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Cytochrome P-450 Monooxygenase Systems in Aquatic Species: Carcinogen Metabolism and Biomarkers for Carcinogen and Pollutant Exposure

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High levels of polynuclear aromatic hydrocarbon (PAH) carcinogens commonly occur in aquatic systems where neoplasms arise in fish and other animals. Enzymes that transform PAHs can act in initiating these diseases and can indicate the contamination of fish by carcinogens and other pollutants. Cytochrome P-450 has similar roles in activating PAH carcinogens in fish and mammalian species. PAHs and many chlorinated hydrocarbons, e.g., polychlorinated biphenyls (PCBs) induce a form of cytochrome P-450 in fish that is the primary catalyst of PAH metabolism. The induction of this P-450 in fish can accelerate the disposition of hydrocarbons, but can also enhance the formation of carcinogenic derivatives of PAHs. Invertebrates have lower rates of PAH metabolism than fish. These rates are not obviously inducible by exposure to PAHs or PCBs. The lower rates of foreign compound metabolism contribute to higher pollutant residue levels in bivalve mollusks (clams, mussels, etc.) than in fish and may limit the involvement of some procarcinogens (requiring activation) in disease processes in invertebrates.

The induction of P-450 forms can indicate the exposure of fish to PAHs, PCBs, and other toxic compounds. This is not restricted to carcinogens. Environmental induction has been detected in fish from contaminated areas by use of catalytic assay, antibodies to fish P-450, and cDNA probes that hybridize with P-450 messenger RNA. Application of these methods can provide sensitive biological monitoring tools that can detect environmental contamination of fish by some carcinogens and tumor promoters. The potential for using P-450 induction to detect direct-acting carcinogens and tumor promoters that are noninducers is limited, although such compounds can be expected to co-occur with pollutants that are inducers. Further study of the P-450 genes and their products in different species could identify biochemical features related to the presence and action of additional chemicals involved in carcinogenesis in fish and will provide insight into the evolution and genetic regulation of the multigene family.

Introduction

Histologically identifiable diseases including liver neoplasms are found at relatively high frequencies in some teleost fish from highly contaminated sites, including Puget Sound, Washington, Boston Harbor, Massachusetts, and the Black River, Ohio. These areas have high concentrations of aromatic hydrocarbons, chlorinated hydrocarbons, pesticides and/or metals in the sediments (*1–3*). Bivalve mollusks, which inhabit similarly contaminated sites, also bear proliferative diseases that may be neoplastic (*4*). The co-occurrence of high levels of environmental chemicals and neoplastic diseases in animals from these locations suggests that the chemicals could be causing these diseases.

Polynuclear aromatic hydrocarbons (PAHs) present in high amounts at these sites include benzo(*a*)pyrene, benzofluoranthenes, benzanthracenes, and others (*1,5*). Studies have shown that some compounds of this group are carcinogenic in mammalian species. Benzo(*a*)pyrene (BaP) and other PAHs have been shown to be carcinogenic also in some fish species (*6,7*), and extracts of sediments from sites highly contaminated with PAHs have elicited neoplasms in two species of fish artificially exposed to such extracts (*8*). Such evidence strongly indicates that these compounds may be involved in some environmental neoplasms in fishes.

Many chemical carcinogens are procarcinogens, requiring activation to carcinogenic derivatives by metabolic processes. Oxidative metabolism is frequently the initial step in biotransformation. It can lead not only to activation of certain procarcinogens and noncarcinogenic protoxicants but also to the inactivation of toxic compounds. This oxidation is catalyzed mainly by two major groups of microsomal monooxygenase or mixed-function oxidase enzymes: the heme protein cytochrome P-450 monooxygenases and the flavoprotein monooxygenases.

The essential role of biotransformation in activation of aromatic hydrocarbon carcinogens was most elegantly demonstrated for activation of BaP by cytochrome P-450. Sequential oxygenation and hydration steps catalyzed by a

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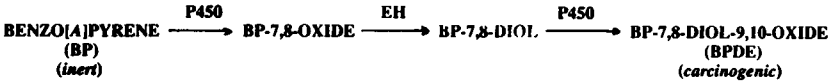

FIGURE 1. Scheme showing metabolic pathway of benzo(*a*)pyrene activation. Enzymes responsible for steps are above the lines P-450, cytochrome P-450; EH, epoxide hydrolase. One of four possible BPDE structures, the +(anti) 7,8-diol-9,10-oxide is the most potent carcinogenic product of BaP metabolism.

particular isozyme of cytochrome P-450 and by epoxide hydrolase lead to formation of several isomeric dihydrodiol-epoxide structures (*9*), one of which is known to be the most potent carcinogenic derivative of BaP (*10*) (Fig. 1). This activation pathway has been confirmed for other polynuclear aromatic structures as well, leading to the bay region theory of carcinogenesis involving these compounds (*10*). However, hydrocarbons lacking bay region structures can also be activated by cytochrome P-450, and compounds other than aromatic hydrocarbons can be activated by cytochrome P-450 or by other monooxygenase catalysts. Some examples of procarcinogenic compounds and catalysts responsible for their activation are listed in Table 1.

This paper summarizes the biochemistry of monooxygenases in fishes as it relates to initiation of environmental chemical carcinogenesis. The paper describes what the function of the monooxygenase system reveals about a possible chemical origin for environmental neoplastic diseases in fish and invertebrates and suggests how changes in levels of one form of cytochrome P-450 might indicate the exposure of fish to carcinogens and other compounds (such as tumor promoters). The implications of these enzyme changes for consumers of these organisms will also be considered.

Cytochrome P-450 Systems in Fish

The roles of cytochrome P-450 and flavoprotein monooxygenases in activating carcinogens has been defined primarily in mammalian systems. However, fish and invertebrates possess microsomal enzymes, including cytochrome P-450 and flavoprotein monooxygenases, that are similar to those in mammals. The basic biochemistry of the microsomal cytochrome system and its functions in aquatic species has been described in considerable detail in earlier reviews (*15–17*).

A key feature of cytochrome P-450 systems in both fish and mammals is their inducibility by chemical substrates for the enzymes/and by structurally related compounds. The induction of cytochrome P-450 has been demonstrated in numerous studies in which hepatic microsomes of fish treated with aromatic hydrocarbons show enhanced rates of catalytic activity with selected substrates. Table 2 indicates the diversity of compounds that can act as inducers in fish and the catalytic activities that are most strongly induced by these compounds. Among the many compounds that experimentally induce monooxygenase activity in fish liver are PAH carcinogens that are also found where tumor-bearing fish have been taken. The chlorinated hydrocarbons shown in Table 2 that are potent inducers of cytochrome P-450 in fish (polychlorinated biphenyls, chlorinated dibenzofurans, and chlorinated dibenzodioxins) also occur in many of the same sites.

Some catalytic activities induced are specific in their response to these compounds. Ethoxyresorufin *O*-deethylase (EROD) and aryl hydrocarbon (BaP) hydroxylase (AHH) activities are often undetectable in control or untreated animals, but are highly induced by treatment with hydrocarbons. On the other hand, hydrocarbons will not induce many other monooxygenase activities. Selectively induced catalytic activities should be useful for indicating the exposure to compounds that induce them.

Studies in several fish species have revealed multiple cytochrome P-450 proteins that have different physicochemical as well as catalytic properties (*18*). One form purified from liver of several species has been identified as the P-450 form primarily induced by PAHs and chlorinated hydrocarbons (Table 3). The three enzymes best studied to date, scup P-450E, trout P-450LM$_{4b}$, and cod P-450*c*, are structurally similar. These enzymes have also been identified as the catalysts responsible for those monooxygenase activities that are srongly induced in fish by hydrocarbons. The comments below focus on the catalytic functions of that PAH-inducible cytochrome P-450 in metabolizing PAHs in fish. We also describe results showing that the induction of these forms of cytochrome P-450 can indicate the exposure of fish to carcinogens and other toxic compounds in the environment.

Table 1. Common carcinogens activated by mammalian monooxygenases (*11–14*).

Carcinogen	Activating catalysts[a]
Benzo(*a*)pyrene	P450 IA1
Benzanthracene	P450 IA1
Diethylnitrosamine	P450 IIA1
Aflatoxin B_1	P450 IIIA3
2-Acetylaminofluorene	Various

[a]There may be more than one catalyst for each.

Table 2. Selected aromatic and chlorinated hydrocarbons tested as inducers of microsomal monooxygenase activity in fish.[a]

Active inducers	Inactive as inducers
Benzo(*a*)pyrene	DDT
Dibenzanthracene	2,2′,4,4′-Tetrachlorobiphenyl
Methylcholanthrene	2,2′,4,4′,5,5′-Hexachlorobiphenyl
3,3′,4,4′-Tetrachlorobiphenyl	
2,3,7,8-Tetrachlorodibenzodioxin	
β-Naphthoflavone	

[a]Information reviewed in Stegeman (*16*).

Table 3. PAH-inducible cytochrome P-450 forms fish.

Species	Cytochrome P-450 designation[a]	Major catalytic functions[b]	Reference[c]
Scup	P-450E	EROD, AHH	(*19*)
Rainbow trout	P-450LM$_4$	EROD, AHH	(*20*)
Cod	P-450$_c$	EROD, AHH	(*21*)

[a]These enzymes are now known to be specifically induced by active inducers listed in Table 2.

[b]EROD, ethoxyresorufin *O*-deethylase; AHH, aryl hydrocarbon hydroxylase.

[c]References refer to the original description of these P-450s. Other information concerning them is summarized in Stegeman and Kloepper-Sams (*18*).

Table 4. Benzo(*a*)pyrene metabolites formed *in vitro* by scup microsomal preparations and cytochrome P-450E.

	Microsome source			
B(a)P metabolite	Liver[a]	Kidney[a]	Gill[a]	Purified P-450E plus epoxide hydrolase[b]
9,10-Diol	29[c]	31	28	42
4, 5-Diol	—	—	—	—
7, 8-Diol	28	21	25	34
Quinones	15	6	9	21
9-OH	3	6	11	3
3-OH	26	35	35	—
Total activity	1.19	0.228	0.032	

[a]Data are from Stegeman et al. (*26*).

[b]Data are from Koltz et al. (*19*).

[c]Values are percent of total metabolites. Total activity refers to nanomole/minute/milligram microsomal protein.

Rates and Patterns of Hydrocarbon Metabolism

The rates of *in vitro* aromatic hydrocarbon metabolism by fish liver preparations vary greatly depending on the species and particularly on the degree of induction of P-450. But regardless of total activity, microsomal preparations from liver and other organs of many teleost fish species produce a similar suite of BaP metabolites. Studies over the past 10 years have demonstrated that liver microsomes of numerous fish species preferentially oxidize BaP *in vitro* at those sites on the benzo-ring that are associated with activation of BaP to a carcinogenic derivative (*16*). Furthermore, microsomal preparations of teleost liver (including species showing environmental neoplasms) can efficiently activate BaP and other PAHs to mutagenic products (*22,23*). Preparations of fish liver also activate BaP to products that bind covalently to DNA (*24*). The structures of those adducts include one derived from a 7,8-diol-9,10-epoxide of BaP (*25*).

Table 4 lists BaP metabolites formed *in vitro* by microsomal preparations from several organs of the marine fish scup (*Stenotomus chrysops*). Cytochrome P-450E can be induced in all these organs in scup (*27*). The profile of metabolites formed by P-450E purified from scup (Table 4) shows that this enzyme could account for the formation of these particular metabolites in these respective organs.

Cytochrome P-450E has a preference for metabolism on the benzo-ring of BaP. This same preference for benzo-ring metabolism is also exhibited by the PAH-inducible P-450 purified from rainbow trout (*28*), and by the major PAH-inducible forms from mammalian species (*9,29*). The catalytic properties of PAH-inducible cytochrome in P-450 fish suggest that this protein could activate at least some PAH carcinogens that are activated according to the scheme in Figure 1. Antibodies that inhibit fish PAH-inducible have P-450s confirmed that these P-450s are primarily responsible for metabolizing aromatic hydrocarbon carcinogens in teleost microsomal systems. For example, antibodies to scup cytochrome P-450E almost completely inhibit the metabolism of BaP by liver microsomes of various fish species.

Antibodies and cDNA Probes

Reciprocal studies with monoclonal and/or polyclonal antibodies prepared to scup P-450E, trout P-450LM$_4$, and cod P-450$_c$ have demonsrated a close immunological relationship between the teleost proteins, consistent with their catalytic similarities and their similar response to inducers (*27,30*). Antibodies to teleost P-450s cross-react with proteins specifically induced by PAHs or PCBs in a large number of fish species. Moreover, these teleost P-450 forms show similarities to the mammalian PAH-inducible P-450 forms such as rat P-450*c*. Monoclonal antibody made against scup P-450E recognizes single proteins induced by PAHs or PCBs in every vertebrate species examined to date, including fish, reptiles, birds, and mammals (*27*).

Recently, a DNA probe (a cDNA) derived from 3-methylcholanthrene-treated rainbow trout liver has been cloned and sequenced (*31*). The sequence analysis confirmed that the hydrocarbon-inducible cytochrome P-450s from fish can be classified with the hydrocarbon-inducible cytochrome P-450 IA enzymes from mammals (*27*).* The fish P-450s are apparently counterparts of hydrocarbon-inducible mammalian P-450 IA1 enzymes, such as rat P450*c* and mouse P$_1$-450. The DNA probe hybridizes with genomic DNA and mRNAs induced by PAHs from various species including brook trout, scup, catfish, Fundulus, garter snake, turtle, bullfrog, quail, and rat (*32,33*). These results show that sequence similarities occur in P-450 IA1 genes in many vertebrates, a structural similarity that corroborates the antigenic similarities of the proteins.

Some mammalian P-450 IA1 proteins have been proven to transfom PAHs to carcinogens. Similarities between the mammalian and fish P-450s further suggest a role for fish P-450s in PAH carcinogen activation. P-450 IA proteins also occur in humans (*34*), indicating there may be common pathways of PAH carcinogen activation from fish to man.

Induction Evaluated with Antibody and cDNA Probes

The results described above indicate that the cross-reactive antibodies to the teleost P-450, and the cDNA probe, may be used for analysis of P-450 in many vertebrate species. Induction of cytochrome P-450 IA forms in fish can thus be evaluated by analysis of specific mixed function oxidase catalytic activity (e.g., EROD activity) protien detected immunochemically, and mRNA detected with cDNA probes.

A number of studies have analyzed the induction response in fish in order to define the characteristics of induction and to validate and compare the different methods for detecting induction in various species. Detailed studies have now been accomplished in several fish species including rainbow trout, scup, and the killifish (*Fundulus heteroclitus*). Figure 2 presents results of such a study in scxup. Increases in P-450 protein and P-450 mRNA are readily apparent within 1 to 2 days after a single treatment with β-naphathoflanone (BNF).

*The nomenclature of teleost P-450s is not established. Based on catalytic, regulatory, immunological, and sequence properties, scup P-450E and trout P-450LM$_4$ are considered to be teleost representatives of P-450 IA1 (*27*). PAH-inducible P-450 in other fish species are considered to be in the P-450 IA subfamily, but cannot be identified as P-450 IAa without further characterization. We refer to these as P-450 IA proteins, or as "P-450E" counterparts when anti-P-450E was used in their analysis.

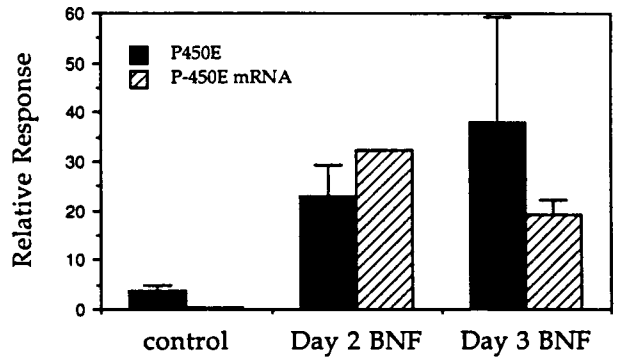

FIGURE 2. Induction of cytochrome P-450E protein and mRNA in scup. Scup were treated IP at day 0 with 20 mg/kg BNF in corn oil, and microsomes and RNA fractions were prepared and analyzed as described elsewhere (*32*).

More detailed studies in killifish and rainbow trout showed that the induction of P-450 protein followed the induction of P-450 mRNA with a considerable lag time of 12 hr or more (*32,33*). The lag times in induction of protein levels are greater than in mammalian systems. Yet, the results are consistent with transcriptional regulation of the initial stages of P-450 IA1 induction in fish, as it is in mammals. Changes in the levels of EROD activity parallel almost exactly the changes in P-450 IA protein (*32*), consistent with the identity of this protein as the EROD catalyst.

The studies in killifish have also revealed that P-450 IA levels induced by PAH-type inducer BNF remain high long after the levels of mRNA have returned to control values (*32*). The mechanism(s) for maintaining these enzyme levels is unknown. Studies in scup have indicated that elevated mRNA levels also persist in fish treated with PCBs (*35*). A distinction between mRNA persistence in hydrocarbon (BNF) and PCB-treated fish may be related to the slower metabolism and elimination of the chlorobiphenyl inducer, which could continue to stimulate mRNA synthesis. Assessing induction by use of catalytic activity, antibodies, and cDNA probes may yield different results depending on the nature of the inducing compound and how long after exposure or treatment the activity is measured.

Environmental Induction and Monitoring

Monitors or early warning sentinels to identify and define areas of contamination could be extremely important in analysis of groundwater aquifers, surface water lakes, reservoirs, rivers, and oceanic systems. Many of the indices proposed years ago are begining to yield fruit. More than a decade ago techniques were proposed that analyzed pollutants or their metabolites in fish bile (*36*). More recent studies have supported the idea of using fish bile analysis as a direct measure of contaminant exposure (*37*) and the relative degree of chemical contamination of aquatic systems.

Monooxygenase (P-450) induction has also long been suggested to indicate the exposure of organisms to contaminants in the environment (*38,39*). Earlier studies on environmental induction of cytochrome P-450 emphasized the analysis of catalytic activity. More recently, antibodies to the PAH-inducible cytochrome P-450 from fish have been used to demonstrate unambiguously that P-450 IA forms are elevated in fish from contaminated regions. These more recent studies also sought to relate the degree of induction to the levels of suspected inducing agents in the organisms themselves.

Several recent studies with different fish species and in different parts of the world have revealed correlations between the levels of induced cytochrome P-450 and levels of PCBs either in the organisms or in their immediate environment. Studies in the flounder *Platichthys flesus* from Langsundsgfjord, Norway (*40*), in starry flounder (*Platichthys stellatus*) from San Francisco Bay (Stegeman et al., unpublished observations), and in rattail' (*Coryphaenoides armatus*) from the deep ocean (*41*) have all shown close correlation between the levels of induction of "P-450E" in liver microsomes and the levels of total PCB residues (Table 5). In the two flounder species, the relationship between P-450E and PCB content in fish from 4 to 5 sites was 0.992 (for *P. flesus*) and 0.996 (*P. stellatus*). Results in lake trout larvae have also correlated mixed-function oxidase induction with PCB content (*42*). Other fish studies have shown that levels of liver microsomal cytochrome P-450E also correlate with contamination by PAH (*43,44*). The growing number of such studies provides a consistent picture, confirming the idea that the levels

Table 5. Selected field studies of P-450 induction.

Location and species	Sites	Individuals per site	"P-450E"[a] associated with		Relationship	Reference
San Francisco Bay, *P. stellatus* (starry flounder)	5	12–24	PCB (liver)[b]		$r^2 = 0.996$	(*41*)
Langesundfjord, *P. flesus* (flounder)	4	10–12	PCB (bioavailable)		$r^2 = 0.992$	(*40*)
Northwest Atlantic, *C. armatus* (rattail)	2	4–8	PCB (liver)	7:1[c] 7:1	P-450E residues	(*42*)
Narragansett Bay, *F. heteroclitus*	2	16	PCB; PAH (whole body)	3.5:1[c] 3.0:1	P-450E residues	(*44*)

[a]"P-450E" refers to P-445E counterpart detected in liver microsomes.
[b]Parentheses indicate where contaminant residues were analyzed. Bioavailable refers to residues in bivalve mollusks at these sites.
[c]Ratio of polluted site to reference site.

of a specific cytochrome P-450 protein can reflect the levels of contaminants in the environment and/or in the organisms themselves.

P-450 induction does not always correlate with the presence of neoplasms. Fish from populations afflicted with liver neoplasms, such as winter flounder from Boston Harbor, have been analyzed for induction of cytochrome P-450. Levels of cytochrome "P-450" in these flounder were no greater than in winter flounder from other regions (*45*) where neoplastic disease is believed absent. Furthermore, fish with tumors can have lower levels of monooxygenase activity (P-450) than fish from the same sites but which lack tumors (*46*).

There is no *a priori* reason to expect that animals with tumors would have higher liver "P-450E" content than animals without tumors. The carcinogen metabolism leading to initiation of carcinogenesis would be expected to occur months or years in advance of the end-stage disease. One might actually predict that livers with advanced disease would have less P-450 activity. In some mammals, neoplastic and preneoplastic cells in diseased organs have diminished capacity for metabolizing foreign chemicals (*47*). Studies using immunohistochemistry to analyze winter flounder from Boston Harbor have shown abnormal cells (abnormally vacuolated cells and basophilic cells) in livers of diseased flounder have reduced levels of cytochrome "P-450E" (*48*). Similar observations have been obtained with experimental tumors in trout (*49*). Regardless of correlation between the levels of induced cytochrome P-450 and the presence of neoplastic disease, there is strong evidence that levels of induced of P-450 in fish correlate with levels of aromatic and chlorinated hydrocarbons in the environment.

Monooxygenases and Carcinogen Metabolism in Invertebrates

The properties of microsomal enzyme systems, including the rates and patterns of xenobiotic metabolism, in marine invertebrates have been detailed in a number of recent reviews (*16,50–52*). There are some features of microsomal electron transport components and monooxygenase activity that have been seen consistently in mollusks and crustaceans.

First, the levels of cytochrome P-450 in mollusks and crustaceans are comparable to those seen in some untreated fish (although as discussed earlier, the levels of cytochrome P-450 in fish vary markedly with exposure to inducers). Second, molluscan and crustacean microsomal enzymes also transform a diverse suite of foreign chemicals, including aromatic hydrocarbons. Third, the rates of hydrocarbon metabolism detected *in vitro* in these invertebrate systems are usually lower (1 to 2 orders of magnitude) than those seen in most teleost fish liver preparations. Studies have repeatedly found low rates of PAH metabolism in bivalve mollusks. However, difficulties involved in the preparation of catalytically competent microsomes from some crustacean tissues (*51*) complicate interpretations of their relative rates of *in vitro* hydrocarbon metabolism. Nevertheless, data obtained using conditions that circumvent the presence of endogenous inhibitors and/or the possibly low rates of NADPH-cytochrome P-450 reductase (*51*) indicate that the potential rates of PAH metabolism in crustaceans fall between those in molluscan and fish groups.

The patterns of PAH metabolism are also different between some invertebrates and vertebrates. Metabolite profiles for BaP have been obtained for several mollusk species, particularly the mussel *Mytilus edulis*. Microsomal preparations from *M. edulis* mainly form quinone derivatives of BaP *in vitro* (*53,54*). Fish, on the other hand, form the hydroxylated derivatives described above.

The different patterns of metabolites probably reflect different mechanisms acting in PAH transformation in mollusks and fish. Fish rely predominantly on cytochrome P-450 acting in epoxide formation, while PAH metabolism in mussels has been suggested to occur by radical oxidation, possibly involving oxygen radicals (*53,55*). Cytochrome P-450 could participate in such metabolism, but the catalysts involved in formation of BaP-quinones by mollusks have yet to be identified.

Variable amounts of other *in vitro* BaP metabolites made by mollusks have been identified. They include dihydrodiols at the 7,8- and the 9,10-positions (*53,54*). These products are presumably formed by some action of cytochrome P-450. Their rates of formation appear to be extremely low in *M. edulis*, further indicating a minor involvement of cytochrome P-450 in metabolism of aromatic hydrocarbons. However, there are reports of high percentages of BaP dihydrodiol formed by some hydrocarbon-treated mollusks, a phenomenon that merits further study (*56*).

Crustacean cytochrome P-450 fractions form a suite of BaP products, including benzo-ring dihydrodiols, quinones, and phenolic derivatives (*51*). The formation rates may be artificial, since the P-450 preparations were fortified with reductase or involved hydroperoxides. The patterns nonetheless indicate the potential for crustacean P-450 to be involved in activation of such compounds.

Mollusks metabolize PAH quite slowly, but they transform some other types of foreign compounds, notably some aromatic amines, more efficiently. Activation of promutagenic compounds such as acetylaminofluorene by molluscan enzyme preparations provided the first evidence for aromatic amine metabolism in this group (*57,58*). In mammalian systems, some aromatic amines are activated by flavoprotein monooxygenases as well as by cytochrome P-450 (*14*). Studies have now shown that flavoprotein monooxygenase systems are present in mollusks and that catalytic rates with some substrates are relatively high.

Involvement of procarcinogen activation in invertebrate diseases has not yet been shown. Since mollusks can only minimally metabolize BaP to benzo-ring derivatives, activation of aromatic hydrocarbons by diol-epoxide pathways is probably insignificant. BaP quinones could exert some mutagenic activity (*60*). In addition, alternate pathways possibly involving peroxidase activity might activate PAHs to diolepoxides (*61*). But it is not yet known whether these pathways of PAH metabolism operate in mollusks *in vivo*.

Compounds other than PAHs including aromatic amines, could be involved in disease processes in mollusks. As stated earlier, several investigators have reported that molluscan tissue preparations can activate aromatic amines to mutagenic derivatives (*57,58*). Furthermore, DNA adducts of some of these compounds have been detected in mollusks (*62*). Although epidemiological evidence might support a relationship between environmental levels of contaminant residues and the appearance

of proliferative lesions in mollusks (*63*). But possible underlying mechanisms are not known.

Mollusks are proven, useful indicators of bioavailable levels of contamination, involving direct analysis of pollutant residues, due to the low activity of their metabolic systems. But based on our present knowledge, there is little potential for using monooxygenase activity or cytochrome P-450 levels in mollusks, or crustaceans, to indicate their exposure to compounds such as the aromatic and chlorinated hydrocarbons. This is due to the lack of any convincing evidence for induction of specific cytochrome P-450 isozymes or of monooxygenase activity, by any of the hydrocarbon inducers known to be active in the vertebrates. Cytochrome P-450 forms have been partially purified from crustaceans (*64,65*), but the relationship of these crustacean cytochrome P-450s to those in fish or mammals is unknown. The presence of any cytochrome P-450 related to PAH-inducible vertebrate proteins is also unknown in mollusks. DNA and RNA related to the clofibrate-inducible mammalian P-450 IVA1 form have been identified in mussels (*52*), but the function and possible regulation of this invertebrate P-450 are unknown.

Consequences of Cytochrome P-450 Induction

The rates and pathways of PAH metabolism in fish and invertebrates can have an impact on the organisms themselves and could be important for the consumers of these organisms, who may be ingesting carcinogens as well. The first impact derives from the fact that foreign compound metabolism can determine carcinogen activation. These same processes can determine the identity and levels of parent compound and of metabolite residues in these organisms. Induction of P-450 can influence both aspects.

As detailed above, activation of many procarcinogens requires the function of cytochrome P-450. A cell or organ devoid of the requisite catalyst will not transform a compound into a carcinogen. Evidence indicates that cytochrome P-450IA proteins catalyze PAH activation in fish and that this protein is synthesized primarily and possibly solely in response to the presence of exogenous inducers. Some degree of P-450IA induction may therefore be a prerequisite for the activation of procarcinogenic hydrocarbons in the environment.

Carcinogenic compounds such as PAH that are active inducers are the prominent compounds in some regions. Greater P-450 induction could contribute to a higher steady-state level of activated carcinogens and consequently to a higher degree of persistent and relevant DNA adduct formation or to enhanced oxidative DNA damage. Greater induction could therefore enhance the initial steps involved in carcinogenesis. It is noteworthy that there is a correlation between induction and carcinogenesis in mammals (*66*), but highly induced P-450 levels are not necessarily associated with a greater risk of carcinogenesis. Formation and persistence of critical genetic lesions may be influenced as much by detoxication or repair processes as by the oxidative metabolism creating the activated carcinogenic derivative. In addition, carcinogenesis is a multistage process, including chemical carcinogenesis. Processes subsequent to initiation and neoplastic transformation of a cell can determine the survival or further selection of that cell type leading to cancer. A variety of nongenotoxic carcinogens (promoters) could enhance these processes.

Significance to Consumer

The risk associated with consuming fish from contaminated environments will depend largely on the type and amount of those compounds accumulated ftom the environment. Rates of xenobiotic metabolism can affect the identity and levels of carcinogenic and noncarcinogenic compounds remaining in the animals. Induced levels of P-450 in fish can also alert us to the presence of such contaminants. Certainly, the correlations noted above indicate that the levels of "P-450E" can be closely related to levels of some foreign chemical residues in fish. But interpretations regarding residues still present in the fish could differ for PAHs as opposed to PCBs and other chlorinated hydrocarbons.

PAH Carcinogens

In environments where there are carcinogenic PAHs induction could indicate exposure to those compounds. Induction does not require that the unmetabolized parent compounds would still be at high levels in the tissues of fish showing high induction. Studies carried out more than 10 years ago clearly showed that animals with active hydrocarbon metabolism accumulate lower levels of parent PAH (*67*). The higher rates of metabolism associated with induction of the hydrocarbon metabolizing P-450 can enhance the rates of PAH elimination. The detection of high levels of hydrocarbon metabolites in the bile of fish exposed to hydrocarcons is consistent with this (*68,69*). Highly induced animals can be expected to have lower levels of PAHs in their tissues than either animals which are not induced or those that have inherently lower rates of hydrocarbon metabolizing activity.

The relative concentrations of PAHs in invertebrates and fish are in keeping with the idea that metabolic rates will influence these concentations. Numerous investigations have reported relatively high levels of PAH residues in molluscan tissues, intermediate levels of these compounds in crustaceans, and low levels in fish. This was illustrated in a study by Dunn et al. (*70*), who reported the levels of BaP in commercial samples of fish and shellfish. Table 6 shows that content of BaP is inversely related to the rates of *in vitro* BaP metabolism expected in fish and shellfish. The potential risk associated with parent PAH carcinogens in seafood would thus appear to be greater with invertebrates, which are less able to metabolize these compounds.

There could still be risk associated with consumption of fish

Table 6. Benzo(*a*)pyrene content in commerical seafoods.[a]

Species group	Number of samples	Percent below detection (< 0.1 ng/g)	Mean concentration in remainder, ng/g wet weight	Highest value detected
Mollusks[b]	34	14	5.9 ± 8.3	36
Crustaceans[c]	19	37	2.3 ± 2.3	2.9
Fish[d]	14	64	1.3 ± 0.9	2.6

[a]Data from Dunn and Fee (*71*). Commercial samples from various world markets were either fresh or packed in oil.
[b]Clams, cockles, mussels, oysters, scallops.
[c]Crabs, lobsters, prawns, shrimp.
[d]Char, cod, sole, mackerel, salmon, trout, sardines, tuna.

from highly contaminated sites. While fish may contain lesser and even undetectable amounts of parent compound, carcinogenic metabolic products of these may be present. There is evidence that metabolites of hydrocarcons are retained in fish tissues (*71*). Fish also efficiently form 7,8-dihydrodiol of BaP, a key intermediate on the pathway to formation of the carcinogenic diol-epoxide derivatives of BaP. Unfortunately, there is little information concerning the presence of compounds such as the diol derivatives of BaP, or of similar products of other PAHs, in environmentally contaminated fish tissues. Whether such compounds would occur in a state in which they would be available for accumulation by people who eat fish is also not known.

Chlorinated Hydrocarbons and Promoters

Rates of PAH metabolism can apparently influence the levels of PAH carcinogens in marine species and therefore modify the risk associated with their consumption. However, other classes of compounds that are not readily metabolized could contribute to carcinogenic risk. Some environments with relatively low levels of PAH contain high levels of PCBs, dibenzofurans, and dioxins. These latter compounds may include carcinogens (*73*), but many are known to be tumor promoters (*73*). The presence of these compounds in fish or shellfish could pose a risk of carcinogenesis for consumers, whether or not carcinogens are also present in that same component of the diet. Although the metabolism of these chlorinated compounds in fish is less well known than that of PAHs, some are metabolized much more slowly than PAHs. These slowly metabolized compounds can accumulate to appreciable levels in fish (*74*) as well as in mollusks and crustaceans. PCBs, dioxins, and dibenzofurans are also potent inducers of P-450 IA forms in fish. Induced levels of such P-450 forms can alert us to the presence of these compounds, as well as to PAHs.

In addition to compounds that induce P-450 IA, there are compounds present in many polluted environments and seafood that may be promoters or initiators of carcinogenesis in mammals, but which do not induce P-450 IA proteins in fish. Such compounds include some chlorinated pesticides and metals (Table 7). Direct-acting carcinogens (not requiring transformation) that are not P-450 inducers might also occur in aquatic resources. However, it is probable that in most environments where contamination is of concern, compounds that induce P-450 IA would co-occur with carcinogens or promoters that do not act as inducers.

The involvement of tumor promoters and direct-acting carcinogens in the development of tumors in fish in contaminated regions is an unexplored subject. As with PAHs the danger these compounds may pose to seafood consumers is also unknown. Many noncarcinogenic compounds in seafood could pose a greater risk to health than carcinogens. Induction of P-450 IA forms in tissues of fish can clearly indicate contamination by many potentially hazardous compounds. Further studies are needed to identify the specific compounds actually responsible for P-450 induction in various environments and the sensitivity of fish to these compounds. Studies are also needed on P-450 forms other than P-450 IA1 and their responses to pollutants in fish and invertebrates. Such studies will improve our ability to interpret P-450 induction as an indicator of contaminant levels and possible risk to seafood resources and consumers.

Table 7. Response of cytochrome P-450 in fish to known carcinogens and tumor promoters identified in aquatic systems.

Compound	Initiating carcinogen	Promoter	Induction of "P-450E"[a]
Benzo(*a*)pyrene	+	?	+
Benzanthracene	+	?	+
TCDD	?	+	+
PCBs			
Aroclor 1254	?	+	+
3,3',4,4'-TCB	?	?	+
2,2',4,4',5,5'-HCB	?	+	−
DDT	Weak?	+	−
Lead	−	+	−(?)

[a]Induction of "P-450E" (IA1) in fish based on reports of catalytic activity induction (*16*). Studies have not been done with lead but metals do not induce P-450 IA1 in mammals.

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