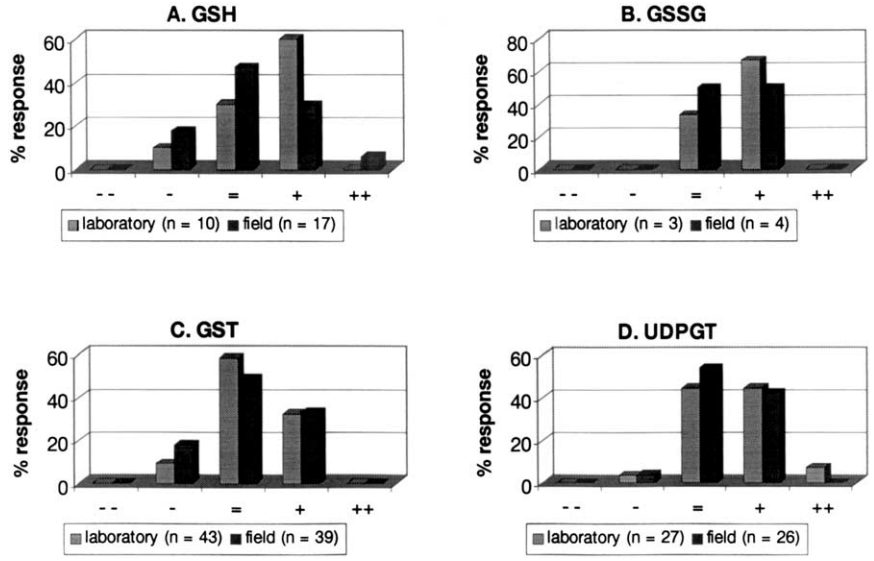

Fig. 8. Frequencies of pollutant-induced responses of phase II enzymes and cofactors in fish: (A) reduced glutathione (GSH); (B) oxidized glutathione (GSSG); (C) glutathione S-transferase (GST); (D) UDP-glucuronyl transferase (UDPGT); --: strong decrease ($<20\%$ of control); $-$: decrease; $=$: no (significant) response; $+$: increase; $++$: strong increase ($>500\%$ of control).

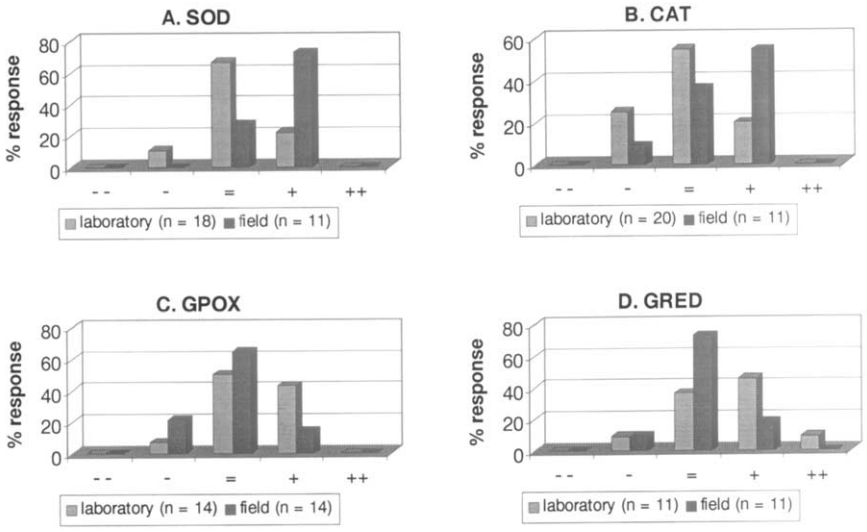

Fig. 9. Frequencies of pollutant-induced responses of antioxidant enzymes in fish: (A) superoxide dismutase (SOD); (B) catalase CAT; (C) glutathione peroxidase (GPOX); (D) glutathione reductase (GRED). $--$, strong decrease ($<20\%$ of control); $-$, decrease; $=$, no (significant) response; $+$, increase; $++$, strong increase ($>500\%$ of control).

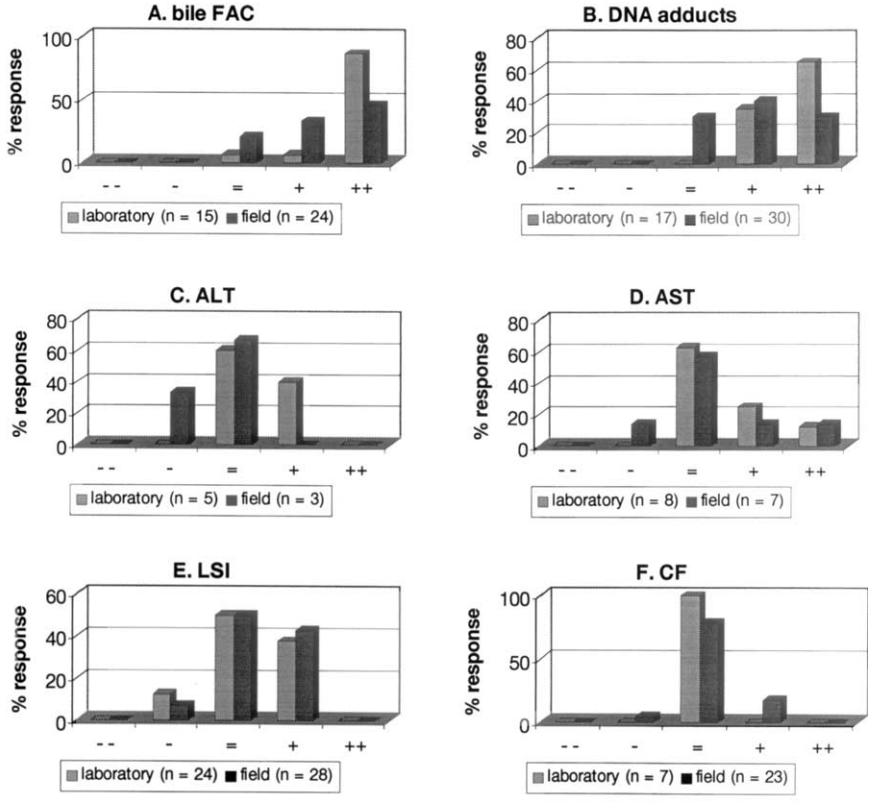

Fig. 10. Frequencies of pollutant-induced responses of fish biotransformation products, serum transaminases and morphological parameters: (A) fluorescent PAH metabolites in bile (bile FAC); (B) DNA adducts; (C) alanine transaminase (ALT) in plasma; (D) aspartate transaminase (AST) in plasma; (E) liver somatic index (LSI); (F) condition factor CF. $--$, strong decrease ($<20\%$ of control); $-$, decrease; $=$, no (significant) response; $+$, increase; $++$, strong increase ($>500\%$ of control).

cycling. Redox-active compounds include aromatic diols and quinones, nitroaromatics, aromatic hydroxylamines, bipyridyls and certain transition metal chelates (Winston and Di Giulio, 1991). In the redox cycle, the parent compound is typically first enzymatically reduced by a NADPH dependent reductase (such as cyt P450 RED) to yield a xenobiotic radical. This radical donates its unshared electron to molecular O_2, yielding $O_2^{-\bullet}$ and the parent compound. Thus, at each turn of the cycle, two potentially deleterious events have occurred: a reductant has been oxidized and an oxyradical has been produced (Winston and Di Giulio, 1991; Goeptar et al., 1995).

Oxidant-mediated effects with a potential suitability as biomarkers include either adaptive responses, such as increased activities of antioxidant enzymes and concentrations of non-enzymatic compounds, or manifestations of oxidant-mediated toxicity such as oxidations of proteins, lipids and nucleic acids, as well as perturbed tissue redox status (Winston and Di Giulio, 1991; Filho, 1996). Defence systems that tend to inhibit oxyradical formation include the antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), glutathione-dependent peroxidase (GPOX) and glutathione reductase (GRED). SOD, CAT and GPOX are critically important in the detoxification of radicals to non-reactive molecules. Numerous low-molecular-weight antioxidants, such as GSH, β-carotene (vitamin B), ascorbate (vitamin C), α-tocopherol (vitamin E) and ubiquinol$_{10}$ have been described (Stegeman et al., 1992; Lopez-Torres et al., 1993). In aquatic ecosystems, dissolved oxygen and temperature are environmental variables that are likely to influence oxidative processes (Parihar et al., 1997). These variables must be carefully controlled in laboratory experiments examining oxidative stress, and similarly considered in field studies including this phenomenon in aquatic animals (Winston and Di Giulio, 1991). Differences in antioxidant capacities were observed between rainbow trout that were adapted to seawater or freshwater (Kolayli and Keha, 1999). Species differences in the efficiency of antioxidant defenses may partly explain prevalence of pathological lesions observed in certain species of fish (Vigano et al., 1998). In experiments with hepatocytes of male and female flounder it was demonstrated that many responses to oxidative stress were sex-related (Winzer et al., 2001). The pollutant-induced responses of fish antioxidant enzymes in laboratory and field studies are listed in Tables 9 and 10, respectively.

6.3.1. Superoxide dismutase (SOD)

The SODs are a group of metalloenzymes that catalyse the conversion of reactive superoxide anions ($O_2^{-\bullet}$) to yield hydrogen peroxide (H_2O_2), which in itself is an important ROS as well. H_2O_2 is subsequently detoxified by two types of enzymes: CATs and glutathione dependent peroxidases (GPOXs). SODs are considered to play a pivotal antioxidant role; their importance is indicated by their presence in all aerobic organisms examined (Stegeman et al., 1992). Additionally, the rate of SOD-catalysed $O_2^{-\bullet}$ dismutation approximates the diffusion limit, making it one of the most active enzymes described (Fridovich, 1986). Most techniques for the measurement of SOD activity are indirect assays in which an indicating scavenger competes with endogenous SOD for $O_2^{-\bullet}$. A unit of SOD activity is defined as the amount that causes 50% inhibition of the reduction of the scavenger under specified conditions (Sazuka, 1989; Stegeman et al., 1992). With the use of certain inhibitors the activities of SODs with different metal centers can be distinguished.

Some laboratory studies have reported an increased SOD activity in fish exposed to paraquat, 2,3,7,8-TCDF or HCB-contaminated food, but most studies could not demonstrate any significant alterations (Table 9). In eight of the 11 field studies considered, however, a significant increase of hepatic SOD activity was observed, i.e. in brown bullhead, carp, dab, grey mullet, Nile tilapia, red mullet, sardine and spot from polluted environments (Table 10). Significant decreases in SOD activity were only observed in rainbow trout exposed to BKME-containing sediments or paraquat (Table 9). The SOD responses for all fish species from 18 laboratory studies and 11 field studies are summarized in Fig. 9A. A significant increase in SOD activity was observed in 22% of the laboratory studies and 73% of the field studies, while a strong increase ($>500\%$ of control) was not observed in any of the laboratory or field studies.

More information is required before the hepatic SOD activity in fish can be considered a valid biomarker for **ERA**. The literature survey revealed a notable difference between responses observed in laboratory studies and in the field. A significant SOD induction was observed in most of the reported field surveys, while most of the laboratory studies did not report any significant responses (Table 9).

6.3.2. Catalase (CAT)

CATs are hematin-containing enzymes that facilitate the removal of hydrogen peroxide (H_2O_2), which is metabolized to molecular oxygen (O_2) and water. Unlike some peroxidases that can reduce various lipid peroxides as well as H_2O_2, CATs can only reduce H_2O_2 (Stegeman et al., 1992; Filho, 1996). It was demonstrated that peroxisome-proliferating compounds (a class of non-genotoxic carcinogens) induce both the activities of H_2O_2-generating fatty acid oxidases and CAT in rodents (Reddy and Lalwani, 1983; Halliwell and Gutteridge, 1999). Since CATs are localized in the peroxisomes of most cells and are involved in fatty acid metabolism, changes in activities may often be difficult to interpret (Stegeman et al., 1992). Therefore, CAT activities in

erythrocytes may be a more appropriate marker for oxidant exposures in vertebrates. A transitory increase in erythrocyte CAT activity was observed in crucian carp exposed to paraquat (Gabryelak and Klekot, 1985). A commonly employed assay for the measurement of CAT activity follows the disappearance of exogenous H_2O_2 spectrophotometrically (Sazuka, 1989; Stegeman et al., 1992).

Increases in hepatic CAT activity were only observed in some experiments with fish exposed to PCBs, BKME or PAH-containing sediments, but most laboratory studies could not demonstrate any significant alterations (Table 9). Increased hepatic CAT activity was, however, demonstrated in six of the 11 field studies considered (Table 10). A significantly decreased CAT activity was observed some lab studies after PAH, DNOC, 3,3′,4,4′-TCB, engine exhaust extracts or cadmium exposure (Table 9) and in a field study with Nile tilapia (Table 10). The CAT responses for all fish species from 20 laboratory studies and 11 field experiments are summarized in Fig. 9B. A significant increase in CAT activity was observed in 20% of the laboratory studies and 55% of the field studies, while a strong increase (> 500% of control) was not observed in any of the laboratory or field studies considered. Similar as for SOD, more CAT responses were observed in the field than in lab studies.

In general, CAT activity cannot be considered a valid biomarker for **ERA** since both induction and inhibition are observed after exposure to environmental pollutants. More research is required to elucidate the mechanism behind these effects on selected fish species.

6.3.3. Glutathione peroxidase (GPOX)

Peroxidases (POXs) are enzymes that reduce a variety of peroxides to their corresponding alcohols. While CAT employs one molecule of H_2O_2 as donor in the reduction of another H_2O_2 molecule, peroxidases employ other reductants. The principal peroxidase in fish is a selenium-dependent tetrameric cytosolic enzyme that employs GSH as a cofactor. GPOX catalyses the metabolism of H_2O_2 to water, involving a concomitant oxidation of reduced GSH to its oxidized form (GSSG). GPOX is considered to play an especially important role in protecting membranes from damage due to LPOX. This observation led to the view that the major detoxification function of GPOX is the termination of radical chain propagation by quick reduction to yield further radicals (Lauterburg et al., 1983). The use of peroxidases in plants may be used to detect early oxidant responses, but the use of GPOX received relatively little attention as a biomarker in animals (Stegeman et al., 1992).

An increased GPOX activity was observed in experiments with fish exposed to paraquat, PAH (3MC), PCBs and HCB-contaminated food, while a decrease was only observed after 3MC exposure (Table 9). The few field experiments reported, showed a significant increase in hepatic GPOX activity only in grey mullet and chub from polluted sites (Table 10). A significant decrease in GPOX activity was observed in three field experiments, i.e. with rainbow trout, red mullet and seabream exposed at contaminated sites (Table 10). The GPOX responses for all fish species from 14 laboratory studies and 14 field experiments are summarized in Fig. 9C. A significant increase in GPOX activity was observed in 43% of the laboratory studies and 14% of the field studies, while a strong increase (> 500% of control) was not observed in any of the laboratory or field studies considered.

The available information suggests that GPOX activity in animals may be less responsive to pro-oxidants than AsPOX in plants (Stegeman et al., 1992). More research is required to determine the potential utility of GPOX activity in fish liver as a biomarker for **ERA** purposes.

6.3.4. Glutathione reductase (GRED)

Although perhaps not involved in antioxidant defence in the same way as the enzymes previously described, GRED merits attention because of its importance in maintaining GSH/GSSG homeostasis under oxidative stress conditions (Winston and Di Giulio, 1991). GRED catalyses the transformation of the oxidized disulfide form of glutathione (GSSG) to the reduced form (GSH), with the concomitant oxidation of NADPH to $NADP^+$. GRED activity can be measured spectrometrically by following the decrease in NADPH levels (Worthington and Rosemeyer, 1974).

The responses of fish GRED to pollutants have apparently received little attention. An increased GRED activity was observed in laboratory experiments with fish exposed to PCBs, PAHs, DDE and HCB-contaminated food (Table 9). A significant increase in hepatic GRED activity was observed in two of the reported field experiments, i.e. with Atlantic salmon and shorthorn sculpin (Table 10). Significant decreases in GRED activity were only observed in laboratory studies with red mullet exposed to PCBs (Rudneva-Titova and Zherko, 1994), and in a field experiment with Nile tilapia from a contaminated site (Bainy et al., 1996). The GRED responses for all fish species from 11 laboratory studies and 11 field experiments are summarized in Fig. 9D. A significant increase in GRED activity was observed in 55% of the laboratory studies and in 18% of the field studies, while a strong increase (> 500% of control) was only observed in a laboratory study with PCB-exposed rainbow trout (Otto and Moon, 1995).

The ratio between hepatic levels of reduced and oxidized glutathione (GSH:GSSG) has been suggested as a potential fish biomarker (Section 6.2.1). Research on altered GRED activity may, therefore, be important in this context. The feasibility of GRED activity as a

biomarker in **ERA** processes, however, remains questionable since the enzyme does not seem to be responsive to contaminant exposure in 82% of the considered field studies.

6.3.5. Non-enzymatic antioxidants

The potential use of levels of reduced and oxidized glutathione (GSH and GSSG) as biomarkers has been discussed under the phase II cofactors (Section 6.2.1). Ascorbate (vitamin C) is an important water-soluble antioxidant, and may additionally serve as a cofactor for enzymes involved in collagen biosynthesis or neurotransmitter conversions (Stegeman et al., 1992; Lopez-Torres et al., 1993). Studies addressing the feasibility of ascorbate as a biomarker are very scarce. The utility of ascorbate as a biomarker is limited to plants and animals that can synthesize it. In a large-scale field study in Sweden, perch inhabiting waters contaminated with BKME consistently displayed higher ascorbate concentrations than fish from a reference site (Andersson et al., 1988). α-Tocopherol (vitamin E) is a lipid-soluble antioxidant that is synthesized by plants, but required in the diets of animals (Stegeman et al., 1992). This compound appears to play a major role in protecting membranes from LPO. Its direct application as a biomarker for **ERA** is probably restricted to plants; however, as is the case for ascorbate, measurements of animal tissue levels may sometimes be useful.

6.3.6. Biochemical indices of oxidative damage

A large number of biochemical and physiological effects have been associated with increased fluxes of oxyradicals. Some biochemical perturbations that seem particularly promising in relation to biomarkers are LPOX, the total oxyradical scavenging capacity (TOSC) assay, DNA oxidation, methemoglobinemia and redox status.

- *Lipid peroxidation*, or the oxidation of polyunsaturated fatty acids is a very important consequence of oxidative stress and has been investigated extensively (Stegeman et al., 1992; Hageman et al., 1992). The process of LPOX proceeds by a chain reaction and, as in the case of redox cycling, demonstrates the ability of a single radical species to propagate a number of deleterious biochemical reactions. The actual chemistry of LPOX and associated production of various free-radical species is extremely complex (Kappus, 1987) and beyond the scope of this discussion. Numerous studies have demonstrated enhancements of LPOX in various tissues from fish species exposed in vivo to a variety of chemicals, e.g. paraquat-exposed carp (Gabryelak and Klekot, 1985), channel catfish and brown bulhead exposed to *t*-butyl hydroperoxide (Ploch et al., 1999), sea bass exposed to heavy metals (Romeo et al., 2000) and bluegill sunfish exposed to anthracene and UV-light (Choi and Oris, 2000). New trends in the demonstration of LPOX by measurement of degradation products such as aldehydes, acetone and malondialdehyde have been described by De Zwart et al. (1997). LPOX appears to have considerable potential as a biomarker for **ERA** (Stegeman et al., 1992; Hai et al., 1995), although it can occur as a consequence of cellular damage due to a variety of insults other than exposure to xenobiotics causing oxidative stress (Kappus, 1987).
- A recently developed analytical method for measuring and quantifying the capability of biological samples to neutralize ROS is the so-called *total oxyradical scavenging capacity (TOSC) assay* (Winston et al. 1998; Regoli et al., 2000). By generating different ROS at a constant rate, the assay provides an index of specific biological resistance to various types of ROS. Since the relative efficiency of antioxidants may considerably vary towards different ROS, the TOSC assay has been standardized for measuring the scavenging capacity of cellular antioxidants with respect to various ROS (Regoli and Winston, 1999; Winzer et al., 2001).
- *DNA oxidation* may be the result of $OH^{\bullet}$ attack at various sites of DNA bases, thus generating hydroxylated bases (Stegeman et al., 1992; Chipman et al., 1998). A sensitive but relatively complicated method of detecting these DNA alterations involves HPLC separation and electrochemical detection. Malins et al. (1990) observed oxidized guanine bases in DNA of hepatic neoplasms of English sole, which were not detected in non-neoplastic livers. Treatment with *t*-butyl hydroperoxide alone or in combination with GSH depletion, however, did not affect levels of DNA oxidation in channel catfish and brown bullhead, indicating that these species are relatively insensitive for this effect (Ploch et al., 1999). Pollutant-mediated effects on DNA integrity are discussed in more detail under the genotoxic parameters (Section 6.10).
- Several compounds (such as aromatic hydrazines, quinones, nitrite and some transition metals) have been shown to enhance the formation of *methemoglobin (MetHb)*, a form of hemoglobin in which the heme iron is in the oxidized state (Fe^{3+}) and which is unable to bind and transport O_2 (Stegeman et al., 1992; Gonzalez et al., 2000). Increases in MetHb formation have been observed in channel catfish exposed to naphthoquinones (Andaya and Di Giulio, 1987) and in feral perch inhabiting BKME-contaminated waters (Andersson et al., 1988).
- Oxyradical-generating compounds can influence the *redox status* of cells by imposing a drain on intracellular reducing equivalents, potentially affecting a variety of metabolic processes (Stegeman et al.,

1992). These effects were discussed previously under phase II cofactors (Section 6.2.1).

6.4. Biotransformation products

The exposure to xenobiotic compounds which are easily biodegradable in fish, such as PAHs and aromatic amines, generally cannot be assessed by simply measuring their tissue levels (Melancon et al., 1992). Depending on the xenobiotic, its metabolism may lead to a form that is more easily monitored and thus may be used as a biomarker of exposure. Biomonitoring via metabolites of xenobiotic chemicals requires knowledge of the extent of metabolism and the types of metabolites of a particular compound produced by an organism. It is, however, beyond the scope of this section to review the metabolism of all xenobiotic chemicals. Substances like PCBs and DDTs are very resistant to metabolism in fish (Melancon et al., 1992; Van der Oost et al., 1996a). PAHs, on the other hand, can be biotransformed by many aquatic organisms, but most effectively in the liver of fish. PAHs are metabolized by the phase I enzymes to more hydrophilic products such as phenols, dihydrodiols, quinones and epoxides (Lech and Vodicnik, 1985; Bucheli and Fent, 1995). The MFO system may thus efficiently detoxify a large number of xenobiotics by converting the parent compounds to easily excretable products. However, not only detoxification occurs, since certain compounds may be biotransformed to reactive intermediates that are highly toxic, mutagenic or carcinogenic to the fish. The oxidative metabolism of PAHs, for instance, proceeds via highly electrophilic intermediate arene oxides, some of which bind covalently to cellular macromolecules such as DNA, RNA, and protein (Neff, 1985). It is generally accepted that metabolic activation by the MFO system is a prerequisite for PAH-induced carcinogenesis (Van Schooten, 1991). Pulp and paper mill effluents generally contain high concentrations of resin and fatty acids, as well as chlorophenolics. Elevated fish bile levels of these substances have been used as biomarkers (Leppänen et al., 1998). The possible uses of metabolites of both xenobiotic chemicals (PAHs, chlorinated phenols, resin acids, etc.) and endogenous metabolites (vitellogenin (VTG), GSH, porphyrins, reproductive hormones, etc.) as biomarkers for exposure and/or effects have been reviewed in more detail by Melancon et al. (1992). The formation of DNA adducts, together with other DNA alterations, will be discussed under the genotoxic parameters (Section 6.10.1).

6.4.1. PAH metabolites in bile

In order to determine the exposure to and possible effects of PAHs in fish, the fate of PAH metabolites should be investigated so as to quantify the PAH flux (uptake and excretion) in the animals (Collier and Varanasi, 1991; Varanasi and Stein, 1991). Some PAHs are excreted as polar metabolites via the gallbladder (in bile), but most PAHs are excreted after conjugation by phase II enzymes (Vermeulen et al., 1992). Metabolite levels in bile can be determined either by analyzing the total level of PAH metabolites as fluorescent aromatic compounds (FAC), or by selecting a single metabolite as a marker for total PAH metabolism. 1-Hydroxypyrene (1-OH pyrene) has been selected for this purpose because, first of all, relatively high levels of pyrene have been detected in most sediments; secondly, pyrene is biotransformed predominantly into a single, strongly fluorescent metabolite (1-OH pyrene); and thirdly, the bioavailability of pyrene is relatively high for aquatic organisms (Ariese et al., 1993a). 1-OH pyrene, accounts for a large percentage of the total PAH metabolites in the bile of PAH-exposed fish (Krahn et al., 1987). PAH metabolites can be determined using synchronous fluorescence spectrometry (SFS), fixed wavelength fluorescence (FF) or HPLC (Aas et al., 2000). The results of the 1-OH pyrene SFS determination in fish bile agreed very well with HPLC/fluorescence data (Ariese et al., 1993b). Generally, the levels of bile metabolites are indicative of short-term exposure (1 week), and therefore, provide information on recent exposure only. It was demonstrated that the PAH metabolite levels in bile as well as the bile volumes were highly influenced by the feeding status of the fish (Collier and Varanasi, 1991; Brumley et al., 1998). In order to reduce variations in PAH metabolite bile levels due to feeding status, they proposed a procedure in which the metabolite concentrations are related to the biliary pigment contents.

A strong increase ($>500\%$ of control) in the biliary levels of PAH metabolites has been observed in most of the laboratory studies in which various fish species were exposed to PAHs (Table 11). These results are confirmed by field studies, in which a significant increase of FAC levels was observed in bile of fish from polluted environments (Table 12). A significant decrease in FAC levels was not observed in any of the laboratory or field studies (Tables 11 and 12). The FAC responses for all fish species from 15 laboratory studies and 24 field studies are summarized in Fig. 10A. A significant increase in biliary FAC levels was observed in 93% of the laboratory studies and 79% of the field studies, while strong increases ($>500\%$ of control) were observed in 87% and 46% of the laboratory and field studies, respectively.

Levels of biliary PAH metabolites are certainly sensitive biomarkers to assess recent exposure to PAHs. Since PAH exposure cannot be reliably determined by measuring fish tissue levels, this parameter is a valid fish biomarker for **ERA** processes concerning PAH-contaminated sites.

6.5. Stress proteins, metallothioneins and multixenobiotic resistance

Environmental stress, as well as a variety of physical conditions, may sometimes induce the synthesis of certain proteins in fish. Some of these proteins are believed to play a role in protecting the cell from the damage, which may result from environmental perturbations, while others are involved in the regulation of various genes. The best-known representatives of this group are the stress proteins (also known as heat shock proteins, (HSPs)), metallothioneins (MTs) and multixenobiotic resistance (MXR) transmembrane proteins.

6.5.1. Stress proteins (HSPs)

The stress proteins comprise a set of abundant and inducible proteins which are involved in the protection and repair of the cell in response to stress and harmful conditions, including high or low temperature, ultraviolet light, oxidative conditions, anoxia, salinity stress, heavy metals, and xenobiotics such as teratogens and hepatocarcinogens (Stegeman et al., 1992; Di Giulio et al., 1995). They are part of the cell's strategy to protect itself from damage. The stress protein response includes two major, closely related groups of gene products: the HSP group and the glucose-regulated protein (GRP) group. Synthesis of HSPs is dramatically increased by exposure to heat and other physical and chemical stresses, while synthesis of GRP is increased in cells by such factors as deprivation of glucose or oxygen (Stegeman et al., 1992). Sanders (1990) proposed a third class of stress proteins, the stressor-specific stress proteins. These proteins appear to participate in specific biochemical pathways involved in the metabolism of chemicals, metabolites or harmful by-products that are the result of a particular chemical or physical condition rather than being a part of the cell's protective system in response to general cellular damage. Two of these stressor-specific stress proteins are well characterized: heme oxygenase and MTs (see Section 6.5.2). Each stress protein comprises a multigene family in which some proteins, often called cognates, are constitutively expressed while others are highly inducible in response to environmental stresses. The cognates play a role in basic cellular physiology, and are present in the cell under normal conditions (Sanders, 1990).

From a mechanistic viewpoint, the strategy of selecting potential stress protein biomarkers based on the molecular mechanisms underlying protection against environmentally induced damage is sound and offers promise for identifying environmentally relevant biomarker assays (Stegeman et al., 1992). The regulation of the heat shock transcriptional response has been reviewed by Morimoto (1998), who described the mechanism as a 'crosstalk between a family of heat shock factors, molecular chaperones, and negative regulators'. However, little is yet known about the environmental relevance of stress protein responses in fish exposed to environmental contaminants. The baseline features of stress proteins or their responses towards various stressors (such as heavy metals) have been characterized in a number of laboratory studies with fish and fish cell cultures (e.g. Kothary and Candido, 1982; Kothary et al., 1984; Chen et al., 1988; Misra et al., 1989; Dyer et al., 1993; Sanders et al., 1994; Grøsvik and Goksøyr, 1996; Janz et al., 1997; Rabergh et al., 2000), but thus far very few studies have been reported in which stress proteins were used as biomarkers in field situations (e.g. Sanders and Martin, 1993; Triebskorn et al., 1997). The latter study revealed that HSP70 induction is more suitable as a biomarker of exposure at low temperatures. More research in both the laboratory and the field will be required before their usefulness as biomarkers in **ERA** monitoring programs can be accurately evaluated. The mechanisms linking tissue level stress responses and impairment of function at the organismal level will also be important for this evaluation (Stegeman et al., 1992). Since nutrition and water quality (pH, temperature, salinity and dissolved oxygen) can affect the HSP response, these factors should be monitored during laboratory experiments and field collections.

6.5.2. Metallothioneins (MTs)

MTs constitute a family of low-molecular-weight, cysteine-rich proteins functioning in the regulation of the essential metals Cu and Zn, and in the detoxication of these and other, non-essential, metals such as Cd and Hg (Roesijadi and Robinson, 1994). The cellular interactions involving MTs are expected to follow two general lines, the first being the interception and binding of metal ions that are initially taken up by the cell and the second being the removal of metals from non-thionein ligands that include cellular targets of toxicity. The latter may represent a detoxication function for structures, which have been reversibly impaired by inappropriate metal binding. The role of MTs in sequestering metals is well established, while their induction by exposure to a wide variety of metals (e.g. Cd, Cu, Zn, Hg, Co, Ni, Bi, and Ag) is associated with their protective function (Stegeman et al., 1992; Viarengo et al., 2000). Studies on the regulation of MT gene expression provided evidence that induction by metals is a direct response to increases in the intracellular metal concentration which is mediated through the action of metal-binding regulatory factors (Thiele, 1992). The capacity for MT induction is greatest in tissues that are active in uptake, storage and excretion, e.g. the small intestine, liver and gills of fish (Roesijadi and Robinson, 1994).

Since MTs have no known catalytic function, measurements of their concentrations are based upon quantitative assays of the protein itself (Stegeman et

al., 1992). Several existing methods (e.g. ion-exchange chromatography coupled with atomic adsorption spectrometry, metal substitution assays, polarographic and immunochemical techniques) have been reviewed by Engel and Roesijadi (1987). In several species of fish, MT levels have been demonstrated to increase in a dose-responsive (e.g. George and Young, 1986; George, 1989; Hogstrand and Haux, 1991; George et al., 1992a; Castaño et al., 1998) or a time-responsive (Beyer et al., 1997) manner after administration of heavy metals. The potency of metals to induce MT may depend upon fish species, tissue and experimental conditions. In gill tissue of carp, for instance, the following order of potency for MT induction was determined: Hg > Cd > Ag > Zn (Cosson, 1994). Several field studies with feral fish have supported the experimental data that MTs sequester heavy metals and that MT levels correlate with tissue levels of heavy metals (e.g. Roch et al., 1982; Roch and McCarter, 1984; Olsson and Haux, 1986; Hylland et al., 1992; Schlenk et al., 1995; Olsvik et al., 2000). In mammals, insects and crustaceans it was demonstrated that MT is induced under many other conditions besides metal exposure (Stegeman et al., 1992; Muto et al., 1999). Both glucocorticoid and peptide hormones have been found to induce MT synthesis, while factors like temperature and nutritional status affect binding of metals to MT. It has been suggested that MT synthesis may be reduced in the presence of high levels of organic contaminants due to an increased demand for cysteine residues for GSH synthesis. Estradiol and estrogenic PCBs appeared to inhibit cadmium-mediated MT induction in Arctic char (Gerpe et al., 2000).

Generally, the need to search for and validate biomarkers for the potential deleterious effects of (heavy) metals seems less important than for organic xenobiotics, since metals are a relatively small group of chemicals which can be easily detected by chemical analyses. On the other hand, it is quite clear that knowledge of intracellular metal compartmentation is essential to understanding the mechanisms of metal-induced cell injury (Fowler, 1987). The feasibility of MTs as biomarkers for metal exposure or metal-induced stress has been discussed by Stegeman et al. (1992). Given the extensive scientific information base and available methods for measuring changes in MT synthesis and its metal composition, these proteins show a strong potential for use as biomarkers for **ERA** of toxic metals. Since MT isoforms seem to be differentially induced by various heavy metals, MT isoform-specific nucleotide probes to quantify the expression of these isoforms need to be developed. The biological function of MT is by no means fully understood. It is, therefore, still not possible to link changes in MT levels to injury at the cellular or organismal level. Before MTs can be used to assess organismal health or fitness, the knowledge concerning their normal physiological function and the factors that control the levels of MT in selected species of fish needs to be extended.

6.5.3. *Multixenobiotic resistance (MXR)*

Many fish are able to survive in environments containing high levels of multiple anthropogenic pollutants or natural product toxins. Tissue levels of certain contaminants are often maintained at levels below those observed in the environment. It has been suggested that this fact is mediated by the MXR phenomenon, which is similar to the multidrug resistance (MDR) phenomenon that was first observed in tumour cell lines resistant to anti-cancer drugs (Juliano and King, 1976). The MXR mechanism acts as an energy-dependent pump that removes both endogenous and xenobiotic chemicals from the cell, thus preventing their accumulation and cytotoxic effects (Kurelec, 1992; Epel, 1998). The protein responsible for this transport function is the transmembrane *P*-glycoprotein (PGP). PGPs are found endogenously in specialized epithelial tissues involved in secretion and excretion, such as gut, liver and kidney, as well as on endothelial cells of capillary blood vessels at the blood–brain barrier (Bard, 2000). The physiological importance of PGPs in mammals has been established with knockout mice, in which an increased accumulation of xenobiotics together with an elevated sensitivity toward these chemicals was observed (Schinkel et al., 1994). PGPs are able to translocate a wide variety of structurally and functionally diverse substrates. These compounds tend to be moderately hydrophobic, planar, natural products, which are often substrates for or metabolites of detoxification enzymes such as cytochromes P450 (Sharom, 1997). In addition to toxin evasion, phospholipid and steroid transport, PGPs endogenous functions may include a role in development and osmotic control (Bard, 2000). The PGP-mediated MXR in aquatic organisms was first reviewed by Kurelec (1992). In order to place ecotoxicological data in context of the larger MDR field of study, Bard (2000) recently reviewed the MXR as a cellular defence mechanism in aquatic organisms.

Numerous studies have reported induction of MXR transport activity and elevated PGP levels in field populations of pollutant exposed aquatic organisms or after laboratory exposures. Both natural products and anthropogenic contaminants found in the aquatic environment, as well as biotransformation products of phase I enzymes, appear to be substrates and inducers of the MXR transporter in aquatic organisms (Bard, 2000). PGP induction may be a generalized response to stressful conditions such as xenobiotic exposure or cellular injury. Elevated *P*-glycoprotein expression may occur via multiple mechanisms, including gene amplification, transcriptional and post-transcriptional controls (Roninson, 1992). The factors that regulate

PGPs are not fully understood. Recent studies suggest that PGP expression may be regulated by protein kinase C (PKC) mediated phosphorylation (Chaudhary and Roninson, 1992), thyroid hormones, and cystic fibrosis transmembrane conductance regulator (CFTR) protein expression (Bard, 2000). In addition to well characterized detoxification systems (phase I, II and III enzymes, HSPs, etc.), the induction of a multixenobiotic defence mechanism in organisms living in polluted environments may explain why contaminant spills cause more severe adverse affects at pristine sites than in already polluted areas (Bard, 2000).

The protective role of the MXR defence mechanism appears to be fragile. As opposed to MXR induction, it was also demonstrated that many classes of chemicals, referred to as 'chemosensitizers', are capable of competitively inhibiting the MXR function (Kurelec, 1997). Since these chemicals may block one of the basic biologic defence mechanisms and cause reversion of natural resistance to pathobiologic sensitivity, they should be considered as environmentally hazardous chemicals. MXR-inhibition causes increased bioaccumulation of xenobiotics and, therefore, elevated internal levels of toxins that may exert cytotoxic, genotoxic or neurotoxic effects at environmental levels not otherwise considered harmful. Due to the clinical importance of acquired MDR in cancer cells, many studies were performed in order to discover novel agents that inhibit *P*-glycoprotein-mediated efflux of cytotoxic drugs (Sarkadi and Muller, 1997). A wide variety of compounds have been shown to reverse MDR in vitro (Kurelec, 1997). Several methods have been described to test environmental samples for the presence of MXR-inhibiting substances (Smital and Kurelec, 1997).

Examples of interactions of responses on PGP and cytochromes P450 are reported for intertidal fish (*Anoplarchus sp.*), upon field and laboratory exposures to crude oil, containing substances that are inducers for both CYP1A and PGP (Bard et al., 1998). After 3 weeks of exposure to contaminated sediments and food PGP expression was demonstrated in bile canaliculi. PGP expression was highly correlated to hepatic CYP1A in these fish. In another study, in which carp were exposed to water with low concentrations of diesel-2 oil induction of CYP1A was stimulated when the PGP mechanism was inhibited in the presence of 20 μM verapamil, a well-known MXR inhibitor (Kurelec, 1995). In marine field study it was demonstrated that the CYP1A induction in grey mullet due to XAD-7 water concentrates was highly correlated with the expression of MDR gene in sponges (Krasko et al., 2001). In some other studies with fish the relationship between CYP1A induction and elevated PGP expression could not be demonstrated (Bard, 2000). Some mammalian studies suggest that CYP3A and PGP may be co-induced in some circumstances (Bard, 2000). The large individual variability in hepatic PGP levels in feral fish suggests that fish may have variable abilities to respond to PGP inducers (Bard et al., 1998).

The discovery of the MXR mechanism in aquatic organisms may have important implications on **ERA** studies, since it interferes with important phenomena such as uptake, bioavailability, bioaccumulation and toxicity of xenobiotic chemicals and their metabolites. However, it has yet to be established how the induction and inhibition of MXR activity can be applied as a biomarker of environmental pollution. Therefore, much work remains to be done to characterize the function of PGPs in wild fish populations, and to determine how PGPs interact with other detoxification systems. The literature on this topic indicates that MXR expression in aquatic organisms other than fish, such as bivalves, worms and sponges, can be reliably used as an indicator of contaminant exposure and/or resistance.

6.6. Haematological parameters

Especially in relation to a reduction in the use of test animals, haematological components may be promising fish biomarkers. Blood samples can regularly be obtained from test organisms, thus allowing the use of a non-destructive approach in effect assessment. Typically, haematological parameters are non-specific in their responses towards chemical stressors. Nevertheless, they may provide important information in effect assessment studies, e.g. by providing an indication as to the general physiology and health status of the organism under investigation (Beyer, 1996).

6.6.1. Serum transaminases

The aminotransferases, alanine transaminase (ALT or GPT) and aspartate transaminase (AST or GOT), constitute a group of enzymes that catalyze the interconversion of amino acids and α-ketoacids by transfer of amino groups. The α-ketoglutarate/L-glutamate couple serves as an amino group acceptor and donor pair in amino-transfer reactions (Moss et al., 1986). ALT catalyses the transfer of the amino group from alanine to α-ketoglutarate to form glutamate and pyruvate, while AST catalyses the transfer of the amino group from aspartate to α-ketoglutarate to form glutamate and oxaloacetate (Moss et al., 1986).

An increase of enzyme activity in the extracellular fluid or plasma is a sensitive indicator of even minor cellular damage since the levels of these enzymes within the cell exceed those in the extracellular fluids by more than three orders of magnitude (Moss et al., 1986). The measurement of enzyme activities in the serum is, therefore, frequently used as a diagnostic tool in human medicine (Adolph and Lorenz, 1978; Goetz, 1980). Most research on the use of serum transaminase activities as an indicator of tissue damage has, therefore, been

performed on humans, since both ALT and AST activities are of great clinical significance (Moss et al., 1986). Following myocardial infarction, an increased AST activity appears in the serum, as may be expected from the relatively high AST concentration in heart muscle. ALT levels are within normal ranges or are only marginally increased in uncomplicated myocardial infarction, since the concentration of ALT in heart muscle is only a fraction of that of AST. Although the serum levels of both AST and ALT become elevated whenever disease processes affect liver cell integrity, ALT is the more specific enzyme for the liver. In humans, elevations of serum ALT activity are rarely observed in conditions other than parenchymal liver disease (Moss et al., 1986). In viral hepatitis and other forms of liver disease associated with hepatic necrosis, serum AST and ALT are elevated even before the clinical signs and symptoms of disease appear. It has been suggested that serum levels of GSTs (Section 6.2.2) may be used as an alternative for AST and ALT activities to indicate tissue damage (Hayes and Pulford, 1995).

Not many of the field and laboratory studies considered reported on the activities of the serum transaminases ALT and AST in fish blood. An increase in both serum ALT and AST activity was observed in experiments in which fish were acutely exposed to domestic waste water, while after prolonged exposure transaminase activities in blood plasma dropped to the control level (Bucher and Hofer, 1990). An increased ALT level was also observed in PAH exposed bluegill sunfish, while increased AST plasma levels were observed in carp exposed to deltamethrin and in PAH-exposed flounder (Table 11). Other laboratory studies could not demonstrate any significant alterations in plasma transaminase activities. Fish serum transaminase activities were reported in few field experiments only. A significant increase in plasma AST activity was only observed in flounder and carp, caged at polluted sites (Table 12). A significant decrease in ALT activity was observed in roach exposed at a BKME-polluted site (Table 12). The ALT responses for all fish species from five laboratory studies and three field experiments are summarized in Fig. 10C. A significant increase in ALT activity was observed in three laboratory studies (60%) and none of the field studies, while a strong increase ($>$ 500% of control) was not observed in any of the studies considered. The AST responses for all fish species from eight laboratory studies and seven field experiments are summarized in Fig. 10D. A significant increase in AST activity was observed in 38% of the laboratory studies and in two field studies (29%), while a strong increase ($>$500% of control) was only observed in one laboratory study (13%) with PAH-exposed flounder (Beyer et al., 1997) in and one field study (14%) with caged flounder (Beyer et al., 1997).

Determinations of ALT and AST activities in blood plasma have incidentally been applied in fish research to indicate bacterial, viral and parasitic infections, intoxications and water pollution (Bucher and Hofer, 1990). However, the application of serum enzyme determinations in fish, as an indicator of chronic intoxication, seems to be questionable. In general, necrotic cells do not contribute to an increased enzyme activity in the serum, since the greater part of the increase originates from damaged but still living cells, and membrane defects causing increased permeability (Adolph and Lorenz, 1978). On the other hand, toxicants can also inhibit the activity or synthesis of enzymes (Goetz, 1980), resulting in decreased activities in the blood. Consequently, serum transaminases in fish cannot be considered reliable biomarkers to assess the effects of chronic pollutant exposure in **ERA**. AST in fish plasma, however, may be a promising effect biomarker when used in short-term caging experiments, as was demonstrated by Beyer et al. (1997), Van der Oost et al. (1998).

6.6.2. *Other haematological parameters*

Utilization of the chemically induced alterations in the heme pathway as biomarkers of both exposure and effect has been the subject of intense study over the past 20 years in mammalian toxicology. These studies have delineated a number of chemical-specific responses that have proven extremely useful in the early detection of low-dose chemical effects in mammals (Stegeman et al., 1992). Chemically induced disturbances in the heme pathway, which is essential for the biosynthesis of haemoproteins (e.g. hemoglobin) and various cytochromes (e.g. cyt P450), have been reviewed by Silbergeld and Fowler (1987). In a number of fish species similar specific changes in heme pathway enzyme activities have been observed following waterborne exposure to lead or cadmium (reviewed by Stegeman et al., 1992). Since a number of relationships between these indicators and cell injury are already known in mammals, interpretation of the alterations in a given heme pathway enzyme activity in relation to an injurious process in fish will also be more reliable. Other haematological parameters, such as hematocrit, hemoglobin, corpuscular volume, corpuscular hemoglobin concentration, plasma osmolality, plasma lipids, albumin, total protein and glucose, are generally less specific than the serum enzymes, and may also be influenced by natural factors such as bacterial challenges (Iwama et al., 1986). However, in specific situations, these parameters may still be useful as biomarkers of toxicant effects in fish (e.g. Van Vuren, 1986; Tort et al., 1987; Reddy et al., 1991; Boon et al., 1992; Ghazali, 1992; Allen, 1993).

Particularly when measured in concert with other biomarkers with more specific response patterns, such as

CYP1A-mediated responses, blood parameters may be valuable biomarkers for effect assessment.

6.7. Immunological parameters

The immune system comprises a network of cells capable of rapid proliferation and differentiation, regulated by a variety of soluble factors, and is closely integrated with other organ systems and functions. As such, it is extremely vulnerable to insult from exogenous chemicals, especially after chronic exposure or repeated short exposures (Weeks et al., 1992). In mammals it was observed that both cell-mediated and humoral (antibody-mediated) immunity may be depressed by pollutants such as PAHs, PCBs, PBBs, OCPs (e.g. dieldrin, lindane, DDTs and HCB), organometals (e.g. methylmercury and organotins) and heavy metals such as Pb and Cd (Vos et al., 1989). The immune system, therefore, was considered to be a promising field in which new candidate biomarkers for environmental monitoring could be found. It should, however, be emphasized that the immune system can be influenced by a large variety of stressors, which implies that immunological biomarkers may be useful and sensitive, but often non-specific (reviewed by Weeks et al., 1992).

The immunology of fish species is less well-characterized than in mammalian species, although the know ledge has increased rapidly in recent years. As in mammals, the immune system biomarkers in fish are considered to have considerable potential for application in pollution biomonitoring (Wester et al., 1994). An increasing number of studies have demonstrated immune effects in fish after exposure to environmental pollutants (e.g. Robohm, 1986; Payne and Fancey, 1989; Secombes et al., 1991; Pulsford et al., 1992; Zelikoff, 1993; Arkoosh et al., 1994; Dunier et al., 1994; Lemaire-Gony et al., 1995; Sanchez-Dardon et al., 1999; Aaltonen et al., 2000a,b). Several immunological parameters may potentially be used as biomarkers in fish, e.g. white blood cell (leukocyte) and lymphocyte status (measured as blood cell or differential counts), non-specific defence factors (such as lysosomal activity and levels of acute phase proteins in body fluids), weight and morphology of leukocyte producing organs (such as spleen, thymus and kidney), melanomacrophage centers (number, size and histopathological examination), macrophage function (chemotaxis, phagocytosis, pinocytosis and chemiluminescence), increased susceptibility to bacterial infections, and others (reviews of Weeks et al., 1992; Wester et al., 1994). In recent studies with roach exposed to pulp and paper mill effluents (Aaltonen et al. 2000a,b), fish were immunized with bovine and gamma-globulin (BGG) 3 weeks before sampling. Specific anti-BGG antibody secreting cells (ASC) and immunoglobulin secreting cells (ISC) were suppressed and the plasma levels of anti-BGG antibody were lower in the exposed animals. In addition, a decreased migration of granulocytes was observed. Sex-related differences in the immune responses were evident in many parameters, e.g. in the number of blood ISC and splenic ASC. The studies by Aaltonen et al. (2000a,b) suggest that steroids may contribute to immunemodulation in fish. The causal relationships between immunotoxic pollutants and fish diseases as well as the ecological significance of such effects in the field still remain unclear. It is possible that immunosuppressive effects of pollutants serve as causal factors in the origin of fish diseases with multifactorial etiology, e.g. various skin diseases such as lymphocystis, papillomas, fin erosion, fin rot and skin ulcers (Vethaak, 1993). In certain situations, however, the opposite effect, i.e. protection of the fish against pathogens, may be observed after pollutant exposure (MacFarlane et al., 1986).

As with other biomarker responses, immune responses provide an integrated measure of exposure over time and may reflect the combined results of simultaneous exposure to several chemicals. It is, however, not possible to determine which chemical has caused the observed effect as none of the changes in immune function can be attributed to a specific compound or class of chemicals (Wester et al., 1994). It should be taken into account that a number of other stresses, such as handling, transportation or social interactions among individuals, may cause immunological disturbances in fish as well. In addition, three main categories of hormones (i.e. corticoids, catecholamines and opioid peptides) have direct and indirect effects on various aspects of the immune system (Weeks et al., 1992). Although highly reliable and reproducible assays of immune function exist, much research has to be carried out in order to establish the most responsive immunological alterations and to validate their ecotoxicological relevance. Nevertheless, immunological biomarkers have an important role to play in monitoring the health of fish prior to the occurrence of devastating disease outbreaks and as early-warning indicators for the potential harm of environmental chemicals. Due to the fundamental physiological role of the immune system, any kind of impairment in the immunological resistance of fish may be interpreted as an important signal in **ERA**. Since many parameters of the immune system are similar in different organisms, fish may serve as sentinels of potential environmental hazards for humans (Weeks et al., 1992).

6.8. Reproductive and endocrine parameters

Decreased reproductive capability in feral organisms may be considered as one of the most damaging effects of persistent pollutants released by man. A number of xenobiotics with widespread distribution in the environ-

ment are reported to have endocrine activity which might affect reproduction and thus might threaten the existence of susceptible species (Colborn et al., 1993; Peterson et al., 1993; White et al., 1994). Animals at high trophic levels, generally having limited reproduction rates, are likely to be the most vulnerable in this regard. An example is formed by the indications that PCB pollution of Swedish waters is linked to uterine occlusions and stenoses in female ringed seal, resulting in reproductive failure and, consequently, a rapid decrease of the seal population (Helle et al., 1976a,b). Another example is the occurrence of imposex (development of male sexual characteristics in female gastropods) in the common whelk due to organotin exposure (Mensink, 1999).

Effects on reproductive competence as a response to pollution stress have also been demonstrated in fish. There is increasing evidence that low-level pollution may decrease the fecundity of fish populations, leading to a long-term decline and eventually extinction of important natural resources. This, together with overfishing, may account for a decline in major fisheries, such as those in the North Sea, in the industrialized West. In an extensive review, Kime (1995) presented an overview of the effects of sublethal pollution, both industrial and agricultural, on all aspects of fish reproduction, from gonadal development through to spawning, together with a discussion of how some of these effects may be a result of disturbance of the reproductive endocrine system. Evidence has been presented demonstrating that pollutant effects may occur at multiple sites of the reproductive system. They may cause lesions, hemorrhage, or malformations in the gonads, pituitary, liver and the brain. Production and secretion of hormones of the hypothalamus, pituitary, and gonads is usually inhibited and their metabolism by the liver can be altered. There is also a considerable literature on the survival of eggs, larvae and fry, which are particularly susceptible to pollutants, and may have a major impact on population dynamics (Kime, 1995; Hugla and Thome, 1999).

Xenobiotics may reduce reproductive success of fish by interacting directly with the germ cells (Armstrong, 1990), resulting in a high rate of mitotic chromosome abnormalities (Crosby Longwell et al., 1992). Negative correlations between hatching success and ovarian tissue burdens of chlorinated hydrocarbons have been observed, for instance, in North Sea whiting and in flounder from the Baltic Sea (von Westernhagen et al., 1981; von Westernhagen et al., 1989). Spies et al. (1988) investigated relationships between impairment of the reproductive success of the starry flounder from the Pacific coast of North America and environmental pollution. It was found that the hepatic CYP1A activity was inversely related to the egg viability, the fertilization success and the successful development from fertilization through hatching. These findings suggested that the responses in CYP1A activity interfered with the ability of the general class of cyt P450 enzymes to regulate sex steroids. Due to their broad substrate specificity, cyt P450 isoenzymes may accelerate testosterone clearance rates and affect estradiol levels in contaminant-exposed fish (Spies et al., 1990). Binding of xeno-estrogenic xenobiotics (e.g. DDTs, HCHs and alkylphenols) to the estrogen receptor also causes estrogenic effects (Harrison et al., 1995). A national fish survey in the US, which sampled fish from 25 streams in 13 states, suggests that water-soluble pesticides such as atrazine and other herbicides in current use may be affecting the endocrine systems of fish (Renner, 1997). The pesticides do not accumulate in fish, but lab studies demonstrated that they are able to permanently change steroidal pathways. Although no cause and effect could be established, the variation in unusual hormone levels in carp (determined as the median ratio of 17β-estradiol to 11-ketotestosterone) appeared to be best explained by high concentrations of pesticides in the water. Reproductive steroids were also affected in feral perch and roach exposed to BKME effluents in Finland (Karels et al., 1998).

The synthesis of VTG, a precursor of yolk proteins, is affected by estradiol. It was demonstrated that PCB-exposed fish were less capable of producing VTG (Spies et al., 1990). An impaired reproductive function due to decreased plasma VTG levels was reported for female rainbow trout exposed to Cd (Haux et al., 1988). VTG synthesis can also be induced in male fish exposed to endocrine disrupting chemicals such as alkylphenols, thus leading to a so-called feminisation of male fish (Gimeno et al., 1996). The VTG response in fish may thus be used as a sensitive biomarker of exposure to estrogenic compounds. A pronounced increase in plasma VTG levels in male fish was observed in many laboratory studies (e.g. Sumpter and Jobling, 1995; Arukwe et al. 2000; Lindholst et al., 2000; Hemmer et al., 2001) and field experiments (e.g. Purdom et al., 1994; Mellanen et al., 1999; Lye et al., 1999; Larsson et al., 1999). The latter field study revealed that a substantial part of the observed estrogenic effects was due to 17α-ethinyloestradiol, a synthetic oestrogen used in contraceptives that was present in effluents of sewage treatment plants receiving (mainly) domestic wastewater. The occurrence of hermaphrodite fish in the sites receiving this type of waste water might thus be due to the presence of (xeno-)estrogenic compounds in the effluents (e.g. Sumpter, 1995; Jobling et al., 1998). However, vitellogenesis may also be affected by pollutants with known affinity for the estrogenic receptor, such as nonylphenol, bisphenol A, PCBs and PAHs. Nicolas (1998) reviewed the present understanding of the effects of PAHs on vitellogenesis in fish. Another potential biomarker for estrogenic effects in male fish might be the induction of zona radiata proteins (ZRP),

also known as vitelline envelope proteins (Hyllner et al., 1991). Recent studies with juvenile salmon indicated that the ZRP response was more sensitive to various environmental pollutants than the VTG response (Arukwe and Goksøyr, 1997; Arukwe et al., 2000), thus providing a sensitive means of detecting exposure to environmental estrogens. Both VTG and ZRP can be analyzed by measuring mRNA expression or plasma protein levels.

After years of exposure to low levels of environmental contaminants, the sexual competence of both male and female fish may be impaired, which might eventually lead to a decrease in fitness or even extinction of the population. There is, surprisingly, little difference between the effects of different classes of pollutants (e.g. heavy metals, organophosphorous pesticides, organochlorine pollutants and [poly-]aromatic compounds). The literature covered in a review of Kime (1995) leaves no doubt that all types of pollutants have a serious inhibitory effect on fish reproduction, even when in minute quantities. Taylor and Harrison (1999) presented an overview of the main evidence for endocrine disruption in wildlife, focussing on reproduction effects. They concluded that in most cases a causal link between the observed abnormalities and chemical exposure has not been established. In addition they described priority research projects for the UK, ultimately aimed at determining the population-level significance of endocrine disruption. It is assumed that fish make excellent bioindicators of the harmful effects that might be expected in mammals in general, and human populations in particular (Kime, 1995). It is, therefore, of paramount importance for a reliable **ERA**, that sensitive reproductive biomarkers have to be developed and validated for their ecotoxicological significance. An overview of the methods and strategies to monitor the impact of endocrine-disrupting chemicals is given by Sadik and Witt (1999). In vitro VTG assays may also be used to assess the estrogenic potency of newly developed chemicals or existing environmental pollutants (Pellisero et al., 1993; Folmar et al., 2000).

6.9. Neurotoxic parameters

With respect to neural functions, enzymes of interest are cholinesterases (CHE) (Payne et al., 1996). Two types of CHE are recognized; firstly, those with a high affinity for acetylcholin (ACHE), and secondly, those with affinity for butyrylcholin (BCHE), also known as non-specific esterases or pseudocholinesterases (Walker and Thompson, 1991; Sturm et al., 2000). Fish brain contains ACHE, but no BCHE, while muscle tissues contain both ACHE and BCHE (Sturm et al., 2000). ACHE is involved in the deactivation of acetylcholin at nerve endings, preventing continuous nerve firings, which is vital for normal functioning of sensory and neuromuscular systems (e.g. Murphy, 1986). It is, therefore, assumed that this enzyme is more important than the non-specific esterases. This assumption, however, can only be confirmed when the physiological functions of non-specific esterases are elucidated more fully.

Many organophosphate (OP) and carbamate pesticides are reported to be effective ACHE inhibitors. The ACHE inhibition has, therefore, been used to assess the nature and extent of the exposure of wildlife to agricultural and forest sprays (e.g. Greig-Smith, 1991; Zinkl et al., 1991). Evidence for variation of ACHE in the muscle tissues of dab and flounder was demonstrated along a pollution gradient in the North Sea (Galgani et al., 1992). A significant depression of ACHE-activity in fish from OP-polluted sites was also observed in muscle tissues of brown trout and flounder in Newfoundland, Canada (Payne et al., 1996), in muscle tissue of the three-spined stickleback from parathion polluted streams Germany (Sturm et al., 1999, 2000), and in brain tissue of menhaden and mummichog from polluted rivers in South Carolina (Fulton and Key, 2001). It was unknown whether inhibition observed in field studies was due to pesticides, other factors, or a combination of both. Payne et al. (1996) suggested that complex mixtures of contaminants, other than pesticides, could be important sources of ACHE-inhibiting compounds in the aquatic environment; they provided preliminary evidence for ACHE-inhibiting activity present in extracts of used engine oil and wood leachate. Since the in vitro sensitivity of ACHE and BCHE and their respective in vivo responses in the field differed significantly, these enzymes should be considered separately in studies with fish. In a study with juvenile rainbow trout it was demonstrated that malathion induced CHE inhibition correlated significantly with behavioral measures, such as deviations in swimming speed and turning rate (Beauvais et al., 2000).

Although a number of field studies have successfully used CHE inhibition in fish as a biomarker of pesticide exposure, more research is required before this parameter can be used in **ERA** programs. Additional research is needed to better explain the species-specific differences in the relationship between ACHE inhibition and mortality and to investigate other physiological perturbations associated with ACHE inhibition (Fulton and Key, 2001).

6.10. Genotoxic parameters

The exposure of an organism to genotoxic chemicals may induce a cascade of events (Shugart et al., 1992). Genetic ecotoxicology can be defined as the study of pollutant-induced changes in the genetic material of biota in nature and has two aspects: (1) initially, the genotoxicity of pollutants, such as structural alterations

of the DNA, and (2) consequently, the procession and expression of DNA damage in mutant gene products, resulting in long-term heritable effects, such as changes in gene frequency within exposed populations, mutational events, etc. (Shugart, 1996). The detection and quantification of various events in this sequence may be employed as biomarkers of exposure and effects in organisms environmentally exposed to genotoxic substances. Within the discipline of genetic toxicology, research has taken place at three levels (Maccubbin, 1994):

- identifying the kind and determining the frequency of genetic diseases;
- studying the mechanisms of how chemical and physical agents cause genetic disorders;
- evaluating agents for their potential to cause genetic damage.

The study of DNA adducts in human and animal models has been an important part of the latter two levels of research. A more general approach involves the detection of DNA strand breaks that are produced, either directly by the toxic chemical (or its metabolite) or by the processing of structural damage (Shugart et al., 1992). DNA base composition, oncogene activation, cytogenetic effects and tumorigenesis also have the potential to be used as biomarkers. The study of genetic toxicology in aquatic systems is mainly focused on carcinogenesis in fish and shellfish.

6.10.1. DNA adducts

Paradoxically, the same metabolic processes that are responsible for the efficient elimination of biodegradable substances such as PAHs from the organism are also able to activate environmental carcinogens to DNA-reactive forms (Dunn, 1991). In environments with high PAH levels, greater P450 induction could contribute to higher steady-state levels of activated carcinogens and, consequently, to a greater formation of DNA adducts or to enhanced oxidative DNA damage (Stegeman and Hahn, 1994). Watson et al. (1998) demonstrated that the induction of CYP1A (due to BNF pre-treatment) significantly increased the in vivo DNA adduct formation in channel catfish after exposure to 2-amino-anthracene (AA), indicating the involvement of CYP1A in the bioactivation of AA. Similar observations were reported for CYP1A induced zebrafish exposed to aflatoxin B1 (Troxel et al., 1997). Greater P450 induction, however, is not necessarily associated with a greater risk of carcinogenesis, since the formation and persistence of critical genetic lesions is also influenced by phase II defence and repair processes. It is, nevertheless, most likely that liver PAH-DNA levels in fish reflect the extent of exposure to carcinogenic or mutagenic PAHs.

DNA adducts are generally determined in the liver, since this is the key organ for biotransformation of xenobiotics. Levels of hepatic DNA adducts may be indicative of cumulative exposure of fish to genotoxic compounds over a longer period of time (several months), as opposed to bile metabolites, which are indicative of recent exposure (several days) (Varanasi et al., 1989b). In a recent study by Malmström et al. (2000) it was demonstrated that DNA adducts could be detected in both liver tissue and leucocytes. However, the adduct levels in liver were higher and the adduct patters were different in leucocytes. Further investigations are needed to convincingly elucidate whether the use of non-invasive sampling methods will be appropriate for environmental biomonitoring of DNA adducts in fish. Currently, methods of varying sensitivity exist for the measurement of DNA adducts, including ^{32}P-postlabeling, HPLC/fluorescence spectrometry and immunoassays using adduct-specific antibodies (Shugart et al., 1992). With the most sensitive method, the ^{32}P-postlabeling assay, it is possible to determine levels as low as one adduct per 10^9 nucleotides. Even in wild brook trout from remote areas in Newfoundland (without a known input of pollutants) elevated DNA adduct levels were detected in liver and brain tissues (Ray et al., 1995).

Since the formation of PAH–DNA adducts is thought to be a necessary step in the carcinogenic action of PAHs, it is possible that there is a relationship between the amount of DNA adducts and cancer risk (Dunn, 1991). There is a good (but not perfect) correlation between the level of chemical binding to DNA and carcinogenic potency and organ specificity (Maccubbin, 1994). In general, however, it is difficult to demonstrate consistent quantitative relationships between DNA adduct levels in organisms, and formation of tumours or histologically related lesions and environmental contamination. Adduct formation is likely to be a complex function of various factors that may influence carcinogen metabolism (Dunn, 1991). Apart from pollutant levels, these factors may be related to the age and sex of the organisms, as well as to season, water temperature and food availability. Kurelec et al. (1989) indicated that the overwhelming majority of DNA modifications in various freshwater fish species are caused by natural factors rather than by man-made chemicals, since they did not observe any statistically significant differences between the DNA adduct levels of fish from polluted and unpolluted sites. However, since statistically significant differences in hepatic DNA adduct levels have been unambiguously demonstrated in other field studies (Table 12), this theory is evidently not valid for all fish species. Maccubbin (1994) reviewed DNA adduct formation in fish, both in vitro and in vivo, and concluded that fish have been shown to activate carcinogens to products that form DNA

adducts, which are the same ones as described in rodent studies. Since both phase I and II enzyme activities may influence DNA adduct formation, a thorough characterization of the metabolic pathways of the carcinogen is necessary to evaluate adduct formation relative to species sensitivity (Maccubbin, 1994). In addition, the route and duration of the exposure are important aspects to be considered when attempting to extrapolate results from laboratory studies to real-world situations, in order to predict the ultimate biological effect of a chemical. With respect to field studies, more work is needed in identifying adducts observed in wild fish.

Both laboratory and field studies on DNA adduct formation in fish have been reviewed by Pfau (1997). A strong increase (> 500% of control) in the hepatic levels of DNA adducts was observed in most laboratory studies in which various species of fish were exposed to PAHs (Table 11). These results are confirmed by a lot of field studies, in which a significant increase of DNA adduct levels was observed in the liver of fish from polluted environments (Table 12). A significant decrease in DNA adduct levels was not observed in any of the laboratory or field studies (Tables 11 and 12). The DNA adduct responses for all fish species from 17 laboratory studies and 30 field studies are summarized in Fig. 10B. A significant increase in hepatic DNA adduct levels was observed in 100% of the laboratory studies and 70% of the field studies, while strong increases (> 500% of control) were observed in 65% and 30% of the laboratory and field studies, respectively.

Due to the strong and consequent responses of hepatic DNA adduct levels to PAHs exposure, this parameter is considered to be an excellent biomarker for the assessment of PAH exposure as well as a sensitive biomarker for the assessment of potentially genotoxic effects. It is important to combine the measurements of DNA adduct formation as a molecular dosimeter with analysis of carcinogen metabolism and determination of tumour formation to provide insights in the mechanisms involved in chemical carcinogenesis (Maccubbin, 1994). This type of integrated study may be used in **ERA** to provide information about potential exposure and risk of environmental carcinogenesis that may be found in contaminated waterways. In addition to their use as a biomarker for exposure and effects of genotoxins, DNA adducts may provide information about the biological effect and potential risk of a chemical, since it has been suggested that any chemical that forms DNA adducts, even at very low levels, should be considered to have carcinogenic and mutagenic potential (Maccubbin, 1994). The first criterion for a valid biomarker, i.e. the relative ease with which it can be measured, is not met when DNA adducts are determined using the ^{32}P-postlabeling assay, which is expensive and time consuming. In this connection it might be interesting to improve immunological methods, which are less sensitive but much easier to perform, like the ELISA assay of Van Schooten et al. (1992) which uses polyclonal antibodies against benzo[a]pyrene-7,8-diol-9,10-epoxide-DNA.

6.10.2. Secondary DNA modifications

Exposure to toxic chemicals may cause secondary concomitant types of DNA alterations. Besides direct adduct formation, damage may include strand breaks in the DNA polymer, changes in the DNA's minor base composition or an increase in the level of DNA repair (Shugart et al., 1992). Apoptosis, also known as programmed cell death, is a physiological and irreversible process in tissue homeostasis that leads to DNA fragmentation of multiples of 180–200 basepairs. Apoptosis could be demonstrated by an increased number of small DNA fragments in liver of dab exposed to PCBs and cadmium (Piechotta et al., 1999) and in ovarian follicular cells from prespawning white sucker exposed to BKME (Janz et al., 1997), thus indicating its possibility to be used as a biomarker of effects

Many toxic chemicals cause *strand breaks* in DNA, either directly or indirectly. Alkaline unwinding and COMET assays are able to estimate the increase in the level of breaks above background resulting from exposure to these chemicals (Shugart, 1990a). This method is well suited for routine, in situ monitoring of feral fish because of its ease, speed and low cost. In laboratory studies, an increase in strand breaks was detected in the fathead minnow and the bluegill sunfish, which were chronically exposed to waterborne benzo[a]pyrene (Shugart, 1988). The feasibility of utilizing this technique on environmental species as a general biomarker for pollution-related genotoxicity is being evaluated (Everaarts et al., 1994).

Chemical carcinogens have been shown to produce *hypomethylation* of DNA as a result of their effect on certain maintenance enzymes (Shugart et al., 1992). This loss of DNA integrity can be easily measured using ion-exchange chromatographic techniques. Hypomethylation, as measured by the loss of 5-methyl-deoxycytidine, has been demonstrated in fish exposed to benzo[a]pyrene (Shugart, 1990b). The onset and persistence of this phenomenon was found to be correlated with other types of DNA-damaging events, such as strand breaks and adduct formation.

6.10.3. Irreversible genotoxic events

The consequence of structural perturbations to the DNA molecule, such as adducts and secondary modifications, may result in lesions that become permanent. Affected cells often exhibit altered function indicative of a subclinical manifestation of genotoxic disease. These irreversible genotoxic events have been reviewed by Shugart et al. (1992):

- *Cytogenetic assays*, like standard chromosome analysis, sister-chromatid exchange (SCE) and erythrocitic nuclear abnormalities (ENA), are sensitive non-specific indicators for mutagenic damage, which have shown promising, results in laboratory experiments. An increase in ENA frequency was observed in eel exposed to certain PAHs and resin acids (Pacheco and Santos, 1997) and cyclophosphamide (Pacheco and Santos, 1998). The micronucleus assay is less sensitive than the former assays, but it reveals the consequences of both spindle anomalies and chromosomal breakage and has been applied to feral fish. Most of these techniques are labor-intensive, so they have limited utility as routine screening assays.
- *Flow cytometric measurement* (*FCM*) is a technique that measures several cellular variables, including DNA, RNA, protein, specific chemicals and numerous morphological attributes in suspended cells. The efficiency of measuring the effects of chronic mutagen exposure with this technique has been demonstrated in both laboratory and field studies. Due to its low cost and high speed and sensitivity this method has a tremendous potential for use as an initial screening procedure. The main advantages and limitations of flow cytometry for biochemical studies are reviewed by O'Connor et al., 2001.
- *Oncogene activation* and *mutation rates* are two parameters, which may be used in the future to assess the impact of DNA mutations, although much research is still needed to validate the assays for their use as potential biomarkers. Oncogene activation is a DNA-based assay to measure specific nucleotide changes in oncogenes and other appropriate genes of chemically exposed animals. The use of DNA sequencing and restriction fragment length polymorphism data in the study of environmental mutagens has yet to be investigated. The p53 gene has been sequenced for several fish species with a view to the possible use of mutations in the highly conserved domains of p53 to identify genotoxins in the aquatic environment (Cachot et al., 1998; Bhaskaran et al., 1999).

The involvement of xenobiotic-metabolizing enzymes in carcinogenesis has been demonstrated in some mammalian studies, which reported altered levels and activities of these enzymes in preneoplastic and neoplastic lesions (Stegeman et al., 1992). The decrease in phase I enzymes involved in electrophile production from chemical carcinogens, combined with the increases in phase II enzymes involved in the detoxification and elimination of electrophiles, suggests an adaptive response to a toxic environment. In the few reported studies with fish, however, GST levels in liver neoplasms were either depressed or unaffected (Stegeman et al., 1992).

As an overall conclusion, it can be stated that the detection of structural DNA changes and ensuing events has been demonstrated and documented as a viable scientific tool for in situ BM of the genotoxicity of chemicals in **ERA**.

6.11. Physiological and morphological parameters

As opposed to most of the biochemical parameters that have been discussed in this review, physiological and morphological parameters are higher-level responses following chemical and cellular interaction, which are generally indicative of irreversible damage (Hinton et al., 1992). The main objective of this review is to discuss the feasibility of biological parameters in ERA, so that most emphasis is placed on the use of biomarkers as an early-warning system for potential hazards due to environmental contaminants. Therefore, the use of physiological and morphological parameters as biomarkers will be discussed only briefly.

6.11.1. Histopathology

The feasibility of using histopathological parameters in fish as a biomarker for aquatic pollution has been reviewed by Hinton et al. (1992), Hinton (1994). Epidemiological studies on the occurrence of fish disease in relation to their usefulness in monitoring marine pollution have been reviewed by Vethaak and ap Rheinallt (1992). They concluded that, on a worldwide scale, the most convincing examples of a causal relationship between fish disease and pollution was provided by intensive and detailed studies carried out in North America, particularly on liver pathology. Mix (1986) critically reviewed the neoplastic and cancerous diseases in aquatic organisms and their relationship to environmental pollution. Only a small number of studies could be identified in which the data were considered to support an association between pollution and neoplasia (Brown et al., 1977; Kimura et al., 1984; Malins et al., 1985; Myers et al., 1994). Triebskorn et al. (1997), Schramm et al. (1998) described methods to study the liver ultrastructure using quantitative and semi-quantitative electron microscopy. The biomarker responses observed in these studies were correlated with the results obtained by behavioral, limnological and analytical investigations, and reflected the levels of pollution at each site. The species specific sensitivity for histopathological lesions were demonstrated in a study with rubberlip surfperch and rainbow surfperch exposed to a natural petroleum seep (Spies et al., 1996). Total lesion scores were not different between two groups of rubberlip surfperch, while pronounced differences in gill lesions were observed between exposed and reference rainbow surfperch. It was suggested that certain lesions (e.g. abnormal branching of gill filaments) are biological

markers for xenobiotics that induce CYP1A (Spies et al., 1996).

It is generally assumed that histopathological biomarkers are valuable as indicators of the general health of fish and mirror the effects of exposure to a variety of anthropogenic pollutants (Hinton et al., 1992). No geographic or ecosystem limitations are apparent. Acute changes are seen when contaminant levels are sufficiently high, while chronic duration is required to determine sublethal aspects of change. Many alterations persist even after exposure to a toxicant has ceased so that host responses to prior toxicity can also be used to determine effects. Responses are relatively easily recognized, provided that proper reference and control data are available. Sufficient information is at hand to assemble cellular or histopathological biomarker approaches and to apply them in integrated field studies (Hinton, 1994).

6.11.2. Gross indices

Gross indices are sometimes indicative of toxicant effects (Mayer et al., 1992). Plant or animal condition as determined by morphology, appearance and other gross characteristics should not be overlooked in assessing contaminant impact. A first-level screen to identify potential pollutant exposure and effect can be accomplished on the basis of overt and relatively simple measures of condition. Such measures may serve to identify the most sensitive members of a fish population. In addition, they may provide information on energy reserves and possibly the ability of animals to tolerate toxicant challenges or other environmental stresses (Mayer et al., 1992). Morphological parameters that are often determined in field research are the liver somatic index (LSI), to identify possible liver diseases, and the condition factor (CF), to assess the general condition of fish.

The LSI is the ratio between the weight of the liver and the total body weight of the fish: 100 × liver weight (g)/body weight (g) (Slooff et al., 1983). Slooff et al. (1983) summarized a number of studies, which supported the hypothesis that there was a causal relationship between liver enlargement and exposure to chemical pollutants. Based on both biochemical analyses and histological observations it was concluded that liver enlargement in bream from polluted sites was mainly caused by hypertrophy (increase in cell size). In contrast to this, Poels et al. (1980) found the liver enlargement in young rainbow trout that were experimentally exposed to polluted Rhine water to be the result of hyperplasia (increase in cell number). It was suggested that this discrepancy might be explained by age differences of the fish, since the fast growing liver tissue of juvenile fish will respond more readily by a hyperplasic reaction than the liver tissue of full-grown fish.

Several laboratory studies considered for this review reported a significantly increased LSI value in fish exposed to PCBs, OCPs, BKME, PCDDs and PAHs (Table 11). Increased LSI values were also reported in field studies with bream, brown bullhead, chub, cod, dab, English sole, perch, plaice, shorthorn sculpin and white sucker from contaminated sites (Table 12). Significantly decreased LSI values were observed in laboratory studies with fish exposed to OCPs, PCBs and PAHs (BNF) and in field studies with redbreast sunfish and tilapia at polluted sites (Tables 11 and 12). The LSI responses for all fish species from 24 laboratory studies and 28 field studies are summarized in Fig. 10E. A significant increase in LSI values was observed in 38% of the laboratory studies and 43% of the field studies, while strong increases (> 500% of control) were not observed.

Bagenal and Tesch (1978) proposed a CF for fish, based upon the ratio between body weight and length: 100 × body weight (g)/(length (cm))^3. This factor may be affected if the availability of food is limited or if the food consumption of the fish is impaired due to stress factors.

None of the laboratory studies considered for this review reported a CF value that was significantly different from the controls (Table 11). Increased CF values were reported in field studies with brown bullhead, dab, English sole and perch from contaminated sites (Table 12). Significantly decreased CF values were only observed in a field study with white sucker (Hodson et al., 1992). The CF responses for all fish species from 7 laboratory studies and 23 field studies are summarized in Fig. 10F. A significant increase in CF values was observed in none of the laboratory studies and 17% of the field studies, while strong increases (> 500% of control) were not observed.

The condition of the liver and of the whole body, as measured with the LSI and CF values, can provide information on potential pollution impacts. Although these parameters are not very sensitive and may be affected by non-pollutant factors (e.g. season, disease, nutritional level), they may serve as an initial screening biomarker to indicate exposure and effects or to provide information on energy reserves (Mayer et al., 1992). The condition indices are quite general and non-specific, but their low cost, ease and rapidity still make them valuable **ERA** tools.

6.12. Toxicological significance of fish biomarkers

A major challenge of biomarker development with respect to **ERA** is to define the significance of biomarker responses in terms of ecological effects of the pollutants. For **ERA** it is not sufficient to show that biomarker levels differ among sites or even that a biomarker level is abnormally high at a site (Suter, 1990). The relationships between responses at different levels of biological organization as well as the relevance and time scales of

these biomarker responses are illustrated in Fig. 11 (Adams et al., 1989). Responses at each level provide information that helps to understand and interpret the relationship between exposure and adverse effects. It is generally accepted that ecological relevance is inversely related to criteria like sensitivity and specificity (De Zwart, 1995). Effects at a higher level of biological organization (population, community, etc.) have a high biological and toxicological relevance, but may be insensitive due to the presence of alternative pathways in an ecosystem.

The question whether or not biochemical alterations, like hepatic EROD induction, are indicative of the development of irreversible toxic effects after prolonged or elevated exposure to the causative agents, is not easy to answer. It is hard to predict, therefore, to what extent the biochemical alterations in a certain population may eventually influence the health of this population or the entire ecosystem. Several comprehensive studies, however, have demonstrated clear relationships between environmental pollution and fish disease (Section 6.11.1). In a study with English sole from the Puget Sound area on the Pacific coast of the USA, for instance, cause-and-effect relationships could be demonstrated between pollution levels (PAHs), exposure (biliary FACs), biomarker responses (increased CYP1A activities) and biological consequences, such as preneoplastic hepatic lesions and hepatic neoplasms (Myers et al., 1994). Similar relationships were reported by Vethaak et al. (1996), who observed increased biliary FAC levels, EROD induction and skin and liver diseases in flounder after long-term exposure to PAH- and PCB-contaminated sediments in a mesocosm study.

One effect that has been clearly demonstrated to be a consequence of enzyme induction is an increased clearance of endogenous substrates (Stegeman and Hahn, 1994). There are, however, numerous possible toxic mechanisms involving altered levels and activities of biotransformation enzymes and receptors. An example is the link between routes I and II of Fig. 6, i.e. increased phase I biotransformation due to the induction of cyt P450 isozymes. Although primarily a pathway for detoxification, there are various ways whereby cyt P450 activity or regulation can elicit toxic effects (Stegeman and Hahn, 1994):

- Many protoxicants, promutagens, and procarcinogens are converted to reactive, toxic products by cyt P450. In some cases, if the capacity of the phase II enzymes (e.g. GSTs and UDPGTs) is insufficient to conjugate these products, electrophilic metabolites can bind to nucleophilic centers in cellular macromolecules (DNA, RNA, proteins) leading to membrane impairment, cellular toxicity, mutations or even carcinogenesis.
- Induction of cyt P450 isozymes can alter the rates of endogenous substrate metabolism, either directly as catalysts or indirectly by competition for reducing equivalents from P450 RED. Since cyt P450 isozymes are involved in the metabolism of steroid hormones, such effects may eventually result in reproductive failure.
- Direct or indirect inhibition or inactivation of P450 by substrates or non-substrates can affect the capacity for xenobiotic metabolism or for endogenous functions, depending on the P450 form inhibited.
- Failure to complete a catalytic cycle following substrate binding, electron transfer and O_2 binding can result in the formation and release of oxygen radicals, which are toxic and mutagenic themselves.
- Receptor mechanisms involved in the regulation of some P450 genes can act in toxic mechanisms independent of the catalytic role of P450.

Establishing the network of interactions between P450 enzyme function and/or regulation and other molecular processes will reveal the full significance of these enzymes in toxic mechanisms (Stegeman and Hahn, 1994). Linkages exist between the P450 system and other biochemical systems, including those involving heme synthesis and degradation, HSPs, steroid receptors, antioxidant enzymes, MTs, oncogenes, tumour suppressor genes, etc. (Fig. 12). These complex interactions illustrate the difficulty of evaluating and assessing the toxicological consequences of impaired biotransformation processes due to exposure to environmental pollutants. Chronically intoxicated fish can reduce the inflow of toxicants by building up morphological barriers and they may activate mechanisms of detoxification (Lindström-Seppä and Pesonen, 1986; Andersson et al., 1988), which make the fish more resistant to toxicants (Bucher and Hofer, 1990). Moreover, many variables without any association to pollution may have an additional impact on the various enzyme systems (see Section 6.13).

There are indications that some phase II enzymes may be induced by the same AhR-mediated mechanism as the phase I enzymes (Owens, 1977; Pickett and Lu, 1989). The ultimate toxicity of any specific xenobiotic chemical is related to the status of induction of phase I as well as phase II enzymes and to the balance between bioactivation and detoxification reactions (i.e. the balance between formation of toxic and non-toxic metabolites) (Vermeulen, 1996). Since the extent of phase II enzyme induction is usually lower than that of cyt P450, the rate of removal of toxic metabolites by conjugation may be reduced relative to the rate of their formation by oxidative reactions (Hodgson, 1994). Formation and persistence of critical genetic lesions may be influenced as much by detoxification and repair processes as by the oxidative metabolism creating the

activated carcinogenic derivative (Stegeman and Hahn, 1994). This balance between bioactivation and detoxification is crucial for the overall assessment of potential hazards due to the exposure to toxic substances, and thus for the **ERA** using fish biomarkers. We would, therefore, like to emphasize the importance of using combinations of existing fish biomarkers, both for the classification of the environmental quality and for the identification of potential environmental risks. An example of an *integrated biomarker* approach, indicative of the balance between bioactivation and detoxification, was recently presented as the *biotransformation index* (*BTI*), expressing the ratio between phase I and II activities as EROD:UDPGT or EROD:GST ratios (Van der Oost et al., 1998). A suggestion for an integrated biomarker for oxidative stress might be to express the ratio between SOD or CAT activities and LPOX, as proposed by Peters et al. (1994). However, these indices, together with other potential integrated biomarkers, should first be validated in both laboratory and field research before they can be incorporated in **ERA** programs.

More research is required before the toxicological significance of changes in levels and activities of stress proteins, MTs and hematological parameters can be clearly evaluated, validated and qualified. The toxicological significance of other biomarkers, such as immunological, reproductive, genotoxic, physiological and morphological parameters, is more evident (Fig. 11).

Despite indications that certain biomarker responses are an early warning for adverse effects on the health or fitness of individual organisms, it will be hard to correlate these responses with effects on population, community or ecosystem levels. The sensitivity of population and community responses to naturally varying environmental factors implies that observed field responses to stress, even in the most carefully selected cases to compare references and polluted sites, cannot be attributed solely to the action of the stressor in question (see Section 6.13). These difficulties have led to the suggestion that generalized risk assessment may be impossible, because each population and community is so tightly integrated into its own particular ecosystem that it is unique (Power and McCarty, 1997).

6.13. Limitations of biomarkers

A successful implementation of biomarkers in environmental monitoring programs requires a good understanding of the mechanisms underlying the responses. The same attributes that make the biomarker approach a powerful tool for biological (effect) monitoring also caution against its rapid and indiscriminate application without the benefit of carefully accumulated experience. Biomarker responses are powerful because they integrate a wide array of environmental, toxicological and ecological factors that control and modulate exposure to, as well as effects of, environmental contaminants. However, these same factors may also complicate interpretation of the significance of the biomarker responses in ways that may not always be anticipated (McCarthy, 1990). Many non-pollution-related variables may have an additional impact on the various enzyme systems, and may thus interfere with biomarker responses when experimental conditions are not thoroughly analyzed or controlled. Examples of such 'confounding' or 'modifying' factors are the organisms' health, condition, sex, age, nutritional status, metabolic activity, migratory behavior, reproductive and developmental status, and population density, as well as factors like season, ambient temperature, heterogeneity of the environmental pollution, etc. Unfortunately, most available toxicity data rarely quantify the potency that confounding factors are likely to exhibit in natural environments (De Kruijf, 1991). Moreover, estimates of confounding factor interactions are scarce, as evidenced by the extensive use of uncertainty factors in risk assessment to address unknowns (Power and McCarty, 1997).

Several examples of the impact of confounding factors on CYP1A indices have been reported in recent years. Highly elevated CYP1A levels were observed in mature male dab collected from off-shore stations with low water temperature due to stratification, while considerably lower CYP1A levels were observed at stations with higher water temperatures in vertically mixed areas, including polluted coastal stations (Sleiderink et al., 1995a). The effect of the water temperature, which was inversely related to the CYP1A levels, appeared to dominate over the effect of PCB contamination, which showed a positive correlation with CYP1A levels. Elevated water temperatures caused changes in antioxidant defenses by increasing SOD activity and decreasing GSH levels and GPOX activity in gills of the freshwater catfish (Parihar et al., 1997). Eggens et al. (1996) demonstrated significant seasonal fluctuations in CYP1A indices in flounder. Sex-related differences in EROD activity were observed during the spawning period (January–March), while sex-related differences in CYP1A protein levels were observed during the post-spawning period (March–June). The activity of endogenous antioxidant systems (GPOX, CAT, GST) in rainbow trout and black bullhead are influenced by age and maturation (Otto and Moon, 1996a). A 7 weeks food deprivation also affected detoxification enzyme activities (notably GST, UDPGT and GRED) in the liver of rainbow trout (Blom et al., 2000). Moreover, nutritional status (long-term food deprivation) influenced PCB tissue levels and biomarker responses (EROD and CYP1A) in arctic charr (Jørgensen et al., 1999). It has been demonstrated that bacterial infections seriously affect the activity of biotransforma-

tion enzymes in carp. Levels of cyt P450 and activities of EROD, UDPGT and GST as well as enzyme induction of these enzymes after 3MC exposure were significantly decreased after infection with Listeria monocytogenes (Chambras et al., 1999) or the bacterial endotoxin lipopolysaccharide (Marionnet et al., 1998). Similar effects have been observed when Mediterranean scorpio fish were maintained under laboratory conditions in the presence of a tropical alga (*Caulerpa taxifolia*), which is known to produce repulsive toxic compounds, such as caulerpenyne derivatives (Uchimura et al., 1999).

A further limitation of using biomarkers for **ERA** is the fact that relationships between biomarker responses and field population-level effects are (presently) not well defined. Ecosystems respond in aggregate to the anthropogenic and natural influences acting on them, as is illustrated in Fig. 13. In addition, since various substances may affect the same biomarkers, most biomarker responses are not specific for individual compounds. Their dose–response behavior is often not predictable due to inadequate basic research. For mobile species such as fish, the actual duration of exposure is uncertain. It is, therefore, important to carefully define reference conditions. In order to avoid potential artefacts, particular care in sampling and handling of samples is required.

7. Summary and conclusions

In the preceding chapters a wide array of bioaccumulation markers and biomarkers, which can be or are being used to demonstrate exposure to and effects of environmental contaminants, have been discussed. Based upon the data presented in this review, there is little doubt that measurement of bioaccumulation and biomarker responses in organisms from contaminated sites offers great promises for providing information that can contribute to environmental monitoring programs designed for surveillance, hazard assessment, regulatory compliance or documenting remediation. The biomarker approach clearly permits acquisition of information that cannot be obtained from the measurement of chemical residues in environmental and biological media. However, in some cases it still has to be demonstrated that biomarkers respond in a regular and predictable manner to increasing exposure and that higher-level effects are predictable from biomarker levels and activities (Suter, 1990). At present, most routine programs on environmental or ecological risk assessment are still based upon measurement of a selected group of chemicals in various environmental compartments, and on comparing the results with legislative threshold values or safety standards (Suter, 1993; De Zwart, 1995). However, xenobiotic chemicals, in the forms found in the environment, often do not by themselves constitute a hazard to indigenous organisms. Once exposure has occurred and substances are bioavailable, a sequence of biological responses may take place. Whether the well-being of the organism is eventually affected will depend upon many factors, some intrinsic (e.g. age, sex, health and nutritional status of the organism) and others extrinsic (e.g. dose, duration, route of exposure to the contaminant and the presence of other chemicals). These intrinsic and extrinsic factors represent barriers to the assessment of exposure and subsequent risk from that exposure (Shugart et al., 1992; Heugens et al., 2001). However, biomarker responses can help to circumvent these problems to a large extent by focusing on relevant molecular events that occur after exposure and metabolism. It is, therefore, of major importance to incorporate the use of biomarkers in assessing potential hazards and risks due to the presence of toxic environmental pollutants.

7.1. Conclusions on fish bioaccumulation markers

In order to elucidate the aquatic behavior of environmental contaminants and to assess exposure of aquatic organisms, fish bioaccumulation markers may be applied. It is virtually impossible to predict the fate of xenobiotic substances with simple partitioning models. When performing risk assessment on potentially hazardous substances the mere consideration of the BCF is insufficient and may be completely misleading (Franke, 1996). Instead, the complexity of bioaccumulation should be considered, which means that data on the overall bioaccumulation process, including toxicokinetics, metabolism, BSAFs, organ-specific bioaccumulation and bound residues, are of greater significance and should be related to critical body burden concentrations for ecotoxicological endpoints. Predictive models may eventually become powerful tools for realistic simulation of pollutant behavior and may be used in the future to protect ecosystems and human health (Farrington, 1991). However, caution must be exercised that the elegance and complexity of a series of coupled mathematical equations does not evoke a false sense that accurate predictive capabilities of wide-ranging applicability are a proven reality. Most models have been tested on relatively few chemicals, biota and ecosystems, and for relatively short periods of time only. Whereas more complex sets of equations provide accurate output at the cost of much input, simple functions are parameter-scarce but yield less precise outcomes (Hendriks, 1995b). The complex of site-specific factors which may influence the bioconcentration, biomagnification, biotransformation and bioavailability of chemicals (e.g. sediment organic carbon, particulate OM, presence of other chemicals), as discussed in Section 5, make it almost inevitable that

serious discrepancies will exist between model-estimated and actual body burdens. Predictive models will, therefore, remain of limited value until they have been applied and validated in 'real world' situations. Rather than relying on models to estimate exposure, BAM (in fish, SPMDs or invertebrates) may allow actual measurement of exposure and a more accurate assessment of adverse health outcomes to the aquatic community (Valberg et al., 1996).

The ideal bioaccumulation marker of exposure is one that is chemical-specific, accurately measurable in trace quantities, measurable in easily sampled biological media or by non-invasive techniques, and is well correlated with previous exposure (Valberg et al., 1996). Contaminant levels in fish may be used as indicators of exposure, although confounding factors like age, sex, season and mobility may have an additional impact on bioaccumulation. Since pollutant levels in fish blood are hard to detect, the most promising fish bioaccumulation markers for exposure assessment are body burdens (generally muscle, liver tissue or whole body) of persistent organic pollutants, like PCBs and OCPs (e.g. HCB, DDTs, drins and HCHs). Since PCDD ad PCDF levels in fish tissues are very low as compared with the sediment levels, their value as bioaccumulation markers remains questionable. Easily biodegradable compounds, such as PAHs and chlorinated phenols, do not tend to accumulate in fish tissues in quantities that reflect the exposure. Exposure to these compounds may be assessed by measurement of biliary metabolite levels. In order to make the results of different studies more comparable, future bioaccumulation research should preferably be carried out using standardized procedures. BSAFs should, therefore, be determined as the ratio between LW-based tissue levels and OM-based sediment levels. A study combining bioaccumulation and ecotoxicity testing, including measurement of biota tissue levels, is considered a promising approach which should be pursued in future research (Franke, 1996). In other words, a reliable ERA procedure preferably encompasses both bioaccumulation markers and biomarkers.

7.2. Conclusions on fish biomarkers

It would be ideal for ERA purposes to have a limited set of specific biomarkers indicating the exposure and assessing the hazards of all major classes of pollutants as well as non-specific biomarkers that assess accurately and completely the health condition of the organism and the ecosystem (Peakall and Walker, 1994). In the 'real world' we have some promising biomarkers to assess exposure and effects of toxic substances, but much more research is required before we approach the ideal situation. It should be emphasized that the most sensitive or selective biomarkers should also be validated for their ecotoxicological relevance before they can be used in standardized biomonitoring programs. In reverse, for the biomarkers with a clear ecological relevance, research should be focused on increasing their sensitivity and selectivity. Since it seems unlikely that biomarkers will replace all chemical analyses, biomonitoring should always be performed in concert with CM. In addition, it is of paramount importance that the potential impact of confounding factors (as discussed in Section 6.13) has to be taken into account when interpreting biomarker data.

In order to assess exposure to or effects of environmental pollutants on aquatic ecosystems, the following suite of biomarkers (biological and biochemical parameters) may be examined in fish:

- biotransformation enzymes (phase I and II);
- oxidative stress parameters;
- biotransformation products;
- stress proteins, MTs and MXR proteins;
- hematological parameters;
- immunological parameters;
- reproductive and endocrine parameters;
- genotoxic parameters;
- neuromuscular parameters;
- physiological, histological and morphological parameters.

All fish biomarkers discussed in this review are evaluated for their potential use in ERA programs, based upon the six criteria that were proposed in Section 2 of the present paper. The evaluation is summarized in Table 13 and elaborated in the following paragraphs.

- The phase I biotransformation enzymes, notably CYP1A, definitely belong to the most sensitive fish biomarkers known at present. The value and feasibility of these biomarkers have been demonstrated in numerous laboratory and field studies with various fish species. Consequently, there is growing interest in using CYP1A induction in fish as a biomarker to indicate the exposure of aquatic organisms to CYP1A-inducing compounds and to evaluate the degree and possible risk of environmental contamination. CYP1A protein levels and EROD activity can be incorporated in **ERA** programs, provided that the experimental design considers all intrinsic and extrinsic variables that may potentially influence this parameter, as well as xenobiotics that inhibit CYP1A activity (e.g. organotins). No sensitive pollution-related responses have been observed in fish for other P450 isozymes (e.g. CYP3A) thus far. At present, CYP1A levels and activities have been validated for use in various areas of (environmental) toxicological research, such as:
  – research on toxic mechanisms of xenobiotics;

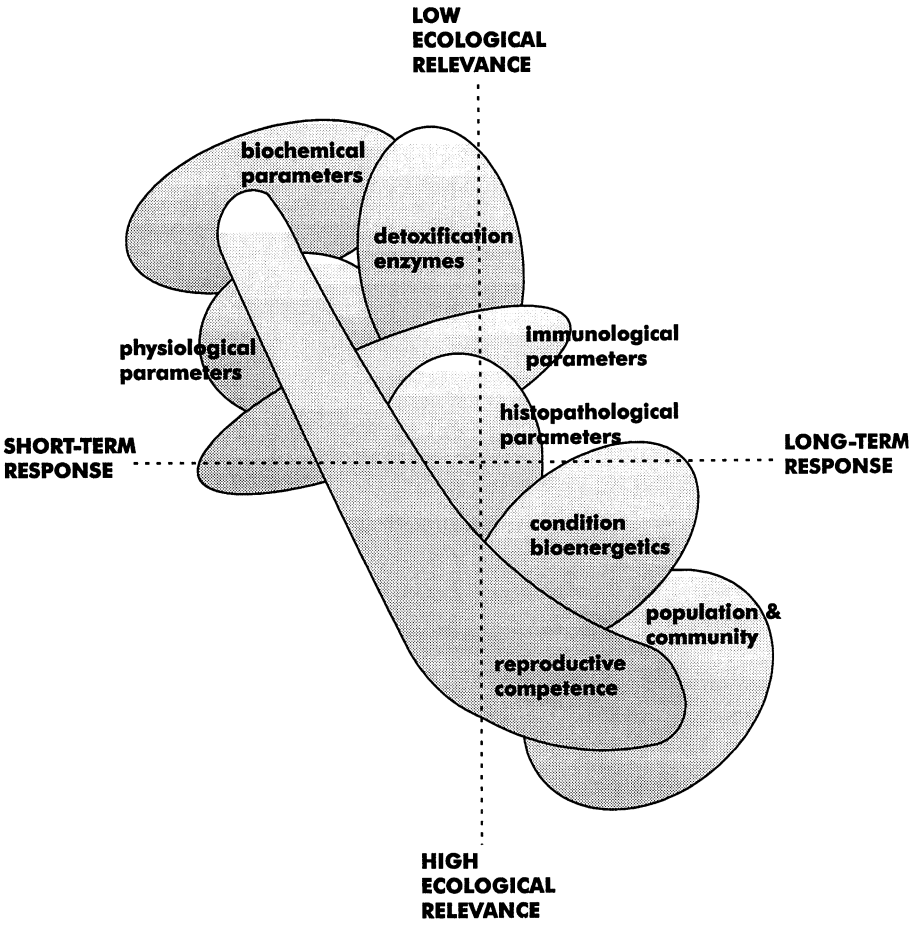

Fig. 11. A theoretical visualization of the relationships between ecological relevance and time-scales of pollutant-induced biomarker responses. Adapted from Adams et al. (1989).

  - toxicity screening;
  - identification of exposure to specific compounds;
  - quantification of impact and exposure of a suite of organic trace pollutants;
  - identifying subtle early effects ('early-warning');
  - health and ecosystem monitoring;
  - triggering of regulatory action.
- Due to the important role of phase II enzymes in detoxification they should be considered in future biomonitoring programs as well, although their sensitivity towards pollutant exposure is limited. An integrated biomarker approach, using combinations of biomarkers such as the biotransformation index (BTI, reflecting the ratio between phase I and II activities), may be more useful for monitoring since this index reflects the balance between bioactivation and detoxification (Van der Oost et al., 1998). The BTI may also be indicative of the susceptibility of organisms to toxic xenobiotics with carcinogenic properties. Further studies are required, however, to improve the usefulness of phase II enzymes as biomarkers in **ERA** procedures; e.g. with regard to baseline activities, non-pollution confounding factors, linkage between enzyme activities and higher levels of organization (e.g. organ or whole animal) and the basic chemistry of purified forms. Current research on the latter topic may reveal specific isoforms of GST and UDPGT that are more sensitive indicators of exposure or effects than the measurement of total activity.
- Since many environmental contaminants exert toxic effects related to oxidative stress, this phenomenon may be another important feature for biomarker development. However, antioxidant enzymes are generally less responsive to pollutants than phase I and II enzymes and the relationships between response and contaminant exposure are still less well established. Their function in detoxification processes, however, motivates continued research on the potential use of SOD, CAT and GRED in **ERA** programs. Other promising parameters that may be useful to indicate oxidative stress are the GSH:GSSG ratio and LPOX products such as aldehydes.
- Biliary levels of (conjugated) metabolites of easily biodegradable xenobiotics, such as PAHs, chlorinated phenols and resin acids, have been validated

as fish biomarkers of exposure and may now be used in **ERA** monitoring programs. Additional research has to be performed to establish the impact of confounding factors and to define clear relationships between biomarker responses and the organisms' health.

- The feasibility of stress proteins (HSPs), MTs and multi xenobiotic resistance (MXR) as fish biomarkers for **ERA** monitoring needs to be further evaluated with additional research, especially as to baseline data on their normal physiological function and the influences of non-environmental factors in the field.
- Increased enzymatic activity, such as that of transaminases, in the blood of fish may be indicative of impaired membranes or cell damage in specific organs, but the initial effects tend to decrease with time. In short-term caging studies, however, the AST activity may be a feasible biomarker for **ERA** purposes. More research is needed, however, to establish clear relationships between transaminase responses and pollutant exposure. Other hematological parameters may be valuable for effect assessment when measured in concert with more sensitive and selective biomarkers.
- Assays to determine adverse effects of xenobiotics on the immune system (e.g. leukocyte and lymphocyte status) may become important non-specific effect biomarkers because of their high ecological significance. More research on cause-and-effect relationships and the influences of confounding non-pollution-related stressors is required, however, before these parameters can be used in **ERA** programs.
- Many environmental contaminants are known to have endocrine-disrupting properties. Effects of pollutants on the endocrine system may be of major importance in **ERA** programs, since impairments in reproductive capability may have a serious impact on fish populations. Sensitive assays, such as levels of VTG and ZRPs are promising, but they have to be further validated for their ecological significance.
- The inhibition of ACHE in fish tissues may be a promising biomarker for the assessment of exposure and effects of complex mixtures of contaminants. Compounds such as OP and carbamate pesticides, have been demonstrated to depress ACHE activity, but various other organic trace pollutants may cause the same effects. More research on the impact of confounding factors and the identification of the (classes of) compounds responsible for observed effects in field trials is required, before this parameter can be used in **ERA** programs.
- The formation of hepatic DNA adducts in fish is considered to be a valid biomarker for exposure to PAHs and for the assessment of potential genotoxic effects. ^{32}P-postlabeling is a very sensitive assay for measuring DNA adduct levels, but it may be considered as too expensive and time-consuming to be applied in routine **ERA** programs. Other DNA modifications, such as strand breaks, may also be feasible as biomarkers for genotoxic chemicals (e.g. COMET assay and DNA unwinding assay).
- When physiological, histological and morphological parameters in fish are affected, this is generally indicative of irreversible damage or disease. These parameters are, therefore, not suitable as early-warning signals in **ERA** programs. Histopathological parameters are, however, relatively easy determined and valuable to evaluate the ecotoxicological significance of other biomarkers. Gross indices, such as the LSI and the CF, can be easily measured and may be used to assess the condition of the liver and the general health of fish, respectively. Immunhistochemical detection of cellular and tissue related abnormalities offer 'earlier' warning signals than are provided by normal pathological and histopathological examination of tissue samples.

A visual overview of the biomarker evaluation (Fig. 14) demonstrates that phase I enzymes (e.g. hepatic EROD and CYP1A), biotransformation products (e.g. biliary PAH metabolites), reproductive parameters (e.g. plasma VTG) and genotoxic parameters (e.g. hepatic DNA adducts) are currently the most valuable fish biomarkers for **ERA** purposes. It has to be emphasized, however, that the value for other biomarkers will be elevated when additional research on certain topics has been performed successfully. Moreover, certain biomarkers will be considered more valuable for specific situations, other that determining the overall environmental quality in **ERA** programs. The overview is simplified, since all criteria are given the same value (good [+], 1; fair [±], 0.5 and poor [−], 0) and no weight factors are included to indicate the importance of the different criteria. It is not possible to propose weight factors that are generally applicable, since these will depend upon the purpose of the investigations the biomarkers are applied for. It is, for instance, not advisable to use the ^{32}P-postlabeling assay for DNA adduct determinations in routine monitoring programs with large amounts of samples because of the expensive and time-consuming assay. If, on the other hand, only a small amount of samples has to be investigated, e.g. to confirm the presence of potentially carcinogenic substances in the environment, this assay will be quite suitable since the first criterion is less important.

The main role of biomarkers in environmental assessment is to determine whether or not, in a specific environment, organisms are physiologically normal (Peakall and Walker, 1994). The use of biomonitoring methods in the control strategies for chemical pollution has several advantages over chemical monitoring (De Zwart, 1995). Firstly, these methods measure effects in

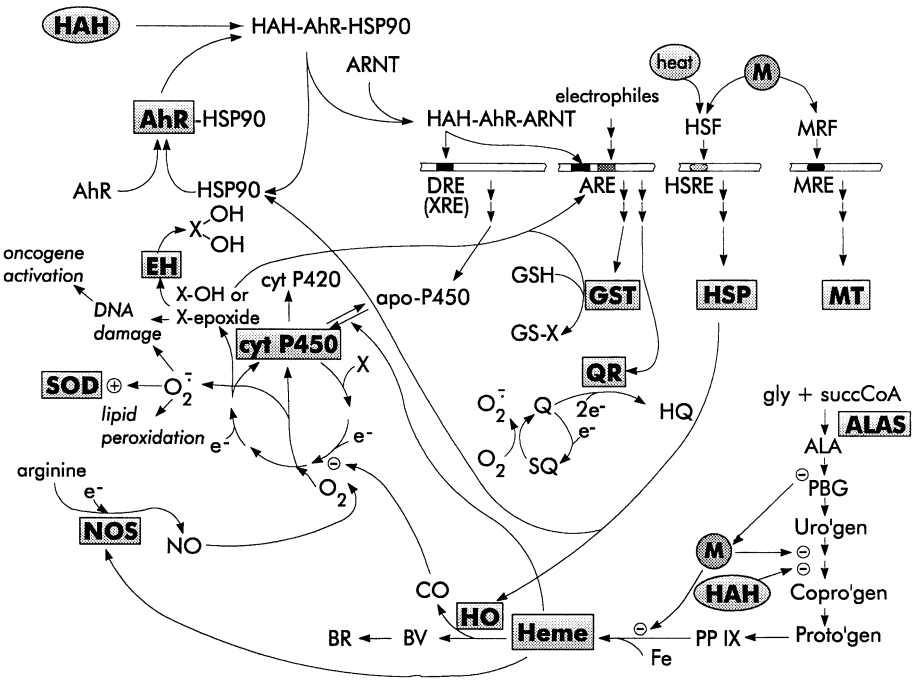

Fig. 12. Linkage between P450 and other biochemical systems. This figure illustrates the complex interactions that are known to occur between biochemical systems involved in responses to pollutant exposure. Further linkages remain to be discovered. AhR, Ah receptor; ALAS, δ-amino-levulinic acid synthase; ARE, antioxidant responsive element (electrophilic response element); ARNT, Ah receptor nuclear translocator; BR, bilirubin; BV, biliverdin; CO, carbon monoxide; DRE, dioxin responsive element; EH, epoxide hydrolase; GSH, glutathione; GST, glutathione *S*-transferase; HAH, halogenated aromatic hydrocarbon; HO, heme oxygenase; HQ, hydroquinone; HSF, heat shock factor; HSP90, 90 kDa heat shock protein; HSRE, heat shock response element; M, metal; MRE, metal responsive element; MRF, metal response factor; MT, metallothionein; NO, nitric oxide; NOS, nitric oxide synthase; cyt P450, cytochrome P450; PP, protoporphyrin; Q, quinone; QR, quinone reductase (a.k.a. DT-diaphorase); SOD, superoxide dismutase; SQ, semiquinone radical; XRE, xenobiotic response element. Adapted from Stegeman and Hahn (1994)

which the bioavailability of the compound(s) of interest is integrated with the concentration of the compounds and their intrinsic toxicity. Secondly, most biological measurements form the only way of integrating the effects on a large number of individual and interactive processes. A disadvantage of most of the biological effect measurements is that it may be very difficult to relate the observed effects to specific aspects of pollution or to effects on the level of populations, communities or ecosystems. In view of the present chemically oriented pollution abatement policies and the need to reveal specific chemical problems, it is most probable that biological effect analysis will never totally replace chemical analyses. The biomarker approach, therefore, should not be considered as a replacement for conventional assessment techniques, but as an important supplementary approach of great ecological relevance (Depledge and Fossi, 1994).

8. Perspectives

McCarthy (1990) proposed a research plan for focusing and coordinating resources necessary to develop and implement a biomarker-based environmental monitoring program, consisting of five major tasks:

- **Task I: preliminary survey: proof of the principle.** The primary objective of this research is to compare the qualitative pattern and quantitative responses of a suite of biomarkers in sentinel species from sites polluted with specific types of contaminants, compared with the responses of organisms from pristine reference sites.
- **Task II: development, standardization and validation of key biomarkers.** The main objective of this research is to standardize protocols for existing biomarker measurements, develop and modify new biomarkers as needed and acquire the fundamental understanding of the relationship between exposure, biomarker responses and adverse effects in sentinel species at the cellular, organismal or population level.
- **Task III: environmental monitoring: biomarkers of exposure.** The objective of this research is to increase understanding of the qualitative pattern and quantitative responses of a suite of biomarkers in sentinel species until a capability is developed to identify the extent of exposure and the nature of the contaminant to which the organisms are being exposed. The emphasis of this task is on developing a capability

to assess *exposure* rather than to evaluate the adverse effects or long-term biological significance of that exposure.

- **Task IV: linking biomarker responses to community level effects.** The objective of this task is to establish and verify the relationships between exposure, changes in rapidly-responding suborganismal biomarkers and long-term adverse effects at the population or community level. The successful completion of this objective will validate the use of molecular, biochemical and physiological level biomarkers as short-term predictors of long-term adverse effects.
- **Task V: linking biomarker responses in sentinel species to human epidemiology.** The objective of this research is to determine if there are correlations between patterns in the extent, nature and geographic distribution of biomarker responses in sentinel species and patterns of epidemiological evidence of increased risk to human health.

The environmental monitoring program that emerges from the research plan proposed by McCarthy (1990) should be the final development of the field monitoring in Task III, illuminated by the laboratory research and development described in Task II, with ecological insights provided by Task IV. In terms of budget and effort McCarthy (1990) anticipated three phases: the *initial phase* (i.e. the implementation of the preliminary survey of Task I), the *learning phase* (i.e. the laboratory studies and field sampling of Tasks II and III, and accumulation of field data for Tasks IV and V) and the *routine monitoring phase* (i.e. the mature phase of the environmental monitoring program). At present, the research on most of the biomarkers described in this review is either in the initial phase (novel biomarkers) or in the learning phase. Peakall and Walker (1994) stated that the biomarkers are in the same stage of development as the analyses of environmental chemicals were in the 1960s, that is, that reasonably precise measurements were made on local samples. For chemical analyses this eventually led to national and international programs, supported by QA and quality control, being set-up. At present, several international programs (e.g. BEEP, CITY FISH and BEQUALM) are being carried out in order to standardize methods for a suite of (fish) biomarkers.

Den Besten (1998) discussed the concepts for implementation of biomarkers in environmental monitoring. Although it has been demonstrated that (fish) biomarkers are useful monitoring tools, it is clear that more information is needed about the relationships between biomarker responses and the health and fitness of organisms, and even more so between biomarker responses and the risks for the ecosystem. With respect to future biomarker research it is important to realize that different concepts are needed for the specific

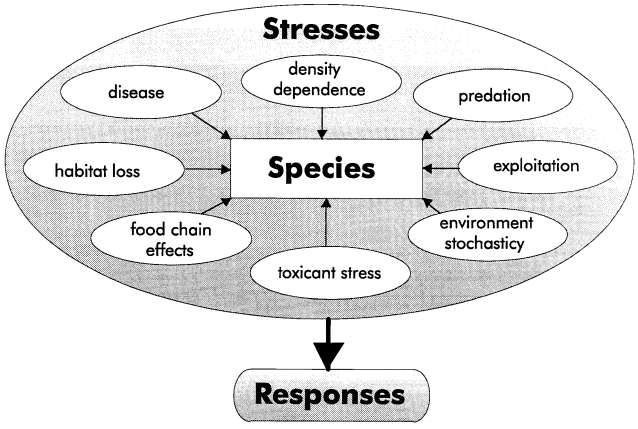

Fig. 13. The complexity of stress–response relationships. The dose–response paradigm, although necessarily simple for experimental practice, does not adequately account for the multiple, simultaneous stressors to which all species are subjected in natural environments. Adapted from Power and McCarty (1997).

purposes of environmental monitoring programs, such as first carrying out cost-effective measurements in a stepwise approach, obtaining insights into the cause of observed effects in the field, studying trends in time or spatial variation, or using biomarker responses as signals of negative effects on the ecosystem. The characteristics and specific research needs for the application of biomarkers to perform screening, diagnosis, trend monitoring (both in time and space) or risk assessment are outlined in Table 14 (adapted from Den Besten, 1998). The use of biomarkers for risk assessment at the community and ecosystem level is still rather ambitious. In the assessment of site-specific risks,

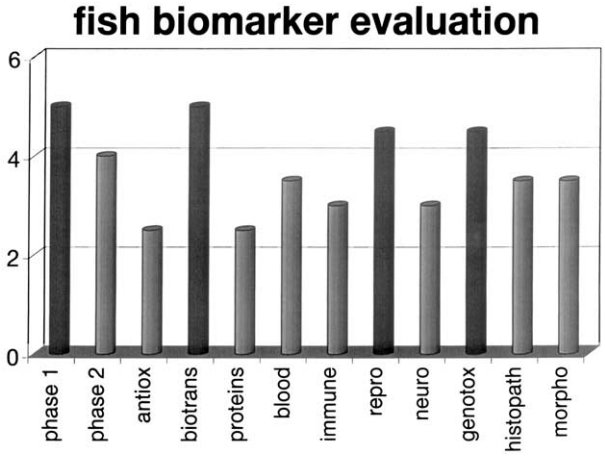

Fig. 14. Visualization of the evaluation of 12 groups of fish biomarkers by adding up the results of a judgment (good, 1; fair, 0.5 and poor, 0) using six biomarker criteria. phase I, phase I biotransformation enzymes; phase II, phase II biotransformation enzymes; antiox, antioxidant enzymes; biotrans, biotransformation products; proteins, stress proteins, metallothioneins & MXR-proteins; blood, serum transaminases; immune, immunological parameters; repro, reproductive parameters; genotox, genotoxic parameters; histopath, histopathological parameters; morpho, morphological parameters.

Table 13
Fish biomarker evaluation, using six biomarker criteria

Criteria	Biomarkers >											
	Phase 1	Phase 2	Antiox	Biotrans	Proteins	Blood	Immune	Repro	Neurotox	Genotox	Histopath	Morpho
	EROD CYP1A	GST UDPGT	SOD CAT	bile FAC	HSP MT MXR	AST ALT	Leuko lympho	VTG ZRP	ACHE	DNA add DNA dam	Tissue	LSI CF
Reliable and easy assay	+	+	+	+	+	+	±	+	+	−	±	+
Sensitive to pollution	+	±	−	+	±	±	±	+	±	+	−	−
Known baseline data	+	±	±	+	−	±	±	±	±	+	+	+
Confounding factors	±	±	±	±	−	±	±	±	−	±	±	±
Response mechanism	+	±	−	+	±	−	−	+	±	+	±	±
Toxicological significance	±	+	±	±	±	+	+	±	±	+	+	±
Overall	5.0+	4.0+	2.5+	5.0+	2.5+	3.5+	3.0+	4.5+	3.0+	4.5+	3.5+	3.5+

Symbols: +, good; ±, fair; −, poor. Abbriviations: phase I, phase I biotransformation enzymes; phase II, phase II biotransformation enzymes; antiox, antioxidant enzymes; biotrans, biotransformation products; proteins, stress proteins, metallothioneins & MXR-proteins; blood, serum transaminases; immune, immunological parameters; repro, reproductive parameters; genotox, gemotoxic parameters; histopath, histopathological parameters; morpho, morphological parameters (individual biomarker abbriviations in text of Section 6).

Table 14
Concepts for the implementation of biomarkers in environmental monitoring (adapted from Den Besten, 1998)

Concept	Purpose	Use of biomarkers	Specific needs for further implementation of this concept
Screening	Cost-effective use of biomarkers as a first screening step	Simple biomarker measurements in a tiered approach (together with chemical analyses and bioassays)	Sensitive biomarkers with specificity for certain types of contaminants or certain types of effect mechanism
	Signalling effects (early-warning system)	Biomarkers of exposure and of toxic effect Biomarkers incorporated in bioassays	
Diagnosis	To signal the possible cause for observed adverse effects in populations of a certain species	Large batteries of biomarkers (effect indicators, in part non-invasive) are applied in studies on animals from affected populations; responses are compared with those in reference populations	Development of a suite of biomarkers
			Insight in the relationship between change in homeostasis (healthy state), biomarker response and fitness or performance of the organism
Trend monitoring	Study biomarker responses in time	Repeated sampling and biomarker measurements in time	Knowledge of the influence of confounding factors
	To compare changes of biomarker responses in time with quality objectives; check improvement after remedial action		Translation of existing quality objectives in biomarker response criteria
Monitoring of spatial variation	Assessment of site-specific biomarker responses (gradients)	Comparison of biomarker responses between sites	Biomarkers must be applicable in common species (for a region considered) Knowledge on confounding factors; reference values
Site-specific risk assessment	Study of contaminant bioavailability and related risks at polluted sites (hot spots)	Biomarkers as indicators of exposure	Biomarkers must be applicable in local species or in situ bioassays
	Signalling effects at higher levels of organisation	Use of selected biomarkers as early-warning signals of adverse effects on the individual, population or community level	Responses of predictive biomarkers must be related to effects on higher organisation levels
Risk assessment: ecosystem structure and ecosystem function	Predicting effects on ecosystem level	Application of selected biomarkers in a range of species	Biomarkers must be applicable in different species (including key organisms)
		Estimation of the fraction potentially affected species (PAF) Application of selected biomarkers in key organisms	Sensitivity comparisons between species

information from biomarkers should be used in combination with other biological data (e.g. species abundance) and chemical data (Den Besten, 1998). Ellis (2000) discussed the advantages and limitations of four different risk assessment approaches (chemical specific limits, biological assessment, direct toxicity assessment [DTA] and biomarker techniques) in urban receiving waters. The inability of DTA procedures to satisfactorily evaluate chronic, sub-lethal risks increased the interest in using in situ biomarkers for the fingerprinting of stress-response properties as a means of diagnosing risk assessment for integrated urban runoff management (Ellis, 2000).

Due to the interdisciplinary nature of biomarker studies and the need for integration of numerous research specialties, long-term progress will be accelerated by general agreement on a common research strategy. Future research should be focused on the possible implementation of biomarkers in environmental monitoring programs. However, since monitoring information requirements and monitoring objectives are very situation-specific and are strongly dependent on national water management policies, it is unlikely that the near future will show a global trend towards unification of standard biomonitoring protocols (De Zwart, 1995). The ultimate objective for applied envir-

onmental research should be to make biomarkers more usable in ERA. We, therefore, want to propose some guidelines for ecotoxicological research programs, in order to actually incorporate fish biomarkers in **ERA** monitoring:

- Efforts have to be made to design a set of fish biomarkers, covering the exposure and/or early effects of the entire spectrum of potentially toxic substances which may be present in the aquatic environment.
- All biological and biochemical parameters that may be used to assess exposure to and effects of environmental pollutants have to be objectively evaluated according to the six biomarker criteria, as proposed in Section 2.
- It is essential that more research should be carried out to demonstrate relationships between biomarker responses and effects on pathology, survival, growth or reproduction at the level of individual organisms (Den Besten, 1998). The knowledge on these relationships should then be used to design numerical standards for biomarkers.
- Standard procedures should be developed with regard to sampling, sample treatment, assay conditions, etc. preferably in international programs such as BEEP, CITY FISH and BEQUALM.
- The selection of indicator species is going to be, at least to some extent, site-specific. Instead of investigating the same responses in thousands of fish species, however, national as well as international agreements should be made on the selection of a few sentinel species. In these indicator species a suite of candidate biomarkers must be thoroughly investigated and validated in both laboratory and field experiments.
- Interpretation of results obtained by research on feral fish will remain complicated since the impact of the various factors that are possibly affecting biomarker responses cannot always be established due to the unknown life history of the fish specimens. In this respect the feasibility of caging or mesocosm experiments with cultured fish should be examined further.
- In order to facilitate a reliable comparison of results of caging and mesocosm studies between research groups, it is recommended to apply recently developed standardized fish lines, e.g. the mirror carp (Gimeno et al., 1996), consisting of genetically identical animals of the same age and gender. Responses in these caged individuals introduced in polluted sites would: (A). confirm the relationship between specific sites and the pattern of biomarkers observed in wild animals; (B). provide a finer level of geographic resolution in distinct areas of the site; and/or (C). test specific hypotheses about the contribution of different routes of exposure (McCarthy and Shugart, 1990).
- In order to reduce the amount of fish that has to be killed for biomarker research, emphasis has to be put on the development of non-destructive and non-invasive biomarkers. As an alternative to fish biomarkers, artificial devices, such as passive sampling (e.g. SPMD) combined with cell-line bioassays, may be used to monitor the water quality. Pilot experiments in this field have to be compared with studies on fish, in order to correlate the results to data from the 'real world'.
- New and promising developments in the biomarker field are the so-called 'genomics' and 'proteomics'. Genomics is based upon the application of DNA microarrays that allow the expression of hundreds to many thousands of genes to be monitored simultaneously, thus providing a broad and integrated picture of the way an organism responds to a changing environment (Gracey et al., 2001). The entire protein complement of the genome, the 'proteome', can now be analyzed for changes associated with specific treatments, using 'peptide mass profiling', a combination of two-dimensional gel electrophoresis and mass spectrometry (Shepard et al., 2000). Proteomics research provided certain protein expression signatures (PESs), which are specific sets of proteins, present or absent, indicating specific toxicity profiles.

In conclusion, it can be stated that fish biomarkers are promising tools for ERA, as supplements to existing chemical measures. Much work has to be done, however, in order to test and interpret biomarker responses and to develop acceptable QA procedures. Only when both scientific and legal credibility of this information is established, the biomarker techniques can be fully applied in routine monitoring programs. It seems obvious that CM alone is insufficient for a reliable classification of water quality. Therefore, the efforts to incorporate biological compounds to the **ERA** research will eventually be worthwhile.

References

Aaltonen, T.M., Jokinen, E.I., Salo, H.M., Markkula, S.E., Lammi, R., 2000a. Modulation of immune parameters of roach, *Rutilus rutilus*, exposed to untreated ECF and TCF bleached pulp effluents. Aquat. Toxicol. 47, 277–289.

Aaltonen, T.M., Jokinen, E.I., Lappivaara, J., Markkula, S.E., Salo, H.M., Leppänen, H., Lammi, R., 2000b. Effects of primary- and secondary-treated bleached kraft mill effluents on the immune system and physiological parameters of roach. Aquat. Toxicol. 51, 55–67.

Aas, E., Baussant, T., Balk, L., Liewenborg, B., Andersen, O.K., 2000. PAH metabolites in bile, cytochrome P4501A and DNA adducts as

environmental risk parameters for chronic oil exposure: a laboratory experiment with Atlantic cod. Aquat. Toxicol. 51, 241–258.

Adams, S.M., Shepard, K.L., Greeley, M.S., Jr, Ryon, M.G., Jimenez, B.D., Shugart, L.R., McCarthy, J.F., Hinton, D.E., 1989. The use of bioindicators for assessing the effects of pollutant stress in fish. Mar. Environ. Res. 28, 459–464.

Adams, S.M., Shugart, L.R., Southworth, G.R., Hinton, D.E., 1990. Application of bioindicators in assessing the health of fish populations experiencing contaminant stress. In: McCarthy, J.F., Shugart, L.R. (Eds.), Biomarkers of Environmental Contamination. Lewis Pub, pp. 333–353.

Adams, S.M., Crumby, W.D., Greeley, M.S., Jr, Shugart, L.R., Saylor, C.F., 1992. Responses of fish populations and communities to pulp mill effluents: a holistic assesment. Ecotoxicol. Environ. Safe 24, 347–360.

Addison, R.F., Edwards, A.J., 1988. Hepatic microsomal monooxygenase activity in flounder *Platichthys flesus* from polluted sites in Langesundfjord and from mesocosms experimentally dosed with diesel oil and copper. Mar. Ecol. Prog. Ser. 46, 51–54.

Addison, R.F., Sadler, M.C., Lubet, R.A., 1987. Absence of microsomal pentyl- or benzyl-resorufin *O*-dealkylase induction in rainbow trout (*Salmo gairdneri*) treated with phenobarbitone. Biochem. Pharmacol. 36, 1183–1184.

Adolph, L., Lorenz, R., 1978. Enzymdiagnostik bei Herz- Leber- und Pankreaserkrankungen (in German). Karger AG, Basel, Switserland.

Agradi, E., Baga, R., Cillo, F., Ceradini, S., Heltai, H., 2000. Environmental contaminants and biochemical response in eel exposed to Po river water. Chemosphere 41, 1555–1562.

Ahokas, J.T., Holdway, D.A., Brennan, S.E., Goudey, R.W., Bibrowska, H.B., 1994. MFO activity in carp (*Cyprinus carpio*) exposed to treated pulp and paper mill effluent in Lake Coleman, Victoria, Australia, in relation to AOX, EOX, and muscle PCDD/PCDF. Environ. Toxicol. Chem. 13, 41–50.

Allen, P., 1993. Effects of acute exposure to cadmium (II) chloride and lead (II) chloride on the haematological profile of *Oreochromis aureus* (Steindachner). Comp. Biochem. Physiol. 105C, 213–217.

Andaya, A.A., Di Giulio, R., 1987. Acute toxicities and hemotological effects of two substituted naphtoquinones in channel catfish. Arch. Environ. Contam. Toxicol. 16, 233–238.

Andersson, T., Pesonen, M., Johansson, C., 1985. Differential induction of cytochrome P-450-dependent monooxygenase, epoxide hydrolase, glutathione transferase and UDP glucuronosyl transferase activities in the liver of rainbow trout by β-naphthoflavone or Clophen A50. Biochem. Pharmacol. 34, 3309–3314.

Andersson, T., Förlin, L., Härdig, J., Larsson, Å., 1988. Physiological disturbances in fish living in coastal waters polluted with bleached kraft pulp mill effluents. Can. J. Fish. Aquat. Sci. 45, 1525–1536.

Ankley, G.T., Blazer, V.S., Reinert, R.E., Agosin, M., 1986. Effects of Aroclor 1254 on cytochrome P450-dependent monooxygenase, glutathione *S*-transferase, and UDP-glucuronosyltransferase activities in channel catfish liver. Aquat. Toxicol. 9, 91–103.

Ankley, G.T., Cook, P.M., Carlson, A.R., Call, D.J., Swenson, J.A., Corcoran, H.F., Hoke, R.A., 1992. Bioaccumulation of PCBs from sediments by oligochaetes and fishes: comparison of laboratory and field studies. Can. J. Fish. Aquat. Sci. 49, 2080–2085.

Arcand-Hoy, L.D., Metcalfe, C.D., 1999. Biomarkers of exposure of brown bullheads (*Ameiurus nebulosos*) to contaminants in the lower Great Lakes, North America. Environ. Toxicol. Chem. 18, 740–749.

Ariese, F., Kok, S.J., Verkaik, M., Gooijer, C., Velthorst, N.H., Hofstraat, J.W., 1993a. Synchronous fluorescence spectrometry of fish bile; a rapid screening method for the biomonitoring of polycyclic aromatic hydrocarbons in the aquatic environment. Aquat. Toxicol. 26, 273–286.

Ariese, F., Kok, S.J., Verkaik, M., Hoornweg, G.P., Gooijer, C., Velthorst, N.H., Hofstraat, J.W., 1993b. Chemical derivatization and Shpol'skii spectrofluorimetric determination of benzo[a]pyrene metabolites in fish bile. Anal. Chem. 65, 1100–1106.

Arkoosh, M.R., Clemons, E., Meyers, M., Casillas, E., 1994. Suppression of B-cell mediated immunity in juvenile chinook salmon (*Oncorhynchus tshawytscha*) after exposure to either a polycyclic aromatic hydrocarbons or to polychlorinated biphenyls. Immunopharmacol. Immunotoxicol. 16, 293–314.

Armstrong, D.T., 1990. Environmental stress and ovarian function. Biol. Reprod. 34, 29–39.

Arnold, H., Pluta, H.-J., Braunbeck, T., 1995. Simulteneous exposure of fish to endosulfan and disulfoton in vivo: ultrastructural, stereological and biochemical reactions in hepatocytes of male rainbow trout (*Oncorhynchus mykiss*). Aquat. Toxicol. 33, 17–43.

Arukwe, A.E., Goksøyr, A., 1997. Fish zona radiata (egg shell) protein—a sensitive biomarker for environmental estrogens. Environ. Health Perspect. 105, 418–422.

Arukwe, A.E., Celius, T., Walther, B.T., Goksøyr, A., 2000. Effects of xenoestrogen treatment on *zona radiata* protein and vitellogenin expression in Atlantic salmon (*Salmo salar*). Aquat. Toxicol. 49, 159–170.

Baan, R.A., Steenwinkel, M.-J.S.T., Van den Berg, P.T.M., Roggeband, R., Van Delft, J.H.M., 1994. Molecular dosimetry of DNA damage induced by polycyclic aromatic hydrocarbons; relevance for exposure monitoring and risk assessment. Hum. Exp. Toxicol. 13, 880–887.

Bagenal, T.B., Tesch, F.W., 1978. Methods for assessment of fish production in fresh waters. In: Bagenal, T.B. (Ed.), Age and Growth. Blackwell Scientific Publications, Oxford, pp. 101–136.

Bagnasco, M., Camoirano, A., De Flora, S., Melodia, F., Arillo, A., 1991. Enhanced liver metabolism of mutagens and carcinogens in fish living in polluted seawater. Mutat. Res. 262, 129–137.

Bailer, A.J., Walker, S.E., Venis, K.J., 2000. Estimating and testing bioconcentration factors. Environ. Toxicol. Chem. 19, 2338–2340.

Bainy, A.C.D., Saito, E., Carvalho, P.S.M., Junquieira, V.B.C., 1996. Oxidative stress in gill, erythrocytes, liver and kidney of Nile tilapia (*Oreochromis niloticus*) from a polluted site. Aquat. Toxicol. 34, 151–162.

Bainy, A.C.D., Woodin, B.R., Stegeman, J.J., 1999. Elevated levels of multiple cytochrome P450 forms in tilapia from Billings Reservoir—Sao Paulo, Brasil. Aquat. Toxicol. 44, 289–305.

Balint, T., Szegletes, T., Szegletes, Z., Halasy, K., Nemcsók, J., 1995. Biochemical and subcellular changes in carp exposed to the organophosphorus methidathion and the pyrethroid deltamethrin. Aquat. Toxicol. 33, 279–295.

Balk, L., Larsson, Å., Förlin, L., 1996. Baseline studies of biomarkers in the feral female perch (*Perca fluviatilis*) as tools in biological monitoring of anthropogenic substances. Mar. Environ. Res. 42, 203–208.

Banca, L., Deer, K.A., Nemcsok, J., Abraham, M., 1997. In vivo and in vitro effects of deltamethrin on cytochrome P450 monooxygnase activity in carp (*Cyprinus carpio* L.) liver. J. Environ. Sci. Health B 32, 789–802.

Bard, S.M., 2000. Multixenobiotic resistance as a cellular defense mechanism in aquatic organisms. Aquat. Toxicol. 48, 357–389.

Bard, S.M., Woodin, B., Stegeman, J.J., 1998. Induction of the multixenobiotic resistance transporter and cytochrome P450 1A in intertidal fish exposed to environmental contaminants. Toxicol. Sci. 42, 16–26.

Bascietto, J., Hinckley, D., Plafkin, J., Slimak, M., 1990. Ecotoxicity and ecological risk assessment; regulatory applications at EPA. Environ. Sci. Technol. 24, 10–15.

Baumann, P.C., Harshbarger, J.C., 1995. Decline in liver neoplasms in wild brown bullhead catfish after coking plant closes and environmental PAHs plummet. Environ. Health Perspect. 103, 168–170.

Bayne, B.L., Brown, D.A., Burns, K., Dixon, D.R., Ivanovici, A., Livingstone, D.A., Lowe, D.M., Moore, M.N., Stebbing, A.R.D.,

Widdings, J., 1985. The Effects of Stress and Pollution on Marine Animals. Praeger, New York, USA.

Beauvais, S.L., Jones, S.B., Brewer, S.K., Little, E.E., 2000. Physiological measures of neurotoxicity of diazinon and malathion to larval rainbow trout (*Oncorhynchus mykiss*) and their correlation with behavioral measures. Environ. Toxicol. Chem. 19, 1875–1880.

Belfroid, A.C., Seinen, W., van den Berg, M., Hermens, J., van Gestel, C.A.M., 1995. Uptake bioavailability and elimination of hydrophobic compounds in earthworms (*Eisenia andrei*) in field contaminated soil. Environ. Toxicol. Chem. 14, 605–612.

Belfroid, A.C., Sijm, D.T.H.M., van Gestel, C.A.M., 1996. Bioavailability and toxicokinetics of hydrophobic aromatic compounds in benthic and terrestrial invertebrates. Environ. Rev. 4, 276–299.

Bello, S.M., Franks, D.G., Stegeman, J.J., Hahn, M.E., 2001. Acquired resistence to Ah receptor agonists in a population of Atlantic killifish (*Fundulus heteroclitus*) inhabiting a marine superfund site: in vivo and in vitro studies on the inducibility of xenobiotic metabolizing enzymes. Toxicol. Sci. 60, 77–91.

Bergqvist, P.-A., Strandberg, B., Ekelund, R., Rappe, C., Granmo, A., 1998. Temporal monitoring of organochlorine compounds in seawater by semipermeable membranes following a flooding episode in Western Europe. Environ. Sci. Technol. 32, 3887–3892.

Beyer, J., 1996. Fish biomarkers in marine pollution monitoring; evaluation and validation in laboratory and field studies. Academic thesis, University of Bergen, Norway.

Beyer, J., Sandvik, M., Hylland, K., Fjeld, E., Egaas, E., Aas, E., Skaare, J.U., Goksøyr, A., 1996. Contaminant accumulation and biomarker responses in flounder (*Platichthys flesus* L.) and Atlantic cod (*Gadus morhua* L.) exposed by caging to polluted sediments in Sørfjorden, Norway. Aquat. Toxicol. 36, 75–98.

Beyer, J., Sandvik, M., Skaare, J.U., Egaas, E., Hylland, K., Waagbø, R., Goksøyr, A., 1997. Time- and dose-dependent biomarker responses in flounder (*Platichthys flesus* L.) exposed to benzo[a]pyrene, 2,3,3′,4,4′,5-hexachlorobiphenyl (PCB-156) and cadmium. Biomarkers 2, 35–44.

Bhaskaran, A., May, D., Rand-Weaver, M., Tyler, C., 1999. Fish p53 as a possible biomarker for genotoxins in the aquatic environment. Environ. Mol. Mutagen. 33, 177–184.

Blom, S., Förlin, L., 1997. Effects of PCB on xenobiotic biotransformation enzyme activitie in the liver and 21-hydroxylation in the head kidney of juvenile rainbow trout. Aquat. Toxicol. 39, 215–230.

Blom, S., Andersson, T.B., Förlin, L., 2000. Effects of food deprivation and handling stress on head kidney 17α-hydroxyprogesterone 21-hydroxylase activity, plasma cortisol and the activities of liver detoxification enzymes in rainbow trout. Aquat. Toxicol. 48, 265–274.

Boese, B.L., Lee, H., II, Specht, D.T., 1988. Efficiency of uptake from water by the tellinid clam, *Macoma nasuta*. Aquat. Toxicol. 12, 345–356.

Boese, B.L., Lee, H., II, Specht, D.T., Randall, R.C., Winsor, M.H., 1990. Comparison of aqueous and solid-phase uptake for hexachlorobenzene in the tellinid clam, *Macoma nasuta* (Conrad): a mass balance approach. Environ. Toxicol. Chem. 9, 221–231.

Booij, K., Sleiderink, H.M., Smedes, F., 1998. Calibrating the uptake kinetics of semipermeable membrane devices using exposure standards. Environ. Toxicol. Chem. 17, 1236–1245.

Boon, J.P., Everaarts, J.M., Hillebrand, M.T.J., Eggens, M.L., Pijnenburg, J., Goksøyr, A., 1992. Changes in levels of hepatic biotransformation enzymes and haemoglobin levels in female plaice (*Pleuronectes platessa*) after oral administration of a technical polychlorinated biphenyl mixture (Clophen A40). Sci. Total Environ. 114, 113–133.

Braunbeck, T., Völkl, A., 1991. Induction of biotransformation in the liver of eel (*Anguilla anguilla* L.) by sublethal exposure to dinitro-*o*-cresol: an ultrastructural and biochemical study. Ecotoxicol. Environ. Safe 21, 109–127.

Breck, J.E., 1985. Comment on 'Fish/sediment concentration ratios for organic compounds'. Environ. Sci. Technol. 19, 198–199.

Brevik, E.M., Grande, M., Knutzen, J., Polder, A., Skaare, J.U., 1996. DDT contamination of fish and sediments from lake Ørsjøen, southern Norway: comparison of data from 1975 and 1994. Chemosphere 33, 2189–2200.

Brown, J.F., Jr, 1992. Metabolic alterations of PCB residues in aquatic fauna: distributions of cytochrome P4501A- and P4502B-like activities. Mar. Environ. Res. 34, 261–266.

Brown, J.F., Jr, 1994. Determination of PCB metabolic, excretion, and accumulation rates for use as indicators of biological response and relative risk. Environ. Sci. Technol. 28, 2295–2305.

Brown, E.R., Sinclair, T., Keith, L., Beamer, P., Hazdra, J.J., Nair, V., Callaghan, O., 1977. Chemical pollutants in relation to diseases in fish. Ann. New York Acad. Sci. 298, 535–546.

Brumley, C.M., Haritos, V.S., Ahokas, J.T., Holdway, D.A., 1998. The effects of exposure duration and feeding status on fish bile metabolites: implications for biomonitoring. Ecotoxicol. Environ. Safe 39, 147–153.

Bucheli, T.D., Fent, K., 1995. Induction of cytochrome P450 as a biomarker for environmental contamination in aquatic ecosystems. Crit. Rev. Environ. Sci. Technol. 25, 201–268.

Bucher, F., Hofer, R., 1990. Effects of domestic wastewater on serum enzyme activities of brown trout (*Salmo trutta*). Comp. Biochem. Physiol. 97C, 381–385.

Bucher, F., Hofer, R., Krumschnabel, G., Doblander, C., 1993. Disturbances in the prooxidant–antioxidant balance in the liver of bullhead (*Cottus gobio* L.) exposed to treated paper mill effluents. Chemosphere 27, 1329–1338.

Buchmann, A., Wannemacher, R., Kulzer, E., Buhler, D.R., Bock, W., 1993. Immunohistochemical localization of the cytochrome P450 isoenzymes LMC2 and LM4B (P4501A1) in 2,3,7,8-tetrachlorodibenzo-*p*-dioxin-treated zebrafish (*Brachidanio rerio*). Toxicol. Appl. Pharmacol. 123, 160–169.

Burchell, B., Coughtrie, M.W.H., 1989. UDP-glucuronosyltransferases. Pharmacol. Ther. 43, 261–271.

Burgeot, T., Bocquené, G., Pingray, G., Godefroy, D., Legrand, J., Dimeet, J., Marco, F., Vincent, F., Henocque, Y., Jeanneret, H.O., Galgani, F., 1994. Monitoring biological effects of contamination in marine fish along French coasts by measurement of ethoxyresorufin-*O*-deethylase activity. Ecotoxicol. Environ. Safe 29, 131–147.

Burgeot, T., Bocquéné, G., Porte, C., Dimeet, J., Santella, R.M., Dimeet, J., Santella, R.M., Garcia de la Parra, L.M., Pftol-Leszkowicz, A., Raoux, C., Galgani, F., 1996. Bioindicators of pollutant exposure in the northwestern Mediterranean Sea. Mar. Ecol. Prog. Ser. 131, 125–141.

Burke, M.D., Mayer, R.T., 1974. Ethoxyresorufin: direct fluorometric assay of a microsomal *O*-dealkylation which is preferentially inducible by 3-methylcholanthrene. Drug Metab. Dispos. 2, 583–588.

Burkhard, L.P., 1998. Comparison of two models for predicting bioaccumulation of hydrophobic organic chemicals in a Great Lakes food web. Environ. Toxicol. Chem. 17, 383–393.

Burkhard, L.P., Lukasewycz, M.T., 2000. Some bioaccumulation factors and biota-sediment accumulation factors for polycyclic aromatic hydrocarbons in lake trout. Environ. Toxicol. Chem. 19, 1427–1429.

Burns, K.A., 1976. Microsomal mixed-function oxidases in an estuarine fish, *Fundulus heteroclitus*, and their induction as a result of environmental contamination. Comp. Biochem. Biophys. 53B, 443–446.

Butte, W., Fox, K., Zauke, G.P., 1991. Kinetics of bioaccumulation and clearance of isomeric hexachlorocyclohexanes. Sci. Total Environ. 109/110, 377–382.

Cabana, G., Rasmussen, J.B., 1994. Modelling food chain structure and contaminant bioaccumulation using stable nitrogen isotopes. Nature 372, 255–257.

Cachot, J., Galgani, F., Vincent, F., 1998. Production of polyclonal antibody raised against recombinant flounder p53 protein. Comp. Biochem. Physiol. C Pharmacol. Toxicol. Endocrinol. 120, 351–356.

Calabrese, E.J., 1991. Multiple Chemical Interactions. Lewis Publishers, Chelsea, MI, USA, p. 704.

Campbell, P.M., Devlin, R.H., 1996. Expression of CYP1A1 in livers and gonads of Pacific salmon: quantification of mRNA levels by RT-cPCR. Aquat. Toxicol. 34, 47–69.

Cairns, J. Jr., McCormick, P.V., 1992. Developing an ecosystem-based capability for ecological risk assessments. Eviron. Profession 14, 186–196.

Castaño, A., Carbonelli, G., Carballo, M., Fernandez, C., Boleas, S., Tarazona, J.V., 1998. Sublethal effects of repeated intraperitoneal cadmium injections on rainbow trout (*Oncorhynchus mykiss*). Ecotoxicol. Environ. Safe 41, 29–35.

Celander, M., 1993. Induction of cytochrome P450 in teleost fish; with emphasis on the CYP1 gene family. Academic thesis, University of Göteborg, Sweden.

Celander, M., Förlin, L., 1991. Catalytic activity and immunochemical quantification of hepatic cytochrome P450 in *b*-naphtoflavone and isosafrol treated rainbow trout (*Oncorhynchus mykiss*). Fish Physiol. Biochem. 9, 189–197.

Celander, M., Ronis, M., Förlin, L., 1989. Initial characterisation of a constitutive cytochrome P450 isoenzyme in rainbow trout liver. Mar. Environ. Res. 28, 9–13.

Celander, M., Leaver, M.J., George, S.G., Förlin, L., 1993. Induction of cytochrome P450 1A1 and conjugating enzymes in rainbow trout (*Oncorhynchus mykiss*) liver: a time course study. Comp. Biochem. Physiol. 106C, 343–349.

Celander, M., Näf, C., Broman, D., Förlin, L., 1994. Temporal aspects of induction of hepatic cytochrome P450 1A and conjugating enzymes in the viviparous blenny (*Zoarces viviparus*) treated with petroleum hydrocarbons. Aquat. Toxicol. 29, 183–196.

Chambers, J.E., 1979. Induction of microsomal mixed-function oxidase system components in striped mullet by short-term exposure to crude oil. Toxicol. Lett. 4, 227–230.

Chambras, C., Marionnet, D., Taysse, L., Deschaux, P., Moreau, J., Bosgiraud, C., 1999. Xenobiotic-metabolizing enzymes in carp (*Cyprinus carpio*) liver, spleen, and head kidney following experimental Listeria monocytogenes infection. J. Toxicol. Environ. Health 56, 205–219.

Chapman, P.M., 1990. The sediment quality Triad approach to determining pollution-induced degradation. Sci. Total Environ. 97/98, 815–825.

Chaudhary, P.M., Roninson, I.B., 1992. Activation of MDR1 (*P*-glycoprotein) gene expression in human cells by protein kinase C agonists. Oncol. Res. 4, 281–290.

Chipman, J.K., March, J.W., Livingstone, D.R., Evans, B., 1992. Genetic toxicology in dab, L*imanda limanda*, from the North Sea. Mar. Ecol. Prog. Ser. 91, 121–126.

Chipman, J.K., Davies, J.E., Parsons, J.L., Nair, J., O'Neill, G., Fawell, J.K., 1998. DNA oxidation by potassium bromate: a direct mechanism or linked to lipid peroxidation. Toxicology 126, 93–102.

Chen, J.D., Yew, F.H., Li, G.C., 1988. Thermal adaptation and heat shock response of tilipia ovary cells. J. Cell. Physiol. 134, 189–199.

Chen, G., Xu, Y., Xu, L., Zheng, Y., Schramm, K.W., Kettrup, A., 1998. Influence of dioxin and metal-contaminated sediment on phase I and II biotransformation enzymes in silver crucian carp. Ecotoxicol. Environ. Safe 40, 234–238.

Choi, J., Oris, J.T., 2000. Evidence of oxidative stress in bluegill sunfish (*Lepomis macrochirus*) liver microsomes simultaneously exposed to solar ultraviolet radiation and anthracene. Environ. Toxicol. Chem. 19, 1795–1799.

Clark, T., Clark, K., Paterson, S., Mackay, C., Norstrom, R.J., 1988. Wildlife monitoring, modeling and fugacity. Environ. Sci. Technol. 22, 120–127.

Clarke, D.J., Burchell, B., George, S.C., 1992. Differential expression and induction of UDP-glucuronosyltransferase isoforms in hepatic and extrahepatic tissues of a fish, *Pleuronectus platessa*: immunochemical and functional characterisation. Toxicol. Appl. Pharmacol. 115, 130–136.

Colborn, T., vom Saal, F.S., Soto, A.M., 1993. Developemental effects of endocrine-disrupting chemicals in wildlife and humans. Environ. Health Perspect. 101, 378–384.

Collier, T.K., Varanasi, U., 1991. Hepatic activities of xenobiotic metabolizing enzymes and biliary levels of xenobiotics in English sole (*Parophrys vetulus*) exposed to environmental contaminants. Arch. Environ. Contam. Toxicol. 20, 462–473.

Collier, T.K., Singh, S.V., Awasthi, Y.C., Varanasi, U., 1992. Hepatic xenobiotic metabolizing enzymes in two species of benthic fish showing different prevalences of contaminant-associated liver neoplasms. Toxicol. Appl. Pharmacol. 113, 319–324.

Collier, T.K., Stein, J.E., Goksøyr, A., Myers, M.S., Gooch, J.W., Huggett, R.J., Varanasi, U., 1993. Biomarkers of PAH exposure in oyster toadfish (*Opsanis tau*) from the Elisabeth River, Virginia. Environ. Sci. 2, 161–177.

Collier, T.K., Anulacion, B.F., Stein, J.E., Goksøyr, A., Varanasi, U., 1995. A field evaluation of cytochrome P450 1A as a biomarker of contaminant exposure in three species of flatfish, Environ. Toxicol. Chem. 14, 143–152.

Commandeur, J.N.M., Stijntjes, G.J., Vermeulen, N.P.E., 1995. Enzymes and transport systems involved in the formation and disposition of glutathione S-conjugates. Role in bioactivation and detoxication mechanisms of xenobiotics. Pharmacol. Rev. 47, 271–330.

Connor, M.S., 1984. Fish/sediment concentration ratios for organic compounds. Environ. Sci. Technol. 18, 31–35.

Cormier, S.M., Lin, E.L.C., Fulk, F., Subramanian, B., 2000a. Estimation of exposure criteria values for biliary polycyclic aromatic hydrocarbon metabolite concentrations in white suckers (*Catostomus commersoni*). Environ. Toxicol. Chem. 19, 1120–1126.

Cormier, S.M., Millward, M.R., Mueller, C., Subramanian, B., Johnson, R.D., Tiedge, J.E., 2000b. Temporal trends in ethoxyresorufin-*O*-deethylase activity of brook trout (*Salvelinus fontinalis*) fed 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. Environ. Toxicol. Chem. 19, 462–471.

Cosson, R.P., 1994. Heavy metal intracellular balance and relationship with metallothionein induction in the gills of carp. After contamination by Ag, Cd, and Hg following pre-treatment with Zn or not. Biol. Trace Elem. Res. 46, 229–245.

Courtenay, S.C., Grunwald, C.M., Kreamer, G.-L., Fairchild, W.L., Arsenault, J.T., Ikonomou, M., Wirgin, I.I., 1999. A comparison of the dose and time response of CYP1A1 mRNA induction in chemically treated Atlantic tomcod from two populations. Aquat. Toxicol. 47, 43–69.

Crosby Longwell, A., Chang, S., Hebert, A., Hughes, J.B., Perry, D., 1992. Pollution and developemental abnormalities of Atlantic fishes. Environ. Biol. Fish. 35, 1–21.

Curtis, L.R., Carpenter, H.M., Donohoe, R.M., Williams, D.E., Hedstrom, O.R., Deinzer, M.L., Bellstein, M.A., Foster, E., Gates, R., 1993. Sensitivity of cytochrome P450-1A1 induction in fish as a biomarker for distribution of TCDD and TCDF in the Willamette River, Oregon. Environ. Sci. Technol. 27, 2149–2157.

Dabrowska, H., Fisher, S.W., Ciereszko, R., Dabrowski, K., Woodin, B.R., Stegeman, J.J., 2000. Hepatic P4501A activity, plasma sex steroids, and gonadal steroidogenesis in vitro in yellow perch

exposed to 3,3′,4,4′,5-pentachlorobiphenyl. Environ. Toxicol. Chem. 19, 3052–3060.
Da Costa, E.G., Curtis, L.R., 1995. Bioaccumulation of dietary 2,2′,4,4′,5,5′-hexachlorobiphenyl and induction of hepatic arylhydrocarbon hydroxylase in rainbow trout (*Oncorhynchus mykiss*). Environ. Toxicol. Chem. 14, 1711–1717.
De Boer, J., Stronck, C.J.N., Traag, W.A., Van der Meer, J., 1993. Non-ortho and mono-ortho substituted chlorobiphenyls and chlorinated dibenzo-*p*-dioxins and dibenzofurans in marine and freshwater fish and shellfish from the Netherlands. Chemosphere 26, 1823–1842.
De Boer, J., Van der Valk, F., Kerkhoff, M.A.T., Hagel, P., 1994. 8-Year study on the elimination of PCBs and other organochlorine compounds from eel (*Anguilla anguilla*) under natural conditions. Environ. Sci. Technol. 28, 2242–2248.
De Kruijf, 1991. Extrapolation through hierarchical levels. Comp. Biochem. Physiol. 100C, 291–299.
De Maagd, P.G.-J., 1996. Polycyclic aromatic hydrocarbons: fate and effects in the aquatic environment. Academic thesis, Rijksuniversiteit Utrecht, The Netherlands.
De Maagd, P.G.-J., 2000. Bioaccuulation tests applied in whole effluent assessment: a review. Environ. Toxicol. Chem. 19, 25–35.
De Rooij, B.M., Boogaard, P.J., Commandeur, J.N.M., Van Sittert, N.J., Vermeulen, N.P.E., 1997. Allymercapturic acid as urinary biomarker of human exposure to allyl chloride. Occup. Environ Med. 54, 653–661.
De Zwart, D., 1995. Monitoring water quality in the future, Volume 3: Biomonitoring. National Institute of Public Health and Environmental Protection (RIVM), Bilthoven, The Netherlands.
De Zwart, L.L., Venhorst, J., Groot, M., Commandeur, J.N.M., Hermanns, R.C.A., Meerman, J.H.M., van Baar, B.L.M., Vermeulen, N.P.E., 1997. Simultaneous determination of eight lipid peroxidation degradation products in urine of rats treated with carbon tetrachloride using gas chromatography with electron-capture detection. J. Chromatogr. B 694, 227–287.
Den Besten, P.J., 1998. Concepts for the implementation of biomarkers in environmental monitoring. Mar. Environ. Res. 46, 253–256.
Depledge, M.H., Fossi, M.C., 1994. The role of biomarkers in environmental assessment (2). Ecotoxicology 3, 161–172.
Devault, D.S., Hesselberg, R., Rogers, P.W., Feist, T.J., 1996. Contaminant trends in lake trout and walleye from the Laurentian Great Lakes. J. Great Lakes Res. 22, 884–895.
Devillers, J., Bintein, S., Domine, D., 1996. Comparison of BCF models based on log *P*. Chemosphere 33, 1047–1065.
Di Giulio, R.T., Washburn, P.C., Wenning, R.J., Winston, G.W., Jewell, C.S., 1989a. Biochemical responses in aquatic animals: a review of determinants of oxidative stress. Environ. Toxicol. Chem. 8, 1103–1123.
Di Giulio, R.T., Habig, C., Wolfe, T., 1989b. Phase I and phase II biotransformation enzyme activities in channel catfish exposed to contaminated sediments. Toxicologist 9, 43abs..
Di Giulio, R.T., Benson, W.H., Sanders, B.M., van Veld, P.A., 1995. Biochemical mechanisms: metabolism, adaptation, and toxicity. In: Rand, G.M. (Ed.), Fundamentals of Aquatic Toxicology: Effects, Environmental fate, and Risk Assessment, second ed.. Taylor and Francis, London, UK, pp. 523–562.
Donohoe, R.M., Wang-Buhler, J.-L., Buhler, D.R., Curtis, L.R., 1999. Effects of 3,3′,4,4′,5,5′-hexachlorobiphenyl on cytochrome P4501A and estrogen-induced vitellogenesis in rainbow trout (*Oncorhynchus mykiss*). Environ. Toxicol. Chem. 18, 1046–1052.
Duke, L.D., Taggart, M., 2000. Uncertainty factors in screening ecological risk assessments. Environ. Toxicol. Chem. 19, 1668–1680.
Dunier, M., Siwicki, A.K., Scholtens, J., Molin, S.D., Vergnet, C., Studnicka, M., 1994. Effects of lindane exposure on rainbow trout (*Oncorhynchus mykiss*) immunity. Ecotoxicol. Environ. Safe 27, 324–334.
Dunn, B.P., 1991. Carcinogen adducts as an indicator for the public health risks of consuming carcinogen-exposed fish and shellfish. Environ. Health Perspect. 90, 111–116.
Dunn, B.P., Black, J.J., MacCubbin, A., 1987. ^{32}P-postlabeling analysis of aromatic DNA adducts in fish from polluted water. Cancer Res. 47, 6543–6548.
Dunn, B.P., Fitzsimmons, J., Stalling, D., MacCubbin, A.E., Black, J.J., 1990. Pollution-related aromatic DNA adducts in liver from populations of wild fish. Proc. Am. Assoc. Cancer Res. 31, 570.
Dyer, S.D., Brooks, L.G., Dickson, K.L., Sanders, B., Zimmerman, E.G., 1993. Synthesis and accumulation of stress proteins in tissues of arsenite-exposed fathead minnows (*Pimephalus promelas*). Environ. Toxicol. Chem. 12, 913–924.
ECETOC, 1993. Environmental hazard assessment of substances. European Centre for Ecotoxicology and Toxicology of Chemicals: Technical Report No. 51. Brussels, Belgium.
Egaas, E., Skaare, J.U., Svendsen, N.O., Sandvik, M., Falls, J.G., Dauterman, W.C., Collier, T.K., Netland, J., 1993. A comparative study of effects of atrazine on xenobiotic metabolizing enzymes in fish and insect, and of the in vitro phase II atrazine metabolism in some fish, insects, mammals and one plant species. Comp. Biochem. Physiol. 106C, 141–149.
Egaas, E., Sandvik, M., Fjeld, E., Kallqvist, T., Goksøyr, A., Svensen, A., 1999. Some effects of the fungicide propiconazole on cytochrome P450 and glutathione *S*-transferase in brown trout (*Salmo trutta*). Comp. Biochem. Physiol. C. Pharmacol. Toxicol. Endocrinol. 122, 337–344.
Eggens, M.L., Galgani, F., Klungøyr, J., Everts, J., 1992. Hepatic EROD activity in dab, *Limanda limanda*, in the German Bight using an improved plate-reader method. Mar. Ecol. Prog. Ser. 91, 71–75.
Eggens, M.L., Bergman, A., Vethaak, D., Van der Weiden, M., Celander, M., Boon, J.P., 1995. Cytochrome P450 1A indices as biomarkers of contaminant exposure: results of a field study with plaice, *Pleuronectus platessa* and flounder, *Platichtys flesus*, from the southern North Sea. Aquat. Toxicol. 32, 211–225.
Eggens, M.L., Opperhuizen, A., Boon, J.P., 1996. Temporal variation of CYP1A indices, PCB and 1-OH pyrene concentration in flounder, *Platichthys flesus*, from the Dutch Wadden Sea. Environ. Toxicol. Chem.
El Adlouni, C., Tremblay, J., Walsh, P., Lageux, J., Bureau, J., Lamberte, D., Keith, G., Nadeau, D., Poirier, G.G., 1995. Comparative study of DNA adduct levels in white sucker fish (*C. commersoni*) from the basin of the St. Lawrence river (Canada). Mol. Cell. Biochem. 148, 133–138.
Ellis, J.B., 2000. Risk assessment approaches for ecosystem responses to transient pollution events in urban receiving waters. Chemosphere 41, 85–91.
Ellis, G.S., Huckins, J.N., Rostad, C.E., Schmitt, C.J., Petty, J.D., McCarthy, P., 1995. Evaluation of lipid-containing semipermeable membrane devices for monitoring organochlorine contaminants in the upper Mississippi river. Environ. Toxicol. Chem. 14, 1875–1884.
Elskus, A.A., Stegeman, J.J., 1989. Induced cytochrome P-450 in *Fundulus heteroclitus* associated with environmental contamination by polychlorinated biphenyls and polynuclear aromatic hydrocarbons. Mar. Environ. Res. 27, 31–45.
Elskus, A.A., Pruell, R.J., Stegeman, J.J., 1992. Endogenously-mediated, pretranslational suppression of cytochrome P4501A in PCB-contaminated flounder. Mar. Environ. Res. 34, 97–101.
Elskus, A.A., Stegeman, J.J., Gooch, J.W., Black, D.E., Pruell, R.J., 1994. Polychlorinated biphenyl congener distributions in winter flounder as related to gender, spawning site, and congener metabolism. Environ. Sci. Technol. 28, 401–407.

Endicott, D.D., Cook, P.M., 1994. Modelling the partitioning and bioaccumulation of TCDD and other hydrophobic organic chemicals in Lake Ontario. Chemosphere 28, 75–87.

Engel, D.W., Roesijadi, G., 1987. Metallothioneins: a monitoring tool. In: Vernberg, F.J., Thurberg, F.P., Calabrese, A., Vernberg, W.B. (Eds.), Pollution and Physiology of Estuarine Organisms. University of South Carolina Press, Columbia, USA, pp. 421–438.

Engwall, M., Brunström, B., Brewer, A., Norrgren, L., 1994. Cytochrome P450IA induction by a coplanar PCB, a PAH mixture, and PCB-contaminated sediment extracts following microinjection of rainbow trout sac-fry. Aquat. Toxicol. 30, 311–324.

Epel, D., 1998. Use of multidrug transporters as first lines of defense against toxins in aquatic organisms. Comp. Biochem. Physiol. A 120, 23–28.

Ericson, G., Åkerman, G., Liewenborg, B., Balk, L., 1996. Comparison of DNA damage in the early life stages of cod, *Gadus morhua*, originating from the Barents Sea and Baltic Sea. Mar. Environ. Res. 42, 119–123.

Ericson, G., Noaksson, E., Balk, L., 1999a. DNA adduct formation and persistence in liver and extrahepatic tissue of northern pike (*Esox lucius*) following oral exposure to benzo[a]pyrene, benzo[k]fluoranthene and 7H-dibenzo[c,g]carbazole. Mutat. Res. 427, 135–145.

Ericson, G., Liewenborg, B., Lindesjöö, E., Näf, C., Balk, L., 1999b. DNA adducts in perch (*Perca fluviatilis*) from a creosote contaminated site in the river Angermanälven, Sweden. Aquat. Toxicol. 45, 181–193.

Everaarts, J.M., Sleiderink, H.M., den Besten, P.J., Halbrook, R.S., Shugart, L.R., 1994. Molecular responses as indicators of marine pollution: DNA damage and enzyme induction in *Limanda limanda* and *Asterias rubens*. Environ. Health Perspec. 102 (suppl. 12), 37–43.

Farrington, J.W., 1991. Biogeochemical processes governing exposure and uptake of organic pollutant compounds in aquatic organisms. Environ. Health Perspect. 90, 75–84.

Fendick, E.A., Mather-Mihaich, E., Houck, K.A., St. Clair, M.B., Faust, J.B., Rockwell, C.H., Owens, M., 1990. Ecological toxicology and human health effects of heptachlor. Rev. Environ. Contam. Toxicol. 111, 61–142.

Fenet, H., Casellas, C., Bontoux, J., 1998. Laboratory and field-caging studies on hepatic enzymatic activities in European eel and rainbow trout. Ecotoxicol. Environ. Safe 40, 137–143.

Fent, K., Bucheli, T.D., 1994. Inhibition of hepatic microsomal monooxygenase system by organotins in vitro in freshwater fish. Aquat. Toxicol. 28, 107–126.

Fent, K., Woodin, B.R., Stegeman, J.J., 1998. Effects of triphenyltin and other organotins on hepatic monooxygenase system in fish. Comp. Biochem. Physiol. C Pharmacol. Toxicol. Endocrinol. 121, 277–288.

Fiedler, H., Cooper, K., Bergek, S., Hjelt, M., Rappe, C., Bonner, M., Howell, F., Willett, K., Safe, S., 1998. PCDD, PCDF, and PCB in farm-raised catfish from souteast United States—concentrations, sources, and CYP1A induction. Chemosphere 37, 1645–1656.

Filho, D.W., 1996. Fish antioxidant defenses—a comparative approach. Braz. J. Med. Biol. Res. 29, 1735–1742.

Fisk, A.T., Norstrom, R.J., Cymbalisty, C.D., Muir, D.C.G., 1998. Dietary accumulation and depuration of hydrophobic organochlorines: bioaccumulation parameters and their relationship with the octanol/water partition coefficient. Environ. Toxicol. Chem. 17, 951–961.

Flammarion, P., Garric, J., 1997. Cyprinids EROD activities in low contaminated rivers: a relevant statistical approach to estimate reference levels for EROD biomarker. Chemosphere 35, 2375–2388.

Folmar, L.C., Hemmer, M., Hemmer, R., Bowman, C., Kroll, K., Denslow, N.D., 2000. Comparative estrogenicity of estradiol, ethynyl estradiol and diethylstilbestrol in an in vivo, maloe sheepshead minnow (*Cyprinodon variegatus*), vitellogenin bioassay. Aquat. Toxicol. 49, 77–88.

Ford, W.M., Hill, E.P., 1991. Organochlorine pesticides in soil sediments and aquatic animals in the Upper Steele Bayou of Mississippi. Arch. Environ. Contam. Toxicol. 20, 161–167.

Förlin, L., Celander, M., 1993. Induction of cytochrome P450 1A in teleosts: environmental monitoring in Swedish fresh, brackish and marine waters. Aquat. Toxicol. 26, 41–56.

Förlin, L., Andersson, T., Bengtsson, B.E., Härdig, J., Larsson, A., 1985. Effects of pulp bleach plant effluents on hepatic xenobiotic biotransformation enzymes in fish: laboratory and field studies. Mar. Environ. Res. 17, 109–112.

Förlin, L., Balk, L., Celander, M., Bergek, S., Hjelt, M., Rappe, C., de Wit, C., Jansson, B., 1992. Biotransformation enzyme activities and PCDD/PCDF levels in pike caught in a Swedish lake. Mar. Environ. Res. 34, 169–173.

Förlin, L., Andersson, T., Balk, L., Larsson, A., 1995. Biochemical and physiological effects in fish exposed to bleached kraft mill effluents. Ecotoxicol. Environ. Safe 30, 164–170.

Förlin, L., Blom, S., Celander, M., Sturve, J., 1996. Effects on UDP glucuronosyl transferase, glutathione transferase, DT-diaphorase and glutathione reductase activities in rainbow trout liver after long-term exposure to PCB. Mar. Environ. Res. 42, 213–216.

Fossi, M.C., Marsili, L., 1997. The use of non-destructive biomarkers in the study of marine mammals. Biomarkers 2, 205–216.

Foster, E.P., Vrolijk, N.H., Chen, T.T., Curtis, L.R., 1998. Interaction of 2,2′,4,4′,5,5′-hexachlorobiphenyl with hepatic cytochrome P-4501A in rainbow trout. J. Toxicol. Environ. Health 53, 313–325.

Fowler, B.A., 1987. Intracellular compartmentation of metals in aquatic organisms: relationships to mechanisms of cell injury. Environ. Health Perspect. 71, 121–128.

Fragoso, N.M., Hodson, P.V., Kozin, I.S., Brown, R.S., Parrot, J.L., 1999. Kinetics of mixed function oxygenase induction and retene excretion in retene-exposed rainbow trout (*Oncorhynchus mykiss*). Environ. Toxicol. Chem. 18, 2268–2274.

Franke, C., 1996. How meaningful is the bioconcentrationfactor for risk assessment. Chemosphere 32, 1897–1905.

Franke, C., Studinger, G., Berger, G., Böhling, S., Bruckmann, U., Cohors-Fresenborg, D., Jöhncke, U., 1994. The assessment of bioaccumulation. Chemosphere 29, 1501–1514.

Fridovich, I., 1986. Superoxide dismutases. Adv. Enzymol. 58, 61–97.

Fulton, M.H., Key, P.B., 2001. Acetylcholinesterase inhibition in estuarine fish and invertebrates as an indicator of organophosphorus insecticide exposure and effects. Environ. Toxicol. Chem. 20, 37–45.

Gabryelak, T., Klekot, J., 1985. The effect of paraquat on the peroxide metabolism enzymes in erythrocytes of freswater fish species. Comp. Biochem. Physiol. 81C, 415–418.

Gadagbui, B.K.-M., Goksøyr, A., 1996. CYP1A and other biomarker responses to effluents from a textile mill in the Volta river (Ghana) using caged tilapia (*Oreochromis niloticus*) and sediment-exposed mudfish (*Clarias anguillaris*). Biomarkers 1, 252–261.

Gagnon, M.M., Holdway, D.A., 2000. EROD induction and biliary metabolite excretion following exposure to the water accommodated fraction of crude oil and chemically dispersed crude oil. Arch. Environ. Contam. Toxicol. 38, 70–77.

Galassi, S., Guzzella, L., Battegazzore, M., Carrieri, A., 1994. Biomagnification of PCBs, *pp′*-DDE, and HCB in the river Po ecosystem (northern Italy). Ecotoxicol. Environ. Safe 29, 174–186.

Galassi, S., Vigano, L., Sanna, M., 1996. Bioconcentration of organochlorine pesticides in rainbow trout caged in the river Po. Chemosphere 32, 1729–1739.

Gale, R.W., Huckins, J.N., Petty, J.D., Peterman, P.H., Williams, L.L., Morse, D., Schwartz, T.R., Tillitt, D.E., 1997. Comparison of the uptake of dioxin-like compounds by caged channel catfish and semipermeable membrane devices in the Saginaw river, Michigan. Environ. Sci. Technol. 31, 178–1187.

Galgani, F., Bocquene, G., Lucon, M., Grzebyk, D., Letrouit, F., Claisse, D., 1991. EROD measurements in fish from the northwest part of France. Mar. Pollut. Bull. 22, 494–500.

Galgani, F., Bocquene, G., Cadiou, Y., 1992. Evidence of variation in cholinesterase activity in fish along a pollution gradient in the North Sea. Mar. Ecol. Prog. Ser. 13, 77–82.

Gallagher, E.P., Di Giulio, R.T., 1989. Effects of complex waste mixtures on hepatic monooxygenaseactivities in brown bullheads (*Ictalurus nebulosus*). Environ. Pollut. 62, 113–128.

Gallagher, E.P., Di Giulio, R.T., 1991. Effects of 2,4-dichlorophenoxyacetic acid and picloram on biotransformation, peroxisomal and serum enzyme activities in channel catfish (*Ictalurus punctatus*). Toxicol. Lett. 57, 65–72.

George, S.G., 1989. Cadmium effects on plaice liver xenobiotic and metal detoxication systems: dose response. Aquat. Toxicol. 15, 303–310.

George, S.G., 1994. Enzymology and molecular biology of phase II xenobiotic-conjugating enzymes in fish. In: Malins, D.C., Ostrander, G.K. (Eds.), Aquatic Toxicology; Molecular, Biochemical and Cellular perspectives. Lewis Publishers, CRC press, pp. 37–85.

George, S.G., Young, P., 1986. The time course of effects of cadmium and 3-methylcholanthrene on activities of enzymes of xenobiotic metabolism and metallothionein levels in the plaice, *Pleuronectus platessa*. Comp. Biochem. Physiol. 83C, 37–44.

George, S.G., Burgess, D., Leaver, M., Frerichs, N., 1992a. Metallothionein induction in cultured fibroblasts and liver of a marine flatfish, the turbot, *Scophthalmul maximus*. Fish Physiol. Biochem. 10, 43–54.

George, S.G., Groman, D., Brown, S., Holmes, K., 1992b. Studies of a fatal pollutant-induced hyperbilirubinaemia in spawning Atlantic salmon. Mar. Environ. Res. 34, 81–86.

George, S.G., Christiansen, J.S., Killie, B., Wright, J., 1995. Dietary crude oil exposure during sexual maturation induces hepatic mixed function oxygenase (CYP 1A) activity at very low environmental temperatures in Polar cod *Boreogadus saida*. Mar. Ecol. Prog. Ser. 122, 307–312.

Gerpe, M., Kling, P., Berg, A.H., Olsson, P.-E., 2000. Arctic char (*Salvelinus alpinus*) methallothionein: cDNA sequence, expression, and tissue-specific inhibition of cadmium-mediated methallothionein induction by 17β-estradiol, 4-OH-PCB 30, and PCB 104. Environ. Toxicol. Chem. 19, 638–645.

Ghazali, K.S., 1992. Hematological and physiological responses to sublethal concentrations of cadmium in a freshwater teleost, *Tilipia zilii*. Water Air Soil Pollut. 64, 551–559.

Gibbons, W.N., Munkittrick, K.R., Taylor, W.D., 1998. Monitoring aquatic environments receiving industrial effluents using small fish species 1: response of spoonhead sculpin (*Cottus ricei*) downstream of a bleached-kraft pulp mill. Environ. Toxicol. Chem. 17, 2227–2237.

Gimeno, S., Gerritsen, A., Bowmer, T., Komen, H., 1996. Feminazation of male carp. Nature 384, 221–222.

Gobas, F.A.P.C., 1993. A model for predicting the bioaccumulation of hydrophobic organic chemicals in aquatic food webs: application to Lake Ontario. Ecol. Model. 69, 1–17.

Gobas, F.A.P.C., Muir, D.C.G., Mackay, D., 1988. Dynamics of dietary bioaccumulation and feacal elimination of hydrophobic organic chemicals in fish. Chemosphere 17, 943–962.

Gobas, F.A.P.C., Zhang, X., Wells, R., 1993. Gastrointestinal magnification: the mechanism of biomagnification and food chain accumulation of organic chemicals. Environ. Sci. Technol. 27, 2855–2863.

Gobas, F.A.P.C., Wilcockson, J.B., Russell, R.W., Haffner, G.D., 1999. Mechanism of biomagnification in fish under laboratory and field conditions. Environ. Sci. Technol. 33, 133–141.

Goeptar, A.R., Scheerens, H., Vermeulen, N.P.E., 1995. Oxygen reductase and substrate reductase activity of cytochrome P450. Crit. Rev. Toxicol. 25, 25–65.

Goetz, W., 1980. Diagnostik von Lebererkrankungen (in German). G.I.T. Verlag Ernst Giebeler, Darmstadt, Germany.

Goksøyr, A., 1991. A semi-quantitative cytochrome P-450IA1 ELISA: a simple method for studying the monooxygenase induction response in environmental monitoring and ecotoxicological testing of fish. Sci. Total Environ. 101, 255–262.

Goksøyr, A., Larsen, E., 1991. The cytochrome P450 system of Atlantic salmon (*Salmo salar*): I. basal properties and induction of P450 1A1 in liver of immature and mature fish. Fish Physiol. Biochem. 9, 339–349.

Goksøyr, A., Förlin, L., 1992. The cytochrome P450 system in fish, aquatic toxicology and environmental monitoring. Aquat. Toxicol. 22, 287–312.

Goksøyr, A., Husøy, A.M., 1998. Immunochemical approaches to studies of CYP1A localization and induction by xenobiotics in fish. In: Braunbeck, T., Hinton, D.E., Streit, B. (Eds.), Fish Ecotoxicology. Birkhäuser Verlag, Basel, pp. 165–202.

Goksøyr, A., Larsen, H.E., Husøy, A.M., 1991a. Application of a cytochrome P450 1A1-ELISA in environmental monitoring and toxicological testing of fish. Comp. Biochem. Biophys. 100C, 157–160.

Goksøyr, A., Husøy, A.M., Larsen, H.E., Klungsøyr, J., Wilhelmsen, S., Maage, A., Brevik, E.M., Andersson, T., Celander, M., Pesonen, M., Förlin, L., 1991b. Environmental contaminants and biochemical responses in flatfish from the Hvaler Archipelago in Norway. Arch. Environ. Contam. Toxicol. 21, 486–496.

Goksøyr, A., Beyer, J., Husøy, A.M., Larsen, H.E., Westrheim, K., Wilhelmsen, S., Klungsøyr, J., 1994. Accumulation and effects of aromatic and chlorinated hydrocarbons in juvenile Atlantic cod (*Gadus morhua*) caged in a polluted fjord (Sørfjorden, Norway). Aquat. Toxicol. 29, 21–35.

Gonzalez, J.F., Del Valle, P.L., Thohan, S., Kane, A.S., 2000. Effects of waterborne nitrite on phase I–II biotransformation in channel catfish (*Ictalurus punctatus*). Mar. Environ. Res. 50, 29–32.

Gracey, A.Y., Troll, J.V., Somero, G.N., 2001. Hypoxia-induced gene expression profiling in the euryoxid fish *Gillichthys mirabilis*. Proc. Natl. Acad. Sci. 98, 1993–1998.

Greig-Smith, P.W., 1991. Use of cholinesterase measurements in surveillance of wildlife poisoning in farmland. In: Mineau, P. (Ed.), Cholinesterase-Inhibiting Insecticides, Chemicals in Agriculture, vol. 2. Elsevier, Amsterdam, pp. 127–150.

Gronlund, W.D., Stein, J.E., Chan, S.L., Brown, D.W., McCain, B.B., Landahl, J.T., 1991. Multidisciplinary assessment of pollution at three sites in Long Island Sound (USA). Estuaries 14, 299–305.

Grøsvik, B.E., Goksøyr, 1996. Biomarker protein expression in primary cultures of salmon (*Salmo salar* L.) hepatocytes exposed to environmental pollutants. Biomarkers 1, 45–53.

Gustafsson, O., Haghseta, F., Chan, C., MacFarlane, J., Gschwend, M., 1997. Quantification of the dilute sedimentary soot phase: implications for PAH speciation and bioavailability. Environ. Sci. Technol. 31, 203–209.

Haasch, M.L., Quardokus, E.M., Sutherland, L.E., Goodrich, M.S., Lech, J.J., 1993a. Hepatic CYP1A1 induction in rainbow trout by continuous flowthrough exposure to *b*-naphtoflavone. Fundam. Appl. Toxicol. 20, 72–82.

Haasch, M.L., Prince, R., Wejksnora, P.J., Cooper, K.R., Lech, J.J., 1993b. Caged and wild fish: induction of hepatic cytochrome P-450 (CYP1A1) as an environmental biomonitor. Environ. Toxicol. Chem. 12, 885–895.

Haasch, M.L., Graf, W.K., Quardokus, E.M., Mayer, R.T., Lech, J., 1994. Use of 7-alcoxyphenoxazones, 7-alkoxycoumarins and 7-alkoxyquinolines as fluorescent substrates for rainbow trout hepatic microsomes after treatment with various inducers. Biochem. Pharmacol. 47, 893–903.

Hageman, J.J., Bast, A., Vermeulen, N.P.E., 1992. Monitoring of oxidative free radical damage in vivo: analytical aspects. Chem.-Biol. Interact. 82, 243–293.

Hahn, M.E., Stegeman, J.J., 1994. Regulation of cytochrome P4501A1 in teleosts: sustained induction of CYP1A1 mRNA, protein, and catalytic activity by 2,3,7,8-tetrachlorodibenzofuran in the marine fish *Stenotomus chrysops*. Toxicol. Appl. Pharmacol. 127, 187–198.

Hahn, M.E., Woodin, B.R., Stegeman, J.J., Tillitt, D.E., 1998. Aryl hydrocarbon receptor function in early vertebrates: inducibility of cytochrome P450 1A in agnathan and elasmobranch fish. Comp. Biochem. Physiol. C Pharmacol. Toxicol. Endocrinol. 120, 67–75.

Hai, D.Q., Varga, I.S., Matkovics, B., 1995. Effects of an organophosphate on the antioxidant systems of fish tissues. Acta Biol. Hungar. 46, 39–50.

Haitzer, M., Höss, S., Traunspurger, W., Steinberg, C., 1999. Relationship between concentration of dissolved organic matter (DOM) and the effect of DOM on the bioconcentration of benzo[a]pyrene. Aquat. Toxicol. 45, 147–158.

Halliwell, B., Gutteridge, 1999. Free Radicals in Biology and Medicine, third ed.. Oxford University Press, Oxford, UK.

Ham, K.D., Adams, S.M., Peterson, M.J., 1997. Application of multiple bioindicators to differentiate spatial and temporal variability from the effects of contaminant exposure on fish. Ecotoxicol. Environ. Safe 37, 53–61.

Harrison, P.T.C., Humfrey, C.D.N., Litchfield, M., Peakall, D., Schuker, L.K., 1995. IEH Assessment on Environmental Oestrogens: Consequences to Human Health and Wildlife. Page Bros, Norwich, UK, p. 107.

Hasspieler, B.M., Behar, J.V., Carlson, D.B., Watson, D.E., Di Giulio, R.T., 1994. Susceptibility of channel catfish (*Ictalurus punctatus*) and brown bullhead (*Ameriurus nebulosus*) to oxidative stress: a comparative study. Aquat. Toxicol. 28, 53–64.

Haux, C., Björnsson, B.T., Förlin, L., Larsson, Å., Deftos, L.J., 1988. Influence of cadmium exposure on plasma calcium, vitellogenin and calcitonin in vitelogenic rainbow trout. Mar. Environ. Res. 24, 199–210.

Hawker, D.W., Connel, D.W., 1985. Relationships between partition coefficient, uptake rate constant, clearance rate constant, and time to equilibrium for bioaccumulation. Chemosphere 14, 1205–1219.

Hayes, J.D., Pulford, D.J., 1995. The glutathione *S*-transferase supergene family: regulation of GST and the contribution of the isoenzymes to cancer chemoprotection and drug resistance. Crit. Rev. Biochem. Mol. Biol. 30, 445–600.

Hektoen, H., Bernloft, A., Ingebrigtsen, K., Skaare, J.U., Goksøyr, A., 1994. Response of hepatic xenobiotic metabolizing enzymes in rainbow trout (*Oncorhynchus mykiss*) and cod (*Gadus morhua*) to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (2,3,7,8-TCDD). Aquat. Toxicol. 28, 97–106.

Helle, E., Olsson, M., Jensen, S., 1976a. DDT and PCB levels and reproduction in ringed seals from the Bothnian bay. Ambio 5, 188–189.

Helle, E., Olsson, M., Jensen, S., 1976b. PCB levels correlated with pathological changes in seal uteri. Ambio 5, 261–263.

Hellou, J., Mackay, D., Banoub, J., 1999. Levels, persistence and bioavailability of organic contaminants present in marine harbor sediments impacted by raw sewage. Chemosphere 38, 457–473.

Hemmer, M.J., Hemmer, B.L., Folmar, L.C., Marcovich, D., Hoglund, M.D., Bowman, C.J., Kroll, K.J., Denslow, N.D., 2001. Effects of *p*-nonylphenol, methoxychlor, and endosulfan on vitellogenin induction and expression in sheepshead minnow (*Cyprinodon variegatus*). Environ. Toxicol. Chem. 20, 336–343.

Henderson, F., Bechtold, W.E., Bond, J.A., Sun, J.D., 1989. The use of biological markers in toxicology. Crit. Rev. Toxicol. 20, 65–82.

Hendriks, A.J., 1995a. Modelling equilibrium concentrations of microcontaminants in organisms of the Rhine delta: can average field residues in the aquatic foodchain be predeicted from laboratory accumulation. Aquat. Toxicol. 31, 1–25.

Hendriks, A.J., 1995b. Modelling non-equilibrium concentrations of microcontaminants in organisms: comparative kinetics as a function of species size and octanol–water partitioning. Chemosphere 30, 265–292.

Hendriks, A.J., Pieters, H., de Boer, J., 1998. Accumulation of metals, polycyclic (halogenated) hydrocarbons, and biocides in zebra mussel and eel from the Rhine and Meuse rivers. Environ. Toxicol. Chem. 17, 1885–1898.

Heugens, E.H., Hendriks, A.J., Dekker, T., van Straalen, N.M., Admiraal, W., 2001. A review of the effects of multiple stressors on aquatic organisms and analysis of uncertainty factors for use in risk assessment. Crit. Rev. Toxicol. 2001, 247–284.

Hinton, D.E., 1994. Cells, cellular responses, and their markers in chronic toxicity of fishes. In: Malins, D.C., Ostrander, G.K. (Eds.), Aquatic Toxicology; Molecular, Biochemical and Cellular Perspectives. Lewis Publishers CRC press, pp. 207–240.

Hinton, D.E., Baumann, P.C., Gardner, G.C., Hawkins, W.E., Hendricks, J.D., Murchelano, R.A., Okihiro, M.S., 1992. Histopathologic biomarkers. In: Huggett, R.J., Kimerly, R.A., Mehrle, P.M., Jr, Bergman, H.L. (Eds.), Biomarkers: Biochemical, Physiological and Histological Markers of Anthropogenic Stress. Lewis Publishers, Chelsea, MI, USA, pp. 155–210.

Hodgson, E., 1994. Chemical and environmental factors affecting metabolism of xenobiotics. In: Hodgson, E., Levi, P.E. (Eds.), Introduction to Biochemical Toxicology, second ed.. Appleton and Lange, Norwalk, Connecticut, pp. 162–175.

Hodson, P.V., McWhirter, M., Ralph, K., Gray, B., Thivierge, D., Carey, J.H., Van der Kraak, G., Whittle, D.M., Levesque, M.C., 1992. Effects of bleached kraft mill effluent on fish in the St. Maurice river, Quebec. Environ. Toxicol. Chem. 11, 1635–1651.

Hogstrand, C., Haux, C., 1991. Binding and detoxification of heavy metals in lower vertebrates with reference to metallothionein. Comp. Biochem. Physiol. 100C, 137–141.

Holdway, D.A., Brennan, S.E., Ahokas, J.T., 1994. Use of hepatic MFO and blood enzyme biomarkers in sand flathead (*Platycephalus bassensis*) as indicators of pollution in Port Phillip Bay, Australia. Mar. Pollut. Bull. 28, 683–695.

Holm, G., Lundström, J., Andersson, T., Norrgren, L., 1994. Influences of halogenated organic substances on ovarian developement and hepatic EROD activity in the three-spined stickleback, *Gasterosteus aculeatus*, and rainbow trout, *Oncorhynchus mykiss*. Aquat. Toxicol. 29, 241–256.

Hsu, T., Deng, F.-Y., 1996. Studies on the susceptibility of various organs of zebrafish (*Brachydanio rerio*) to benzo(a)pyrene-induced DNA adduct formation. Chemosphere 33, 1975–1980.

Huckins, J.N., Tubergen, M.W., Manuweera, G.K., 1990. Semipermeable membrane devices containing model lipid: a new approach to biomonitoring the bioavailability of lipophylic contaminants and estimating their bioconcentration potential. Chemosphere 20, 533–552.

Hugla, J.L., Thome, J.P., 1999. Effects of polychlorinated biphenyls on liver ultrastructure, hepatic monooxygenases and reproductive success in the barbel. Ecotoxical. Environ. Saf. 42, 265–273.

Huuskonen, S., Linström-Seppä, P., 1995. Hepatic cytochrome P4501A and other biotransformation activities in perch (*Perca fluviatilis*): the effects of unbleached pulp mill effluents. Aquat. Toxicol. 31, 27–41.

Huuskonen, S., Lindström-Seppä, P., Koponen, K., Roy, S., 1996. Effects of non-*ortho*-substituted polychlorinated biphenyls (congeners 77 and 126) on cytochrome P4501A and conjugation activities in rainbow trout (*Oncorhyncchus mykiss*). Comp. Biochem. Physiol. 113, 205–213.

Hylland, K., Haux, C., Hogstrand, C., 1992. Hepatic metallothionein and heavy metals in dab *Limanda limanda* from the German Bight. Mar. Ecol. Prog. Ser. 91, 89–96.

Hylland, K., Sandvik, M., Skaare, J.U., Beyer, J., Egaas, E., Goksøyr, A., 1996. Biomarkers in flounder (*Platichtys flesus*): an evaluation of their use in pollution monitoring. Mar. Environ. Res. 42, 223–227.

Hyllner, S.J., Oppen-Berntsen, D.O., Helvik, J.V., Walther, B.T., Haux, C., 1991. Oestradiol-17b induces major vitelline envelope proteins in both sexes in teleosts. J. Endocrinol. 131, 229–236.

Hyotylainen, T., Oikari, A., 1999. Assessment of the bioactivity of creosote-contaminated sediment by liver biotransformation system of rainbow trout. Ecotoxicol. Environ. Safe 44, 253–258.

Iwama, G.K., Greer, G.L., Randell, D.J., 1986. Changes in selected haematological parameters in juvenile chinook salmon subjected to a bacterial challenge and a toxicant. J. Fish. Biol. 28, 563–572.

Jager, T., Vermeire, T.G., Rikken, M.G.J., Van der Poel, P., 2001. Opportunities for a probabilistic risk assessment of chemicals in the European Union. Chemosphere 43, 257–264.

Janz, D.M., McMaster, M.E., Munkittrick, K.R., Van der Kraak, G., 1997. Elevated ovarian follicular apoptosis and heat shock protein-70 expression in white sucker exposed to bleached kraft pulp mill effluent. Toxicol. Appl. Pharmacol. 147, 391–398.

Järnberg, U., Asplund, L., de Wit, C., Grafström, A.-K., Haglund, P., Jansson, B., Lexén, K., Strandell, M., Olsson, M., Jonsson, B., 1993. Polychlorinated biphenyls and polychlorinated naphtalenes in Swedish sediment and biota: levels, patterns and time trends. Environ. Sci. Technol. 27, 1364–1374.

Jedamski-Grymlas, J., Lange, U., Siebers, D., Karbe, L., 1994. Induction of the hepatic biotransformation system of golden ide [*Leuciscus idus* (L.)] after exposure in the river Elbe. Ecotoxical. Environ. Saf. 28, 35–42.

Jedamski-Grymlas, J., Kammann, U., Tempelmann, A., Karbe, L., Siebers, D., 1995. Biochemical responses and environmental contaminants in breams (*Abramis brama* L.) caught in the river Elbe. Ecotoxicol. Environ. Safe 31, 49–56.

Jeney, Z., Valtonen, E.T., Jeney, G., Jokinen, E.I., 1996. Effects of pulp and paper mill effluent (BKME) on physiology and biochemistry of the roach (*Rutilus rutilus* L.). Arch. Environ. Contam. Toxicol. 30, 523–529.

Jensen, E.G., Skaare, J.U., Egaas, E., Goksøyr, A., 1991. Response of xenobiotic metabolizing enzymes in rainbow trout (*Oncorhynchus mykiss*) to endosulfan, detected by enzyme activities and immunochemical methods. Aquat. Toxicol. 21, 81–92.

Jimenez, B.D., Oikari, A., Adams, S.M., Hinton, D.E., McCarthy, J.F., 1990. Hepatic enzymes as biomarkers: interpreting the effects of environmental, physiological, and toxicological variables. In: McCarthy, J.F., Shugart, L.R. (Eds.), Biomarkers of Environmental Contamination. Lewis Pubishers, pp. 123–142.

Jobling, S., Nolan, M., Tyler, C.R., Brightly, G., Sumpter, J.P., 1998. Wide spread sexual disruption in wild fish. Environ. Sci. Technol. 32, 2498–2506.

Jørgensen, E.H., Bye, B.E., Jobling, M., 1999. Influence of nutritional status on biomarker responses to PCB in the arctic charr (*Salvelinus alpinus*). Aquat. Toxicol. 44, 233–244.

Juliano, R.L., King, V.A., 1976. A surface glycoprotein modulating drug permeability in Chinese hamster ovary cell mutants. Biochim. Biophys. Acta 455, 152–162.

Jung, D.K.J., Klaus, T., Fent, K., 2001. Cytochrome P450 induction by nitrated polycyclic aromatic hydrocarbons, azaarenes, and binary mixtures in fish hepatoma cell line PLHC-1. Environ. Toxicol. Chem. 20, 149–159.

Kannan, K., Nakata, H., Stafford, R., Masson, G.R., Tanabe, S., Giesy, J.P., 1998. Bioaccumulation and toxic potential of extremely hydrophobic polychlorinated biphenyl congeners in biota collected at a superfund site contaminated with Aroclor 1268. Environ Sci. Technol. 32, 1214–1221.

Kantoniemi, A., Vahakangas, K., Oikari, A., 1996. The capacity of liver microsomes to form benzo[a]pyrene-diolepoxide-DNA adducts and induction of cytochrome P450 1A in feral fish exposed to pulp mill effluents. Ecotoxicol. Environ. Safe 35, 136–141.

Kappus, H., 1987. A survey of chemicals inducing lipid peroxidation in biological systems. Chem. Phys. Lipids 45, 105–115.

Karels, A., Soimasuo, M., Lappivaara, J., Leppänen, H., Aaltonen, T., Mellanen, P., Oikari, A., 1998. Effects of bleached kraft mill effluent on reproductive steroids and liver MFO activity in populations of perch and roach. Ecotoxicology 7, 123–132.

Karickhoff, S.W., 1984. Organic pollutant sorption in aquatic systems. J. Hydraulic Eng. 110, 707–735.

Kasper, C.B., Henton, 1980. Glucuronidation. In: Jakoby, W.B. (Ed.), Enzymatic basis of Detoxication, vol. II. Academic Press, New York, pp. 3–36.

Kelly, A.G., Wells, D.E., Fryer, R.J., 1994. Sampling strategy to detect a change in concentration of trace organic contaminants in marine sediment. Sci. Total Environ. 144, 217–230.

Kime, D.E., 1995. The effects of pollution on reproduction in fish. Rev. Fish Biol. Fish. 5, 52–96.

Kimura, T., Taniguchi, N., Tomita, I., Kinea, N., Yoshizaki, K., Ito, M., Ishikawa, T., 1984. Correlation of epiziootiological observation with experimental data: chemical induction of chromatophoromas in the croaker, *Nibea mitsukurii*. Natl. Cancer Inst. Monogr. 65, 139–154.

Kirby, M.F., Neall, P., Tylor, T., 1999a. EROD activity measured in flatfish from the area of the Sea Empress oil spill. Chemosphere 38, 2929–2949.

Kirby, M.F., Matthiessen, P., Neall, P., Tylor, T., Allchin, C.R., Kelly, C.A., Maxwell, D.L., Thain, J.E., 1999b. Hepatic EROD activity in flounder (*Platichtys flesus*) as an indicator of contaminant exposure in English estuaries. Mar. Pollut. Bull. 38, 676–686.

Kleinow, K.M., Melancon, M.J., Lech, J.J., 1987. Biotransformation and induction: implications for toxicity, bioaccumulation and monitoring of environmental xenobiotics in fish. Environ. Health Perspect. 71, 105–119.

Kloepper-Sams, P.J., Stegeman, J.J., 1992. Effects of temperature acclimation on the expression of hepatic cytochrome P4501A mRNA and protein in the fish *Fundulus heteroclitus*. Arch. Biochem. Biophys. 299, 38–46.

Kloepper-Sams, P.J., Owens, J.W., 1993. Environmental biomarkers as indicators of chemical exposure. J. Hazard. Mater. 35, 283–294.

Kloepper-Sams, P.J., Benton, E., 1994. Exposure of fish to biologically treated bleached-kraft effluent. 2. induction of hepatic cytochrome P4501A in mountain whitefish (*Prosopium williamson*) and other species. Environ. Toxicol. Chem. 13, 1483–1496.

Knudson, J.C., Poland, A., 1982. Response of murine epidermis to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin: interaction of the *Ah* and *hr* loci. Cell 30, 225–232.

Kolayli, S., Keha, E., 1999. A comparitive study of antioxidant enzyme activities in freshwater and seawater-adapted rainbow trout. J. Biochem. Mol. Toxicol. 13, 334–337.

Kosmala, A., Migeon, B., Flammarion, P., Garric, J., 1998. Impact assessment of a wastewater treatment plant effluent using the fish biomarker ethoxyresorufin-*O*-deethylase: field and on-site experiments. Ecotoxicol. Environ. Safe 41, 19–28.

Kothary, R.K., Candido, E.P.M., 1982. Induction of a novel set of polypeptides by heat shock or sodium arsenite in cultured cells of rainbow trout, *Salmo gairdneri*. Can. J. Biochem. 60, 347–355.

Kothary, R.K., Burgess, E.A., Candido, P.M., 1984. The heat-shock phenomenon in cultured cells of rainbow trout hsp70 mRNA synthesis and turnover. Biochim. Biophys. Acta 783, 137–143.

Krahn, M.M., Burrows, D.G., MacLeod, W.D., Malins, D.C., 1987. Determination of individual metabolites of aromatic compounds in hydrolysed bile of English sole from polluted sites in Puget Sound, Washington. Arch. Environ. Contam. Toxicol. 16, 511–522.

Krasko, A., Kurelec, B., Batel, R., Müller, I.M., Müller, W.E.G., 2001. Potential multidrug resistance gene *POHL*: an ecologically relevant indicator in marine sponges. Environ. Toxicol. Chem. 20, 198–204.

Kristensen, P., Tyle, H., 1991. The assessment of bioaccumulation. In: Nagel, R., Loskill, R. (Eds.), Bioaccumulation in Aquatic Systems.

Contributions to the Assessment. Varlag Chemie, Weinheim, pp. 187–227.

Kuehl, D.W., Cook, P.M., Batternman, A.R., Lothenbach, D., Butterworth, B.C., 1987. Bioavailability of polychlorinated dibenzo-*p*-dioxins from contaminated Wisconsin river sediment to carp. Chemosphere 16, 667–679.

Kurelec, B., 1992. The multixenobiotic resistance mechanism in aquatic organisms. Crit. Rev. Toxicol. 22, 23–43.

Kurelec, B., 1995. Inhibition of the multixenobiotic resistance mechanism in aquatic organisms: ecotoxic consequences. Sci. Total Environ. 171, 197–204.

Kurelec, B., 1997. A new type of hazardous chemical: the chemosensitizers of multixenobiotic resistance. Environ. Health Perspect. 105 (suppl. 4), 855–860.

Kurelec, B., Garg, A., Krca, S., Chacko, M., Gupta, R.C., 1989. Natural environment surpasses polluted environment in inducing DNA damage in fish. Carcinogenesis 10, 1337–1339.

Lake, J.L., McKinney, R., Lake, C.A., Osterman, F.A., Heltshe, J., 1995. Comparison of patters of polychlorinated biphenyl congeners in water, sediment and indigenous organisms from New Bedford Harbor, Massachusetts. Arch. Environ. Contam. Toxicol. 29, 207–220.

Lamoureux, E.M., Brownawell, B.J., 1999. Chemical and biological availability of sediment-sorbed hydrophobic organic contaminants. Environ. Toxicol. Chem. 18, 1733–1741.

Landrum, P.F., Faust, W.R., 1991. Effect of variation in sediment composition on the uptake rate coefficient for selected PCB and PAH congeners by the amphipod, *Diporeia* sp. In: Mayes, M.A., Baron, M.G. (Eds.), Aquatic Toxicology and Risk Assessment, vol. 14, ASTM STP 1124, American Society for Testing and Materials, Philadelphia, pp. 166–180.

Larsson, D.G.J., Adolfsson-Erici, M., Parkkonen, J., Pettersson, M., Berg, A.H., Olson, P.-E., Förlin, L., 1999. Ethinyloestradiol—an undesired fish contraceptive. Aquat. Toxicol. 45, 91–97.

Lauterburg, B.H., Smith, C.V., Hughes, H., Mitchell, J.R., 1983. Determinants of hepatic glutathione turnover: toxicological significance. In: Lamble, J.W. (Ed.), Drug Metabolism and Distribution. Elsevier Biomedical Press, Amsterdam, Netherlands, pp. 166–180.

Leadly, T.A., Balch, G., Metcalfe, C.D., Lazar, R., Mazak, E., Habowsky, J., Haffner, D., 1998. Chemical accumulation and toxicological stress in three brown bullhead (*Ameiurus nebulosus*) populations of the Detroit river, Michigag, USA. Environ. Toxicol. Chem. 17, 1756–1766.

Leadly, T.A., Arcand-Hoy, L.D., Haffner, D., Metcalfe, C.D., 1999. Fluorescent aromatic hydrocarbons in bile as a biomarker of exposure of brown bullheads (*Ameiurus nebulosus*) to contaminated sediments. Environ. Toxicol. Chem. 8, 750–755.

LeBlanc, G.A., 1995. Trophic-level differences in the bioconcentration of chemicals: implications in assessing environmental biomagnification. Environ. Sci. Technol. 29, 154–160.

Lech, J.J., Vodicnik, M.J., 1985. Biotransformation. In: Rand, G.M., Petrocelli, S.R. (Eds.), Fundamentals of Aquatic Toxicology; Methods and Applications. Hemisphere Publishing Corporation, New York, USA, pp. 526–557.

Lehtinen, K.-J., Kierkegaard, A., Jakobsson, E., Wändell, A., 1990. Physiological effects in fish exposed to effluents from mills with six different bleaching processes. Ecotoxicol. Environ. Safe 19, 33–46.

Lemaire, P., Förlin, L., Livingstone, D., 1996. Responses of hepatic biotransformation and antioxidant enzymes to CYP1A-inducers (3-methylcholanthrene, β-naphthoflavone) in sea bass (*Dicentrarchus labrax*), dab (*Limanda limanda*) and rainbow trout (*Oncorhynchus mykiss*). Aquat. Toxicol. 36, 141–160.

Lemaire-Gony, S., Lemaire, P., 1992. Interactive effects of cadmium and benzo(a)pyrene on cellular structure abd biotransformation enzymes of the liver of the European eel *Anguilla anguilla*. Aquat. Toxicol. 22, 145–160.

Lemaire-Gony, S., Lemaire, P., Pulsford, A.L., 1995. Effects of cadmium and benzo[a]pyrene on the immune system, gill ATPase and EROD activity of European sea bass *Dicentrarchus labrax*. Aquat. Toxicol. 31, 297–313.

Lenartova, V., Holovska, K., Pedrajas, J.-R., Martinez-Lara, E., Peinado, J., Lopez-Barea, J., Rosival, I., Kosuth, P., 1997. Antioxidant and detoxifying fish enzymes as biomarkers of river pollution. Biomarkers 2, 247–252.

Leppänen, H., Marttinen, S., Oikari, A., 1998. The use of fish bile metabolite analyses as exposure biomarkers to pulp and paper mill effluents. Chemosphere 36, 2621–2634.

Levine, S.L., Oris, J.T., 1997. Induction of CYP 1A mRNA and catalytic activity in gizzard shad (*Dorosoma cepedianum*) after waterborne exposure to benzo[a]pyrene. Comp. Biochem. Physiol. C. Pharmacol. Toxicol. Endocrinol. 118, 397–404.

Levine, S.L., Oris, J.T., 1999. CYP1A expression in liver and gill of rainbow trout following waterborne exposure: implications for biomarker determination. Aquat. Toxicol. 46, 279–287.

Levine, S.L., Oris, J.T., Wissing, T.E., 1994. Comparison of P-4501A1 monooxygenase induction in gizzard shad (*Dorosoma cepedianum*) following intraperitoneal injection or continuous waterborne-exposure with benzo[a]pyrene: temporal and dose-dependent studies. Aquat. Toxicol. 30, 61–75.

Levine, S.L., Oris, J.T., Denison, M.S., 1999. Modulation of CYP1A expression in rainbow trout by a technical grade formulation of propiconazole. Environ. Chem. Toxicol. 18, 2565–2573.

Lindholst, C., Petersen, K.L., Pedersen, S.N., 2000. Estrogenic response of bisphenol A in rainbow trout (*Oncorhynchus Mykiss*). Aquat. Toxicol. 48, 87–94.

Lindström-Seppä, P., Pesonen, M., 1986. Biotransformation enzymes in fish as tools for biomonitoring the aquatic environment. Acta Biol. Hung. 37, 85–95.

Lindström-Seppä, P., Oikari, A., 1989. Biotransformation and other physiological responses in whitefish caged in a lake receiving pulp and paper mill effluents. Ecotoxical. Environ. Saf. 18, 191–203.

Lindström-Seppä, P., Oikari, A., 1990. Biotransformation and other toxicological and physiological responses in rainbow trout (*Salmo gairdneri* Richardson) caged in a lake receiving effluents of pulp and paper industry. Aquat. Toxicol. 16, 187–204.

Lindström-Seppä, P., Oikari, A., 1991. Biotransformation activities of feral fish in waters receiving bleached pulp mill effluents. Environ. Toxicol. Chem. 9, 1415–1424.

Lindström-Seppä, P., Huuskonen, S., Pesonen, M., Muona, P., Hänninen, O., 1992. Unbleached pulp mill effluents affect cytochrome P450 monooxygenase enzyme activities. Mar. Environ. Res. 34, 157–161.

Lindström-Seppä, P., Korytko, P.J., Hahn, M.E., Stegeman, J.J., 1994. Uptake of waterborn 3,3′4,4′-tetrachlorobiphenyl and organ and cell-specific induction of cytochrome P4501A in adult and larval fathead minnow *Pimephales promelas*. Aquat. Toxicol. 28, 147–167.

Lindström-Seppä, P., Roy, S., Huuskonen, S., Tossavainen, K., Ritola, O., Marin, E., 1996. Biotransformation and glutathione homeostasis in rainbow trout exposed to chemical and physical stress. Mar. Environ. Res. 42, 323–327.

Little, P.J., James, M.O., Pritchard, J.B., Bend, J.R., 1984. Benzo[a]pyrene metabolism in hepatic microsomes from feral and 3-methylcholanthreen-treated southern flounder, *Paralichtys lethostigma*. J. Environ. Pathol. Toxicol. Oncol. 5, 309–320.

Livingstone, D.R., Lemaire, P., Mathews, A., Peters, L., Bucke, D., Law, R.J., 1993. Pro-oxidant, antioxidant and 7-ethoxyresorufin *O*-deethylase (EROD) activity responses in liver of dab (*Limanda limanda*) exposed to sediment contaminated with hydrocarbons and other chemicals. Marine Pollition Bull. 26, 602–606.

Lockhart, W.L., Metner, D.A., 1992. Applications of hepatic mixed function oxidase enzyme activities to northern freshwater fish: I. Burbot, *Lota lota*. Mar. Environ. Res. 34, 175–180.

Loonen, H., Parsons, J.R., Govers, H.A.J., 1991. Dietary accumulation of PCDDs and PCDFs in guppies. Chemosphere 23, 1349–1357.

Loonen, H., Parsons, J.R., Govers, H.A.J., 1994a. Effect of sediment on the bioaccumulation of a complex mixture of polychlorinated dibenzo-*p*-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) by fish. Chemosphere 28, 1433–1446.

Loonen, H., Tonkes, M., Parsons, J.R., Govers, H.A.J., 1994b. Bioconcentration of polychlorinated dibenzo-*p*-dioxins and polychlorinated dibenzofurans in guppies after aqueous exposure to a complex PCDD/PCDF mixture: relationship with molecular structure. Aquat. Toxicol. 30, 153–169.

Lopez-Torres, M., Perez-Campo, R., Cadenas, S., Rojas, C., Barja, G., 1993. A comparative research of free radicals in vertebrates—II. Non-enzymatic antioxidants and oxidative stress. Comp. Biochem. Physiol. 105, 757–763.

Luk, G.K., Brockway, F., 1997. Application of a polychlorinated biphenyls bioaccumulation model to Lake Ontario lake trout. Ecol. Model 101, 97–111.

Lye, C.M., Frid, C.L.J., Gill, M.E., Cooper, D.W., Jones, D.M., 1999. Estrogenic alkylphenols in fish tissues, sediments, and water from the UK Tyne and Tees estuaries. Environ. Sci. Technol. 33, 1009–1014.

Maccubbin, A.E., 1994. DNA adduct analysis in fish: laboratory and field studies. In: Malins, D.C., Ostrander, G.K. (Eds.), Aquatic Toxicology; Molecular, Biochemical and Cellular Perspectives. Lewis Publishers, CRC press, pp. 267–294.

MacFarlane, R.D., Bullock, G.L., McLaughlin, J.J.A., 1986. Effects of five metals on susceptibility of striped bass to *Flexibacter columnaris*. Trans. Am. Fish. Soc. 115, 227–231.

Machala, M., Drabek, P., Neca, J., Kolarova, J., Svobodova, Z., 1998. Biochemical markers for differentiation of exposures to nonplanar polychlorinated biphenyls, organochlorine pesticides, or 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in trout liver. Ecotoxicol. Environ. Safe 41, 107–111.

Mackay, D., 1982. Correlation of bioconcentration factors. Environ. Sci. Technol. 16, 274–278.

Madenjian, C.P., Hesselberg, R.J., Desorcie, T.J., Schmidt, L.J., Stedman, R.M., Quintal, R.T., Begnoche, L.J., Passino-Reader, D.R., 1998a. Estimate of net trophic transfer efficiency of PCBs to Lake Michigan lake trout from their prey. Environ. Sci. Technol. 32, 886–891.

Madenjian, C.P., Elliott, R.F., Schmidt, L.J., Desorcie, T.J., Hesselberg, R.J., Quintal, R.T., Begnoche, L.J., Bouchard, P.M., Holey, M.E., 1998b. Net trophic transfer efficiency of PCBs to Lake Michigan coho salmon from their prey. Environ. Sci. Technol. 32, 3063–3067.

Malins, D.C., Krahn, M.M., Brown, D.W., Rhodes, L.D., Myers, M.S., McCain, B.B., Chan, S.L., 1985. Toxic chemicals in marine sediment and biota from Mukilteo, Washington: relationships with hepatic neoplasms and other hepatic lesions in English sole (*Parophrys vetulus*). J. Natl. Cancer Inst. 74, 487–494.

Malins, D.C., Ostrander, G.K., Haimanot, R., Williams, P., 1990. A novel DNA lesion in neoplastic livers of feral fish: 2,6-diamino-4-hydroxy-5-formamidopyrimidine. Carcinogenesis 11, 1045–1047.

Malmström, C.M., Miettinen, S., Bylund, G., 2000. DNA adducts in liver and leucocytes of flounder (*Platichthys flesus*) experimentally exposed to benzo[a]pyrene. Aquat. Toxicol. 48, 177–184.

Mantel, N., 1967. The detection of disease clustering and a generated regression approach. Cancer Res. 27, 200–209.

Marionnet, D., Chambras, C., Taysse, L., Bosgireaud, C., Deschaux, P., 1998. Modulation of drug-metabolizing systems by bacterial endotoxin in carp liver and immune organs. Ecotoxicol. Environ. Safe 41, 189–194.

Martel, P.H., Kovacs, T.G., O'Connor, B.I., Voss, R.H., 1995. A laboratory exposure procedure for screening pulp and paper mill effluents for the potential of causing increased mixed function oxidase activity in fish. Environ. Pollut. 89, 229–240.

Maruya, K.A., Lee, R.F., 1998. Biota-sediment accumulation and trophic transfer factors for extremely hydrophobic polychlorinated biphenyls. Environ. Toxicol. Chem. 17, 2463–2469.

Masfaraud, J.F., Monod, G., Devaux, A., 1990. Use of the fish cytochrome P450-dependent 7-ethoxyresorufin *O*-deethylase activity as a biochemical indicator of water pollution. Study of the liver and the kidney of male and female nase (*Chondrostoma nasus*) from the river Rhône. Sci. Total Environ. 97/98, 729–738.

Mather-Mihaich, E., Di Giulio, R.T., 1991. Oxidant, mixed-function oxidase and peroxisomal responses in channel catfish exposed to a bleached kraft mill effluent. Arch. Environ. Contam. Toxicol. 20, 391–397.

Mayer, F.L., Versteeg, D.J., McKee, M.J., Folmar, L.C., Graney, R.L., McCume, D.C., Rattner, B.A., 1992. Metabolic products as biomarkers. In: Huggett, R.J., Kimerly, R.A., Mehrle, P.M., Jr, Bergman, H.L. (Eds.), Biomarkers: Biochemical, Physiological and Histological Markers of Anthropogenic Stress. Lewis Publishers, Chelsea, MI, USA, pp. 5–86.

McCarthy, J.F., 1990. Implementation of a biomarker-based environmental monitoring program. In: McCarthy, J.F., Shugart, L.R. (Eds.), Biomarkers of Environmental Contamination. Lewis Publishers, Boca Raton, FL, USA, pp. 429–440.

McCarthy, J.F., Shugart, L.R., 1990. Biological markers of environmental contamination. In: McCarthy, J.F., Shugart, L.R. (Eds.), Biomarkers of Environmental Contamination. Lewis Publishers, Boca Raton, FL, USA, pp. 3–16.

McCarthy, J.F., Halbrook, R.S., Shugart, L.R., 1991. Conceptual strategy for design, implementation, and validation of a biomarker-based biomonitoring capability. Publication no. 3072, ORNL/TM-11783. Environmental Sciences Division, Oak Ridge National Laboratory, Tennessee, USA.

McCarthy, J.F., Southworth, G.R., Ham, K.D., Palmer, J.A., 2000. Time-integrated, flux-based monitoring using semipermeable membrane devices to estimate the contribution of industrial facilities to regional polychlorinated biphenyl budgets. Environ. Toxicol. Chem. 19, 352–359.

McCarty, L.S., 1987. Relationship between toxicity and bioconcentration for some organic chemicals, parts I & II. In: Kaiser, K.L.E., Reidel, D. (Eds.), QSAR in Environmental Toxicology, Tensen Scientific Publ. Co., Dordrecht, The Netherlands, pp. 207–229.

McDonald, S.J., Kennicutt, M.C., Liu, H., Safe, S.H., 1995. Assessing aromatic hydrocarbon exposure in Antarctic fish captured near Palmer and McMurdo stations. Antarctica Arch. Environ. Contam. Toxicol. 29, 232–240.

McMaster, M.E., Munkittrick, K.R., Luxon, P.L., Van der Kraak, G.J., 1994. Impact of low-level sampling stress on interpretation of physiological responses of white sucker exposed to effluent from a bleached kraft pulp mill. Ecotoxicol. Environ. Saf. 27, 251–264.

Meador, J.P., Stein, J.E., Reichert, W.L., Varanasi, U., 1995. Bioaccumulation of polycyclic aromatic hydrocarbons by marine organisms. Rev. Environ. Contam. Toxicol. 143, 79–165.

Meadows, J.C., Echols, K.R., Huckins, J.N., Borsuk, F.A., Carline, R.F., Tillitt, D.E., 1998. Estimation of uptake rate constants for PCB congeners accumulated by semipermeable membrane devices and brown trout (*Salmo trutta*). Environ. Sci. Technol. 32, 1847–1852.

Melancon, M.J., Lech, J.J., 1983. Dose-effect relationship for induction of hepatic monooxygenase activity in rainbow trout and carp by Aroclor 1254. Aquat. Toxicol. 4, 51–61.

Melancon, M.J., Alscher, R., Benson, W., Kruzynski, G., Lee, R.F., Sikka, H.C., Spies, R.B., 1992. Metabolic products as biomarkers. In: Huggett, R.J., Kimerly, R.A., Mehrle, P.M., Jr, Bergman, H.L. (Eds.), Biomarkers: Biochemical, Physiological and Histological Markers of Anthropogenic Stress. Lewis Publishers, Chelsea, MI, USA, pp. 87–124.

Mellanen, P., Soimasuo, M., Holmbom, B., Oikari, A., Santti, R., 1999. Expression of the vitellogenin gene in the liver of juvenile whitefish (*Coregonus lavaretus* L. *s.l.*) exposed to effluents from pulp and paper mills. Ecotoxicol. Environ. Safe 43, 133–137.

Mensink, B.P., 1999. Imposex in the common whelk. Buccinum undatum. Ph.D. thesis, University of Wageningen, The Netherlands.

Metcalfe, T.L., Metcalfe, C.D., 1997. The trophodynamics of PCBs, including mono- and non-ortho congeners, in the food web of North-Central Lake Ontario. Sci. Total Environ. 201, 245–272.

Miranda, C., Wang, J.L., Chang, H.S., Buhler, D., 1990. Multiple effects of 3,4,5,3′,4′,5′-hexachlorobiphenyl administration on hepatic cytochrome P450 isozymes and associated mixed-function oxidase activities in rainbow trout. Biochem. Pharmacol. 40, 387.

Misra, S., Zafarulla, M., Price-Haughey, J., Gedamu, L., 1989. Analysis of stress-induced gene expression in fish cell lines exposed to heavy metals and heat shock. Biochim. Biophys. Acta 1007, 325–333.

Mix, M.C., 1986. Cancerous diseases in aquatic animals and their association with environmental pollutants: a critical literature review. Mar. Environ. Res. 20, 1–141.

Monod, G., Devaux, A., Riviere, J.L., 1988. Effects of chemical pollution on the activities of hepatic xenobiotic metabolizing enzymes in fish from the river Rhône. Sci. Total Environ. 73, 189–201.

Morimoto, R.I., 1998. Regulation of the heat shock transcriptional response: crosstalk between a family of heat shock factors, molecular chaperones, and negative regulators. Genes Dev. 12, 3788–3796.

Morrison, H.A., Gobas, F.A.P.C., Lazar, R., Whittle, D.M., Haffner, G.D., 1997. Development and verification of a benthic/pelagic food web bioaccumulation model for PCB congeners in Western Lake Erie. Environ. Sci. Technol. 31, 3267–3273.

Moss, D.W., Henderson, A.R., Kochmar, J.F., 1986. Enzymes; principles of diagnostic enzymology and the aminotransferases. In: Tietz, N.W. (Ed.), Textbook of Clinical Chemistry. Saunders, Philadelphia, PA, pp. 663–678.

Mosse, P.R.L., Brumley, C.M., Ahokas, J.T., Holdway, D.A., 1996. A preliminary investigation into the use of biomarkers for the monitoring of an Ocean outfall. Environ. Toxicol. Water Qual. 11, 113–119.

Mowrer, J., Aswalt, K., Burgermeister, G., Machado, L., Tarradellas, J., 1982. PCBs in a Lake Geneva ecosystem. Ambio 11, 355–360.

Muir, D.C.G., Lawrence, S., Holoka, M., Fairchild, W.L., Segstro, M.D., Webster, G.R.B., Servos, M.R., 1992a. Partitioning of polychlorinated dioxins and furans between water, sediments and biota in lake mesocosms. Chemosphere 25, 119–124.

Muir, D.C.G., Yarechewski, A.L., Metner, D.A., Lockhart, W.L., 1992b. Dietary 2,3,7,8-tetrachlorodibenzofuran in rainbow trout: accumulation, disposition, and hepatic mixed-function oxidase enzyme induction. Toxicol. Appl. Pharmacol. 117, 65–74.

Muir, D.C.G., Hobden, B.R., Servos, M.R., 1994. Bioconcentration of pyrethroid insecticides and DDT by rainbow trout: uptake, depuration, and effect of dissolved organic carbon. Aquat. Toxicol. 29, 223–240.

Mulder, G.J., Coughty, M.W.H., Burchell, B., 1990. Glucuronidation. In: Mulder, G.J. (Ed.), Conjugation Reactions in Drug Metabolism; an Integrated Approach. Taylor and Francis, London.

Munkittrick, K.R., Van der Kraak, G.J., McMaster, M.E., Portt, B.B., 1992. Response of hepatic MFO activity and plasma sex steroids to secondary treatment of bleached kraft pulp mill effluent and mill shutdown. Environ. Toxicol. Chem. 11, 1427–1435.

Murk, A.J., Denison, M.S., Giesy, J.P., Van der Guchte, C., Brouwer, A., 1996. Chemical-activated luciferase gene expression (*CALUX*): a novel in vitro bioassay for Ah receptor active compounds in sediments and pore water. Fundam. Appl. Toxicol. 33, 149–160.

Murphy, S.D., 1986. Pesticides. In: Doul, J., Klassen, C.D., Anders, M.O. (Eds.), The Basic Science of Poisons. Macmillan, New York, pp. 519–581.

Muto, N., Ren, H.W., Hwang, G.S., Tominaga, S., Itoh, N., Tanaka, K., 1999. Induction of two major isoforms of metallothionein in crucian carp (*Carassius cuvieri*) by air-pumping stress, dexamethasone, and metals. Comp. Biochem. Physiol. C. Pharmacol. Toxicol. Endocrinol. 122, 75–82.

Myers, M.S., Stehr, C.M., Olsen, O.P., Johnson, L.L., McCain, B.B., Chan, S.L., Varanasi, U., 1994. Relationships between toxicopathic hepatic lesions and exposure to chemical contaminants in English sole (*Pleuronectus vetulus*), starry flounder (*Platichthys stellatus*), and white croaker (*Genyonemus lineatus*) from selected marine sites on the Pacific coast, USA. Environ. Health Perspect. 102, 200–215.

Narbonne, J.F., Gallis, J.L., 1979. In vivo and in vitro effect of Phenoclor DP6 on drug metabolizing activity in mullet liver. Bull. Environ. Contam. Toxicol. 23, 338–343.

Narbonne, J.F., Garrigues, P., Ribera, D., Raoux, C., Mathieu, A., Lemaire, P., Salaun, J.P., Lafaurie, M., 1991. Mixed-function oxygenase enzymes as tools for pollution monitoring: field studies on the French coast of the Mediterranean Sea. Comp. Biochem. Physiol. 100C, 37–42.

Nebert, D.W., Peterson, D.D., Fornace, A.J., 1990. Cellular responses to oxidative stress: the [Ah] gene battery as a paradigm. Environ. Health Perspect. 88, 13–25.

Neff, J.M., 1985. Polycyclic Aromatic Hydrocarbons. In: Rand, G.M., Petrocelli, S.R. (Eds.), Fundamentals of Aquatic Toxicology; Methods and Applications. Hemisphere Publishing Corporation, New York, USA, pp. 416–454.

Neskovic, N.K., Elezovic, I., Karan, V., Poleksic, V., Budimir, M., 1993. Acute and subacute toxicity of atrazine to carp (*Cyprinus carpio* L.). Ecotoxicol. Environ. Safe 25, 173–182.

Newsted, J.L., Giesy, J.P., 1993. Effect of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) on the epidermical growth factor receptor in hepatic plasma membranes of rainbow trout (*Oncorhynchus mykiss*). Toxicol. Appl. Pharmacol. 119, 41–51.

Nicolas, J.-M., 1998. Vitellogenesis in fish and the effects of polycyclic aromatic hydrocarbon contaminants. Aquat. Toxicol. 45, 77–90.

Niimi, A.J., Oliver, B.G., 1989. Distribution of polychlorinated biphenyl congeners and other halocarbons in whole fish and muscle among lake Ontario salmonids. Environ. Sci. Toxicol. 23, 83–88.

Nikunen, E., 1985. Toxic impact from petrochemical industry. Ecotoxicol. Environ. Safe 9, 84–91.

NRC: Committee on Biological Markers of the National Research Council, 1987. Biological markers in environmental health research. Environ. Health Perspect. 74, 3–9.

O'Connor, J.E., Callahan, R.C., Escudero, M., Herrera, G., Martinez, A., Monterio, M.D., Momtoliu, H., 2001. The relevance of flow cytometry for biochemical analyses. IUBMB Life 51, 231–239.

O'Hare, D.B., Robotham, P.W.J., Gill, R., 1995. EROD me'asurement using post mitochondrial supernatant (PMS) in roach (*Rutilus rutilus*), a possible biomonitor for PAH contamination in the freshwater environment. Chemosphere 30, 257–264.

Oikari, A., Nakori, T., 1982. Kraft pulp mill effluent components cause liver dysfunction in trout. Bull. Environ. Contam. Toxicol. 28, 266–270.

Oikari, A., Kunnamo-Ojala, 1987. Tracing of xenobiotic contamination in water with the aid of fish bile metabolites: a field study with caged rainbow trout (*Salmo gairdneri*). Aquat. Toxicol. 9, 327–341.

Oikari, A., Jimenez, B., 1992. Effects of hepatotoxicants on the induction of microsomal monooxygenase activity in sunfish liver by b-naphtoflavone and benzo[a]pyrene. Ecotoxicol. Environ. Safe 23, 89–102.

Oikari, A., Holmbom, B., Anäs, E., Miilunpalo, M., Kruzynski, G., Castren, M., 1985. Ecotoxicological aspects of pulp mill effluents discharged to an inland water system: distribution in water, and

toxicant residues and physiological effects in caged fish (*Salmo gairdneri*). Aquat. Toxicol. 6, 219–239.

Olsson, P.E., Haux, C., 1986. Increased hepatic methallothionein content correlates to cadmium accumulation in environmentally exposed perch (*Perca fluviatilus*). Aquat. Toxicol. 9, 231–242.

Olsvik, P.A., Gundersen, Andersen, R.A., Zachariassen, K.E., 2000. Metal accumulation and metallothionein in two populations of brown trout, *Salmo trutta*, exposed to different natural water environments during a run-off episode. Aquat. Toxicol. 50, 301–316.

Omura, T., Sato, R., 1964. The carbon monoxide-binding pigment of liver microsomes, I and II. J. Biol. Chem. 239, 2370–2385.

Opperhuizen, A., 1991. Bioconcentration and biomagnification: is a distinction necessary. In: Nagel, R., Loskill, R. (Eds.), Bioaccumulation in Aquatic Systems. VCH Publishers, Weinheim, pp. 67–80.

Opperhuizen, A., Sijm, D.T.H.M., 1990. Bioconcentration and bioaccumulation of polychlorinated dibenzo-*p*-dioxins and dibenzofurans in fish. Environ. Toxicol. Chem. 9, 175–186.

Orn, S., Andersson, P.L., Förlin, L., Tysklind, M., Norrgren, L., 1998. The impact on reproduction of an orally administered mixture of selected PCBs in zebrafish (*Danio rerio*). Arch. Environ. Contam. Toxicol. 35, 52–57.

Otto, D.M.E., Moon, T.W., 1995. 3,3′,4,4′-tetrachlorobiphenyl effects on antioxidant enzymes and glutathione status in different tissues of rainbow trout. Pharmacol. Toxicol. 77, 281–287.

Otto, D.M.E., Moon, T.W., 1996a. Endogenous antioxidant systems of two teleoast fish, the rainbow trout and the black bullhead, and the effect of age. Fish Physiol. Biochem. 15, 349–358.

Otto, D.M.E., Moon, T.W., 1996b. Phase I and II enzymes and antioxidant responses in different tissues of brown bullheads from relatively polluted and non-polluted systems. Arch. Environ. Contam. Toxicol. 31, 141–147.

Otto, D.M.E., Lindström-Seppä, P., Sen, C.K., 1994. Cytochrome P450-dependent enzymes and oxidant-mediated responses in rainbow trout exposed to contaminated sediments. Ecotoxicol. Environ. Safe 27, 265–280.

Otto, D.M.E., Buttner, J.K., Arquette, D.M., Moon, T.W., 1996. Impaired inducibility of xenobiotic and antioxidant responses in rainbow trout exposed to polychlorinated biphenyl contaminated sediments in the St. Lawrence river. Chemosphere 33, 2021–2032.

Otto, D.M.E., Sen, C.K., Casley, W.L., Moon, T.W., 1997. Regulation of 3,3′,4,4′-tetrachlorobiphenyl induced cytochrome P450 metabolism by thiols in tissues of rainbow trout. Comp. Biochem. Physiol. C Pharmacol. Toxicol. Endocrinol. 117, 29–309.

Owens, I.D., 1977. Genetic regulation of UDP-glucuronyl-transferase induction by polycyclic aromatic hydrocarbon compounds in mice. J. Biol. Chem. 252, 2827–2832.

Pacheco, M., Santos, M.A., 1997. Induction of EROD activity and genotoxic effects by polycyclic aromatic hydrocarbons and resin acids on the juvenile eel (*Anguilla anguilla* L.). Ecotoxicol. Environ. Safe 38, 252–259.

Pacheco, M., Santos, M.A., 1998. Induction of liver EROD and erythrocytic nuclear abnormalities by cyclophosphamide and PAHs in *Anguilla anguilla* L. Ecotoxicol. Environ. Safe 40, 71–76.

Pacheco, M., Santos, M.A., 1999. Biochemical and genotoxic responses of adult eel (*Anguilla anguilla* L.) to resin acids and pulp mill effluent: laboratory and field experiments. Ecotoxicol. Environ. Safe 42, 81–93.

Padros, J., Pelletier, E., Reader, S., Denizeau, F., 2000. Mutual in vivo interactions between benzo[a]pyrene and tributyltin in brook trout (*Salvelinus fontinalis*). Environ. Toxicol. Chem. 19, 1019–1027.

Palace, V.P., Dick, T.A., Brown, S.B., Baron, C.L., Klaverkamp, J.F., 1996. Oxidative stress in Lake sturgeon (*Acipenser fulvescens*) orally exposed to 2,3,7,8-tetrachlorodibenzofuran. Aquat. Toxicol. 35, 79–92.

Parrott, J.L., Hodson, P.V., Dixon, D.G., 1995. Rainbow trout hepatic mixed-function oxygenase induction by polychlorinated dibenzo-*p*-dioxins (PCDDs) as a function of time and tissue concentration. J. Toxicol. Environ. Health 46, 301–316.

Parihar, M.S., Javeri, T., Hemnani, T., Dubey, A.K., Prakash, P., 1997. Responses of superoxide dismutase, glutathione peroxidase and glutathione antioxidant defenses in gills of the freshwater catfish (*Heteropneustes fossilis*) to short-term elevated temperature. J. Thermal Biol. 22, 151–156.

Park, S.S., Erstfeld, K.M., 1997. A numerical kinetic model for bioaccumulation of organic chemicals in sediment–water systems. Chemosphere 34, 419–427.

Payne, J.F., 1976. Field evaluation of benzopyrene hydroxylase induction as a monitor for marine pollution. Science 191, 945–949.

Payne, J.F., Penrose, W.R., 1975. Induction of hydrocarbon (benzo[a]pyrene) hydroxylase in fish by petroleum. Bull. Environ. Contam. Toxicol. 14, 112–116.

Payne, J.F., Fancey, L.F., 1989. Effects of polycyclic aromatic hydrocarbons on immune responses in fish: change in melanomacrophage centers in flounder (*Pleuropleuronectus americanus*) exposed to hydrocarbon-contaminated sediments. Mar. Environ. Res. 28, 431–435.

Payne, J.F., Bauld, C., Dey, A.C., Kiceniuk, J.W., Williams, U., 1984. Selectivity of mixed-function oxygenase enzyme induction in flounder (*Pseudopleuronectes americanus*) collected at the site of the Baie Verte, Newfoundland oil spill. Comp. Biochem. Physiol. 79, 15–19.

Payne, J.F., Fancey, L.L., Rahimtula, A.D., Porter, E.L., 1987. Review and perspective on the use of mixed-function oxygenase enzymes in biological moitoring. Comp. Biochem. Physiol. 86C, 233–245.

Payne, J.F., Mathieu, A., Melvin, W., Fancey, L.L., 1996. Acetylcholinesterase, an old biomarker with a new future? Field trials in association with two urban rivers and a paper mill in Newfoundland. Mar. Pollut. Bull. 32, 225–231.

Peakall, D.W., 1994. Biomarkers: the way forward in environmental assessment. Toxicol. Ecotoxicol. News 1, 55–60.

Peakall, D.W., Walker, C.H., 1994. The role of biomarkers in environmental assessment (3). Ecotoxicology 3, 173–179.

Pedrajas, J.R., Peinado, J., Lopez-Barea, J., 1995. Oxidative stress in fish exposed to model xenobiotics. Oxidatively modified forms of Cu, Zn-superoxide dismutase as potential biomarkers. Chem. Biol. Interact. 98, 267–282.

Pellisero, C., Flouriot, G., Foucher, J.L., Bennetau, B., Dunogues, J., Gac, F.L., Sumpter, J.P., 1993. Vitellogenin synthesis in cultured Hepatocytes; an in vitro test for the estrogenic potency of chemicals. J. Steroid Biochem. Mol. Biol. 44, 263–272.

Pesonen, M., Andersson, T.B., Sorri, V., Korkalainen, M., 1999. Biochemical and ultrastructural changes in the liver of Baltic salmon sac fry suffering from high mortality (M74). Environ. Toxicol. Chem. 18, 1007–1013.

Peters, L.D., Porte, C., Albaigés, J., Livingstone, D.R., 1994. 7-Ethoxyresorufin *O*-deethylase (EROD) and antioxidant enzyme activities in larvae of sardine (*Sardina Pilchardus*) from the North coast of Spain. Mar. Pollut. Bull. 28, 299–304.

Peters, L.D., Morse, H.R., Waters, R., Livingstone, D.R., 1997. Responses of hepatic P450 1A and formation of DNA-adducts in juveniles of turbot (*Scophthalmus maximus* L.) exposed to waterborne benzo[a]pyrene. Aquat. Toxicol. 38, 67–82.

Peterson, R.E., Theobald, H.M., Kimmel, G.L., 1993. Developemental and reproductive toxicity of dioxin and related compounds: cross-species comparisons. Crit. Rev. Toxicol. 23, 283–335.

Petty, J.D., Huckins, J.N., Martin, D.B., Adornato, T.G., 1995. Use of semipermeable membrane devices (SPMDs) to determine bioavailable organochlorine pesticide residues in streams receiving irrigation drainwater. Chemosphere 30, 1891–1903.

Pfau, W., 1997. DNA adducts in marine and freshwater fish as biomarkers of environmental contamination. Biomarkers 2, 145–151.

Pickett, C.B., Lu, A.Y.H., 1989. Glutathione S-transferases: gene structure, regulation, and biological function. Annu. Rev. Biochem. 58, 743–751.

Piechotta, G., Lacorn, M., Lang, T., Kammann, U., Simat, T., Jenke, H.-S., Steinhart, H., 1999. Apoptosis in dab (*Limanda limanda*) as possible new biomarker for anthropogenic stress. Ecotoxicol. Environ. Safe 42, 50–56.

Ploch, S.A., King, L.C., Kohan, M.J., Di Giulio, R.T., 1998. Comparative in vitro and in vivo benzo[a]pyrene–DNA adduct formation and its relationship to CYP1A activity in two species of ictalurid catfish. Toxicol. Appl. Pharmacol. 149, 90–98.

Ploch, S.A., Lee, Y.-P., MacLean, E., Di Giulio, R.T., 1999. Oxidative stress in liver of brown bullhead and channel catfish following exposure to *tert*-butyl hydroperoxide. Aquat. Toxicol. 46, 231–240.

Poels, C.L.M., Van der Gaag, M.A., van de Kerkhoff, J.F.J., 1980. An investigation into the long-term effects of Rhine water on rainbow trout. Water Res. 14, 1029–1035.

Poginsky, B., Blomeke, B., Hewer, A., Phillips, D., Karbe, L., Marquardt, H., 1990. ^{32}P-postlabeling analysis of hepatic DNA of benthic fish from European waters. Proc. Am. Assoc. Cancer Res. 31, 568.

Potter, D., Clarius, T.M., Wright, A.S., Watson, W.P., 1994. Molecular dosimetry of DNA adducts in rainbow trout (*Oncorhynchus mykiss*) exposed to benzo(a)pyrene by different routes. Arch. Toxicol. 69, 1–7.

Powers, D.A., 1989. Fish as model systems. Science 246, 352–358.

Power, M., McCarty, L.S., 1997. Fallacies in ecological risk assessment practices. Environ. Sci. Technol. 31, 370A–375A.

Power, M., McCarty, L.S., 1998. A comparative analysis of environmental risk assessment/risk management frameworks. Environ. Sci. Technol. 32, 224A–231A.

Pulsford, A., Lemaire-Gony, S., Farley, S.R., Burke, D., Dixon, P., 1992. Effects of environmental stress on dab *Limanda limanda* immune system. ICES report, Marine Environmental Quality Comittee.

Purdom, C.E., Hardiman, P.A., Bye, V.J., Eno, N.C., Tyler, C.R., Sumpter, J.P., 1994. Estrogenic effects of effluents from sewage treatment works. Chem. Ecol. 8, 275–285.

Rabergh, C.M., Airaksinen, S., Soltamo, A., Bjorklund, H.V., Johansson, T., Nikinmaa, M., Sistonen, L., 2000. Tissue-specific expression of zebrafish (*Danio rerio*) heat shock factor 1 mRNAs in response to heeat stress. J. Exp. Biol. 203, 1817–1824.

Rantalainen, A.-L., Ikonomou, M.G., Rogers, I.H., 1998. Lipid-containing semipermeable membrane devices (SPMDs) as concentrators of toxic chemicals in the lower Fraser river, Vancouver, British Columbia. Chemosphere 37, 1119–1138.

Rantalainen, A.-L., Cretney, W.J., Ikonomou, M.G., 2000. Uptake rates of semipermeable membrane devices (SPMDs) for PCDDs, PCDFs and PCBs in water and sediment. Chemosphere 40, 147–158.

Ray, S., Bieger, T., Scruton, D.A., 1995. ^{32}P-postlabeling analysis of aromatic DNA-adducts in liver and brain of wild brook trout (*Salvelinus fontinalis*). Chemosphere 30, 773–778.

Raza, H., Otaiba, A., Montague, W., 1995. *b*-Naphtaflavone-inducible cytochrome P4501A1 activity in liver microsomes of the marine Safi fish (*Siganus canaliculatus*). Biochem. Pharmacol. 50, 1401–1406.

Reddy, J.K., Lalwani, N.D., 1983. Carcinogenesis by hepatic peroxisome proliferators: evaluation of the risk of hypolipidemic drugs and industrial plasticizers to humans. CRC Crit. Rev. Toxicol. 12, 1–68.

Reddy, S.J., Reddy, B.V., Ramamurthi, R., 1991. Impact of chronic phosalone toxicity on erythropoietic activity of fish, *Oreochromis mossambicus*. Biochem. Int. 25, 547–552.

Regoli, F., Winston, G.W., 1999. Quantification of total oxidant scavenging capacity (TOSC) of antioxidants for peroxynitrite, peroxyl radicals and hydroxyl radicals. Toxicol. Appl. Pharmacol. 156, 96–105.

Regoli, F., Nigro, M., Bompadre, S., Winston, G.W., 2000. Total oxidant scavenging capacity (TOSC) of microsomal and cytosolic fractions from Antarctic, Arctic and Mediterranean scallops: differentiation between three potent oxidants. Aquat. Toxicol. 49, 13–25.

Renner, R., 1997. National fish survey links pesticides with sex hormone imbalance. Environ. Sci. Technol. 31, 312A–313A.

Renton, K.W., Addison, R.F., 1992. The utilization of cytochrome P4501A mRNA in *Limanda limanda* (dab) as a monitor of chemical exposure in the North Sea. Mar. Environ. Res. 34, 151–155.

Rice, C.D., Roszell, L.E., 1998. Tributyltin modulates 3,3′,4,4′,5-pentachlorobiphenyl (PCB 126)-induced hepatic CYP1A activity in channel catfish, *Ictalurus punctatus*. J. Toxicol. Environ. Health 55, 197–212.

Rice, D.W., Seltenrich, C.P., Keller, M.L., Spies, R.B., Felton, J.S., 1994. Mixed-funcion oxidase-specific activity in wild and caged speckled sanddabs *Citharichthys stigmaeus* in Elkhorn Slough, Moss Landing Harbor and nearshore Monterey Bay, California. Environ. Pollut. 84, 179–188.

Rifkin, E., LaKind, J., 1991. Dioxin bioaccumulation: key to a sound risk assessment methodology. J. Toxicol. Environ. Health 33, 103–112.

Riviere, J.L., Devaux, A., Gonin, O., Monod, G., 1990. Effect of ß-naphtoflavone and MCPA on liver and kidney drug-metabolizing enzymes from the carp, *Cyprinus carpio*. Ecotoxical. Environ. Safe 19, 276–284.

Roberts, M.H., Sved, D.W., Felton, S.P., 1987. Temporal changes in AHH and SOD activities in feral spot from the Elizabeth River, a polluted sub-estuary. Mar. Environ. Res. 23, 89–101.

Robohm, R.A., 1986. Paradoxial effects of cadmium exposure on antibacterial antibody responses in two fish species: inhibition in cuners (*Tautogolabrus adspersus*) and enhancement in striped bass (*Morone saxatilis*). Immunol. Immunopathol. 12, 251–262.

Roch, M., McCarter, J.A., 1984. Hepatic metallothionein production and resistance to heavy metals by rainbow trout (*Salmo gairdneri*) held in a series of contaminated lakes. Comp. Biochem. Physiol. 77C, 77–82.

Roch, M., McCarter, J.A., Matheson, A.T., Clark, M.J.R., Olafson, R.W., 1982. Hepatic metallothionein in rainbow trout (*Salmo gairdneri*) as an indicator of metal pollution in the Campbell River system. Can. J. Fish. Aquat. Sci. 39, 1596–1601.

Rodriguez-Ariza, A., Martinez-Lara, E., Pascual, P., Pedrajas, J.R., Abril, N., Dorado, G., Toribio, F., Barcena, J.A., Peinado, J., Pueyo, C., Lopez-Barea, J., 1993. Biochemical and genetic indices of marine pollution in Spanish littoral. Sci. Total Environ. Suppl. 1, 109–116.

Rodriguez-Ariza, A., Dorado, G., Navas, J.I., Pueyo, C., Lopez-Barea, J., 1994. Promutagen activation by fish liver as a biomarker of littoral pollution. Environ. Mol. Mutagen. 24, 116–123.

Roesijadi, G., Robinson, W.E., 1994. Metal regulation in aquatic animals: mechanisms of uptake, accumulation and release. In: Malins, D.C., Ostrander, G.K. (Eds.), Aquatic Toxicology; Molecular, Biochemical and Cellular Perspectives. Lewis Publishers, CRC press, pp. 387–420.

Romeo, M., Bennani, N., Gnassia-Barelli, M., Lafaurie, M., Girard, J.P., 2000. Cadmium and copper display different responses towards oxidative stress in the kidney of the sea bass *Dicentrarchus labrax*. Aquat. Toxicol. 48, 185–194.

Roninson, I.B., 1992. From application to function: the case of the MDR1 gene. Mutat. Res. 276, 151–161.

Ronis, M.J.J., Celander, M., Förlin, L., Badger, T.M., 1992. The use of polyclonal antibodies raised against rat and trout cytochrome P450 CYP1A1 orthologues to monitor environmental induction in the channel catfish (*Ictalurus punctatus*). Mar. Environ. Res. 34, 181–188.

Rotchell, J.M., Bird, D.J., Newton, L.C., 1999. Seasonal variation in ethoxyresorufin O-deethylase (EROD) activity in European eels *Anguilla anguilla* and flounders *Pleuronectes flesus* from the Severn estuary and Bristol Channel. Mar. Ecol. Prog. Ser. 190, 263–270.

Rotchell, J.M., Steventon, G.B., Bird, D.J., 2000. Catalytic properties of CYP1A isoforms in the liver of an agnathan (*Lampetra Fluviatilis*) and two species of teleost (*Pleuronectus flesus*, *Anguilla anguilla*). Comp. Biochem. Physiol. C 125, 203–214.

Roy, S., Lindström-Seppä, P., Huuskonen, S., Hänninen, O., 1995. Responses on biotransformation and antioxidant enzymes in *Lemna* minor and Oncorhynchus mykiss simultaneously to hexaclorobenzene. Chemosphere 30, 1489–1498.

Rudneva-Titova, I.I., Zherko, N.V., 1994. Effects of polychlorinated biphenyls on the activity of anti-oxidant enzymes and lipid peroxidation in muscle and liver of two Black Sea fish species. Biochemistry (Moscow) 59, 25–31.

Rushmore, T.H., Pickett, C.B., 1990. Transcriptional regulation of the rat glutathione S-transferase Ya subunit gene. J. Biol. Chem. 265, 14648–14653.

Russell, R.W., Gobas, F.A.P.C., Haffner, G.D., 1999. Role of chemical and ecological factors in trophic transfer of organic chemicals in aquatic food webs. Environ. Toxicol. Chem. 18, 1250–1257.

Sabuliunas, D., Lazutka, J., Sabaliuniene, I., Södergren, A., 1998. Use of semipermeable membrane devices for studying effects of organic pollutants: comparison of pesticide uptake by semipermeable membrane devices and mussels. Environ. Toxicol. Chem. 17, 1815–1824.

Sadik, O.A., Witt, D.M., 1999. Monitoring endocrine-disrupting chemicals. Environ. Sci. Technol 33, 368A–374A.

Safe, S., 2001. Molecular Biology of the Ah receptor and its role in carcinogenesis. Toxicol. Lett. 120, 1–7.

Sagelsdorff, P., 1995. Methods for the determination of reactive compounds. In: Thomas, H., Hess, R., Waechter, F. (Eds.), Toxicology of Industrial Compounds. Taylor & Francis, London, pp. 73–89.

Sanchez-Dardon, J., Voccia, I., Hontela, A., Chilmonczyk, S., Dunier, M., Boermans, H., Blakley, B., Fournier, M., 1999. Immunomodulation by heavy metals tested individually or in mixtures in rainbow trout (*Oncorhynchus mykiss*) exposed in vivo. Environ. Toxicol. Chem. 18, 1492–1497.

Sanchez-Hernandez, J.C., Fossi, M.C., Leonzio, C., Focardi, S., Barra, R., Gavilan, J.F., Parra, O., 1998, Use of biochemical biomarkers as a screening tool to focus the chemical monitoring of organic pollutants in Biobio river basin (Chile). Chemosphere 37, 699–710.

Sanders, B.M., 1990. Stress proteins: Potential as multitired biomarkers. In: Shugart, L., McCarty, J. (Eds.), Environmental Biomarkers. Lewis Publishers, Chelsea, MI, pp. 165–191.

Sanders, B.M., 1993. Stress proteins in aquatic organisms: an environmental perspective. Crit. Rev. Toxicol. 23, 49–75.

Sanders, B.M., Martin, L.S., 1993. Stress proteins as biomarkers of contaminant exposure in archived environmental samples. Sci. Total Environ. 139-140, 459–470.

Sanders, B.M., Nguyen, J., Douglass, T.G., Miller, S., 1994. Heat-inducable proteins that react with antibodies to chaperonin 60 are localized in the nucleus of a fish cell line. Biochem. J. 297, 21–25.

Sarkadi, B., Muller, M., 1997. Search for specific inhibitors of multidrug resistance in cancer. Semin. Cancer Biol. 8, 171–182.

Sawyer, T., Safe, S., 1982. PCB isomers and congeners: induction of aryl hydrocarbon hydroxylase and ethoxyresorufin-O-deethylase enzyme activities in rat hepatoma cells. Toxicol. Lett. 13, 87–94.

Sazuka, Y., 1989. Effect of adriamycin on the activities of superoxide dismutase, glutathione peroxidase and catalase in tissues of mice. Jpn. J. Cancer Res. 80, 80–94.

Schinkel, A.H., Smit, J.J., van Tellingen, O., Beijnen, J.H., Wagenaar, E., van Deemter, L., Mol, C.A., Van der Valk, M.A., Robanus-Maandag, E.C., te Riele, H.P., 1994. Disruption of the mouse mdr1a *P*-glycoprotein gene leads to a deficiency in the blood–brain barrier and to increased sensitivity to drugs. Cell 77, 491–502.

Schlenk, D., Zhang, Y.S., Nix, J., 1995. Expression of hepatic metallothionein messenger RNA in feral fish and caged fish species correlates with muscle mercury levels. Ecotoxicol. Environ. Safe 31, 282–286.

Schlezinger, J.J., Stegeman, J.J., 2000. Induction of cytochrome P450 1A in the Americal eel by model halogenated and non-halogenated aryl hydrocarbon receptor agonists. Aquat. Toxicol. 50, 375–386.

Schlezinger, J.J., White, R.D., Stegeman, J.J., 1999. Oxidative inactivation of cytochrome P-450 1A (CYP1A) stimulated by 3,3′,4,4′-tetrachlorobiphenyl: production of reactive oxygen by vertebrate CYP1As. Mol. Pharmacol. 56, 588–597.

Schramm, M., Müller, E., Triebskorn, R., 1998. Brown trout (*Salmo trutta* f. Fario) liver ultrastructure as a biomarker for assessment of small stream pollution. Biomarkers 3, 93–108.

Schrap, S.M., Opperhuizen, A., 1990. Relationship between bioavailability and hydrophobicity: reduction of the uptake of organic chemicals by fish due to the sorption on particles. Environ. Toxicol. Chem. 9, 715–724.

Schuytema, G.S., Krawczyk, D.F., Griffis, W.L., Nebeker, A.V., Robideaux, M.L., 1990. Hexachlorobenzene uptake by fathead minnows and macroinvertebrates in recirculating sediment/water systems. Arch. Environ. Contam. Toxicol. 19, 1–9.

Secombes, C.T., Fletcher, T.C., O'Flynn, J.A., Costello, M.J., Stagg, R., Houlihan, D.F., 1991. Immunocompetence as a measure of the biological effects of sewage sludge pollution in fish. Comp. Biochem. Physiol. 100C, 133–136.

Sharom, F.J., 1997. The P-glycoprotein efflux pump: how does it transport drugs. J. Membr. Biol. 160, 161–175.

Shepard, J.L., Olsson, B., Tedengren, M., Bradley, B.P., 2000. Protein expression signatures identified in *Mytilus edulis* exposed to PCBs, copper and salinity stress. Mar. Environ. Res. 50, 337–340.

Shugart, L.R., 1988. Quantitation of chemically induced damage to DNA of aquatic organisms by alkalyne unwinding assay. Aquat. Toxicol. 13, 43–52.

Shugart, L.R., 1990a. Biological monitoring: testing for genotoxicity. In: McCarthy, J.F., Shugart, L.R. (Eds.), Biomarkers of Environmental Contamination. Lewis Publishers, Boca Raton, FL, USA, pp. 205–216.

Shugart, L.R., 1990b. 5-Methyl deoxycytidine content of DNA from bluegill sunfish (*Lepomis macrochirus*) exposed to benzo[a]pyrene. Environ. Toxicol. Chem. 9, 205–208.

Shugart, L.R., 1996. Molecoular markers to toxic agents. In: Newman, M.C., Jagoe, C.H. (Eds.), Ecotoxicology: a Hierarchial Treatment. CRC Press, Boca Raton, USA, pp. 133–161.

Shugart, L.R., Bickham, J., Jackim, G., McMahon, G., Ridley, W., Stein, J., Steinert, S., 1992. DNA alterations. In: Huggett, R.J., Kimerly, R.A., Mehrle, P.M., Jr, Bergman, H.L. (Eds.), Biomarkers: Biochemical, Physiological and Histological Markers of Anthropogenic Stress. Lewis Publishers, Chelsea, MI, USA, pp. 155–210.

Sijm, D.T.H.M., Opperhuizen, A., 1989. Biotransformation of organic chemicals by fish: enzyme activities and reactions. In: Hutzinger, O. (Ed.), Handbook of Environmental Chemistry Reactions and Processes, vol. 2E. Springer, Berlin, pp. 163–235.

Sijm, D.T.H.M., Wever, H., Opperhuizen, A., 1989a. Influence of biotransformation on the accumulation of PCDDs from fly-ash in fish. Chemosphere 19, 475–480.

Sijm, D.T.H.M., Wever, H., de Vries, P.J., Opperhuizen, A., 1989b. Octan-1-ol/water partition coefficients of polychlorinated dibenzo-p-dioxins and dibenzofurans: experimental values determined with a stirring method. Chemosphere 19, 263–266.

Sijm, D.T.H.M., Seinen, W., Opperhuizen, A., 1992. Life-cycle biomagnification study in fish. Environ. Sci. Technol. 26, 2162–2174.

Sijm, D.T.H.M., Wever, H., Opperhuizen, A., 1993. Congener-specific biotransformation and bioaccumulation of PCDDs and PCDFs from fly ash in fish. Environ. Toxicol. Chem. 12, 1895–1907.

Sikka, H.C., Rutkowski, J.P., Kandaswami, C., Kumar, K., Gupta, R.C., 1990. Formation and persistence of DNA-adducts in the liver of brown bullheads exposed to benzo[a]pyrene. Cancer Lett. 49, 81–87.

Silbergeld, E.K., Fowler, B.A., 1987. Mechanisms of chemical-induced porphyrinopathies. Ann. New York Acad. Sci. 514, 352–354.

Skaare, J.U., Jensen, E.G., Goksøyr, A., Egaas, E., 1991. Response of xenobiotic metabolizing enzyme of rainbow trout (*Oncorhynchus mykiss*) to the mono–ortho substituted polychlorinated PCB congener 2,3′,4,4′,5-pentachlorobiphenyl, PCB-118, detected by enzyme activities and immunochemical methods. Arch. Environ. Contam. Toxicol. 20, 349–352.

Sleiderink, H.M., 1996. Assessment of cytochrome P450 1A in dab as biomarker of exposure to poly-chlorinated biphenyls and related compounds. Academic thesis, Agricultural University of Wageningen, The Netherlands.

Sleiderink, H.M., Boon, J.P., 1995. Cytochrome P450 1A response in North Sea dab, *Limanda limanda*, from offshore and coastal sites. Mar. Pollut. Bull. 30, 660–666.

Sleiderink, H.M., Boon, J.P., 1996. Temporal induction pattern of hepatic cytochrome P450 1A in thermally acclimated dab (*Limanda limanda*) treated with 3,3′,4,4′-tetrachlorobiphenyl (CB77). Chemosphere 32, 2335–2344.

Sleiderink, H.M., Beyer, J., Scholtens, E., Goksøyr, A., Nieuwenhuize, J., van Liere, J.M., Everaarts, J.M., Boon, J., 1995a. Influence of temperature and polyaromatic contaminants on CYP1A levels in North Sea dab (*Limanda limanda*). Aquat. Toxicol. 32, 189–209.

Sleiderink, H.M., Oostingh, I., Goksøyr, A., Boon, J.P., 1995b. Sensitivity of cytochrome P450 1A induction in dab (*limanda limanda*) of different age and sex as a biomarker for environmental contaminants in the southern North Sea. Arch. Environ. Contam. Toxicol. 28, 423–430.

Slooff, W., van Kreijl, C.F., Baars, A.J., 1983. Relative liver weights and xenobiotic-metabolizing enzymes of fish from polluted surface waters in the Netherlands. Aquat. Toxicol. 4, 1–14.

Smital, T., Kurelec, B., 1997. Inhibitors of the multixenobiotic resistance mechanism in natural waters: in vivo demonstration of their effects. Environ. Toxicol. Chem. 16, 2164–2170.

Soimasuo, R., Aaltonen, T., Nikinmaa, M., Pellinen, J., Ristola, T., Oikari, A., 1995. Physiological toxicity of low-chlorine bleached pulp and paper mill effluent on whitefish (*Coregonus lavaretus* L. s.l.): a laboratory exposure simulating lake pollution. Ecotoxicol. Environ. Safe 31, 228–237.

Soimasuo, R., Karels, A.E., Lepanen, H., Santti, R., Oikari, A., 1998a. Biomarker responses in whitefish (*Coregonus lavaretus* L. s.l.) experimentally exposed in a large lake receiving effluents from pulp and paper industry. Arch. Environ. Contam. Toxicol. 34, 69–80.

Soimasuo, R., Lapinvaara, J., Oikari, A., 1998b. Confirmation of in situ exposure of fish to secondary treated bleached-kraft mill effluent using a laboratory simulation. Environ. Toxicol. Chem. 17, 1371–1379.

Sole, M., Porte, C., Barcelo, D., 2000. Vitellogenin induction and other biochemical responses in carp, *Cyprinus carpio*, after experimental injection with 17 alpha-ethynylestradiol. Arch. Environ. Toxicol. 38, 494–500.

Spacie A., Hamelink, J.L., 1982. Alternative models for describing the bioconcentration of organics in fish. Environ. Toxicol. Chem. 1, 309–320.

Spies, R.B., Rice, D.W., Jr, Felton, J., 1988. Effects of organic contaminants on reproduction of the starry flounder *Phatichthys stellatus* in San Francisco Bay, I. Hepatic contamination and mixed-function oxidase (MFO) activity during the reproductive season. Mar. Biol. 98, 181–189.

Spies, R.B., Stegeman, J.J., Rice, D.W., Jr, Woodlin, B., Thomas, P., Hose, J.E., Cross, J.N., Prieto, M., 1990. Sublethal responses of *Platichtus stellatus* to organic contamination in San Francisco Bay with emphasis on reproduction. In: McCarthy, J.F., Shugart, L.R. (Eds.), Biomarkers of Environmental Contamination. Lewis Publishers, Boca Raton, FL, USA, pp. 87–122.

Spies, R.B., Stegeman, J.J., Hinton, D.E., Woodin, B., Smolowitz, R., Okihiro, M., Shea, D., 1996. Biomarkers of hydrocarbon exposure and sublethal effects in embiotocid fishes from a natural petroleum seep in the Santa Barbara channel. Aquat. Toxicol. 34, 195–219.

Stagg, R.M., Rusin, J., McPhail, M.E., McIntosh, A.D., Moffat, C.F., Craft, J.A., 2000. Effects of polycyclic aromatic hydrocarbons on expression of CYP1A in salmon (*Salmo salar*) following experimental exposure and after the Braer oil spill. Environ. Toxicol. Chem. 19, 2797–2805.

Stegeman, J.J., Lech, J.J., 1991. Cytochrome P450 momooxygenase systems in aquatic species: carcinogen metabolism and biomarkers for carcinogen and pollutant exposure. Environ. Health Perspect. 90, 101–109.

Stegeman, J.J., Hahn, M.E., 1994. Biochemistry and molecular biology of monooxygenase: current perspective on forms, functions, and regulation of cytochrome P450 in aquatic species. In: Malins, D.C., Ostrander, G.K. (Eds.), Aquatic toxicology; Molecular, Biochemical and Cellular Perspectives. Lewis Publishers, CRC press, Boca Raton, pp. 87–206.

Stegeman, J.J., Woodlin, B.R., Goksøyr, A., 1988. Apperent cytochrome P-450 induction as an indication of exposure to environmental chemicals in the flounder *Platichthys flesus*. Mar. Ecol. Prog. Ser. 46, 55–60.

Stegeman, J.J., Renton, K.W., Woodin, B.R., Zhang, Y.-S., Addison, R.F., 1990. Experimental and environmental induction of cytochrome P450E in fish from Bermuda waters. J. Exp. Mar. Biol. Ecol. 138, 49–67.

Stegeman, J.J., Brouwer, M., Richard, T.D.G., Förlin, L., Fowler, B.A., Sanders, B.M., van Veld, P.A., 1992. Molecular responses to environmental contamination: enzyme and protein systems as indicators of chemical exposure and effect. In: Huggett, R.J., Kimerly, R.A., Mehrle, P.M., Jr, Bergman, H.L. (Eds.), Biomarkers: Biochemical, Physiological and Histological markers of Anthropogenic Stress. Lewis Publishers, Chelsea, MI, USA, pp. 235–335.

Stein, J.E., Hom, T., Varanasi, U., 1984. Simultaneous exposure of English sole (*Parophrys vetulus*) to sediment-associated xenobiotics: I. Uptake and disposition of ^{14}C-polychlorinated biphenyls and ^{3}H-benzo[a]pyrene. Mar. Environ. Res. 13, 97–119.

Stein, J.E., Collier, T.K., Reichert, W.L., Casillas, E., Hom, T., Varanasi, U., 1992. Bioindicators of contaminant exposure and sublethal effects: studies with benthic fish in Puget Sound, Washington. Environ. Toxicol. Chem. 11, 701–714.

Stein, J.E., Reichert, W.L., French, B., Varanasi, U., 1993. ^{32}P-postlabeling analysis of DNA adduct formation and persistence in English sole (*Pleuronectus vetulus*) exposed to benzo[a]pyrene and 7H dibenzo [c,g]carbazole. Chem. Biol. Interact. 88, 55–69.

Stephensen, E., Svavarsson, J., Sturve, J., Ericson, G., Adolfsson-Erici, M., Förlin, L., 2000. Biochemical indicators of pollution exposure in shorthorn sculpin (*Myoxocephalus scorpius*), caught in

four harbours on the southwest coast of Iceland. Aquat. Toxicol. 48, 431–442.
Streit, B., 1992. Bioaccumulation processes in ecosystems. Experientia 48, 955–970.
Sturm, A., Wogram, J., Hansen, P.-D., Liess, M., 1999. Potential use of cholinesterase in monitoring low levels of organophosphates in small streams: natural variability in three-spined stickleback (*Gasterosteus aculeatus*) and relation to pollution. Environ.Toxicol. Chem. 18, 194–200.
Sturm, A., Wogram, J., Segner, H., Liess, M., 2000. Different sensitivity to organophosphates of acetylcholinesterase and butyrylcholinesterase from three-spined stickleback (*Gasterosteus aculeatus*): application in biomonitoring. Environ. Toxicol. Chem. 19, 1607–1615.
Sumpter, J.P., 1995. Feminized responses in fish to environmental estrogens. Toxicol. Lett. 82, 737–742.
Sumpter, J.P., Jobling, S., 1995. Vitellogenesis as a biomarker for estrogenic contamination of the aquatic environment. Environ. Health Perspect. 103 (Suppl. 7), 173–178.
Suter, G.W., II, 1990. Use of biomarkers in ecological risk assessment. In: McCarthy, J.F., Shugart, L.R. (Eds.), Biomarkers of Environmental Contamination. Lewis Publishers, Boca Raton, FL, USA, pp. 419–428.
Suter, G.W., II, 1993. Ecological Risk Assessment. Lewis Publishers, Boca Raton, FL, USA, p. 538.
Sutter, T.R., Greenlee, W.F., 1992. Classification of members of the Ah gene battery. Chemosphere 25, 223–226.
Taylor, M.R., Harrison, P.T.C., 1999. Ecological effects of endocrine disruption: current evidence and research priorities. Chemosphere 39, 1237–1248.
Thiele, D.J., 1992. Metal-regulated transcription in eukaryotes. Nucl. Acids Res. 20, 1183–1188.
Thomann, R.V., 1989. Bioaccumulation model of organic chemical distribution in aquatic food chains. Environ. Sci. Technol. 23, 699–707.
Thomann, R.V., Connolly, J.P., 1984. Model of PCB in the Lake Michigan lake trout food chain. Environ. Sci. Technol. 18, 65–71.
Thomann, R.V., Komlos, J., 1999. Model of biota-sediment accumulation factor for polycyclic aromatic hydrocarbons. Environ. Toxicol. Chem. 18, 1060–1068.
Thomann, R.V., Conolly, J.P., Parkerton, T.F., 1992. An equilibrium model of organic chemical accumulation in aquatic food webs with sediment interaction. Environ. Toxicol. Chem. 11, 615–629.
Tillitt, D.E., Ankley, G.T., Giesy, J.P., Ludwig, J.P., Kurita-Matsuba, H., Weseloh, D.V., Ross, P.S., Bishop, C.A., Sileo, L., Stromborg, K.L., Larson, J., Kubiak, T.J., 1992. Polychlorinated biphenyl residues and egg mortality in double-crested cormorants from Great Lakes. Environ. Toxicol. Chem. 11, 1281–1288.
Timbrell, J.A., 1991. Principles of Biochemical Toxicology, second ed.. Taylor & Francis, London.
Tjärnlund, U., Ericson, G., Lindesjöö, E., Petterson, I., Åkerman, G., Balk, L., 1996. Further studies of the effects of exhaust from two-stroke outboard motors on fish. Mar. Environ. Res. 42, 267–271.
Tort, L., Torres, P., Flos, R., 1987. Effects on dogfish haematology and liver composition after acute copper exposure. Comp. Biochem. Physiol. 87C, 349–353.
Tracey, G.A., Hansen, D.J., 1996. Use of biota-sediment accumulation factors to assess similarity of nonionic organic chemical exposure to benthically-coupled organisms of differing trophic mode. Arch. Environ. Contam. Toxicol. 30, 467–475.
Triebskorn, R., Köhler, H.-R., Honnen, W., Schramm, M., Adams, S.M., Müller, E.F., 1997. Induction of heat shock proteins, changes in liver ultrastructure, and alterations of fish behaviour: are these biomarkers related and are they useful to reflect the state of pollution in the field. J. Aquat. Ecosyst. Stress Rec. 6, 57–73.
Troxel, C.M., Buhler, D.R., Hendricks, J.D., Bailey, G.S., 1997. CYP1A induction by beta-nephtaflavone, Aroclor 1254 and 2,3,7,8-tetrachlorordibenzo-*p*-dioxin and its influence on aflatoxin B1 metabolism and DNA adduction in zebrafish. Toxicol. Appl. Pharmacol. 146, 69–78.
Tuvikene, A., 1995. Responses of fish to polycyclic aromatic hydrocarbons (PAHs). Ann. Zool. Fennici 32, 295–309.
Tuvikene, A., Huuskonen, S., Koponen, K., Ritola, O., Mauer, U., Lindström-Seppa, P., 1999. Oil shale processing as a source of aquatic pollution: monitoring of the biologic effects in caged and feral freshwater fish. Environ. Health Perspect. 107, 745–752.
Tyle, H., Egsmose, M., Harrit, N., 1991. Mixed-function oxygenase in juvenile rainbow trout exposed to hexachlorobenzene or 3,3′,4,4′-tetrachlorobiphenyl. Comp. Biochem. Physiol. 100C, 161–164.
Uchimura, M., Sandeaux, R., Larroque, C., 1999. The enzymatic detoxifying system of a native Mediterranean scorpio fish is affected by *Caulerpa taxifolia* in its environment. Environ. Sci. Technol. 33, 1671–1674.
Ueng, T.-H., Ueng, Y.-F., Park, S.S., 1992. Comparative induction of cytochrome P-450-dependent monooxygenases in the livers and gills of tilapia and carp. Aquat. Toxicol. 23, 49–64.
Valberg, P.A., Drivas, P.J., McCarthy, S., Watson, A.Y., 1996. Evaluating the health impacts of incinerator emissions. J. Hazard. Mater. 47, 205–227.
Van den Berg, M., De Jongh, J., Poiger, H., Olsen, J.R., 1994. The toxicokinetics and metabolism of polychlorinated dibenzo-*p*-dioxins (PCDDs) and dibenzofurans (PCDFs) and their relevance for toxicity. CRC Crit. Rev. Toxicol. 24, 1–74.
Van der Aar, E.M., Buikema, D., Commandeur, J.N.M., te Koppele, J.M., van Ommen, B., van Bladeren, P.J., Vermeulen, N.P.E., 1996. Enzyme kinetics and substrate selectivities of rat glutathione *S*-transferase isoenzymes towards a series of new 2-substituted 1-chloro-4-nitrobenzenes. Xenobiotica 26, 143–155.
Van der Kooij, L.A., van de Meent, D., van Leeuwen, C.J., Bruggeman, W.A., 1991. Deriving quality criteria for water and sediment from aquatic toxicity tests and product standards: application of the equilibrium partitioning method. Water Res. 25, 697–705.
Van der Oost, R., Heida, H., Opperhuizen, A., 1988. Polychlorinated biphenyl congeners in sediments, plankton, molluscs, crustaceans, and eel in a freshwater lake: implications of using reference chemicals and indicator organisms in bioaccumulation studies. Arch. Environ. Contam. Toxicol. 17, 721–729.
Van der Oost, R., Heida, H., Opperhuizen, A., Vermeulen, N.P.E., 1991a. Bioaccumulation of organic micropollutants in different aquatic organisms: sublethal toxic effects on fish, In: Mayes, M.A., Baron, M.G. (Eds.), Aquatic Toxicology and Risk Assessment ASTM STP 1124, vol. 14, American Society for Testing and Materials, Philadelphia, pp. 166–180.
Van der Oost, R., Heida, H., Opperhuizen, A., Vermeulen, N.P.E., 1991b. Interrelationships between bioaccumulation of organic trace pollutants (PCBs, OCPs and PAHs), and MFO-induction in fish. Comp. Biochem. Physiol. 100c, 43–47.
Van der Oost, R., van Gastel, L., Worst, D., Hanraads, M., Satumalay, K., van Schooten, F.J., Heida, H., Vermeulen, N.P.E., 1994a. Biochemical markers in feral roach (*Rutilus rutilus*) in relation to the bioaccumulation of organic trace pollutants. Chemosphere 29, 801–817.
Van der Oost, R., van Schooten, F.J., Ariese, F., Heida, H., Vermeulen, N.P.E., 1994b. Bioaccumulation, Biotransformation and DNA binding of PAHs in feral eel (*Anguilla anguilla*) exposed to polluted sediments: a field survey. Environ. Toxicol. Chem. 13, 859–870.
Van der Oost, R., Opperhuizen, A., Satumalay, K., Heida, H., Vermeulen, N.P.E., 1996a. Biomonitoring aquatic pollution with feral eel (*Anguilla anguilla*): I. Bioaccumulation: biota-sediment ratios of PCBs, OCPs, PCDDs and PCDFs. Aquat. Toxicol. 35, 21–46.
Van der Oost, R., Goksøyr, A., Celander, M., Heida, H., Vermeulen, N.P.E., 1996b. Biomonitoring aquatic pollution with feral eel

(*Anguilla anguilla*): II. Biomarkers: pollution-induced biochemical responses. Aquat. Toxicol. 36, 189–222.

Van der Oost, R., Vindimian, E., van den Brink, P., Satumalay, K., Heida, H., Vermeulen, N.P.E., 1997. Biomonitoring aquatic pollution with feral eel (*Anguilla anguilla*): III. Statistical analyses of relationships between contaminant exposure and biomarkers. Aquat. Toxicol. 39, 45–75.

Van der Oost, R., Lopes, S.C.C., Komen, H., Satumalay, K., van den Bos, R., Heida, H., Vermeulen, N.P.E., 1998. Assessment of environmental quality and inland water pollution using biomarker responses in caged carp (*Cyprinus carpio*); use of a bioactivation:-detoxication ratio as biotransformation index (BTI). Mar. Environ. Pollut. 46, 315–319.

Van der Weiden, M.E.J., Van der Kolk, J., Bleumink, R., Seinen, W., van den Berg, M., 1992. Concurrence of P450 1A1 induction and toxic effects after administration of a low dose of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) to the rainbow trout (*Oncorhynchus mykiss*). Aquat. Toxicol. 24, 123–142.

Van der Weiden, M.E.J., Tibosch, H.J.H., Bleumink, R., Sinnige, T.L., Van der Guchte, C., Seinen, W., Van der Berg, M., 1993. Cytochrome P450 1A induction in the common carp (*Cyprinus carpio*) following exposure to contaminated sediments with halogenated polyaromatics. Chemosphere 27, 1207–1309.

Van der Weiden, M.E.J., Bleumink, R., Seinen, W., van den Berg, M., 1994. Concurrence of P450 1A1 induction and toxic effects in the mirror carp (*Cyprinus carpio*) after administration of a low dose of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). Aquat. Toxicol. 29, 147–162.

Van Gestel, C.A.M., Van Brummelen, T.C., 1996. Incorporation of the biomarker concept in ecotoxicology calls for a redefinition of terms. Ecotoxicology 5, 217–225.

Van Leeuwen, C.J., Hermens, J.L.M., 1995. Risk Assessment of Chemicals: an Introduction. Kluwer Academic Publishers, Dordrecht, The Netherlands.

Van Schanke, A., Boon, J.P., Aardoom, Y., van Leest, A., van Schooten, F.J., Maas, L., van den Berg, M., Everaarts, J., 2002. Effect of a dioxin-like PCB (CB 126) on the biotransformation and genotoxicity of benzo[a]pyrene in the marine flatfish dab (*Limanda limanda*). Aquat. Toxicol. 50, 403–415.

Van Schooten, F.J. 1991. Polycyclic aromatic hydrocarbon–DNA adducts in mice and humans. Academic thesis, State University of Leiden, The Netherlands.

Van Schooten, F.J., Hillebrand, M.J.X., van Leeuwen, F.E., van Zandwijk, N., Jansen, H.M., den Engelse, L., Kriek, E., 1992. Polycyclic aromatic hydrocarbon–DNA adducts in white blood cells from lung cancer patients: no correlation with adduct levels in lung. Carcinogenesis 13, 987–993.

Van Veld, P.A., Westbrook, D.J., Woodin, B.R., Hale, R.C., Smith, C.L., Huggett, R.J., Stegeman, J.J., 1990. Induced cytochrome P-450 in intestine and liver of spot (*Leiostomus xanthurus*) from a polycyclic aromatic hydrocarbon contaminated environment. Aquat. Toxicol. 17, 119–131.

van Veld, P.A., Vogelbein, W.K., Cochran, M.K., Goksøyr, A., Stegeman, J.J., 1997. Route-specific cellular expression of cytochrome P4501A (CYP1A) in fish (*Fundulus heteroclitus*) following exposure to aqueous and dietary benzo[a]pyrene. Toxicol. Appl. Pharmacol. 142, 348–359.

van Vuren, J.H., 1986. The effects of toxicants on the heamatology of *Labeo umbratus* (Teleostei Cyprinidae). Comp. Biochem. Physiol. 83C, 155–159.

Vanbavel, B., Anderson, P., Wingfors, H., Ahgren, J., Bergqvist, P.A., Norrgren, L., Rappe, C., Tysklind, M., 1996. Multivariate modeling of PCB bioaccumulation in three-spined stickleback (*Gasterosteus aculeatus*). Environ. Toxicol. Chem. 15, 947–954.

Vandeputte, C., Guizon, I., Genestie-Denis, I., Vannier, B., Lorenzon, G.A., 1994. A microtiter plate assay for the total glutathione and glutathione disulfide contents in cultured/isolated cells: performance study of new miniturized protocol. Cell Biol. Toxicol. 10, 415–421.

Varanasi, U., Stein, J.E., 1991. Disposition of xenobiotic chemicals and metabolites in marine organisms. Environ. Health Perspect. 90, 93–100.

Varanasi, U., Nishimoto, M., Reichert, W.L., Le Eberhart, B.-T., 1986. Comparative metabolism of benzo[a]pyrene and covalent binding capacity to hepatic DNA in English sole, starry flounder, and rat. Cancer Res. 46, 3817–3824.

Varanasi, U., Stein, J.E., Nishimento, M., Reichert, W.L., Collier, T.K., 1987. Chemical carcinogenesis in feral fish: uptake, activation, and detoxification of organic xenobiotics. Environ. Health. Perspect. 71, 155–170.

Varanasi, U., Reichert, W.L., Stein, J.E., 1989a. ^{32}P-postlabelling analysis of DNA adducts in liver of wild english sole (*Parophrys vetulus*) and winter flounder (*Pseudopleuronectus americanus*). Cancer Res. 49, 1171–1177.

Varanasi, U., Reichert, W.L., Le Eberhart, B.-T., Stein, J.E., 1989b. Formation and persistence of Benzo[a]pyrene-diolepoxide–DNA adducts in liver of English sole (*Parophrys vetulus*). Chem.-Biol. Interact. 69, 203–216.

Veith, G.D., Kuehl, D.W., Fuglisi, F.A., Glass, G.E., Eaton, J., 1977. Residues of PCBs and DDT in the Western Lake Superior Ecosystem. Arch. Environ. Toxicol. 5, 487–499.

Verbruggen, E.M.J., Vaes, W.H.J., Parkerton, T.F., Hermens, J.L.M., 2000. Polyacrylate-coated SPME fibers as a tool to simulate body residues and target concentrations for estimation of baseline toxicity. Environ. Sci. Technol. 34, 324–331.

Vermeulen, N.P.E., 1996. Role of metabolism in chemical toxicity. In: Ioannides, C. (Ed.), Cytochromes P450: Metabolic and Toxicological Aspects. CRC Press, Boca Raton, FL, USA, pp. 29–53.

Vermeulen, N.P.E., Donné-Op den Kelder, G., Commandeur, J.N.M., 1992. Formation of and protection against toxic reactive intermediates. In: Testa, B., Kyburz, E., Fuhrer, W., Giger, R. (Eds.), Perspectives in Medicinal Chemistry. Verlag Helvetica Chimica Acta, Basel, pp. 573–593.

Vethaak, A.D., 1993. Fish disease and marine pollution; a case study of the flounder (*Platichthys flesus*) in Dutch coastal and estuarine waters. Academic thesis, University of Amsterdam, The Netherlands.

Vethaak, A.D., ap Rheinallt, T., 1992. Fish disease as a monitor for marine pollution: the case of the North Sea. Rev. Fish Biol. Fish. 2, 1–32.

Vethaak, A.D., Jol, J.G., Meijboom, A., Eggens, M.L., ap Reinallt, T., Wester, P.W., van de Zande, T., Bergman, A., Dankers, N., Ariese, F., Baan, R.A., Everts, J.M., Opperhuizen, A., Marquenie, J.M., 1996. Skin and liver diseases induced in flounder (*Platichthys flesus*) after long-term exposure to contaminated sediments in large-scale mesocosms. Environ. Health Perspect. 104, 1218–1229.

Viarengo, A., Burlando, B., Ceratto, N., Panfoli, I., 2000. Antioxidant role of metallothioneins: a comparative overview. Cell. Mol. Biol. 46, 407–417.

Vig, E., Nemcsok, J., 1989. The effects of hypoxia and paraquat on the superoxide dismutase activity in different organs of the carp, *Cyprinus carpio* L. J. Fish Biol. 35, 23–25.

Vigano, L., Galassi, S., Arillo, A., 1994. Bioconcentration of polychlorinated biphenyls (PCBs) in rainbow trout caged in the river Po. Ecotoxicol. Environ. Safe 28, 287–297.

Vigano, L., Arillo, A., Melodia, F., Bagnasco, M., Bennicelli, C., De Flora, S., 1995. Hepatic and biliary biomarkers in rainbow trout injected with sediment extracts from the river Po (Italy). Chemosphere 30, 2117–2128.

Vigano, L., Arillo, A., Melodia, F., Arlanti, P., Monti, C., 1998. Biomarker responses in cyprinids of the middle stretch of the rover Po, Italy. Environ. Toxicol. Chem. 17, 404–411.

Vindimian, E., Namour, P., Munoz, J.F., Gril, J.J., Migeon, B., Garric, J., 1993. Ethoxyresorufin-O-deethylase induction in fish

from a watershed exposed to a non-point source pollution of agricultural origin. Water Res. 3, 449–455.

Von Hofe, E., Puffer, H.W., 1986. In vitro metabolism and in vivo binding of benzo[a]pyrene in the California killifish (*Fundulis parcipinnis*) and speckled sanddab (*Cotharicthys stigmaeous*). Arch. Environ. Contam. Toxicol. 15, 251–256.

Von Westernhagen, H., Rosenthal, H., Dethlefsen, V., Ernest, W., Harms, U.L., Hansen, P.D., 1981. Bioaccumulating substances and reproductive success in Baltic flounder *Plathychthys flesus*. Aquat. Toxicol. 1, 58–99.

Von Westernhagen, H., Dethlefsen, V., Cameron, P., Jansen D., 1989. Chlorinated hydrocarbon residues in gonads of marine fish and effects on reproduction. Sarsia 72, 419–422.

Vos, J., van Loveren, H., Wester, P., Vethaak, D., 1989. Toxic effects of environmental chemicals on the immune system. Trends Parmacol. Sci. 10, 289–292.

Walker, C.H., Thompson, H.M., 1991. Phylogenetic distribution of cholinesterases and related esterases. In: Mineau, P. (Ed.), Cholinesterase-inhibiting Insecticides, Chemicals in Agriculture, vol. 2. Elsevier, Amsterdam, pp. 1–17.

Washburn, B.S., Baden, D.G., Gassman, N.J., Walsh, P.J., 1994. Brevetoxin: tissue distribution and effect on cytochrome P450 enzymes in fish. Toxicon 32, 799–805.

Watson, D.E., Menard, L., Stegeman, J.J., Di Giulio, R.T., 1995. Aminoanthracene is a mechanism-based inactivator of CYP1A in channel catfish hepatic tissue. Toxicol. Appl. Pharmacol. 135, 208–215.

Watson, D.E., Reichert, W., Di Giulio, R.T., 1998. Induction of hepatic CYP1A in channel catfish increases binding of 2-aminoanthracene to DNA in vitro and in vivo. Carcinogenesis 19, 1495–1501.

Weber, S., Karbe, L., 1995. Suitability of the ruffe (*Gymnocephalus cernua* [L.]) for investigations on activity of hepatic enzymes induced by xenobiotics. Ecotoxicol. Environ. Safe 32, 215–218.

Weeks, B.A., Anderson, D.P., DuFour, A.P., Fairbrother, A., Goven, A.J., Lahvis, G.P., Peters, G., 1992. Immunologiocal biomarkers to assess environmental stress. In: Huggett, R.J., Kimerly, R.A., Mehrle, P.M., Jr, Bergman, H.L. (Eds.), Biomarkers: Biochemical, Physiological and Histological Markers of Anthropogenic Stress. Lewis Publishers, Chelsea, MI, USA, pp. 211–234.

Wester, P.W., Vethaak, D., van Muiswinkel, W.B., 1994. Fish as biomarkers in immunotoxicology. Toxicology 86, 213–232.

White, R., Jobling, S., Hoare, S.A., Sumpter, J.P., Parker, M.G., 1994. Environmentally persistent alkylphenolic compounds are estrogenic. Endo 36, 175–182.

White, R.D., Shea, D., Solow, A.R., Stegeman, J.J., 1997. Induction and post-transcriptional suppression of hepatic cytochrome P450 1A1 by 3,3′,4,4′-tetrachlorobiphenyl. Biochem. Pharmacol. 53, 1029–1040.

White, J.C., Hunter, M., Nam, K., Pignatello, J.J., Alexander, M., 1999. Correlation between biological and physiological availabilities of phenanthrene in siols and soil humin in aging experiments. Environ. Toxicol. Chem. 18, 1720–1727.

WHO International Programme on Chemical Safety (IPCS), 1993. Biomarkers and risk assessment: concepts and principles. Environmental Health Criteria 155, World Health Organization, Geneva.

Whyte, J.J., Jung, R.E., Schmitt, C.J., Tillitt, D.E., 2000. Ethoxyresorufin-O-deethylase (EROD) activity in fish as a biomarker of chemical exposure. Crit. Rev. Toxicol. 30, 347–570.

Winston, G.W., Di Giulio, R.T., 1991. Prooxidant and antioxidant mechanisms in aquatic organisms. Aquat. Toxicol. 19, 137–161.

Winston, G.W., Regoli, F., Dugas, A.J., Jr, Fong, J.H., Blanchard, K.A., 1998. A rapid gas-chromatographic assay for determining oxyradicaal scavenging capacity of antioxidants and biological fluids. Free Radic. Biol. Med. 24, 480–493.

Winzer, K., 2001. Oxidative stress in the marine environment—prognostic tools for toxic injury in fish liver cells. Academic Thesis, University of Amsterdam.

Winzer, K., Winston, G.W., Becker, W., van Noorden, C.J.F., Köhler, A., 2001. Sex-related response to oxidative stress in primary cultured hepatocytes of the European flounder (*Platychthys flesus* L.). Aquat. Toxicol., 52, 143–155.

Wolkers, J., Jørgensen, E.H., Nijmeijer, S.M., Witkamp, R.F., 1996. Time-dependent induction of two distinct hepatic cytochrome P4501A catalytic activities at low temperatures in Arctic charr (*Salvelinus alpinus*) after oral exposure to benzo(a)pyrene. Aquat. Toxicol. 35, 127–138.

Wolkers, J., Burkow, I.C., Lydersen, C., Witkamp, R.F., 2000. Chlorinated pesticide concentrations, with an emphasis on polychlorinated camphenes (toxaphenes), in relation to cytochrome P450 enzyme activities in harp seals (*Phoca groenlandica*) from the Barents Sea. Environ. Toxicol. Chem. 19, 1632–1637.

Woodin, B.R., Smolowitz, R.M., Stegeman, J.J., 1997. Induction of cytochrome P4501A in the intertidal fish *Anoplarchus purpurescens* by Prudhoe Bay crude oil and environmental induction in fish from Prince William Sound. Environ. Sci. Technol. 31, 1198–1205.

Worthington, D.J., Rosemeyer, M.A., 1974. Human glutathione reductase: purification of the crystalline enzym from erythrocytes. Eur. J. Biochem. 48, 167–177.

Yang, R., Brauner, C., Thurston, V., Neuman, J., Randall, D.J., 2000. Relationship between toxicant transfer kinetic processes and fish oxygen consumption. Aquat. Toxicol. 48, 95–108.

Yu, Y., Wade, T.L., Fang, J., McDonald, S., Brooks, J.M., 1995. Gas chromatochraphic-mass spectrometric analysis of polycyclic aromatic hydrocarbon metabolites in Antarctic fish (*Notothenia gibberifrons*) injected with Diesel Fuel Arctic. Arch. Environ. Contam. Toxicol. 29, 241–246.

Zaranko, D.T., Griffiths, R.W., Kaushik, N.K., 1997. Biomagnification of polychlorinated biphenyls through a riverine food web. Environ. Toxicol. Chem. 16, 1463–1471.

Zelikoff, J.T., 1993. Metal pollution-induced immunomodulation in fish. In: Annual Review of Fish Diseases. Pergamon Press Ltd, UK, pp. 305–325.

Zhang, H., Davison, W., Knight, B., McGrath, S., 1998. In situ measurements of solution concentration and fluxes of trace metals in soils using DGT. Environ. Sci. Technol. 32, 704–710.

Zinkl, J.G., Lockhart, W.L., Kenny, S.A., Ward, F.J., 1991. The effects of cholinesterase-inhibiting insecticides on fish. In: Mineau, P. (Ed.), Cholinesterase-inhibiting Insecticides, Chemicals in Agriculture, vol. 2. Elsevier, Amsterdam, pp. 151–172.