ISSN: 2347-2723 Impact Factor : 3.3754(UIF) Volume - 5 | Issue - 1 | august - 2017

Review of Literature

RESPONSES OF FISH TO POLYCYCLIC AROMATIC HYDROCARBONS (PAHS)

Shashi Bhushan Singh
Assistant Professor , Dept. Of Zoology , R.B.S. College, Teyai.

ABSTRACT :

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 ctalysed 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.

KEYWORDS :*several mechanisms , polycyclic aromatic hydrocarbons (PAHs).*

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) anthrancene, chrysene, benzo(a) pyrene are considered to be potential human carcinogens (Fig.1)

Polycyclic aromatic hydrocarbons reveal their toxicity following biotransformation to toxic metabolities (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 bio-indicators (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 slowly to stress and have lower toxicological relevance (Adams et al. 1989).

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

2. AQUATIC PAHS: SOURCES AND DISTRIBUTION

PAHs may be formed in three ways (Blumer 1976, Neff 1985): by high-temperature (e.g. 700^{0}C) pyrolysis

of organic materials, by low-to moderate – temperature (e.g. 150^{0}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 & Ratsep 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 process (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 form sediments and can cause adverse effects when organisms accumulate PAHs in the presence of sunlight.

3. ACCUMULATION AND BIOCONCETRATION

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 tropic 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 mater-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 a 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 & Giesy1985): 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 extractable products. The major PAHs-metabolization pathways involve cytochrome P450 monnoxyenase, 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 monoxygenase 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, Lindstrom – Seppa 1993, Pesonen 1992) to produce water – soluble conjugates that are easily excreated by fish. The enzymes involved in phase II are called conjugating enzymes.

5. PAH- METABOLIZING ENZYMES

5.1 Cytochrome P450 system

Cytochrome P450 refers to a family of isoenzymes catalyzing monooxygenase reactions which can transform the structure of organic chemicals; they are classified according of their primary amino acid-sequence alignments (Nebert & Gonzalez 1987). Isoenzymes have been grouped into different families of 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 metabolits (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), P450LM4b from trout (Williams & Buhler 1984) and P450c from cod (Gokyr 1985). The nomenclature, structure, and function of the cytochrome P4501A gene in fishes has been recently reviewed by Stgeman (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 eukaryotic organisms P450 proteins are membrane bound. The ones that metabolize or transform 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), ethoxy-resorufin-O-deethylase (EROD), and 7-ethoxy-coumarin-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 endothelim (Stegeman et al. 1979, Lindstrom-Seppa et al. 1981, Pesonenet al 1985, Andesson and Part 1989, Van Veld et al 1990, Stegeman an Hahn1994). 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 o 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 (Lindstrom-Seppa & Oikari 1989, 1990, Stein et al. 1992, Garrigues et al. 1993). Several field experiments have demonstrated the usefulness of the monoxygenase system as an indicator of contamination (Lindstrom-Seppa & 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 arly hydrocarbon hydroxylase (AHH) and ethoxyresorufin-O-deethylase (EROD) has been observed in a number of fish species (Lindstroom-Seppa 1990, Van Veld et al 1990, Pesonen & Andersson 1991, Pesonen 1992, Garriguses et al. 1993). Many authors have shown the dependence of EROD on the PAH concentration and length of exposure (Saved et al. 1992, Lindstrom-Seppa 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 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 (Lindstrom – Seppa 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 difference between summer and winter).

The cytochrome P450 concentration was significantly higher in the hepatic microsomes of gonadally mature male book 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; 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 shown that estradiol is a regulator of monooxyenase activity (suppreassion 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 kmonooxygenase activities (Lindstrom-Seppa 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 25-85% and 15-77% lower, respectively, in the hepatocullular carcinoma and in the foci of cellular alteration than in non-neoplastic tissue.

6. SUMMARY

Polycylic 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 monooxygennases, 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 melano-macroophage centres in the liver and suppression of proliferative responses of T-lymophocytes

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

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

PAHs have a potential effect on fish reproduction.

REFERENCES

1. Adama, S.M., Shepard, K. L., Greeley Jr. M.S., Jimenez, B. D. Ryon, M.G. oShugart, L.R., McCarthy, J. F. & Hinton, D.E. 1989: the use of bioindicators for assessing the effects of pollutant stress on fish. – Marine Environmental Research 28: 459-64.

2. Ashokas, J.T. 1979: Cytochrom P450 in fish liver microsomes and carcinogen activity. – ACS Symposium Series 99: 279-295.

3. Anderson, T. & Part, P. 1989: Benzo (a) pyrene Metabolism in isolated perfused rainbow trout gills. – Marine Environmental research 25: 3-7.

4. Baumann. P.C., Mac, M.J. Smith, S.B. & Harshbarger, J.C. 1991: Tumor frequencies in walleye (Stizostedion vitreum) and brown bullhead (Ictalurus nebulosus) and sediment contaminants in tributaries of the Laurentain Great Lakes. – Can. J. Fish. Aquat. Sci. 48(9): 1804-1810.

5. Blumer, M. 1976: Polycclic aromatic compound in nature. – Sci. Am. 1:24-45.

6. Bock, K.W., Lipp. H.P. & Bock- Henning. B.S. 1990: Induction of drug-metabolizing enzymes by xenobiotics. – Xenobiotica 20(11): 1101-1111.

7. Catoggio, J.A. 1991: Other organic toxic substances. – In : Guidelines of lake management. toxic substances management in lakes and reservoirs 4: 113-126.

8. Cavalieri, E. & Rogan, E. 1985: Role of radical cations in aromatic hydrocarbon carcinogenesis. – Environ. Health Persp. 64:69 – 84.

9. Clarke, D.J., Burchell, B. & George, S.G. 1992: Differential expression and induction of UDP-glucuronosyltransfearse insoforms in hepatic and extrahepatic tissues of a fish, Pleuronectes platessa: immunochemical and functional characterization. – Toxicol. Appl. Pharmocal. 115 (1): 130-136.

10. Clarke, D.J. George, S.G. & Burchell, B. 1991: Glucuronidation in fish. – Aquatic Toxicology 20:35-56.