Human and Ecological Risk Assessment, 13: 302–316, 2007

ISSN: 1080-7039 print / 1549-7680 online
DOI: 10.1080/10807030701226749

Perspective on Ecotoxicology of PAHs to Fish

Dennis T. Logan
Pasadena, MD, USA

ABSTRACT

This article provides a perspective on recent research on the effects of PAHs on fish. Fish are visible members of aquatic communities that are vulnerable to PAH contamination. The ecotoxicology of fish and PAHs can be complex. Fish are a diverse group that can have complicated life cycles and behavior and can be exposed to PAH-contaminated sediments and water by a variety of routes, including respiration; ingestion of food, sediment, and detritus; and dermal absorption. PAHs are a complex group of chemicals with similar chemical structure but a variety of chemical and physical attributes and are usually produced and occur in the environment as mixtures. Individual PAHs may elicit a variety of effects in different fish species, and different PAHs may elicit different effects in any single fish species. The variation in both fish and PAHs is reflected in the wide range of adverse effects observed in fish exposed to PAHs. Some observed effects include narcosis, mortality in all life stages, decrease in growth, lower condition factor, edema, cardiac dysfunction, a variety of deformities, lesions and tumors of the skin and liver, cataracts, damage to immune systems and compromised immunity, estrogenic effects, bioaccumulation, bioconcentration, trophic transfer, and biochemical changes, some of which can be used as biomarkers.

Key Words: fish, PAHs, ecotoxicology.

INTRODUCTION

This article provides a perspective on recent research on the effects of PAHs on fish. Although past reviews provide a good overview (*e.g.*, Eisler 1987), much research on the toxicological effects of PAHs on fish is relatively recent and ongoing. Because PAHs represents a large class with similar chemical structure but varying chemical and physical characteristics, environmental toxicological effects also vary.

Environmental toxicology of PAHs and fish is complex for several reasons. PAHs in the environment are seldom released singly, and they typically occur as complex mixtures. As a group, PAHs are chemically complex and contain many individual compounds. PAHs, which are defined by having two or more fused benzene

Address correspondence to Dennis T. Logan, 1468 Westcliff Drive, Pasadena, MD 21122, USA. E-mail: dlogan6420@aol.com

(aromatic) rings, include more than 10,000 possible individual compounds. For the purposes of environmental toxicology, the U.S. Environmental Protection Agency (USEPA 2003) now identifies a set of 34 PAHs that are generally the most abundant and commonly measured in environmental monitoring programs as representing "total PAHs" in sediments.

PAH compounds vary in physical (*e.g.*, vapor pressure, solubility, Kow) and chemical (*e.g.*, resistance to oxidation and reduction) characteristics due, in part, to differences in molecular weight (USEPA 2003). These variations affect movement through and fate in the environment. The different size and structure of various PAH compounds also influence the type and magnitude of toxicological effects such as narcotic effects, carcinogenicity, mutagenicity, and genotoxicity. The presence of other chemicals and exposure to light can also affect the toxicity of individual PAHs or PAH mixtures.

Mixtures of PAHs can be produced in various ways and these mixtures vary in toxicity to fish. PAH mixtures can be divided into three or four classes based on environmental source (Neff 1979; USEPA 2003): pyrogenic (formed by combustion of organic matter such as fossil fuels in short time periods, high temperature, and typically low pressure); petrogenic, such as petroleum (formed in the earth by geological process at low temperature, long time periods, and possibly high pressure); diagenic (formed in place in sediments from biogenic compounds, possibly from anaerobic processes); and biogenic (formed directly in sediments by animals, plants, fungi, and bacteria).

Composition of the mixture, including presence of non-PAHs, may affect toxicity in unexpected ways. For example, hatchery-reared California halibut (*Platichthys californicus*) are relatively less sensitive to oil from a natural seep than to PAH mixtures from anthropogenic sources (Seruto *et al.* 2005). Complexity of PAH environmental toxicology, including fish, is explained by the potentially large number of compounds that exhibit different chemical, physical, and toxicological characteristics and that tend to be released in mixtures with compositions that differ by source.

Fish can serve as natural indicators of PAH contamination in surface waters. Because of commercial and recreational importance, fish are often the most visible members of aquatic communities. Damage to the skin, barbels, fins, and eyes from exposure to PAH-contaminated sediments and water can be observed with the naked eye. It is no wonder that such damage has resulted in some of the earliest investigations of the effects of PAHs on fish (*e.g.*, Murchelano and Wolke 1985) and continues to attract attention.

Through their life cycle, fish may be exposed to PAHs in sediments as well as in the water column. Some species excavate nests and many have demersal eggs, which put the eggs in contact with the sediments. Larvae may live close to the bottom and be exposed to sediment contamination. Eggs and larvae of some species also inhabit the surface microlayer where elevated PAH concentrations can occur (Wurl and Obbard 2004). Some adult fish are benthic, such as flatfish and catfish, and come into direct contact with sediments, and many eat benthic invertebrates that accumulate PAHs. All fish have gills comprised of thin membranes to transfer oxygen from water. Fish actively pass large quantities of water, and thus the gill tissues can be exposed to PAHs present in the water.

Potential effects on fish are also of interest because these animals occupy key positions in aquatic and terrestrial food webs. They are preyed upon by numerous species including other fish, humans and other mammals, piscivorous birds, and various scavengers. Therefore, changes in fish populations can translate into changes in other populations dependent on them for food. Fleeger *et al.* (2003) recently reviewed these types of indirect effects of contaminants on fish and identified trophic cascade and competitive release as two consequences.

CHARACTERIZATION OF EXPOSURE

Exposure of fish to PAHs can occur through the organic gill (respiration), gut (ingestion), or integument (dermal contact). Once absorbed through these membranes, the PAHs can be carried to other parts of the fish via the bloodstream. The potential for exposure and accumulation of PAH by fish is influenced by the bioavailability of the compounds. For the more water-soluble PAHs, most uptake is thought to take place through the gills, especially for PAHs with K_{ow}s in the range of 2 to 6.5 (Baussant *et al.* 2001b). The uptake rate of individual PAHs by a particular aquatic species by respiration depends on the K_{ow}, the degree of alkylation (Jonsson *et al.* 2004), the respiration rate, gill structure, and the duration of exposure. For the more hydrophobic PAHs, most of the uptake is thought to be through ingestion of food (Vives *et al.* 2005) or sediment or direct contact with the sediment (Kocan *et al.* 1996; Meador *et al.* 1995). Uptake by ingestion depends on K_{ow}, concentration of the PAH in food, the feeding rate, structure of the gut, and duration of exposure. Uptake of PAHs can be rapid, with maximum concentrations occurring within 7 days of exposure to constant water concentrations (Jonsson *et al.* 2004).

Bioavailability

Bioavailability of PAHs in aquatic environments depends on solubility of these compounds in both water and organic compounds (reviewed in Baussant *et al.* 2001). It is well known, for example, that oil dispersants may affect the bioavailability of the PAHs because they alter the oil matrix containing the compounds (Ramachandran *et al.* 2004; Mielbrecht *et al.* 2005).

The bioavailability of PAHs depends on the source of the PAHs, the media (sediments, water) as well as the degree to which the compounds are adsorbed to particulate matter. For example, petrogenic PAHs are introduced to aquatic environments due to releases of oil and petroleum products. PAHs associated with such releases can occur in slicks, oil droplets, in association with organic carbon on the water surface, in the water column, or in sediments.

The presence of organic matter in the water column or sediments will have a major influence on the bioavailability of PAHs to fish. Increasing levels of particulate organic material and dissolved organic matter such as humic material in the water column result in decreasing bioavailability to fish (Spacie *et al.* 1983; McCarthy and Jimenez 1985; Jonsson *et al.* 2004).

Predicting the bioavailability of PAHs in sediments is largely determined by their molecular weight and hydrophobicity. Because hydrophobicity of PAHs generally increases with molecular weight, the best determinants of PAHs bioavailability in

sediments are the lipid content of the receptor, the organic carbon content of the sediments, and K_{ow} (reviewed in Meador *et al.* 1995; USEPA 2003). Some organic compounds such as black carbon soot, charcoal, and coal that have strong sorption capacities can decrease the bioavailability of PAHs in sediments (USEPA 2003).

Bioaccumulation and Bioconcentration

Bioconcentration of PAHs from water into fish tissue depends on the balance between uptake and elimination rates (Baussant *et al.* 2001b; Jonsson *et al.* 2004). Most fish species (except agnaths) have enzymatic systems that metabolize and detoxify PAHs (Hahn *et al.* 1994), which are eliminated at different rates for different PAHs. The influence of metabolism can result in a pattern of PAH accumulation that differs among fish species. Metabolism also accounts for observed differences between PAHs in fish and in passive samplers intended to mimic biological uptake such as semipermeable membrane devices (SPMS). The composition and concentration of PAHs in these devices is due only to partitioning between water and an organic compound (lipid) and no elimination occurs (Baussant *et al.* 2001a). As a result, the pattern of PAH accumulation that occurs in a particular fish species can differ from that in the environment, in SPMD samplers, and in other species.

Once in an organism, PAHs tend to partition to the most lipid-rich tissues, such as the liver and bile of fish (Meador *et al.* 1995). The highest levels of PAHs in winter flounder (*Pseudopleuronectes americanus*) exposed to crude oil in sediments occurred in the liver followed by muscles (Hellou *et al.* 1994).

Elimination of PAHs from fish depends on both passive diffusion and active biotransformation in the liver followed by excretion with the bile. Passive diffusion across the gills and through the skin is more important for low molecular weight PAHs, and biotransformation and excretion are more important for high molecular weight PAHs. PAHs are transformed into hydroxy-PAHs, which are excreted in the bile (Jonsson *et al.* 2004). The compounds can be detected in the gall bladder and bile (Deshpande 1989) and used as biomarkers for PAH exposure (see later). The time needed to eliminate 95% of the bioaccumulated PAHs in fish tissue by test fish returned to clean water is from 2.1 to 3.8 d for nonalkylated PAHs and 3.8 to 33.3 d for alkylated PAHs (Jonsson *et al.* 2004). The balance of PAH uptake and elimination rates results in increasing bioconcentration factors (BCF) with increasing K_{ow} up to log $K_{ow} = 4$ and decreasing BCF with increasing K_{ow} above that (Baussant *et al.* 2001b). Without active elimination, BCFs would be expected to increase with increasing K_{ow} at all levels.

Fingerling rainbow trout (*Onchorynchus mykiss*) have been found to accumulate retene from contaminated sediments, as evidenced from retene metabolites in bile and liver CYP1A activity in the liver following exposure (Oikari *et al.* 2002). The enzyme CYP1A is involved in metabolizing PAHs and other organic contaminants and measures of its activity are used as biomarkers (see later). Levels of PAH metabolites in the bile of oyster toadfish (*Opsanus tau*), a territorial benthic estuarine fish, increased with increased levels of PAH contamination such that metabolite levels from the most polluted sites were 50 times as great as those from reference sites (Deshpande *et al.* 2002). Mortality of embryos and larvae of shortnose sturgeon (*Acipenser brevirostrum*) exposed to weathered coal tar in sediments resulted from a

direct sediment-tissue transfer of PAH rather than a water-borne transfer of soluble PAHs (Kocan *et al.* 1996).

Trophic Transfer

Fish can be exposed to PAHs present in their food. Bivalves (eastern oyster, *Crassostrea virginica*, and blue mussels, *Mytilus edulis*) can accumulate high levels of PAHs from contaminated sediments, and winter flounder (*Pseudopleuronectes americanus*) fed contaminated blue mussels will in turn be adversely affected (Gardner *et al.* 1991). Laboratory studies have also shown a trophic exposure pathway for benzo(a)pyrene from sediments to deposit-feeding polychaete worms (*Armandia brevis*) to flatfish (English sole, *Peuronectes vetulus*) (Rice *et al.* 2000). After experimentally separating the trophic and direct sediment-fish pathway, the authors concluded that levels of sediment PAHs determined to be safe for invertebrates by bioassay may still have the potential to cause adverse effects to higher trophic levels.

Because fish metabolize PAHs, transfer from fish to the next highest level trophic level (which may also be a fish) should consider PAH metabolites as well as parent compounds (Varanasi and Stein 1991).

CHARACTERIZATION OF EFFECTS

Variability Due to Species, Life Stage, Individual, and Local Population

PAHs may elicit a variety of effects in different fish species, and different PAHs may elicit different effects in any single fish species; effects also depend on the life stage during which the exposure occurs (Aas *et al.* 2001). For example, differences in susceptibility to liver cancer and in production of PAH metabolites differ in two related species of ictalurid catfish (Willet *et al.* 2000) and two species of pleuronectid flounder (Collier *et al.* 1992).

Genetic differences or exposure histories may also affect the expression of PAH toxicity among individuals or populations of the same species (*e.g.*, Deshpande *et al.* 2002; Nacci *et al.* 2002; Ownby *et al.* 2002). In mummichogs (*Fundulus heteroclitus*), a non-migratory estuarine species, induction or suppression of specific genes may be affected by exposure to PAHs and give local populations enhanced tolerance to PAH contamination (Van Veld *et al.* 1992; Peterson and Bain 2004; Meyer *et al.* 2003; Mulvey *et al.* 2003). The increased tolerance appears to be persistent and heritable for one generation following exposure, but then lost in the second and third generations, and not genetically based (Faisal *et al.* 1991; Meyer *et al.* 2002). Resistance of local fish populations to contaminant-induced toxicity has been observed for not only PAHs but also for other contaminants (Wirgin and Waldman 2004), such as PCB congeners categorized as dioxin-like compounds that act through the aryl hydrocarbon receptor (AHR) (Nacci *et al.* 2002).

Mortality, Growth, and Deformities

When chronically exposed to PAHs as embryos, fish may exhibit mortality, decreases in growth, deformities, and edema (Gunderson *et al.* 1996; Barron *et al.* 2004a). Fish embryos exposed to petrogenic PAH mixtures (Incardona *et al.* 2004)

exhibit a syndrome of cardiac dysfunction, edema, spinal curvature, and reduction in the size of the jaw and other craniofacial structures. These effects are caused by exposure to the three-ring PAHs dibenzothiophene and phenanthrene through direct effects on cardiac conduction, with secondary effects on the development of the heart, kidneys, neural tube, and craniofacial skeleton (Incardona *et al.* 2004). The four-ring PAH pyrene in the mixtures induced a different syndrome of anemia, peripheral vascular defects, and neuronal cell death. Juvenile fish exposed to PAHs may show acute mortality or chronic decreases in growth (*e.g.*, Rice *et al.* 2000). Neff (1985) reviewed lethal effects of PAHs to fish.

Narcosis is a generalized toxic effect typically caused by stable, nonpolar organic compounds (Van Leeuwen *et al.* 1992), such as PAHs, and is involved in mortality. Differences between concentrations that result in long-term versus short-term narcotic effects tend to be small. In Pacific herring (*Clupea pallasi*) and pink salmon (*Oncorhynchus gorbuscha*) exposed to PAH mixtures, narcosis associated with the concentration of naphthalenes appears to be the primary contributor to embryo mortality, although the primary contributor to early-life stage toxicity appeared to be alkyl phenanthrene (Barron *et al.* 2004a). Considering narcosis as a common mechanism of toxic action has allowed use of the additive toxicity model to estimate mortality from PAH mixtures to both fish and invertebrates (*e.g.*, French-McCay 2002; USEPA 2003).

Lesions and Tumors

Liver tumors or neoplasms, skin tumors, and other irregularities (*e.g.*, missing, shortened or deformed barbells on bullhead catfish) have been observed in wild fish from habitats characterized by elevated PAH concentrations from a variety of sources (Malins *et al.* 1985, 1987; Thiyagarajah *et al.* 1989; Vogelbein *et al.* 1990; Aas *et al.* 2001; Bauman *et al.* 1991; Myers *et al.* 1991, 1994; Pinkney *et al.* 2001, 2004; Fournie and Vogelbein 1994; Smith *et al.* 1994; Vogelbein and Fournie 1994; Bauman and Harshbarger 1995; Bauman 1998; Khan 2003). Although experimental studies have confirmed that PAHs can cause liver tumors (Hawkins *et al.* 1990; Schiewe *et al.* 1991; Smith *et al.* 1994), such studies have not been reported for skin and oral tumors in bullheads (*Ameiurus nebulosus*), a member of the catfish family Ictaluridae (Pinkney *et al.* 2001). Bullheads and oyster toadfish (*Opsanus tau*) have been suggested as good indicator species for PAH contamination in sediments because of their benthic habits and limited home range (Vogelbein *et al.* 1990; Deshpande *et al.* 2002). Bauman *et al.* (1996) suggested that prevalence of liver tumor in bullheads exceeding 9% could be used as an indicator of contaminant exposure. Working with mummichog (*Fundulus heteroclitus*), Stine *et al.* (2004) provide histological techniques, methods and descriptions for quantitatively monitoring hepatic lesion volumes over time.

Cataract Formation

Exposure of rainbow trout eye lenses to fluoranthene, benzo(a)pyrene, and creosote in combination with exposure to UV irradiation caused changes in the lenses that indicate that PAH exposure may be a contributing factor in cataract formation and lens degradation in fish (Laycock *et al.* 2000; Whyte *et al.* 2000).

Biomarkers

Biomarkers are typically used to indicate the degree to which fish have been exposed to PAH contamination (*e.g.*, Stephensen *et al.* 2002). Biomarkers for PAHs have included levels of PAH parent compounds and PAH metabolites in the bile (*e.g.*, Aas *et al.* 2000; Pointet and Milliet 2000; Rice *et al.* 2000), presence of DNA adducts with PAH metabolites (*e.g.*, Baan *et al.* 1994; Aas *et al.* 2000; Rice *et al.* 2000), presence of hepatic lesions, presence of skin lesions, and levels of PAH in muscle (Hellou *et al.* 1994). Levels of PAH in the muscles of winter flounder (*Pseudopleuronectes americanus*) corresponded more closely than liver levels to exposure levels of crude oil in sediments (Hellou *et al.* 1994). Thyroid endpoints can also serve as biomarkers for PAH exposure, although their physiological consequences are poorly understood (Brown *et al.* 2004). In a recent review, Lee and Anderson (2005) found that although increases of PAH metabolites in fish bile and cytochrome P450 system responses can be sensitive biomarkers of PAH exposure, they saw little unequivocal evidence to suggest a linkage to whole-organism level effects such as toxicity, lesions, and reproductive failure.

PAHs damage DNA through metabolic products that covalently bond to DNA to form carcinogen-DNA adducts (Perlow and Broyde 2001), which may be the initial step in carcinogenesis (Reichert *et al.* 1998; Baan *et al.* 1994; Rose *et al.* 2000). The process can be detected in the liver by measuring biliary levels of the DNA adducts and PAH metabolites (Baan *et al.* 1994; Aas *et al.* 2003). Cytochrome P450 1A (CYP1A) enzymes are active in PAH metabolism and excretion (Stegeman and Lech 1991), and PAH metabolites generated by this system have been implicated in chronic toxicity to larval fish (Hawkins *et al.* 2002). In three estuarine fish species, the most common PAH metabolite was 1-hypoxy pyrene, followed by 1-hydroxy chrysene and 1-hydroxy phenanthrene (Ruddock *et al.* 2002).

Levels of biliary DNA adducts in fish generally correlate with PAH contamination (Baan *et al.* 1994). Bilary PAH metabolites and hepatic DNA adducts in both Atlantic cod (*Gadus morhua*) and corkwing wrasse (*Symphodus melops*) were found to increase in an area highly contaminated with PAHs (Aas *et al.* 2001). Frenzilli *et al.* (2004) found a high level of damaged DNA and bile PAH metabolites associated with eelpout (*Zoarces viviparus*) taken 3 weeks after a spill of bunker oil. They noted recovery in fish taken from the site after 5 months.

Responses of the hepatic aryl hydrocarbon hydroxylase system can also be used as biomarkers (Collier and Varanasi 1991). Aryl hydrocarbon receptor (AhR) agonism is a model for chronic toxicity of PAHs to embryonic fish (Nacci *et al.* 2002; Barron *et al.* 2004a). Analysis of 74 PAHs suggests that some have similar potency to dioxin-like PCBs (Barron *et al.* 2004b).

Compromised Immunity

The potential that PAHs compromise the immune systems of fish has been investigated and shown to occur in some but not all cases. The mechanism, induction of programmed cell death (apoptosis) of lymphocytes and phagocytes, has been observed in *in vitro* studies of common carp (*Cyprinus carpio*) exposed to 3-methylcholanthrene (Reynaud *et al.* 2004). Winter flounder (*Pseudopleuronectes americanus*) taken from a PAH-contaminated area showed external lesions, liver

discoloration, a lower condition factor, lower hemoglobin and lymphocyte levels and higher prevalence of external parasite *Cryptocotyle lingua*, but lower levels of the digestive tract parasite *Steringophorus furciger* compared to reference fish (Khan 2003). Exposure to PAH-contaminated estuarine sediments was found to cause defective macrophage phagocytic and chemocytic activity in spot (*Leiostomus xanthurus*) and hogchoker (*Trinectes maculates*) (Weeks and Warriner 1986). DNA adducts formed by exposure of mummichogs to benzo[a]pyrene were found to persist in the blood, liver and two hematopoietic tissues (anterior kidney and spleen) for at least 96 days, which may lead to disruption of physiological functions such as immunity and hematopoiesis (Rose *et al.* 2001).

Other studies have not shown effects on the immune system. Eel pout (*Zoarces viviparus*) taken from the site of an oil spill did not exhibit apoptosis of nucleated erythrocytes, although other effects were found (Frenzilli *et al.* 2004). Juvenile chinook salmon (*Oncorhynchus tschawytscha*) fed a mixture of 14 PAHs meant to reflect natural estuarine exposure did not show decreased disease resistance or growth when challenged with the bacterium *Listonella (Vibrio) anguillarum* in laboratory tests (Palm *et al.* 2003).

Estrogenic Effects and Reproduction

PAHs and their derivatives may affect estrogenic activity in fish similar to the action of dioxin (Williams *et al.* 1998; Villeneuve *et al.* 2002). Exposure of female starry flounder (*Platichthys flesus*) to phenanthrene altered plasma steroid levels involved in reproduction and indicated antiestrogenic activity capable of impairing reproductive function (Monteiro *et al.* 2000a). The antiestrogenic activity of PAHs in rainbow trout (*O. mykiss*) is mediated through the aryl hydrocarbon receptor (AhR), and AhR-binding PAHs display antiestrogenic activity (Navas and Segner 2000). Phenanthrene, benzo(a)pyrene and chrysene were found to decrease the in vitro synthesis of ovarian steroids in starry flounder (*P. flesus*) and potentially disrupt the reproductive cycle of female fish (Monteiro *et al.* 2000b). Following the *Exxon Valdez* oil spill, abnormal chromosome development in fish embryos and larvae (genotoxicity) was found to correlate with decreases in Pacific herring (*Clupea pallasi*) populations, which may be the first reported example linking genotoxicity to population-level effects (Hose and Brown 1998).

Phototoxicity

Phototoxicity occurs when PAHs present in biological tissue are then exposed to light, and the resulting reaction enhances the PAH toxicity and can cause fish mortality and other effects on aquatic biota (Bowling *et al.* 1983; Oris and Giesy 1985, 1986, 1987; Barron *et al.* 2003). The mode of action is photosensitization wherein toxicity occurs through tissue damage, rather than narcosis, and the phototoxic compounds are 3–5 ring PAHs and heterocycles (Choi and Oris 2000; Barron and Ka'aihue 2001). For many benthic species, exposure to light may be minimal, but photic zone and sea-surface microlayer are important habitats for some fish eggs and larvae where exposure to both light and contaminants may occur (Barron and Ka'aihue 2001; Wurl and Obbard 2004). Such effects have been observed in fish. Brief exposure to sunlight (2.5 hr/da for 2 days) increased the toxicity of weathered

crude oil to Pacific herring (*Clupea pallasi*) 1.5 to 48 times (Barron *et al.* 2003). Chemically dispersed oil may be more phototoxic than undispersed oil (Barron *et al.* 2003), possibly due to increased bioavailability.

SUMMARY AND CONCLUSIONS

Fish are particularly vulnerable and visible receptors for PAH contamination. Their aquatic habitats are naturally close to human habitats and potential sources of contamination, and PAHs tend to move with or in the same direction as surface and groundwater to aquatic habitats. Because of their vulnerability to exposure, the visibility of some adverse effects such as tumors and lesions, their centrality in aquatic food webs, and both recreational and commercial importance, fish and the effects of PAHs on them have been the object of continued investigation.

The ecotoxicology of PAHs and fish is complex. Ecologically, fish are a diverse group with complex life cycles and behavioral patterns that can lead to various routes of exposure even for a single species. PAHs are a complex group of chemicals defined by similar chemical structure but possessing a variety of chemical and physical attributes, and they are usually produced and occur in the environment as PAH mixtures. Possible routes of exposure include: uptake through the gills during respiration; through the gut from ingestion of food, sediment, and detritus; and direct absorption through the integument (dermal), which is thinner and more permeable to many substances than the integument of terrestrial animals. The relative importance of these routes depends on the life stage of the fish, its habits, and the chemical and physical characteristics of the PAHs under consideration. To add to the complexity, after exposure both the PAHs and the products of their metabolic breakdown in tissue can be toxic. These metabolic products may be important in trophic transfer after PAH exposure. Furthermore, once these substances are present in fish tissue, exposure to light may induce phototoxicity.

The wide range of possible adverse effects observed in fish exposed to PAHs reflects the interaction of the complex ecology of fish and the complex nature of PAHs as a stressor. Some of these observed effects include narcosis, mortality in all life stages, decrease in growth, lower condition factor, edema, cardiac dysfunction, a variety of deformities, lesions and tumors of the skin and liver, cataracts, damage to immune systems and compromised immunity, estrogenic effects, bioaccumulation, bioconcentration, trophic transfer, and biochemical changes, some of which are useful as biomarkers. In spite of this complexity, when trying to assess the effects of PAH mixtures on fish, total PAH appears to be a practical measure of mixture toxicity (Barron *et al.* 2004a) just as it is for invertebrates (USEPA 2003). Overall the recent research shows not only the vulnerability of fish to PAH exposure, but also the potential for fish to be a useful sentinel for environmental PAH contamination.

ACKNOWLEDGMENTS

Comments by Charles Menzie and an anonymous reviewer of the draft of this article were very helpful.

REFERENCES

Aas E, Baussant T, Balk L, *et al.* 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 51:241–58

Aas E, Beyer J, Jonsson G, *et al.* 2001. Evidence of uptake, biotransformation and DNA binding of polyaromatic hydrocarbons in Atlantic cod and corkwing wrasse caught in the vicinity of an aluminum works. Mar Environ Res 52:213–29

Aas E, Liewenborg B, Grosvik BE, *et al.* 2003. DNA adduct levels in fish from pristine areas are not detectable or low when analysed using the nuclease P1 version of the 32P-postlabelling technique. Biomarkers 8:445–60

Baan RA, Steenwinkel MJ, van den Berg PT, *et al.* 1994. Molecular dosimetry of DNA damage induced by polycyclic aromatic hydrocarbons; relevance for exposure monitoring and risk assessment. Hum Exp Toxicol 13:880–7

Barron MG and Ka'aihue L. 2001. Potential for photoenhanced toxicity of spilled oil in Prince William Sound and Gulf of Alaska waters. Mar Pollut Bull. 43:86–92

Barron MG, Carls MG, Short JW, *et al.* 2003. Photoenhanced toxicity of aqueous phase and chemically dispersed weathered Alaska North Slope crude oil to Pacific herring eggs and larvae. Environ Toxicol Chem 22:650–60

Barron MG, Carls MG, Heintz R, *et al.* 2004a. Evaluation of fish early life-stage toxicity models of chronic embryonic exposures to complex polycyclic aromatic hydrocarbon mixtures. Toxicol Sci 78:60–7

Barron MG, Heintz R, and Rice SD. 2004b. Relative potency of PAHs and heterocycles as aryl hydrocarbon receptor agonists in fish. Mar Environ Res 58:95–100

Bauman PC. 1998. Epizootics of cancer in fish associated with genotoxins in sediment and water. Mutat Res 411:227–33

Bauman PC and Harshbarger JC. 1995. Decline in liver neoplasms in wild brown bullhead catfish after coking plant closes and environmental PAHs plummet. Environ Health Perspect 103:168–70

Bauman PC, Mac MJ, Smith SB, *et al.*. 1991. Tumor frequencies in walleye (*Stizostedion vitreum*) and brown bullhead (*Ictalurus nebulosus*) in tributaries of the Laurentian Great Lakes. Can J Fish Aquat Sci 48:1804–10

Bauman PC, Smith JR, and Harshbarger JC. 1996. Linkages between chemical contaminants and tumors in benthic Great Lakes fish. J Great Lakes Res 22:131–52

Baussant T, Sanni S, Jonsson G, *et al.* 2001a. Bioaccumulation of polycyclic aromatic compounds: 1. Bioconcentration in two marine species and in semipermeable membrane devices during chronic exposure to dispersed crude oil. Environ Toxicol Chem 20:1175–84

Baussant T, Sanni S, Skadsheim A, *et al.* 2001b. Bioaccumulation of polycyclic aromatic compounds: 2. Modeling bioaccumulation in marine organisms chronically exposed to dispersed oil. Environ Toxicol Chem 20:1185–95

Bowling JW, Leversee GJ, Landrum PF, *et al.* 1983. Acute mortality of anthracene-contaminated fish exposed to sunlight. Aquat Toxicol 3:79–90

Brown SB, Adams BA, Cyr DG, *et al.* 2004. Contaminant effects on the teleost fish thyroid. Environ Toxicol Chem 23:1680–701

Choi J and Oris JT. 2000. Evidence of oxidative stress in bluegill sunfish (*Lepomis macrochirus*) liver microsomes simultaneously exposed to solar ultraviolet radiation and anthracene. Environ Toxicol Chem 19:1795–9

Collier TK and 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 20:462–73

Collier TK, Singh SV, Awasthi YC, *et al.* 1992. Hepatic xenobiotic metabolizing enzymes in two species of benthic fish showing different prevalences of contaminant-associated liver neoplasms. Toxicol Appl Pharmacol 113:319–24

Deshpande AD. 1989. High performance liquid chromatographic separation of fish biliary polynuclear aromatic hydrocarbon metabolites. Arch Environ Contam Toxicol 18:900–7

Deshpande AD, Huggett RJ, and Halbrook RA. 2002. Polycyclic aromatic hydrocarbon metabolites in the bile of a territorial benthic fish, oyster toadfish (*Opsanus tau*) from the Elizabeth River, Virginia. Arch Environ Contam Toxicol 42:43–52

Eisler R. 1987. Polycyclic Aromatic Hydrocarbon Hazards to Fish Wildlife and Invertebrates: A Synoptic Review. Contaminant Hazard Reviews No. 11, Biological Report (1.11). US Fish and Wildlife Service, Patuxent Wildlife Research Center, Laurel, Maryland, USA

Faisal M, Weeks BA, Vogelbein WK, *et al.* 1991. Evidence of aberration of the natural cytotoxic cell activity in *Fundulus heteroclitus* (Pisces: Cyprinodontidae) from the Elizabeth River, Virginia. Vet Immunol Immunopathol. 29:339–51

Fleeger JW, Karman KR, and Nesbet RM. 2003. Indirect effects of contaminants on aquatic ecosystems. Sci Total Environ 317:207–33

Fournie JW and Vogelbein WK. 1994. Exocrine pancreatic neoplasms in the mummichog (*Fundulus heteroclitus*) from a creosote-contaminated site. Toxicol Pathol 22:237–47

French-McCay DP. 2002. Development and application of an oil toxicity and exposure model, OILTOXEX. Environ Toxicol Chem 21:2080–94

Frenzilli G, Scarcelli V, Del Barga I, *et al.* 2004. DNA damage in eelpout (*Zoarces viviparus*) from Goteborg harbour. Mutat Res 552:187–95

Gardner GR, Yevich PP, Harshbarger JC, *et al.* 1991. Carcinogenicity of Black Rock Harbor sediment to the eastern oyster and trophic transfer of Black Rock Harbor carcinogens from the blue mussel to the winter flounder. Environ Health Perspect 90:53–66

Gundersen DT, Kristanto SW, Curtis LR, *et al.* 1996. Subacute toxicity of the water-soluble fractions of Kuwait crude oil and partially combusted crude oil on *Menidia beryllina* and *Palaemonetes pugio.* Arch Environ Contam Toxicol 31:1–8

Hahn ME, Poland A, Glover E, *et al.* 1994. Photoaffinity labeling of the Ah receptor: phylogenetic survey of diverse vertebrate and invertebrate species. Arch Biochem Biophys 310:218–28

Hawkins WE, Walker WW, Overstreet RM, *et al.* 1990. Carcinogenic effects of some polycyclic aromatic hydrocarbons on the Japanese medaka and guppy in waterborne exposures. Sci Total Environ 94:155–67

Hawkins SA, Billiard SM, Tabash SP, *et al.* 2002. Altering cytochrome P4501A activity affects polycyclic aromatic hydrocarbon metabolism and toxicity in rainbow trout (*Oncorhynchus mykiss*). Environ Toxicol Chem 21:1845–53

Hellou J, Payne JF, Upshall C, *et al.* 1994. Bioaccumulation of aromatic hydrocarbons from sediments: a dose-response study with flounder (*Pseudopleuronectes americanus*). Arch Environ Contam Toxicol 27:477–85

Hose JE and Brown ED. 1998. Field applications of the piscine anaphase aberration test: lessons from the Exxon Valdez oil spill. Mutat Res 399:167–78

Incardona JP, Collier TK, and Scholz NL. 2004. Defects in cardiac function precede morphological abnormalities in fish embryos exposed to polycyclic aromatic hydrocarbons. Toxicol Appl Pharmacol 196:191–205

Jonsson G, Bechmann RK, Bamber SD, *et al.* 2004. Bioconcentration, biotransformation, and elimination of polycyclic aromatic hydrocarbons in sheepshead minnows (*Cyprinodon variegatus*) exposed to contaminated seawater. Environ Toxicol Chem 23:1538–48

Khan RA. 2003. Health of flatfish from localities in Placentia Bay, Newfoundland, contaminated with petroleum and PCBs. Arch Environ Contam Toxicol 44:485–92

Kocan RM, Matta MB, and Salazar SM 1996. Toxicity of weathered coal tar for shortnose sturgeon (*Acipenser brevirostrum*) embryos and larvae. Arch Environ Contam Toxicol 31:161–5

Laycock NL, Schirmer K, Bols NC, *et al.* 2000. Optical properties of rainbow trout lenses after in vitro exposure to polycyclic aromatic hydrocarbons in the presence or absence of ultraviolet radiation. Exp Eye Res 70:205–14

Lee RF and Anderson JW. 2005. Significance of cytochrome P450 system responses and levels of bile fluorescent aromatic compounds in marine wildlife following oil spills. Mar Pollut Bull 50:705–23

Malins DC, Krahn MM, Myers MS, *et al.* 1985. Toxic chemicals in sediments and biota from a creosote-polluted harbor: relationships with hepatic neoplasms and other hepatic lesions in English sole (*Parophrys vetulus*). Carcinogenesis 6:1463–9

Malins DC, McCain BB, Myers MS, *et al.* 1987. Field and laboratory studies of the etiology of liver neoplasms in marine fish from Puget Sound. Environ Health Perspect 71:5–16

McCarthy JF and Jimenez BD. 1985. Reduction in the bioavailability to bluegills of polycyclic aromatic hydrocarbons bound to dissolved humic material. Environ Toxicol Chem 4:511–21

Meador JP, Stein JE, Reichert WL, *et al.* 1995. Bioaccumulation of polycyclic aromatic hydrocarbons by marine organisms. Rev Environ Contam Toxicol 143:79–165

Meyer JN, Nacci DE, and Di Giulio RT. 2002. Cytochrome P4501A (CYP1A) in killifish (*Fundulus heteroclitus*): Heritability of altered expression and relationship to survival in contaminated sediments. Toxicol Sci 68:69–81

Meyer JN, Wassenberg DM, Karchner SI, *et al.* 2003. Expression and inducibility of aryl hydrocarbon receptor pathway genes in wild-caught killifish (*Fundulus herteroclitus*) with different contaminant exposure histories. Environ Toxicol Chem 22:2337–43

Mielbrecht EE, Wolfe MF, Tjeerdema RS, *et al.* 2005. Influence of a dispersant on the bioaccumulation of phenanthrene by topsmelt (*Atherinops affinis*). Ecotoxicol Environ Saf 61:44–52

Monteiro PR, Reis-Henriques MA, and Coimbra J. 2000a. Plasma steroid levels in female flounder (*Platichthys flesus*) after chronic dietary exposure to single polycyclic aromatic hydrocarbons. Mar Environ Res 49:453–67

Monteiro PR, Reis-Henriques MA, and Coimbra J. 2000b. Polycyclic aromatic hydrocarbons inhibit in vitro ovarian steroidogenesis in the flounder (*Platichthys flesus* L.). Aquat Toxicol 48:549–59

Mulvey M, Newman MC, Vogelbein WK, *et al.* 2003. Genetic structure and mRNA diversity of *Fundulus heteroclitus* populations from polycyclic aromatic hydrocarbon-contaminated sites. Environ Toxicol Chem 22:671–7

Murchelano RA and Wolke RE. 1985. Epizootic carcinoma in winter flounder, *Pseudopleuronectes americanus*. Science 228:587–9

Myers MS, Landahl JT, Krahn MM, *et al.* 1991. Relationships between hepatic neoplasms and related lesions and exposure to toxic chemicals in marine fish from the U.S. West Coast. Environ Health Perspect 90:7–15

Myers MS, Stehr CM, Olsen OP, *et al.* 1994. Relationship between toxicopathic hepatic lesions and exposure to chemical contaminants in English sole (*Pleuronectes vetulus*), starry flounder (*Platichthys stellatus*), and white croaker (*Genyonemus lineatus*) from selected marine sites on the Pacific coast. Environ Health Perspect 102:2–17

Nacci DE, Kohan M, Pelletier M, *et al.* 2002. Effects of benzo[a]pyrene exposure on a fish population resistant to the toxic effects of dioxin-like compounds. Aquat Toxicol 57:203–15

Navas JM and Segner H. 2000. Antiestrogenicity of beta-naphthoflavone and PAHs in cultured rainbow trout hepatocytes: evidence for a role of the arylhydrocarbon receptor. Aquat Toxicol 51:79–92

Neff JM. 1979. Polycyclic Aromatic Hydrocarbons in the Aquatic Environment. Applied Science Publishers, London, UK

Neff JM. 1985. Polycyclic aromatic hydrocarbons. In: Rand GM and Petrocelli SR (eds), Fundamentals of Aquatic Toxicology, pp 416–54. Hemisphere Publ Corp, New York, NY, USA

Oikari A, Fragoso N, Leppanen H, *et al.* 2002. Bioavailability to juvenile rainbow trout (*Oncorynchus mykiss*) of retene and other mixed-function oxygenase-active compounds from sediments. Environ Toxicol Chem 21:121–8

Oris JT and Giesy JP Jr. 1985. The photoenhanced toxicity of anthracene to juvenile sunfish (*Lepomis* spp.). Aquat Toxicol 6:133–46

Oris JT and Giesy JP Jr. 1986. Photoinduced toxicity of anthracene to juvenile bluegill sunfish (*Lepomis macrochirus rafinesque*): Photoperiod effects and predictive hazard evaluation. Environ Toxicol Chem 5:761–8

Oris JT and Giesy JP Jr. 1987. The photo-induced toxicity of polycyclic aromatic hydrocarbons to larvae of the fathead minnow (*Pimephales promelas*). Chemosphere 16:1395–404

Ownby DR, Newman MC, Mulvey M, *et al.* 2002. Fish (*Fundulus heteroclitus*) populations with different exposure histories differ in tolerance of creosote-contaminated sediments. Environ Toxicol Chem 21:1897–902

Palm RC Jr, Powell DB, Skillman A, *et al.* 2003. Immunocompetence of juvenile chinook salmon against *Listonella anguillarum* following dietary exposure to polycyclic aromatic hydrocarbons. Environ Toxicol Chem 22:2986–94

Perlow RA and Broyde S. 2001. Evading the proofreading machinery of a replicative DNA polymerase: induction of a mutation by an environmental carcinogen. J Mol Biol 309:519–36

Peterson JS and Bain LJ. 2004. Differential gene expression in anthracene-exposed mummichogs (*Fundulus heteroclitus*). Aquat Toxicol 66:345–55

Pinkney AE, Harshbarger JC, May EB, *et al.* 2001. Tumor prevalence and biomarkers of exposure in brown bullhead (*Ictalurus nebulosus*) from the tidal Potomac River, USA, watershed. Environ Toxicol Chem 20:1196–205

Pinkney AE, Harshbarger JC, May EB, *et al.* 2004. Tumor prevalence and biomarkers of exposure in brown bullhead (*Ictalurus nebulosus*) from the Anacostia River, Washington, DC, and the Tuckahoe River, Maryland, USA. Environ Toxicol Chem 23:638–47

Pointet K and Milliet A. 2000. PAHs analysis of fish whole gall bladders and livers from the Natural Reserve of Camargue by GC/MS. Chemosphere 40:293–9

Ramachandran SD, Hodson PV, Khan CW, *et al.* 2004. Oil dispersant increases PAH uptake by fish exposed to crude oil. Ecotoxicol Environ Saf 59:300–8

Reichert WL, Myers MS, Peck-Miller K, *et al.* 1998. Molecular epizootiology of genotoxic events in marine fish: linking contaminant exposure, DNA damage, and tissue-level alterations. Mutat Res 411:215–25

Reynaud S, Duchiron C, and Deschaux P. 2004. 3-Methylcholanthrene induces lymphocyte and phagocyte apoptosis in common carp (*Cyprinus carpio* L) in vitro. Aquat Toxicol 66:307–18

Rice CA, Myers MS, Willis ML, *et al.* 2000. From sediment bioassay to fish biomarker–connecting the dots using simple trophic relationships. Mar Environ Res 50:527–33

Rose WL, French BL, Reichert WL, *et al.* 2000. DNA adducts in hematopoietic tissues and blood of the mummichog (*Fundulus heteroclitus*) from a creosote-contaminated site in the Elizabeth River, Virginia. Mar Environ Res 50:581–9

Rose WL, French BL, Reichert WL, *et al.* 2001. Persistence of benzo[a]pyrene–DNA adducts in hematopoietic tissues and blood of the mummichog, *Fundulus heteroclitus*. Aquat Toxicol 52:319–28

Ruddock PJ, Bird DJ, and McCalley DV. 2002. Bile metabolites of polycyclic aromatic hydrocarbons in three species of fish from the Severn Estuary. Ecotoxicol Environ Saf 51:97–105

Schiewe MH, Weber DD, Myers MS, *et al.* 1991. Induction of foci of cellular alteration and other hepatic lesions in English sole (*Parophrys vetulus*) exposed to an extract of an urban marine sediment. Can J Fish Aquat Sci 48:1750–60

Seruto C, Sapozhnikova Y, and Schlenk D. 2005. Evaluation of the relationships between biochemical endpoints of PAH exposure and physiological endpoints of reproduction in male California Halibut (*Paralichthys californicus*) exposed to sediments from a natural oil seep. Mar Environ Res 60:454–65

Smith SB, Blouin MA, and Mac MJ. 1994. Ecological comparisons of Lake Erie tributaries with elevated incidence of fish tumors. J Great Lakes Res 20:701–16

Spacie, A, Landrum PF, and Leversee GJ. 1983. Uptake, depuration, and biotransformation of anthracene and benzo[a]pyrene in bluegill sunfish. Ecotoxicol Environ Saf 7:330–41

Stegeman JJ and Lech JJ. 1991. Cytochrome P-450 monooxygenase systems in aquatic species: carcinogen metabolism and biomarkers for carcinogen and pollutant exposure. Environ Health Perspect 90:101–9

Stephensen E, Adolfsson-Erici M, Celander M, *et al.* 2002. Biomarker responses and chemical analyses in fish indicate leakage of polycyclic aromatic hydrocarbons and other compounds from car tire rubber. Environ Toxicol Chem 22:2926–31

Stine CB, Smith DL, Vogelbein WK, *et al.* 2004. Morphometry of hepatic neoplasms and altered foci in the mummichog, *Fundulus heteroclitus.* Toxicol Pathol 32:375–83

Thiyagarajah A, Zwerner DE, and Hargis WJ Jr. 1989. Renal lesions in estuarine fishes collected from the Elizabeth River, Virginia. J Environ Pathol Toxicol Oncol 9:261–8

USEPA (US Environmental Protection Agency). 2003. Procedures for the Derivation of Equilibrium Partitioning Sediment Benchmarks (ESBs) for the Protection of Benthic Organisms: PAH Mixtures. EPA-600-R-02-013. Office of Research and Development, Washington, DC, USA

van Leeuwen CJ, Van Der Zandt PTJ, Aidenberg T, *et al.*.1992. Application of QSARs, extrapolation and equilibrium partitioning in aquatic effects assessment. 1. Narcotic industrial pollutants. Environ Toxicol Chem 11:267–82

Van Veld PA, Vogelbein WK, Smolowitz R, *et al.* 1992. Cytochrome P450IA1 in hepatic lesions of a teleost fish (*Fundulus heteroclitus*) collected from a polycyclic aromatic hydrocarbon-contaminated site. Carcinogenesis 13:505–7

Varanasi U and Stein JE. 1991. Disposition of xenobiotic chemicals and metabolites in marine organisms. Environ Health Perspect 90:93–100

Villeneuve DL, Khim JS, Kannan K, *et al.* 2002. Relative potencies of individual polycyclic aromatic hydrocarbons to induce dioxinlike and estrogenic responses in three cell lines. Environ Toxicol 17:128–37

Vives I, Grimalt JO, Ventura M, *et al.* 2005. Distribution of polycyclic aromatic hydrocarbons in the food web of a high mountain lake, Pyrenees, Catalonia, Spain. Environ Toxicol Chem 24:1344–52

Vogelbein WK and Fournie JW. 1994. Ultrastructure of normal and neoplastic exocrine pancreas in the mummichog, *Fundulus heteroclitus.* Toxicol Pathol 22:248–60

Vogelbein WK, Fournie JW, Van Veld PA, *et al.* 1990. Hepatic neoplasms in the mummichog *Fundulus heteroclitus* from a creosote contaminated site. Cancer Res 50:5978–86

Weeks BA and Warinner JE. 1986. Functional evaluation of macrophages in fish from a polluted estuary. Vet Immunol Immunopathol 12:313–20

Whyte JJ, Herbert KL, Karrow NA, *et al.* 2000. Effect of maintaining rainbow trout in creosote microcosms on lens optical properties and liver 7-ethoxyresorufin-O-deethylase (EROD) activity. Arch Environ Contam Toxicol 38:350–6

Willett KL, Gardinali PR, Lienesch LA, *et al.* 2000. Comparative metabolism and excretion of benzo(a)pyrene in 2 species of ictalurid catfish. Toxicol Sci 58:68–76

Williams DE, Lech JJ, and Buhler DR. 1998. Xenobiotics and xenoestrogens in fish: modulation of cytochrome P450 and carcinogenesis. Mutat Res 20:179–92

Wirgin I and Waldman JR. 2004. Resistance to contaminants in North American fish populations. Mutat Res 552:73–100

Wurl O and Obbard JP. 2004. A review of pollutants in the sea-surface microlayer (SML): a unique habitat for marine organisms. Mar Pollut Bull 48:1016–30