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Responses of fish to polycyclic aromatic hydrocarbons (PAHs)

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Fish have several mechanisms that can cope with exposure to polycyclic aromatic hydrocarbons (PAHs). One system involved in defence against PAHs is the induction of drug-metabolizing enzymes. Fish can metabolize PAHs mainly by oxidation, reduction, hydrolysis and conjugation reactions catalysed by various enzymes, for example, cytochrome P450 monooxygenases, glutathione S-transferase (GST), and uridine 5-diphosphate-glucuronosyltransferase (UDP-GT), which are mainly localized in the liver but are also found in extrahepatic tissues. PAHs exert their toxicity following biotransformation to toxic metabolites, which can be bound covalently to cellular macromolecules such as proteins, DNA and RNA, which causes cell damage, mutagenesis, teratogenesis, and carcinogenesis.

1. Introduction

Many aquatic pollutants such as polyaromatic hydrocarbons (PAHs) and their halogenated forms are chemically quite stable; owing to their lipophilic nature they can easily penetrate biological membranes and accumulate in organisms. PAHs are important environmental pollutants because of their ubiquitous presence and carcinogenicity. PAHs are the most toxic among the hydrocarbon families (Catoggio 1991). The United States Environmental Protection Agency (EPA) and World Health Organisation (WHO) have identified 16 PAHs as priority pollutants, while some of these, e.g. benzo(a)anthracene, chrysene, benzo(a)pyrene are considered to be potential human carcinogens (Fig. 1).

Polycyclic aromatic hydrocarbons reveal their toxicity following biotransformation to toxic metabolites (Varanasi & Stein 1991, Stein et al. 1992) through metabolic activation (one- or two-electron oxidation) in the organism (Cavalieri & Rogan 1985).

The degree of ecosystem contamination by toxic organic chemicals can be estimated by the analysis of biochemical changes. The bioindicators (biochemical and physiological) that reflect the health status of fish at lower organizational levels (molecular and cellular) respond relatively rapidly to chemical stress and have high toxicological relevance, while those (e.g. condition indices) that reflect health conditions at higher organizational levels (organism) respond

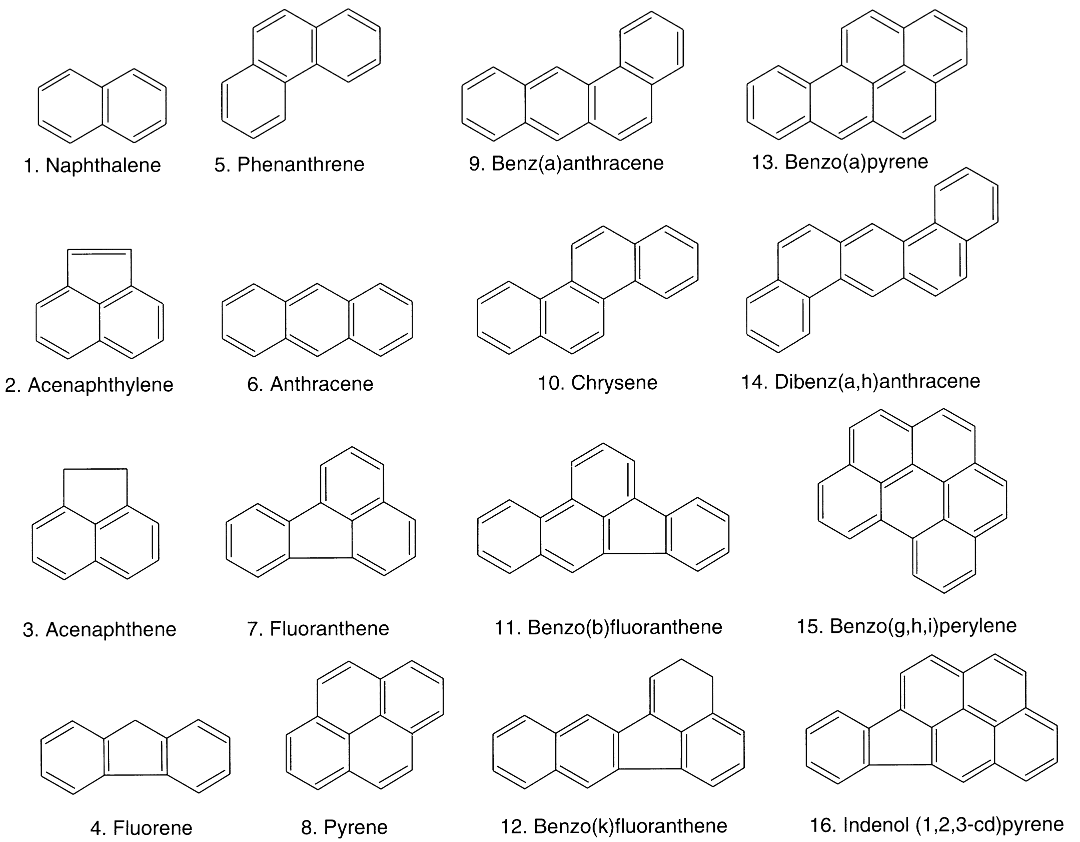

Fig. 1. Structure of sixteen polycyclic aromatic hydrocarbons as US Environmental Protection Agency (EPA) and World Health Organisation (WHO) priority pollutants.

slowly to stress and have lower toxicological relevance (Adams et al. 1989).

2. Aquatic PAHs: sources and distribution

PAHs may be formed in three ways (Blumer 1976, Neff 1985): by high-temperature (e.g. 700°C) pyrolysis of organic materials, by low- to moderate-temperature (e.g. 100–150°C) diagenesis of sedimentary organic material to form fossil fuels, and by direct biosynthesis by microorganisms and plants. They occur in both coal and oil coke. The production and utilization of oil shale has proved to be an important source of PAHs (Ots 1992, Liblik & Rätsep 1994). PAHs are also found as minor components of exhaust gases from diesel engines (Catoggio 1991).

All PAHs are solids, and are sparingly soluble in water. PAHs, especially of higher molecular weight, are relatively immobile because of their large molecular volumes, and low volatility and solubility. After entering water, they quickly become adsorbed on organic and inorganic particulate matter and are mostly deposited in bottom sediments. Most PAHs remain relatively near to the point sources, and their concentrations decrease approximately logarithmically with the distance from the source (Neff 1985). Most of the PAHs entering the aquatic environment are localized in rivers, estuaries, and coastal waters (Collier et al. 1992a, Myers et al. 1992, Rodrigues-Ariza et al. 1993, Hellou et al. 1994), therefore city harbours have a high risk of PAH contamination. Once adsorbed, they are much more stable than pure compounds and are resistant to oxidation and nitration reactions to which they would otherwise be quite sensitive due to photochemical processes (Catoggio 1991). When PAHs are incorporated into anoxic sediments they may persist for a long time, while the phototoxic components of PAHs can be readily released from sediments and can cause adverse effects when organisms accumulate PAHs in the presence of sunlight.

3. Accumulation and bioconcentration

PAHs are readily absorbed by fish and other aquatic animals during exposure to contaminated food, water and sediments, reaching levels higher than those in the ambient medium (Neff 1985). Relative concentrations of PAHs in aquatic ecosystems are generally the highest in sediments, medium in aquatic biota, and the lowest in the water column. Bioaccumulation patterns of different contaminants vary. For example, the amount of PCB is enhanced through the trophic level, whereas PAHs are increasingly metabolized (Van der Oost et al. 1990, Porte & Albaiges 1994). Dissolved organic material in natural waters has a strong effect on the bioavailability of organic pollutants. The bioavailability of several organic pollutants usually decreases with increasing dissolved organic matter-concentration in water (Kukkonen 1991).

Biological membranes are mostly composed of lipids; the majority of organic pollutants are lipophilic. It has been suggested that the larger the lipid content of the biological membrane, the higher is the rate of uptake (Hamelink & Spacie 1977). The rate of pollutant distribution to specific tissues is determined by the regional blood flow through each tissue. Organs with a high blood flow, for example the liver and the kidney, tend to accumulate xenobiotics most readily (Pritchard, 1993). Factors such as plasma protein binding, affinity with specialized cellular uptake mechanisms, metabolism, and excretion, all affect the pattern of distribution, retention, and toxicity.

4. Biotransformation

Most living organisms have at least some ability to metabolize xenobiotics. The oxitative metabolism of PAHs in this system proceeds through highly electrophilic intermediate arene oxides, some of which are covalently bound to cellular macromolecules such as DNA, RNA, and protein (Miller & Miller 1981). A number of factors exist that primarily determine the availability of organic chemicals to fish, while their forms may be greatly modified by physical, chemical and/or biological events. The changing of the chemical form of contaminants by biological (e.g. biotransformation) or physical means (e.g. photo-oxidation) may greatly alter their availability due to changes in their solubility or reactivity (Oris & Giesy 1985). PAHs undergo three types of chemical reactions characteristic of aromatic hydrocarbons (Neff 1985): electrophilic substitution, oxidation, and reduction. Oxidation and reduction reactions destroy the aromatic character of the benzene ring, but electrophilic substitution does not.

The biotransformation of a hydrophobic xenobiotic in fish is a major determinant of its toxicity, distribution and ability to be excreted. The biological half-lives of lipophilic xenobiotics would be markedly prolonged without biochemical processes that convert lipophilic compounds to more readily water-soluble and excretable products. The major PAHs-metabolization pathways involve cytochrome P450 monooxygenase, epoxide hydrolase and several conjugating enzymes (Fig. 2). These transformation processes are mostly enzymatic and are usually classified into two types: phase I and phase II reactions (Jimenez & Stegeman 1990, Pritchard 1993). Phase I enzymes (cytochrome P450 monooxygenase system) introduce a polar group into the xenobiotic molecule via oxidative, reductive or hydrolytic processes.

Phase II reactions involve the conjugation of xenobiotics, or their phase I metabolites, with polar endogenous constituents such as glucuronic acid, sulfate, glutathione or amino acid (Lech & Vodicnic 1985, Lindström-Seppä 1990, Pesonen 1992) to produce water-soluble conjugates that are easily excreted by fish. The enzymes involved in phase II are called conjugating enzymes.

5. PAH-metobolizing enzymes

5.1. Cytochrome P450 system

Cytochrome P450 refers to a family of isoenzymes catalyzing monooxygenase reactions which can tranform the structure of organic chemicals; they

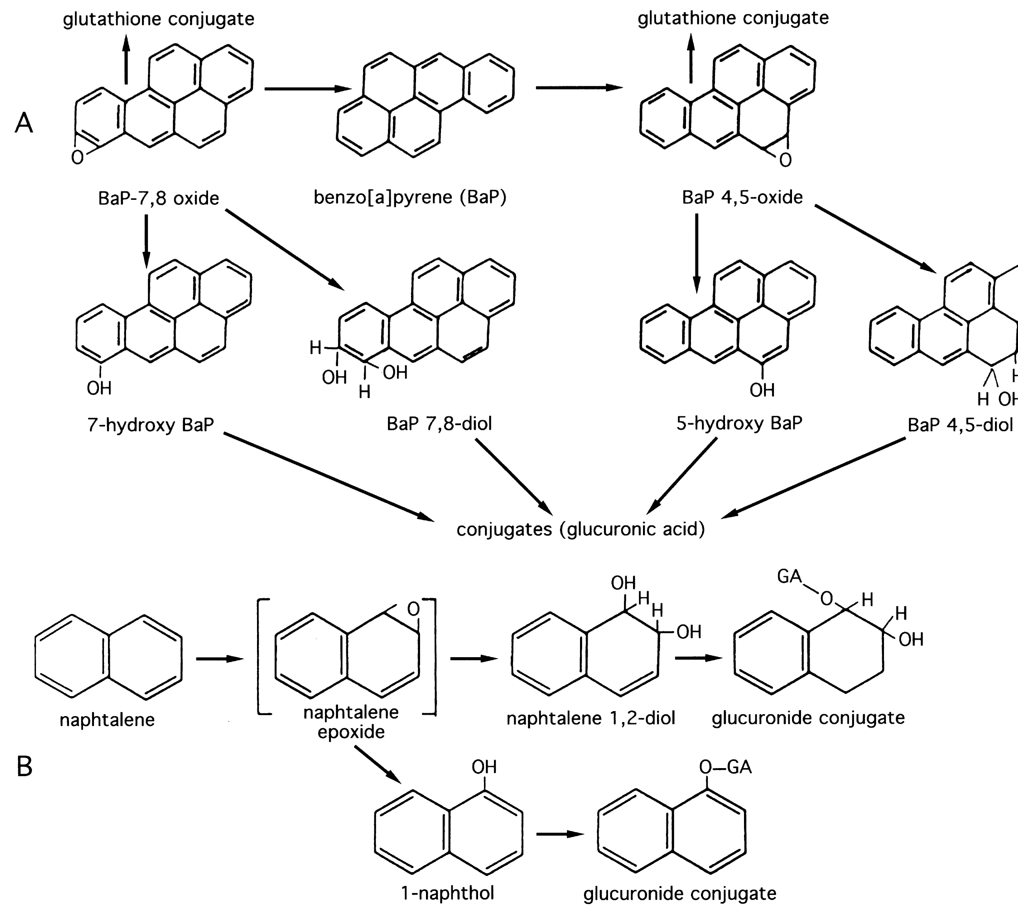

Fig. 2. Biotransformation reactions with representatives of PAHs: A. benzo(a)pyrene (Lee et al. 1972), and B. naphtalene (Khan et al. 1979).

are classified according to their primary amino acid-sequence alignments (Nebert & Gonzalez 1987). Isoenzymes have been grouped into different families if their similarity is under 40%, and into subfamilies if their similarity is 40–75% (Bock et al. 1990). The similarity of one of the trout P450 forms to four mammalian P450 1A1 genes is 57–59% (Heilman et al. 1988). Isoenzymes P4501A1 (or CYP1A1) and P4501A2 (CYP1A2) belong to subfamily CYP1A (Stegeman 1989) and they are induced by various PAH compounds, like BaP and BA and their metabolites (Stegeman & Hahn 1994). In fish, the major PAH-inducible form of P450 has been purified from several species, i.e. P450E from scup (Klotz et al. 1983), P450LM$_{4b}$ from trout (Williams & Buhler 1984) and P450c from cod (Goksøyr 1985). The nomenclature, structure, and function of the cytochrome P450IA gene in fishes has been recently reviewed by Stegeman (1989, 1992), and Stegeman and Hahn (1994).

P450 induction is primarily due to the transcriptional activation of the gene, but can also be caused by post-transcriptional regulation or post-translational regulation (Nebert & Gonzalez 1987, Stegeman & Hahn 1994). The mechanism by which cells recognize inducers and transmit information to genes is well understood in the case of the members of subfamily CYP1A, which are induced by PAHs and their halogenated forms. Being lipophilic, PAHs enter the cell by passive diffusion, where they are specifically bound to the cytosolic Ah-receptor (Stegeman & Hahn 1994).

In eucaryotic organisms P450 proteins are membrane bound. The ones that metabolize or trans-

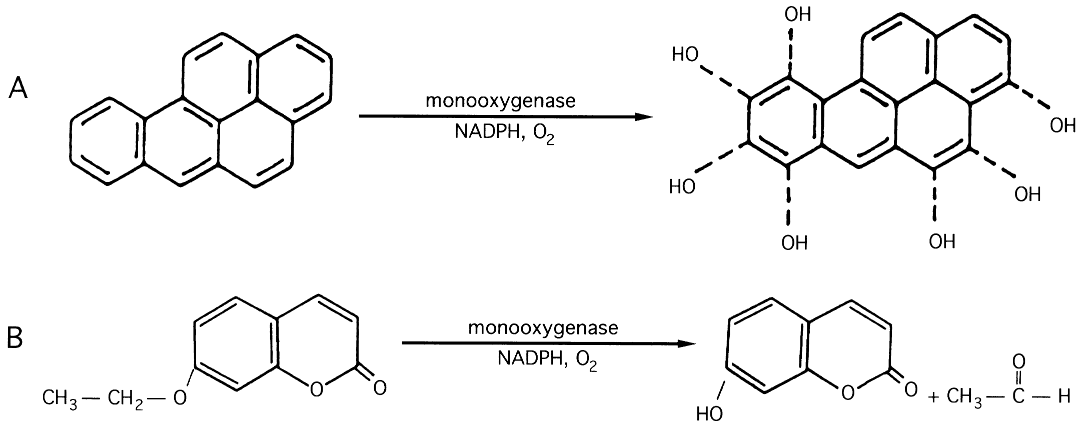

Fig. 3. Monooxygenase reactions with diagnostic substrates: A. benzo(a)pyrene hydroxylase or aryl hydrocarbon hydroxylase (AHH), and B. ethoxyresorufin-O-deethylase (EROD).

form xenobiotic chemicals are primarily located in the endoplasmatic reticulum (Stegeman 1989) and are mostly found in the microsomal fraction of hepatic tissues. Moreover, Lester et al. (1992) found that cytochrome P4501A1 of trout is localized in membranes of the granular endoplasmic reticulum of perinuclear regions in hepatocytes, within specific domains and are not dispersed along all membranes.

Exposure to PAHs results in the induction of specific forms of cytochrome P4501A1 that catalyzes aryl-hydrocarbon-hydroxylase (AHH), ethoxyresorufin-O-deethylase (EROD), and 7-ethoxycoumarin-O-deethylase (ECOD) activity (Fig. 3) (Lech & Vodicnik 1985, Van Veld et al. 1990, Collier et al. 1992b, Pesonen 1992, Di Giulio et al. 1993). The net result of all these enzymatic reactions is the addition of an oxygen atom to the substrate; in most cases, oxygen is further reduced to form a hydroxyl group. Monooxygenases are found at high concentrations in the liver (Masfaraud et al. 1992, Sved et al. 1992). They also occur in many other tissues such as the kidney, intestine, gill, gonad, heart and especially in vascular endothelium (Stegeman et al. 1979, Lindström-Seppä et al. 1981, Pesonen et al. 1985, Andesson and Pärt 1989, Van Veld et al. 1990, Stegeman and Hahn 1994). The highest activity of these enzymes in any nonhepatic tissue examined so far has been found in the kidney. Rainbow trout kidneys have been reported to possess a higher activity than the liver if its level is normalized to the cytochrome P450 content (Pesonen 1992).

5.1.1. Monooxygenases as biomarkers

The use of monooxygenase activity to identify areas of chemical pollution in aquatic environments has lately become more widespread (Lindström-Seppä & Oikari 1989, 1990, Stein et al. 1992, Garrigues et al. 1993). Several field experiments have demonstrated the usefulness of the monooxygenase system as an indicator of contamination (Lindström-Seppä & Oikari 1988, 1990, Kreamer et al. 1991, Masfaraud et al. 1992, Sved et al. 1992, Di Giulio et al. 1993, Huuskonen et al. 1995, Tuvikene et al. 1995). Monooxygenases were induced in the channel catfish *Ictalurus punctatus* exposed in the laboratory to sediments obtained from Black Rock Harbor at the Long Island Sound (Di Giulio et al. 1993) which is highly contaminated with several different aromatic hydrocarbons.

The activation of the (MFO) system, especially the induction of aryl hydrocarbon hydroxylase (AHH) and ethoxyresorufin-O-deethylase (EROD) has been observed in a number of fish species (Lindström-Seppä 1990, Van Veld et al. 1990, Pesonen & Andersson 1991, Pesonen 1992, Garrigues et al. 1993). Many authors have shown the dependence of EROD on the PAH concentration and length of exposure (Sved et al. 1992, Lindström-Seppä 1990, Masfaraud et al. 1992, Di Giulio et al.

1993, Tuvikene et al. 1995). EROD activity increased in rainbow trout after the benzo(a)pyrene treatment (Masfaraud et al. 1992). Differences were not observed on the first day; however after 2–8 days they increased up to 2–3-fold. Van Veld et al. (1990) discovered 8-fold increased liver EROD activity of spot (*Leiostomus xanthurus*) from heavily contaminated sites (96000 mg PAH/kg dry sediment). Huuskonen et al. (1995) discovered 75- and 15-fold increases in EROD and AHH activities, respectively, in rainbow trout treated with b-naphtoflavone (PAH-type inducer). EROD activity is associated with genotoxicity in fish (Masfaraud et al. 1992).

5.1.2. Factors affecting monooxygenase activity

Many endogenous and exogenous factors may influence the activity of the MFO system in fish tissues. The induction response can be modulated by such factors as different fish species, reproduction stage, sex, age and ambient water temperature (Lindström-Seppä 1990, Nishimoto et al. 1992). Steroid hormones (e.g. cortisol) have been suggested to be one of the modulating factors usually promoting induction (Devaux et al. 1992). Some authors have suggested that temperature could play some role in the metabolism of chemicals in biological systems. For example, Garrigues et al. (1993) have found seasonal changes in EROD induction (1.5–2-fold differences between summer and winter).

The cytochrome P450 concentration was significantly higher in the hepatic microsomes of gonadally mature male brook trout, *Salvelinus fontinalis*, and rainbow trout, *Oncorhynchus mykiss*, than in the hepatic microsomes of gonadally mature females of the same species (Stegeman & Chevion 1980). Males of burbot, *Lota lota*, exposed to waters contaminated with oil products at Norman Wells, had higher EROD and AHH activity than females. At the same time, there were no differences in the content of cytochrome P450 between males and females of burbot (Lockhart & Metner 1992). The same authors showed that females with larger gonads had a lower EROD activity; they suggested that if the influence of the state of sexual maturation is removed, body size or the time of collection had little, if any effect, on EROD activity in females. It has been shown that estradiol is a regulator of monooxygenase activity (suppression of P4501A) (Stegeman & Hahn 1994).

Usually, a small increase in the cytochrome P450 concentration accompanies a considerable increase in MFO activity. Some fish are very sensitive to contaminants; this is one explanation of the high level of hepatic monooxygenase activities (Lindström-Seppä 1990). However, an increase in enzyme activity does not necessarily indicate *de novo* synthesis of protein (induction).

Many other factors, too, can influence MFO activities, for example starvation has been shown to limit monooxygenase activities in bluegill liver (Jimenez et al. 1988), and brook trout (Yamauchi et al. 1975). Van Veld et al. (1992) demonstrated that in *Stenotomus chrysops* the content of cytochrome P4501A1 and monooxygenase activity depending on it, measured as EROD, were 28–85% and 15–77% lower, respectively, in the hepatocellular carcinoma and in the foci of cellular alteration than in non-neoplastic tissue.

5.2. Conjugation reactions

Relatively little research has been performed in the area of conjugating (phase II) enzymes as indicators of environmental pollution. The two major enzymes that have been studied in fish are glutathione S-transferase (GST) and uridine 5-diphosphate-glucuronosyltransferase (UDP-GT). Glutathione (GSH) is involved in the metabolism of xenobiotics, serving as a conjugating substrate for many electrophilic compounds. Conjugation with GSH occurs more readily in the presence of GST which is found in the cytoplasm of cells. Glucuronidation in fish has recently been reviewed by Clarke et al. (1991) and S. G. George (1994). Both enzymes are present at significant levels in hepatic and several extrahepatic fish tissues (Clarke et al. 1991, Clarke et al. 1992). Stalker et al. (1991) reported a low level of GST activity in pollution-associated liver neoplasms in white suckers (*Catostomus commersoni*) in comparison with surrounding normal hepatocytes. High UDP-GT activity has also been found in extrahepatic tissues such as the intestine and gills of fish (Koivusaari et al. 1981, Lindström-Seppä et al. 1981, Andersson & Pärt 1989). According to Lindström-Seppä et al. (1981)

UDP-GT activity is higher in the gills than in the liver of vendace, *Coregonus albula*. Sulfation (catalyzed by sulphotransferases) can modulate UDP-GT activity; sulfation is a competing pathway to glucuronidation for metabolites of PAHs, but it is effective at low substrate concentration (George 1994).

6. Metabolism and excretion of PAHs

Contact with biological material can significantly affect the structure of a chemical compound (Lech & Vodicnik 1985, Stegeman 1989, Lindström-Seppä 1990). In general, biotransformation of foreign chemicals serves two purposes: to make them less toxic and/or to make them easier to excrete. Detoxication does not occur at any time. For example, many foreign compounds are metabolized to highly toxic products that are responsible for carcinogenesis and toxic effects (Ahokas 1979). In vitro experiments with benzo(a)pyrene (BaP) indicated that dihydrodiols, precursors of the main BaP metabolite bound to DNA, make up as much as 75% of the metabolites formed by cytochrome P–450E (Stegeman & Kloepper-Sams 1987, Jimenez & Stegeman 1990). The experiments showed that a rise in monooxygenase activity due to pollutants can increase the amount of potentially reactive chemicals that can form adducts with DNA.

Liver microsomes of trout, *Salmo trutta lacustris*, starry flounder, *Platychthys stellatus*, and coho salmon, *Oncorhynchus kisutch,* metabolize BaP to a number of metabolites similar to those produced by rat liver microsomes (Ahokas 1979, Ronis et al. 1992). The major identified metabolites produced by fish microsomes are 7,8- and 9,10-dihydrodiols, which are precursors of major carcinogenic BaP metabolites (Fig. 2) (Steward et al. 1989). Research into the metabolism of BaP by hepatic microsomes of English sole (*Parophyrys vetulus*) has shown that the main BaP metabolites formed are BaP-7,8-dihydrodiol, BaP-10-dihydrodiol, 1-hydroxyBaP and 3-hydroxyBaP (Nishimoto & Varanasi, 1985). For example, the carcinogenicity of BaP is primarily due to 7,8-dihydroxy-9,10-epoxy-7,8,9,10-tetrahydrobenzo(a)pyrene. Ueng et al. (1994) have studied the PAH metabolism in tilapia (*Tilapia* spp.). They demonstrated that tilapia liver microsomes metabolized BaP into BaP-7,8-dihydrodiol (11%), 3-hydroxy-BaP (17%), and 9-hydroxy-BaP (22%) as the major products, and 7-Cl-Benz-(a)anthracene (7-Cl-BA) into 7-Cl-BA-trans-8,9-dihydrodiol as the major metabolite (40%). Fish gills are one way for the absorption of PAHs from the surrounding water. Andersson and Pärt (1989) have found that perfused rainbow trout gills transformed about 20% of the intake of BaP, mainly to 9,10-dihydrodiols and 7,8-dihydrodiols; small quantities of quinones, 4,5-dihydrodiols and 3-hydroxymetabolites were also found.

Bullhead (*Ictalurus nebulosus*) and carp (*Cyprinus carpio*) hepatocytes metabolized BaP predominantly into water-soluble compounds (Steward et al. 1989). BaP-7,8-diol was converted to water-soluble compounds more rapidly (3.3–3.6-fold) than BaP. Carp hepatocytes have been found to metabolize BaP 2-fold faster than bullhead hepatocytes, producing proportionally larger amounts of glutathione (GSH) conjugates and glucuronides (Steward et al. 1989). The same researches established that carp hepatocytes metabolized BaP-7,8-diol 2-fold faster than bullhead hepatocytes. Steward et al. (1989) found that carp liver microsomes metabolized BaP 12-fold faster than brown bullhead microsomes, wherein the main metabolite was the highly carcinogenic BaP-7,8-dihydrodiol. They proposed that the higher rate of GSH conjugate formation by carp hepatocytes is probably caused by the relatively big amount of epoxides (e.g. BaP-7,8-dihydrodiol-9,10-epoxide). In hepatocytes isolated from winter flounder, BaP-7,8-dihydrodiol was metabolized more than four times faster than BaP (McElroy & Kleinow 1992). The data obtained by McElroy and Kleinov (1992) indicated that liver and intestinal mucosal cells of winter flounder had a similar ability to metabolize BaP or BaP-7,8-dihydrodiol (per gram of protein basis). Due to its bigger mass, the liver probably has a greater metabolic potential, at least at higher doses, while at low doses the intestine may be able to metabolize a relatively large portion of the PAH absorbed from food.

The metabolites were mainly conjugated with glucuronic acid. Most conjugates are organic anions which are water-soluble and rapidly excreted (Sipes & Gandolfi 1986). The main routes of excretion of PAHs and metabolites pass directly from the liver into the gastrointestinal tract via the bile, from the kidney into the urine, and through the skin, after which they are bound to mucus (Varanasi et al. 1978). The conjugated metabolites formed in the gills are relatively non-toxic and are rapidly excreted into the urine or bile. Conjugated metabolites predominate in the bile. An increased concentration of the bile metabolites of polycyclic aromatic hydrocarbons measured as fluorescent aromatic compounds (FACs) is a powerful index of exposure to PAHs (Collier et al. 1992b, Di Giulio et al. 1993). Unconjugated metabolites are more abundant in the urine and the skin. The retention of dietary PAHs and metabolites in the tissues of fish increased at low temperatures (Collier et al. 1978).

7. Effects of PAHs and their metabolites

7.1. Toxicity

PAHs are exceedingly toxic to aquatic organisms at concentrations of about 0.2–10 ppm.; deleterious sublethal responses are sometimes observed in aquatic organisms at concentrations in the range of 5–100 ppb (Neff 1985).

The results of many investigations support the conclusions that a major mode of PAHs' toxicity, particularly in the case of compounds with a lower molecular weight, is realized through interference with cellular membrane function and membrane-associated enzyme systems. PAHs may interact physically with hydrophobic sites in the cell, causing molecular deformation and perturbation; however PAH metabolites, being more hydropholic, reactive, and electrophilic, may undergo a variety of spontaneous or enzyme-mediated chemical reactions. The most important of these are the reactions leading to covalent binding of PAHs to cellular macromolecules such as proteins, DNA and RNA, which causes cell damage, mutagenesis, teratogenesis and cancer; moreover, aromatic hydrocarbons appear to be bound selectively to the surface of plasma membranes (Schnitz & O'Connor 1992). Such a binding of aromatic hydrocarbons to the membrane surface causes perturbations in surface organisation, which increases membrane permeability (Andersson & Pärt 1989). Experiments have shown that relatively small doses of PAHs present in the environment can be bound to cellular macromolecules of young fish (Schnitz & O'Connor 1992). A wide variety of histopatologic and physiological responses to naphthalene exposure was described in mummichog, *Fundulus heteroclitus,* for example gill hyperplasia and hemorrhages of gill filaments (Levitan & Taylor 1979). Fish treated with creosote-contaminated sediments refused food and they had several fin erosion and epidermal lesions (Sved et al. 1992).

The four-, five-, and six-ring PAHs appear to be more carcinogenic than PAHs with smaller or larger ring systems (Neff 1985). Highly angular configurations tend to be more carcinogenic than linear ring systems. Parent compounds of PAHs themselves are not carcinogenic; they require metabolic activation to become reactive electrophilic metabolites. The degree of carcinogenicity is related to the structure and reactivity of the major metabolites produced by the cytochrome P450 MFO and epoxide hydratase systems (Neff 1985).

7.1.1. Photo-induced toxicity

PAHs with a small molecular weight have been shown to exhibit acute toxicity to aquatic organisms. Higher molecular weight PAHs are not highly toxic to aquatic organisms; however, in the presence of solar ultraviolet radiation many of them may become acutely toxic. The acute toxicity of PAHs with solar ultraviolet radiation has been demonstrated in juvenile fish (Oris & Giesy 1985, 1986, 1987, Tilghman Hall & Oris 1991).

An increased opercular ventilation rate has been used as an indicator of sublethal photo-induced stress in bluegill sunfish, *Enneacanthus macrochir,* exposed to anthracene and solar ultraviolet radiation; asphyxia caused a high mortality rate in these fish, and further histological examination showed severe damage in gill membranes (Oris & Giesy 1985).

7.2. Immune suppression

Payne and Fancey (1989) found that in winter the number of melanomacrophage centres (MMC) in the liver decreased in flounder, *Pseudopleuronectes americanus*, exposed to sediments contaminated with petroleum-originating PAHs. The amount of MMC was reduced in the liver of fish exposed to PAH concentrations totalling 25 mg/g or more. The authors supported the hypothesis that PAH concentrations in heavily polluted waters may be high enough to affect the immune responses of fish. The decreasing of MMC is usually associated with serious degenerative changes in the liver.

Faisal et al. (1993) showed some suppression of proliferative responses of T-lymphocytes of spot, *Leiostomus xanthurus*, under PAH exposure; Faisal et al. (1991) demonstrated the depression of the tumorilytic activity of anterior kidney and splenic leucocytes of mummichog (*Fundulus heteroclitus*) caught from the polluted Elizabeth River. Moreover, mummichog leucocytes were unable to "recognize" tumour target cells and be bound to them. The authors found this effect to be reversible.

7.3. Effects on DNA and RNA

It is known that in fish as well as in mammals, the cytochrome P450-dependent oxidative metabolism of xenobiotics can generate DNA-reactive species (Masfaraud et al. 1992). The use of DNA adducts as an indicator of the exposure of fish to genotoxic compounds has been investigated relatively recently (Myers et al. 1992). The use of DNA adduct analysis of fishes in laboratory and field studies has been recently reviewed by A. E. Maccubbin (1994). The levels of DNA adducts in benthic flatfish (English sole, *Parophrus vetulus*; rock sole, *Lepidopsetta bilineata*; and starry flounder, *Platichthys stellatus*) were generally related to the level of sediment PAH contamination (Stein et al. 1992). The results of investigators show that fish from contaminated sites have significantly higher DNA-adduct levels than fish from relatively uncontaminated sites; thus the formation of pollution-related DNA adducts correlates with an increased incidence of tumours (Kurelec & Gupta 1993). Moreover, cromatograms of fish from relatively uncontaminated sites generally reveal only a few identifiable adducts suspected of arising from xenobiotic compounds. The observation that the formation of liver DNA adducts is associated with the induction of microsomal EROD activity is consistent with the involvement of cytochrome P4501A in the metabolism of BaP.

Nishimoto et al. (1992) detected two major BaP–DNA adducts in English sole hepatocytes by ^{32}P-postlabelling. These are similar to the conjugated metabolites and BaP–DNA adducts occurring in the bile. Despite the ability of teleost fish to produce mutagenic metabolites from PAH, some of them appear quite resistant to PAH-induced cancer (Steward et al. 1989, Hawkins et al. 1991). Nevertheless, in comparison with brown bullhead hepatocytes, carp hepatocytes produce quite rapidly a large amount of DNA adducts from BaP and BaP-7,8-dihydrodiol, and large amounts of reactive intermediates bound to DNA; however, brown bullhead has a greater susceptibility to PAH-induced carcinogenesis. This phenomenon is not yet known and it needs further investigation.

Many other effects caused by PAHs have been reported, for example, the eye lens cataract is common in some fish species from sites heavily contaminated with PAHs (Williams et al. 1992). The authors have shown that RNA and protein synthesis rates were inhibited by the presence of BaP-diol. Misitano et al. (1994) found that the sublethal effect of PAHs can decrease the DNA content of larval surf smelt, *Hypomesus pretiosus.*

7.4. Liver lesions

A variety of histological changes occur in the liver of fish: depletion of hepatic glycogen reserves, substantial proliferation of smooth and rough endoplasmic reticulum of hepatocytes, mitochondrial swelling, pigment deposition, and nuclear degeneration. Several studies in recent years have demonstrated a relation between the occurrence of hepatic neoplasms in benthic fish and chemical pollution of sediments, mainly due to PAHs (Baumann et al. 1991, Hawkins et al. 1991, Di Giulio et al. 1993).

The prevalence of hepatic neoplasms in English sole (*Parophrys vetulus*) from contaminated areas is statistically associated with exposure to polycyclic aromatic hydrocarbons (Malins et al. 1985). Liver neoplasms have been found to be rare in young fish but a higher prevalence of preneoplastic, regenerative, and degenerative lesions were detected quite often in fish from contaminated sites. Myers et al. (1992) have reported many types of hepatic lesions in subadult English sole (*Parophrus vetulus*), rock sole (*Lepidopsetta bilineata*) and starry flounder (*Platichthys stellatus*) caught from sites with sediments moderately to severely contaminated by aromatic hydrocarbons. Simultaneous lesion prevalences were directly related to the mean bile level of fluorescent aromatic compounds. Hawkins et al. (1990) demonstrated that hepatic neoplastic lesions occur only in the case of treatment with a high concentration (1200 ppb) of 7,12-dimethylbenz(a)anthracene and not with a high concentration (200 ppb) of BaP, whereby sheepshead minnow was less sensitive than Japanese medaka (*Oryzias latipes*) and guppy (*Poecilia reticulata*), both of which developed hepatic neoplasm after exposure to BaP, and both hepatic and extrahepatic neoplasms after 7,12-dimethylbenz(a)anthracene treatment. Moreover, they did not find extrahepatic neoplastic lesions. In mummichog, *Fundulus heteroclitus,* inhabiting creosate-contaminated waters, many extrahepatic neoplasms were found (Vogelbein 1993).

7.5. Effects on the liver somatic index

The liver somatic index (LSI) of sunfish and hardhead catfish from contaminanted areas was increased (Everaarts et al. 1993); moreover, in bluegill sunfish a 2-fold increase of the LSI was reported in the case of PAH-rich sediment treatment (Theodorakis et al. 1992). Baumann et al. (1991) demonstrated an elevated hepatosomatic index in the case of brown bullhead from polluted sites; however some reported data have not shown any enlargement of the fish liver from polluted areas (Van der Oost et al. 1991).

7.6. Effects on hematological parameters

Everaarts et al. (1993) demonstrated increased hemoglobin and mean corpuscular hemoglobin concentrations in sunfish, and decreased hemoglobin and mean corpuscular hemoglobin concentrations in hardhead catfish from PAH-contaminated sites. Levitan and Taylor (1979) found increased blood cortisol and glucose concentrations, and also osmoregulatory imbalance in mummichog exposed to naphthalene (4 mg/l). On the contrary, perch (*Perca fluviatilis*) and pike (*Esox lucius*) from sites highly polluted with PAHs were unable to increase their serum cortisol in response to acute stress (capture), and their pituitary glands were atrophied (Hontela et al. 1992). The same authors suggest that long exposure to xenobiotics may cause exhaustion of the endocrine system producing cortisol.

7.7. Influence on the reproductive potential

Reproduction is one of the most vital functions performed by any organism. Many studies have shown that contaminant exposure causes a negative impact on some reproductive processes in fish. Some studies with petroleum and crude oil have demonstrated the potential effects of PAHs on fish reproduction. These studies have shown decreased gametogenesis, decreased gonad size and lowered egg production (Payne et al. 1978, Hedtke & Puglisi 1980, Fletcher et al. 1982, Kahn & Kiceniuk 1984, Walton et al. 1983, Kiceniuk &Kahn 1987).

It was revealed experimentally that anthracene is bioconcentrated more in the ovaries of fathead minnows than in the testes (Tilghman Hall & Oris 1991). Reproductive output, measured as the mean number of eggs laid, was also reduced in the anthracene-treated fish. Hatching success may be lowered in eggs treated with anthracene when subsequently exposed to solar ultraviolet radiation (SUVR) during development; also elevated developmental deformities including edema, yolk sac malformations, and eye deformities occurred. It is suggested that the impact of PAHs plus SUVR exposure on reproduction is severe. Although anthracene is registered by the U.S. EPA as a nontoxic compound (Sittig 1981), several studies have shown that anthracene is acutely toxic under SUVR (Oris & Giesy 1986, Tilghman Hall & Oris 1991). A recent study of Tilghman Hall and Oris (1991) demonstrated that anthracene in the absence of SUVR has quite a strong influence on the reproductive potential of fish. According to Adams et al. (1989) the influence of PAHs on lipid metabolism is such that the amount of physiologically available energy for gonad maturation is reduced (decreased fecundity). Collier and coworkers (Collier et al. 1992c) demonstrated that early stages of the reproductive process in female English sole are disrupted by PAH-contaminant exposure. Moreover, Collier et al. (1992c) found that hepatic AHH activity correlated negatively with fertilization success. They hypothesized that female English sole exposed to sublethal contaminant levels may at least partially be excluded from the spawning population.

8. Summary

Polycyclic aromatic hydrocarbons reveal their toxicity following biotransformation to toxic metabolites which can be bound covalently to cellular macromolecules such as DNA, RNA, and protein.

The major PAH-metabolizing enzymes are cytochrome P450 monooxygenases, epoxide hydrolase and several conjugating enzymes (e.g. UDP-GT, GST). Exposure to PAHs causes an induction of cytochrome P4501A1, which catalyzes AHH, EROD and ECOD activities; this system is located primarily in the microsomal fraction of hepatic tissue, as well as in the kidney and the gut.

Highly carcinogenic 7,8- and 9,10-dihydrodiols are the major PAH metabolites produced by fish microsomes.

The metabolites of PAHs are mainly conjugated with glucuronic acid. Most conjugates are organic anions which are water-soluble and are rapidly excreted mostly via the gall bladder or the in urine.

PAHs with a higher molecular weight are not acutely toxic to fish; however, in the presence of solar ultraviolet radiation many of them (e.g. anthracene) are acutely toxic.

Exposure to PAHs causes suppression of the immune system: a decreased number of melanomacrophage centres in the liver and suppression of proliferative responses of T-lymphocytes.

After PAH exposure, there is an increase in the number of DNA adducts, as well as some inhibition in RNA and protein synthesis.

A variety of PAH-dependent histological changes (e.g. different types of lesions) occur in the liver of fish.

PAHs have a potential effect on fish reproduction.

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