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Biotransformation and Induction: Implications for Toxicity, Bioaccumulation and Monitoring of Environmental Xenobiotics in Fish

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Biotransformation of xenobiotics in fish occurs by many of the same reactions as in mammals. These reactions have been shown to affect the bioaccumulation, persistence, residue dynamics, and toxicity of select chemicals in fish. P-450-dependent monooxygenase activity of fish can be induced by polycyclic aromatic hydrocarbons, but phenobarbital-type agents induce poorly, if at all. Fish monooxygenase activity exhibits ideal temperature compensation and sex-related variation. Induction of monooxygenase activity by polycyclic aromatic hydrocarbons can result in qualitative as well as quantitative changes in the metabolic profile of a chemical. Induction can also alter toxicity. In addition, multiple P-450 isozymes have been described for several fish species. The biotransformation products of certain chemicals have been related to specific P-450 isozymes, and the formation of these products can be influenced by induction. Exposure of fish to low levels of certain environmental contaminants has resulted in induction of specific monooxygenase activities and monitoring of such activities has been suggested as a means of identifying areas of pollutant exposure in the wild.

It has been clearly established over the past 20 years that fish possess the ability to perform a wide variety of biotransformation reactions (*1–3*). Recent studies on the biotransformation of xenobiotic chemicals in fish have been focused on the specific metabolites produced, since these metabolic reactions affect distribution, accumulation, and toxicity of chemicals (*4*).

Xenobiotic chemicals may also affect the distribution, accumulation, and toxicity of other chemicals by modifying the activity of enzymes that carry out these biotransformation processes. In particular, hepatic microsomal monooxygenase (MO) activity may be increased or decreased by inducing agents (*5*) and by inhibitors, respectively. Induction of MO activity, which can affect the biotransformation of a xenobiotic chemical both qualitatively and quantitatively, may be important environmentally because induction can be effected by ubiquitous chemicals such as polychlorinated biphenyls (PCBs) and polynuclear aromatic hydrocarbons (PAHs). In addition, the modification of MO activity itself may be a sensitive response to certain environmental pollutants and may serve as a biological monitor for exposure to certain classes of xenobiotic chemicals.

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The present report will review, by use of pertinent examples, the relationship of biotransformation and induction to the disposition and toxicity of xenobiotic chemicals. In this review we will discuss those factors influencing this interrelationship as well as the environmental significance of biotransformation and induction as related to aquatic toxicology and monitoring.

Biotransformation in Fish

Table 1 lists the biotransformation reactions that have been demonstrated *in vivo* in fish. Xenobiotic biotransformation in fish can occur via the cytochrome P-450-dependent monooxygenase system, various conjugating enzymes and enzymes which catalyze hydrolytic and reductive reactions. It is evident from these studies that fish are capable of both phase I (nonsynthetic) and phase II (synthetic) reactions. The scope of these biotransformation reactions appear to be similar to those in mammals. Although the types of biotransformation reactions are similar, differences do exist between fish and mammals in the metabolic handling of chemicals. Among these are differences in reaction rates, the relative contribution of a given pathway and the products formed. The biotransformation of aflatoxin B_1 (AFB) is a case in point. In trout, the glucuronide of aflatoxicol

Table 1. Biotransformation reactions demonstrated in fish *in vivo*.

Reaction	Compound	Species	Reference
Acetylation	Ethyl *m*-aminobenzoate	Dogfish shark	(*6*)
	Ethyl *m*-aminobenzoate	Rainbow trout	(*7*)
Glutathione conjugation	Molinate	Carp	(*8*)
	1-Chloro-2, 4-dinitrobenzene	Rainbow trout	(*9*)
Sulfate conjugation	Pentachlorophenol	Goldfish	(*10*)
Taurine conjugation	2,4-D	Flounder	(*11*)
Glucuronide conjugation	3-Trifluoromethyl-4-nitrophenol	Rainbow trout	(*12*)
	Pentachlorophenol	Rainbow trout	(*13*)
	Aflatoxin B_1	Rainbow trout	(*14*)
Glycine, glucuronide conjugation	Aminobenzoic acid	Flounder, Goosefish	(*15*)
Hydrolysis	2,4-D esters	Catfish, bluegill	(*16*)
	Diethylhexylphthalate	Rainbow trout	(*17*)
	Malathion	Pinfish	(*18*)
N-Dealkylation	Dinitramine	Carp	(*19*)
O-Dealkylation	*p*-Nitrophenylethers	Fathead minnow	(*20*)
	Pentachloroanisole	Rainbow trout	(*13*)
	Fenitrothion	Rainbow trout	(*21*)
Oxidation	Naphthalene, benzo(a)pyrene	Mudsucker, sculpin	(*22*)
	Naphthalene	Coho salmon	(*23*)
	Methylnaphthalene	Rainbow trout	(*24*)
	Rotenone	Carp	(*25*)
	Rotenone	Bluegill	(*26*)
	4-(2,4-DB)	Bluegill	(*27*)
	Aldrin, dieldrin	Mosquito fish	(*28*)

was the major aflatoxin conjugate in bile while the glutathione conjugate of aflatoxin was only a minor biotransformation product (*14*). In contrast, the aflatoxin glutathione conjugate was found to be the major biliary metabolite in the rat (*29*). Interspecies differences have also been noted for the unconjugated metabolites of AFB (*30–33*). These differences may be related to the extreme sensitivity of rainbow trout to aflatoxin-induced carcinogenesis. Also, differences in the metabolism of 2-methylnaphthalene (2-MeN) have been demonstrated between rats and rainbow trout (*34,35*). The *in vivo* methyl group oxidation of 2-MeN was an important route of biotransformation only in the rat while formation of dihydrodiols was of importance in both organisms. These examples can be extended to the biotransformation of steroids. Androstenedione has been shown to be hydroxylated by trout microsomes primarily in the 6β position, while the rat microsomes yield hydroxylation in the 6β, 16α, and 7α positions (*36,37*). Species differences were also evident in the relative contribution of a given benzo[α]pyrene (BαP) biotransformation pathway (*38–40*). These studies have indicated that a greater proportion of BαP was converted to BαP-7,8-diol by liver microsomes of the sole and flounder than by rat microsomes.

Although considerable variation exists between aquatic species with regard to basal MO enzyme activities and cytochrome(s) P-450 content (*1*), few studies have comprehensively addressed differences in xenobiotic biotransformation patterns among fish species. A review of biotransformation of BαP in fish shows that fish species can differ substantially in the extent of metabolism at various positions on the molecule (*41*). As a generalization, microsomes from teleost fish (*42*) *in vitro* produce more ring diols than those from species such as the little skate, which forms more phenolic metabolites (*43*).

Large variations in biotransformation pathways between individual fish of the same species have also been demonstrated (*44*). Results of these studies, involving biotransformation in feral scup, indicated that the variation in metabolite profiles was correlated with the epoxide hydrolase activity required for dihydrodiol formation. The authors suggest that environmental factors were influencing epoxide hydrolase, thus causing variation in the metabolite profile.

Although fish and mammals clearly possess many of the same mechanisms for biotransformation, differences do exist in the utilization of these pathways for the biotransformation of a number of compounds. Differences between fish species in biotransformation also appear to exist. It is not yet clear which metabolic differences in certain species are inherent and which are a result of factors derived from the previous life history of the animals. The noted differences in biotransformation, however, may form the basis for differential sensitivities of various species to xenobiotics.

Induction in Fish

Research focusing on the inducibility of biotransformation enzymes in fish has accelerated rapidly in recent years. Studies utilizing prototype substrates have demonstrated the inducibility of fish monooxygenase enzymes in many species (Table 2). The evidence for induction in fish has come largely from experiments with inducers of the PAH P_1-450 type (i.e., BαP, 3-methylcholanthrene [3-MC]). In addition, rainbow trout have been shown to respond to the inducer isosafrole (*55*), while variable results are obtained with novel inducer pregnenolone-16α-carbonitrile (*53,68,69*).

In contrast to PAH induction, the evidence for phenobarbital (PB)-type induction in fish is ambiguous. Although some workers have demonstrated induction in fish with prototype substrates following treatment with PB-type inducers, i.e., dichlorodiphenyltrichloroethane (DDT), noncoplanar PCBs or PBBs, and phenylbutazone (Table 3), the majority of studies have indicated a general lack of response to PB-type induction (Table 4). The factors responsible for these apparently disparate results have not yet been resolved, but the apparent refractile nature of fish to PB-type induction is probably not due to a lack of bioavailability or to inhibition of monooxygenation by the inducers (*55*).

It is of interest to note that for contrasting studies

Table 2. Induction of monooxygenase activity in fish.

Species	Tissue	Inducer[a]	Reference
Brook trout	Liver	PCB, 3-MC	(*45*)
Brown trout	Liver	Petroleum	(*46*)
Coho salmon	Liver	PCB	(*47*)
Rainbow trout	Liver	PCBs	(*48–50*)
		PBBs	(*51*)
		3-MC	(*52–54*)
		PCN	(*53*)
		DMBA, βNF	(*52*)
		TCDD, ISOS	(*55*)
Northern pike	Liver	3-MC	(*56*)
Walleye	Liver	βNF	b
Catfish	Liver	PCB	(*57*)
Goldfish	Liver	βNF	(*58*)
Carp	Liver	PCB	(*49*)
		PB, AFB, crude oil	(*59*)
	Liver, kidney	PCB, βNF	(*60*)
Flounder	Liver	3-MC	(*61*)
Sculpin	Liver	Refinery effluent	(*62*)
Scup	Liver	3-MC, βNF	(*54, 63*)
Sheepshead	Liver	3-MC	(*64*)
		PCBs	(*65*)
Killifish		PCBs, #2 fuel oil	(*66*)
Mullet	Liver	PCB	(*67*)
Croaker	Liver	3-MC	(*54*)

[a] PCBs, polychlorinated biphenyls; PBBs, polybrominated biphenyls; 3-MC, 3-methylcholanthrene; PCN, pregnenolone-16α-carbonitrile; DMBA, dimethylbenzanthracene; βNF, β-naphthoflavone; TCDD, tetrachlorodibenzodioxin; ISOS, isosafrole; BP, benzo(a)-pyrene; AFB, aflatoxin.

[b] Melancon et al., unpublished data.

which grossly lend themselves to comparison [for 2,4,5-2′,4′,5′-HCB (*55,72*) and dieldrin (*71,76*), PB-type induction was demonstrated with dosages lower than those utilized in studies lacking an inductive response. However, the lower dosage of the inducer has not proven to be a significant factor in a subsequent study with rainbow trout exposed 10 days to 1 mg/L of PB (*78*). Alternatively, it has been suggested that contamination of the inducer with PAH-type agents and/or lack of sufficient substrate specificity may be involved in the observed induction by PB-type agents in certain studies (*65*).

Recent studies have examined the incorporation of [^{35}S]-methionine into hepatic microsomal proteins of rainbow trout following *in vivo* exposure to various types of inducers (*78,79*). As shown in Figure 1, increased methionine incorporation provided evidence of *de novo* synthesis in conjunction with elevated catalytic activity following PAH-type inducers. Thus, monooxygenase induction, at least in part, is due to induction of new enzymes rather than activation of existing proteins. In contrast, these trout did not respond to PB-type induction at the translational level (Fig. 1) or at the catalytic level (*78*).

Recent successes in the purification of cytochrome P-450 from scup (*80*) and rainbow trout (*81,82*) have confirmed earlier data, suggesting the existence of multiple enzyme forms in fish. The major P-450 isozymes purified from βNF-induced rainbow trout and untreated scup were labeled LM_{4b} and P-450_E, respectively. Both forms were active towards BαP, and they exhibited immunochemical cross reactivity. In addition, another P-450, LM_2, has been identified in rainbow trout. Immunochemical analysis has indicated that trout LM_2 P-450 is not inducible following exposure to PAH agents (βNF, 3,4,5,3′,4′,5′-HCB, Aroclor 1254) or PB-type agents (PB, 2,4,5,2′,4′,5′-HCB; *78,83*). In contrast, LM_4 content has been shown to increase with PAH-type exposure (Fig. 2). Although constitutive trout P-450 (LM_2) does not appear to be inducible, it has been

Table 3. Phenobarbital-type induction in fish.

Species	Compound	Dose	Route	Effects: Type[a]	Effects: Change[b]	Reference
Mummichog	Phenylbutazone	10 ppb	Waterborne/static,	P-450	+	(*70*)
		100 ppb	10 days	Aldrin epoxidation	+	
Mummichog	Dichlorodiphenyl-trichloroethane (DDT)	0.1 ppm	Waterborne-transferred to DDT-free water for 4 days, then sacrificed	BEND	*	(*61*)
				Benzpyrene hydroxylase	*	
				ECOD	+	
Rainbow trout	Dichlorodiphenyl-trichloroethane	0.25 mg/100 g every 2 days	Diet, 36 days	APD	+	(*71*)
				Aniline hydroxylase	*	
Rainbow trout	Dieldrin	0.25 mg/100 g every 2 days	Diet, 36 days	APD	*	
				Aniline hydroxylase	+	
Rainbow trout	2,4,5,2′,4′,5′-Hexachlorobiphenyl	10 mg/kg	IP, sacrificed after 14 days	P-450	+	(*72*)
				PNA-*O*-demethylase	+	
				NADPH cytochrome *c* reductase	*	
Carp	Phenobarbital	75 mg/kg	IP 3 to 7 days	*N*-Demethylation of *p*-chloro-*N*-methylaniline at 7°C	+	(*73*)

[a] BEND, benzphetamine-*N*-demethylase; ECOD, ethoxycoumarin-*O*-deethylase; APD, aminopyrine-Na, demethylase; PNA, *p*-nitroanisole.
+ = significant induction; − = decline in activity; * = no change in activity.

Table 4. Studies that have not demonstrated phenobarbital-type induction in fish.

Species	Compound	Dose	Route	Effects: Type[a]	Effects: Change[b]	Reference
Brook trout	Dichlorodiphenyl-trichloroethane (*p, p'*-DDT)	15 mg/mL	Oral, 3 × weekly 10 doses	Aldrin epoxidase	*	(*74*)
				ECOD	*	
				P-450	*	
				Aniline hydroxylase	*	
	Dichlorodiphenyl-dichloroethylene (*p,p'*-DDE)	16.7 mg/mL	Oral, 3 × weekly 12 doses	Aldrin epoxidase	*	
				ECOD	*	
				P-450	*	
				Aniline hydroxylase	*	
Rainbow trout	Kepone[c]	5 mg/kg	IP, sacrificed after 5 days	BEND	*	(*55*)
				ECOD	–	
				EROD	–	
				P-450	–	
	Mirex[c]	25 mg/kg 40 mg/kg	IP, sacrificed after 5 days	BEND	–	
				ECOD	*	
				EROD	*	
				P-450	*	
	2,4,5,2',4',5'-Hexachlorobiphenyl	150 mg/kg	IP, sacrificed after 5 days	BEND	*	
				ECOD	–	
				EROD	–	
				P-450	–	
					–	
Rainbow trout	Phenobarbital	65 mg/kg	IP, sacrificed after 72 hr	BEND	*	(*50*)
				ECOD	*	
				EROD	*	
				EMD	*	
Rainbow trout	Phenobarbital	500 ppm	Oral diet for 3 weeks	BEND	*	(*75*)
				ECOD	*	
				EROD	*	
				Cytochrome B_5	+	
				NADPH-cytochrome c reductase	*	
Rainbow trout	Dichlorodiphenyl-trichloroethane	10 or 25 mg/100 g diet	Oral, assayed at 1, 2, 4 weeks	Aniline hydroxylation	*	(*76*)
				APD	*	
				Acetanilide hydroxylation	*	
				Phenacetin dealkylation		
	Dichlorodiphenyl-trichloroethane	50 mg/kg daily	IP, sacrificed at day 5	Aniline hydroxylation	*	
				APD	*	
				Acetanilide hydroxylation	*	
				Phenacetin dealkylation		
Rainbow trout	Phenobarbital	50 mg/kg daily	Oral, sacrificed at day 5	Aniline hydroxylation	*	
				APD	*	
				Acetanilide hydroxylation	*	
				Phenacetin dealkylation		
	Phenylbutazone	50 mg/kg daily	Oral, sacrificed at day 5	Aniline hydroxylation	*	
				APD	*	
				Acetanilide hydroxylation	*	
				Phenacetin dealkylation		
Rainbow trout	Phenobarbital	80 mg/kg daily	IP, 7 days	P-450	*	(*68*)
				Androstenedione hydroxylation	*	
				Androstenedione hydroxylation	*	
Rainbow trout	Phenobarbital	80 mg/kg daily for 5 days	IP, sacrificed at day 14	P-450	*	(*53*)
				PNA-*O*-demethylase	*	
				Benzo(a)pyrene	*	
Carp	2,4,2',4'-Tetrachlorobiphenyl	100 mg/kg	IP, sacrificed after 10 days	ECOD	–	(*60*)
				EROD	*	
				BEND	*	
				P-450	*	
Northern pike	Phenobarbital	20 mg/kg daily	IP, 3 days	P-450	*	(*77*)
				NADPH cytochrome c reductase	*	
				Benzo(a)pyrene	*	
				Epoxide hydaolase	*	

Table continues next page.

Table 4. *Continued*

Species	Compound	Dose	Route	Effects: Type[a]	Change[b]	Reference
Sheepshead	2,4,5,2′,4′,5′-Hexabromobiphenyl	20 mg/kg	IP, sacrificed on day 17	P-450	*	(65)
				NADPH cytochrome c reductase	*	
				AHH	*	
				BEND	*P	
				ECOD	*	
				EROD	*	
	Phenobarbital	100 mg/kg days 1, 4, 7	IP, sacrificed day 10 or 17	P-450	*	
				NADPH cytochrome c reductase	*	
				AHH	*	
				BEND	*	
				ECOD	*	
				EROD	*	

[a] AHH, aryl hydrocarbon hydroxylase; BEND, benzphetamine-*N*-demethylase; ECOD, 7-ethoxycoumarin-*O*-deethylase; EROD, 7-ethoxyresorufin-*O*-deethylase; APD, aminopyrine-*N*-demethylase; PNA, *p*-nitroanisole.
[b] + = Significant induction; − = Decline in activity; * = No change in activity.
[c] Novel inducers.

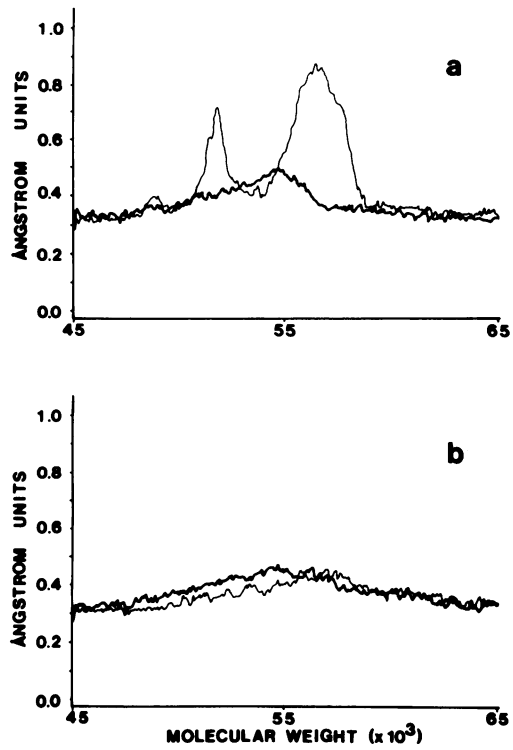

FIGURE 1. Effect of inducer pretreatment (βNF – 100 mg/kg, PB – 75 mg/kg for three daily doses) upon *in vivo* [^{35}S]-methionine incorporation into hepatic microsomal protein of rainbow trout. Laser densitometric scans of fluorgraphs resultant from SDS-PAGE of microsomal protein: (a) ——— corn oil control; ▬▬ βNF; (*b*) ——— saline control; ▬▬ phenobarbital. Adapted from Kleinow et al. (*78*).

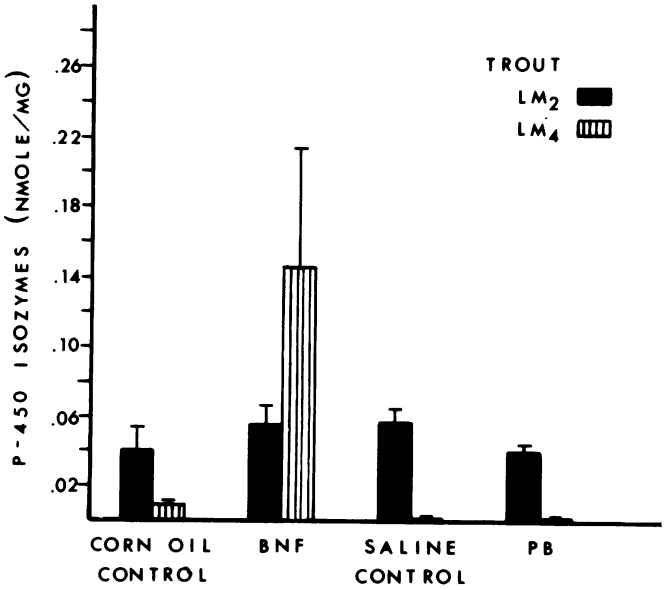

FIGURE 2. Hepatic microsomal P-450 isozyme content in rainbow trout following intraperitoneal administration of either 100 mg/kg β-naphthoflavone (βNF) or a series of three 75 mg/kg phenobarbital (PB) injections at 0, 24, and 48 hr. The animals were sacrificed 66 and 90 hr following the initial inducer pretreatment for βNF and PB, respectively. Adapted from Kleinow et al. (*78*).

shown to be effective in activating AFB to the carcinogenic AFB, 2,3-epoxide (*84*) and is active toward lauric acid (*85*). Trout LM_2 is similar to P-450 from PB-treated rats with respect to the activation of AFB to the epoxide (*86*).

Induction of aryl hydrocarbon hydroxylase activity in mammals has been shown to be regulated by the *Ah* locus and mediated by the cytosolic *Ah* receptor (*87*). Subsequent studies with rainbow trout, Atlantic salmon, and lake trout have not been able to identify

an analogous cytosolic receptor, despite induction with PAH agents (*88*).

Modulators of Biotransformation and Induction

Additional work is needed to further delineate and define the inductive response in fish. The significance of induction by select agents or lack of induction has not been fully realized on a mechanistic and practical basis.

Temperature

A number of physiological factors have been identified that modulate xenobiotic metabolizing enzyme activities in fish. Temperature, sex, and age all influence hepatic biotransformation enzyme activity directly and/or by affecting induction. The liver microsomal P-450-dependent MO system in fish has a lower temperature optimum than mammalian MO systems (*89*). In addition, fish hepatic monooxygenase activity responds to acclimation temperature in a compensatory manner (*71,90–92*). Fish acclimated to colder temperatures exhibit greater enzymatic activity than those acclimated to warmer temperatures. Studies in mature rainbow trout have indicated that some of these variations can be related to seasonal changes (*93*). Hepatic microsomal aryl hydrocarbon hydroxylase (AHH) and ethoxycoumarin O-deethylase (ECOD) activities were nearly identical when measured at environmental temperatures in August (20°C) and November (5°C). When these monooxygenase activities were measured at an incubation temperature of 18°C, specific and total activities were considerably higher in the fish acclimated to 5°C.

Studies examining the effects of acclimation temperature upon metabolism of BαP by liver extracts of the trout incubated at 29°C indicate that substantially more BαP was metabolized from fish acclimated at 7°C than at 16°C. Lower environmental temperature not only increased biotransformation, it also resulted in production of a larger proportion of ethyl acetate-soluble metabolites than water-soluble metabolites. It was suggested that the greater conversion of BαP by liver extracts from rainbow trout of lower temperatures was related to higher levels of polyunsaturated fatty acids in microsomal membranes (*94*).

It has been recently proposed that the composition of the phospholipid annulus surrounding the active site of membrane-bound cytochrome P-450 may determine the optimal temperature of cytochrome P-450 systems (*89*). These studies demonstrated that the optimal temperatures for both NADPH-cytochrome P-450 reductase and cytochrome P-450 in the rat and trout were 37°C and 26°C, respectively. The respective reductases had similar temperature optima when added to microsomes from the same species. However, the temperature optimum of trout reductase was changed to 37°C when it was added to rat microsomes. Similarly, the temperature optimum of rat reductase was reduced to 26°C when it was added to trout microsomes. Parallel shifts in the optimal temperature for O-deethylation of 7-ethoxycoumarin also occurred when these reductases were added to rat or trout microsomes.

Low temperature also influences the process of monooxygenase induction by increasing the time necessary to reach the maximal enzyme activity (*94–96*). It has been suggested that this prolonged response may be due to altered inducer pharmacokinetics and/or a low rate of protein synthesis (*95*). With regard to the former, reduced absorption rates for inducing agents have been described in cold acclimated fish (*96*).

Temperature has been shown to have a pronounced effect on hydrocarbon accumulation and retention in the major organs of coho salmon (*23*). This study demonstrated an inverse relationship between environmental temperature and naphthalene retention in brain, liver, kidney, and blood. Similar findings were evident for naphthalene in the flounder which demonstrated much greater muscle and liver naphthalene residues at 4°C than at 12°C (*97*). It is evident that temperature plays an important role in biotransformation, induction and ultimately persistance. Considering the effect of temperature on other processes, further work must define the effect of compositional lipid changes on biotransformation as well as the implications of acclimation temperature upon MO activity and other physiological processes.

Age and Sex

Pronounced sex differences have been noted in content of hepatic cytochrome P-450 and MO activities in a number of fish species (*93,98*). In general, male fish have higher MO activities and P-450 content than females. Stegeman et al. (*98*) have demonstrated the hepatic microsomal cytochrome P-450 levels in juvenile brook trout were depressed by administration of estradiol 17β and elevated by testosterone (*99*). It was suggested that androgens and estrogens were involved in the regulation of hepatic cytochrome P-450 in the brook trout. In addition, hormone effects on induction have been noted in fish. Estradiol benzoate pretreatment of rainbow trout has been shown to decrease the magnitude of induction with βNF (*69*). Other studies have demonstrated differential effects of inducing agents dependent upon age and sex (*53*). These studies showed that both *p*-nitroanisole-O-demethylase and benzo(a)pyrene hydroxylase activities were higher in male trout than in females following induction with 3-MC and Clophen A 50 (Cl_{50}). The total P-450 content was increased for both sexes with 3-MC, but only in females with Cl_{50}. Greater induction was observed with 3-MC and Cl_{50} in older fish.

Age and sex have also been shown to influence the inducibility of 6β-hydroxylase by 3-MC and Cl_{50} in rainbow trout (*68*). 6β-Hydroxylase activity was unaffected by the inducers in prespawning females or spawning fish of both sexes. In contrast, enzyme activity in maturing females was induced by 3-MC and Cl_{50}; in juveniles Cl_{50} also caused induction. Hormonal factors

were thought to be involved. Sexual differences have also been demonstrated in the P-450 isozyme content in some organs of the rainbow trout (*100*). Immunoquantitation indicated that the kidney of the male rainbow trout contained much higher levels of the P-450 isozyme, LM_2, than found in the juvenile or female kidney. The elevated levels of LM_2 in the kidney of the male appear to be responsible for the higher male cytochrome P-450 content and for greater hydroxylation rates of AFB, progesterone, testosterone, and lauric acid. Further studies on the influence of sex upon MO activity will undoubtedly reveal additional P-450 isozyme and hormonal interactions.

Biotransformation and Induction: Implications for Toxicity and Bioaccumulation

Although it has been known for many years that biotransformation reactions are important modulators of xenobiotic toxicity and bioaccumulation in mammals, only recently has this relationship been investigated in fish. Biotransformation produces metabolites that may have different fates and dispositions than the parent compound. Elimination may involve the parent compound or any of the biotransformation products with rate constants for metabolite elimination being greater or less than the parent compound. The extent of chemical biotransformation may appreciably affect the concentration of the chemical in the fish as well as its persistence. Biotransformation reactions, through alterations in chemical structure, may also significantly modify the toxicological properties of a chemical. These alterations may represent detoxification or lethal synthesis from the parent compound. It has become evident that along with factors influencing physiological considerations, these biologically catalyzed conversions form the basis for the mechanisms that modulate bioaccumulation, persistence, residue dynamics and toxicity of a chemical in aquatic organisms.

Data relating biotransformation to toxicity have primarily come from studies using inhibitors on specific biotransformation reactions. These studies show significant changes in toxicity of a number of compounds when biotransformation pathways such as glucuronide conjugation (*12*) and oxidation (*101*) are inhibited. Inhibition of 3-trifluoromethyl-4-nitrophenol (TFM) glucuronide formation by pretreatment with salicylamide, a glucuronyl transferase inhibitor, increases the toxicity of TFM to rainbow trout (Fig. 3). Increased toxicity was accompanied by decreased biliary elimination of the TFM glucuronide and increased levels of TFM in muscle, brain, blood and heart. Studies with an organochlorine-insecticide resistant population of mosquito fish have also shown increased tolerance to rotenone (*103*) and pyrethroids (*102*), which was attributed to increased monooxygenase activity. These conclusions arose from experiments showing that treatment of resistant fish with the MO inhibitor, sesamex, increased

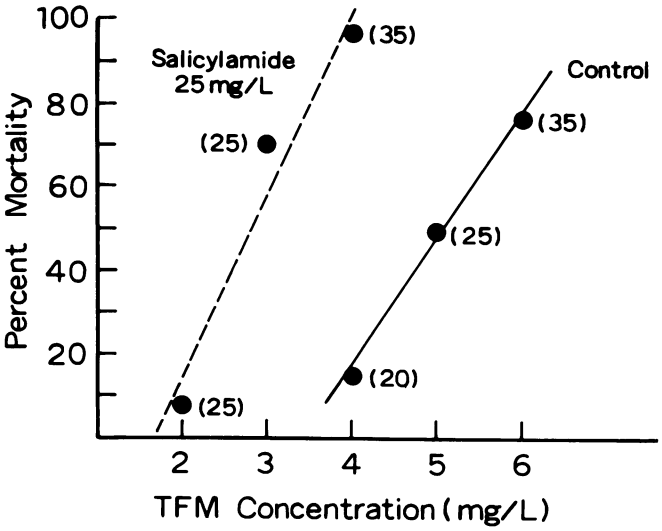

FIGURE 3. Effect of salicylamide upon the toxicity of waterborne 3-trifluoromethyl-4-nitrophenol (TFM) in rainbow trout. Adapted from Lech (*12*).

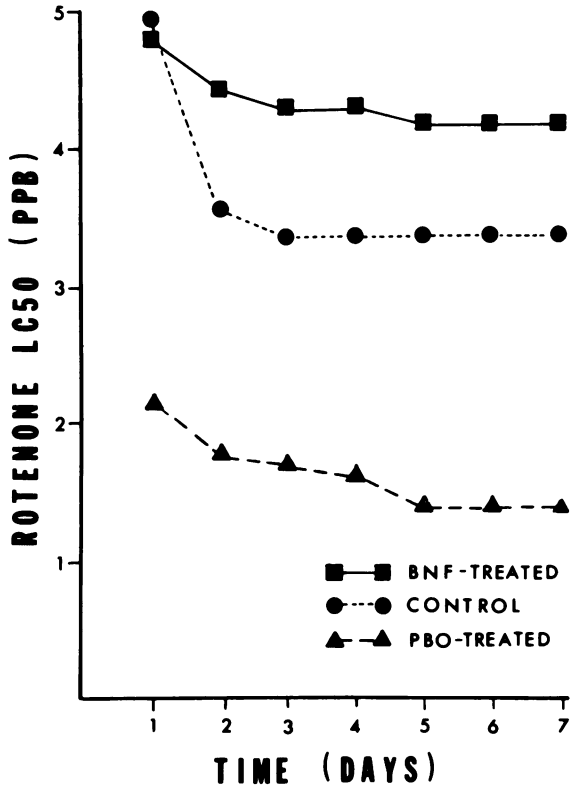

FIGURE 4. Daily LC_{50} values for rotenone in rainbow trout. Adapted from Erickson et al. (*104*).

rotenone toxicity to that found with organochlorine-susceptible fish. In addition, recent work has directly implicated the induction of biotransformation enzymes as one determinant of xenobiotic toxicity in fish (*104*). Figure 4 shows the effect of piperonyl butoxide (PBO), an

inhibitor of P-450-dependent MO activity, and βNF, a PAH-type inducer of P-450-dependent MO activity, upon the toxicity of rotenone over a 7-day exposure to juvenile rainbow trout. When compared to the controls, the 120-hr LC_{50} of rotenone was decreased approximately 60% by 10 ppb PBO exposure and increased 23% by 100 mg/kg βNF administration. Thus, biotransformation reactions play a role in the toxicity of rotenone and the status of the biotransformation process may be altered significantly.

Few studies have considered the effect of induction upon the metabolic profiles of xenobiotic chemicals in fish. One such study has demonstrated alterations in the metabolic profile of aflatoxin in rainbow trout with *in vivo* βNF exposure (*14*). These studies indicate that aflatoxin-M_1 (AFL-M_1) and aflatoxicol (AFL) are the major biliary glucuronides from βNF-pretreated and control trout, respectively. Similarly, changes in the metabolic profile of BαP have been shown to occur with the overall increase of biotransformation following induction. *In vivo* induction of little skate enzymes with 1,2,3,4-dibenzanthracene, for example, resulted in hepatic microsomes that produced greater amounts of BαP quinones and lesser amounts of phenols when compared to the controls (*43*). Changes in BαP metabolic profiles after induction were also noted in coho salmon and starry flounder (*105*). In these species, induction increased the relative proportion of BαP 9,10-diol while decreasing the 7,8-diol.

Only recently has the influence of induction upon quantitative aspects of biotransformation been correlated to specific P-450 isozymes in fish (*106*). These studies on the biotransformation of 2-MeN indicate that *in vivo* βNF pretreatment of rainbow trout influences the relative abundance of dihydrodiol and 2-hydroxymethyl metabolites formed by isolated hepatic microsomes and cytochromes P-450 (Table 5). βNF stimulated the formation of the 5,6- and 7,8-dihydrodiols relative to the 3,4-dihydrodiol and 2-hydroxymethylnaphthalene. The ability of characterized rainbow trout cytochrome P-450 isozymes to metabolize 2-MeN varied considerably. The formation of 2-hydroxymethylnaphthalene was largely associated with the LM_2 P-450-like isozyme, while the 7,8-dihydrodiol was predominantly linked with the P_1-450-like LM_{4b} isozyme. These studies suggest that the products formed from the biotransformation of 2-MeN in the trout can be related, at least in general, to specific P-450 isozymes and their induction.

These examples serve to illustrate that the induction of biotransformation enzymes in fish may significantly alter both metabolism and toxicity of a xenobiotic chemical. The consequences of induction in each specific instance depend upon the degree of induction, the isozyme induced, the isozyme(s) responsible for metabolism of the chemical, and the nature of the metabolites formed.

Biotransformation may ultimately affect the disposition of xenobiotic chemicals in fish. Alterations in disposition are largely a result of changes in chemical and physical properties of the compound. These alterations influence not only elimination and hence bioaccumulation, but also compartmentalization within the animal. The effects of biotransformation upon disposition of di-2-ethylhexyl phthalate (DEHP) were investigated in the rainbow trout (*107*). This study indicated that piperonyl butoxide (PBO), a P-450 inhibitor, was effective in modifying the metabolism of DEHP *in vivo* by diminishing oxidation and hydrolysis. Table 6 indicates that PBO caused significant increases in tissue levels of ^{14}C-DEHP-derived label as well as a decrease in the biliary ^{14}C content. The alteration of disposition with PBO appears to be largely the result of decreased metabolite formation coupled with increased retention of the parent compound (Table 7).

Studies with the use of the inducers βNF and Aroclor 1254 have demonstrated that the extent of induction may influence the metabolism and subsequent disposition of certain xenobiotics in salmonids (*108,109*). Data presented in Table 8 illustrate the effect of Aroclor 1254 upon the distribution of 2,6-dimethylnaphthalene (DMN) in the coho salmon. With 100 mg/kg Aroclor, DMN-derived radioactivity in muscle, brain and blood

Table 5. 2-Methylnaphthalene metabolism by hepatic microsomes and cytochromes P-450 from rainbow trout.[a]

	Metabolite formed, nmole/min/nmole cytochrome			
Enzyme and source[b]	2-Hydroxymethyl metabolite	3,4-Dihydrodiol	5,6-Dihydrodiol	7,8-Dihydrodiol
Control (Mic)	0.000	0.021	0.018	0.032
βNF-fed (Mic)	0.000	0.024	0.044	0.092
LM_2 and EH	0.160	0.011	0.027	0.016
LM_{4b} and EH	0.000	0.004	0.020	0.049

[a]Adapted from Melancon et al. (*106*).
[b]LM, liver microsomes; LM_2 P-450 isozyme involved in aflatoxin B_1 metabolism; LM_{4b} (P_1-450, P-448), isozyme inducible with PAHs. EH, epoxide hydrolase.

Table 6. The *in vivo* effect of piperonyl butoxide on ^{14}C-di-2-ethylhexyl phthalate (DEHP) disposition in rainbow trout.[a]

	^{14}C concentration, as μg/g DEHP	
Tissue	Control	With piperonyl butoxide
Muscle	0.021 ± 0.003	0.041 ± 0.006*
Blood	0.142 ± 0.017	0.234 ± 0.01*
Bile	51.4 ± 5.5	26.2 ± 2.8*
Liver	0.86 ± 0.08	1.08 ± 0.15

[a]Adapted from Melancon et al. (*107*).
*Significantly different from control, $p > 0.01$.

Table 7. Effect of piperonyl butoxide *in vivo* on accumulation of di-2-ethylhexyl phthalate and mono-2-ethylhexyl phthalate (MEHP) in rainbow trout muscle.[a]

	% of total ^{14}C	
Treatment	DEHP	MEHP
Control	46.6	41.8
Piperonyl butoxide (1 ppm)	76.5	18.4

[a]Adapted from Melancon et al. (*107*).

Table 8. Effects of PCB on ^{14}C-dimethylnaphthalene levels in coho salmon tissues.[a]

	^{14}C-Dimethylnaphthalene level, DPM/mL[b]	
	0.0 mg PCB/kg body weight	100 mg PCB/kg body weight
Bile	4400 ± 1000 (15)	9100 ± 1800 (16)*
Liver	160 ± 24 (17)	170 ± 31 (18)
Brain	44 ± 8.6 (19)	29 ± 6.6 (18)*
Muscle	40 ± 9.2 (19)	24 ± 5.4 (18)*
Blood	11 ± 2.9 (16)	8.8 ± 2.0 (18)*

[a] Adapted from Collier et al. (*108*).
[b] X ± SEM (*N*).
* Significant effect due to PCB exposure.

decreased, but biliary levels of DMN metabolites increased. Table 9 illustrates similar effects of βNF upon the disposition of 2-MeN and naphthalene in rainbow trout (*109*). For these compounds, βNF induction resulted in lower ^{14}C residues in muscle and blood, while increasing biliary excretion.

Although bioconcentration of organic xenobiotics in fish is largely a function of lipophilicity, it is evident from the foregoing discussion that biotransformation can play an important role in modifying accumulation. A number of studies have demonstrated that biotransformation can effect fish bioconcentration factors (BCF) for xenobiotics (*110,111*). One such study with *Gambusia affinis* in a model ecosystem indirectly considered the effects of biotransformation upon the bioconcentration of two xenobiotics: DDT [2-bis(*p*-chlorophenyl)-1,1,1-trichloroethane] and 2-bis(*p*-methylthiophenyl)-1,1,1-trichloroethane (*110*). Although these structurally related compounds have similar lipid solubilities, BCFs were 84,500 and 5.5 for DDT and 2-bis-(*p*-methylthiophenyl)-1,1,1-trichloroethane, respectively. However, the latter compound was readily metabolized. As one might expect, such discrepancies occur when experimentally determined BCF values are compared to values predicted from water solubility alone. Another study, of bluegills exposed to an alkylbenzene compound, reported a BCF of 35 (*111*). Again, the difference between this value and the predicted BCF values of 6300 could be attributed to metabolism.

Environmental Induction

Several experimental approaches have been used to demonstrate the induction of MO activity in fish exposure to low levels of inducers present in the environment at large. Among these, the most convincing evidence of environmental induction has come from well controlled studies of changes in hepatic microsomal MO activities in fish exposed to polluted water in the laboratory or in the field. Exposure to pollutants was accomplished either by pumping contaminated water into tanks in the laboratory (*112*) or by suspending cages of fish in polluted water *in situ* (*113*). Laboratory studies maintained similar temperature, photoperoid and diet for treatment groups and controls. They provide evidence of environmental induction irrespective of other factors that may influence MO activity (*112*). Figure 5 shows the results of one such study. The data indicate an initial rapid increase in enzyme activity followed by a stabilization at a level significantly higher than controls. When the induced fish were removed to clean water, the enzyme activity dropped to near control levels in approximately 25 days. This was presumably due to the lack of continued induction and the turnover of existing enzyme.

Additional evidence of environmental induction comes from comparisons of MO activity in fish from areas classified as clean or polluted (Table 10). Most of these studies indicate elevated MO activities with exposure to polluted environments. In contrast, one study with northern pike showed a significant decline in MO activity with exposure in a polluted lake (*114*). The authors suggest that the decline was associated with hepatic injury. Similar studies have been performed with embryos and fry derived from eggs collected from fish with PCB burdens. These studies demonstrated vertical transmission of the inducer (*117*) and the subsequent induction of MO activity in early developmental stages (*118*). Hepatic AHH activities in embryos and fry from PCB-contaminated eggs were 4- to 8.6-fold higher than controls. In addition to providing evidence of environmental induction, these studies illustrate that age and pollutant transfer dynamics may significantly influence metabolism and toxicity of xenobiotics in the environment at large.

Table 9. Metabolism and disposition of ^{14}C-labeled chemicals in rainbow trout as a result of preadministration of β-naphthoflavone (βNF).[a]

		Control[b]		βNF-Treated[b]	
Chemical	Tissue	Tissue level of parent chemical metabolites, μg/g or mL	Metabolites, %	Tissue level of parent chemical metabolites, μg/g or mL	Metabolites, %
2-Methyl-naphthalene	Bile	150 ± 24	96	1233 ± 201	100
	Muscle	4.9	2	2.6	10
	Liver	10.8	10	5.0	40
	Blood	3.3 ± 0.2		1.9 ± 0.1	
Naphthalene	Bile	67.2 ± 5.1	98	308.8 ± 21.1	99
	Muscle	2.25 ± 0.23	5.1 ± 0.4	1.25 ± 0.16	12.3 ± 0.9
	Liver	2.05 ± 0.12	8.5 ± 0.5	1.72 ± 0.01	24.0 ± 1.8
	Blood	1.83 ± 0.23		0.97 ± 0.08	

[a] Adapted from Melancon and Lech (*109*).
[b] Values are means ± SE.

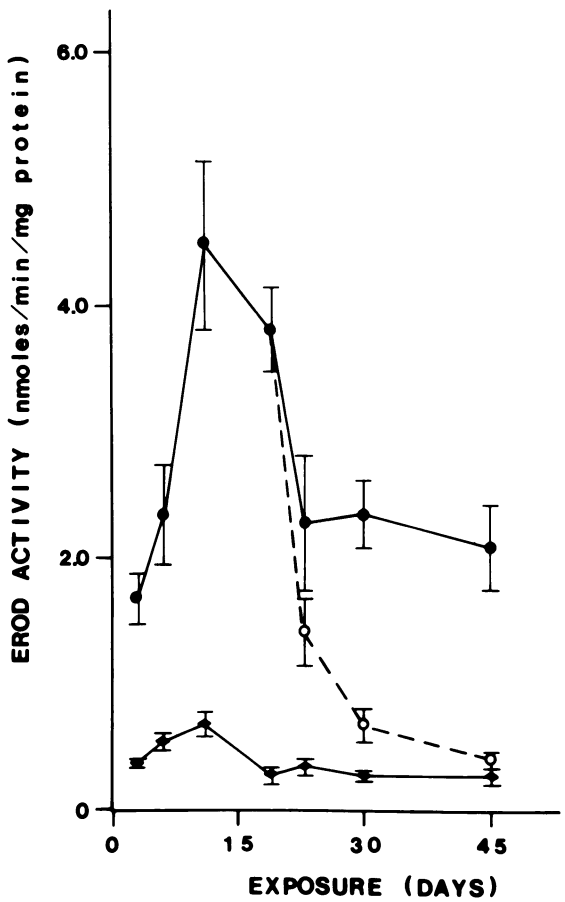

FIGURE 5. Effect of river water exposure on carp hepatic microsomal ethoxyresorufin *O*-deethylase activity: (♦) carp maintained in dechlorinated city water; (●) carp exposed to river water; (○) carp exposed to river water, then transferred to dechlorinated city water. Adapted from Melancon et al. (*112*).

Several studies have utilized the MO inhibitor α-naphthoflavone (αNF) to investigate induction in experimentally (*53,65*) and environmentally exposed animals (*54*). In some cases, αNF inhibits 3-MC-inducible MO activity, but constitutive activity is stimulated or unaffected. Winter flounder collected from a single area in New England waters exhibited wide variation in AHH and EROD activities (*119*). Those fish exhibiting low AHH activities were stimulated by αNF exposure, whereas those with elevated AHH activities were inhibited. Based on earlier studies on the specificity of αNF on hepatic MO activity in the flounder, these fish were considered "noninduced" and "induced," respectively. Discrimination of induced animals from noninduced has been demonstrated in several species, including the little skate (*64*), croaker (*54*), and chub (*113*), by using αNF. However, results with sheepshead (*65*), carp (*60,113*), rainbow trout (*50*), and lake trout (*118*) did not always conform to this pattern. Furthermore, all scup collected from the marine environment have had elevated AHH activity which was responsive to inhibition by αNF (*54*). Apparently, all of these animals were induced, or the scup constitutive P-450 isozymes were of the 3-MC-induced forms. Clearly, it cannot be routinely assumed that αNF will discriminate induced from control fish for every combination of tissue, species, and MO assay.

Induction of MO activity by low levels of chemicals in the environment has generated interest in the dose–response relationship and the minimum effective dose necessary to cause induction in fish. Several studies have demonstrated dose-dependent (MO) induction (*70,120,121*). Table 11 summarizes the minimum effective dose for induction in these studies. Although it is clear that induction can occur experimentally and environmentally at similar levels, the relationship of effects from single dose administrations to those from chronically acquired environmental body burdens is still unknown.

Biotransformation, Induction, and Monitoring

Evidence of biotransformation and induction in fish has generated interest in the use of these mechanisms to monitor xenobiotic chemicals in the environment. At present, induction as an environmental monitoring tool appears to have its greatest utility as a general indicator of contamination rather than for the identification of specific compounds. However, recent advances with immunochemical techniques in conjunction with inhibitor and protein separation methods may in the future provide the basis for definition of classes of inducers by correlations with specific P-450 isozyme induction.

Recent studies have identified various factors such as inducer response, temperature (*94*), sex, and age (*53*) which may complicate the utility of induction as a monitoring tool. For example, numerous studies have indicated that P-450-dependent MO activity in fish appears to respond largely (or solely) to 3-MC-type inducers (Table 4). If this is true, lack of MO induction would not rule out contamination by other classes of agents. Conversely, MO induction would indicate, at least grossly, the presence of a 3-MC-type or novel inducing agent in the environment. However, certain fish species do not demonstrate a response to inducers when treated with certain prototype substrates. For example, carp exposed to Aroclor 1254 and βNF did not exhibit elevations in MO activity assayed with ECOD (*60*). In contrast, AHH and EROD induction in carp and increased ECOD activities in other species (*50*) were observed. As a final caution, recent evidence has suggested that with certain agents, P-450 induction may be tissue-selective. Payne et al. (*123*) showed that flounder exposed to an oil spill exhibited induction of MO activity in kidney, but not in liver. These data suggest that before the presence of induction (or lack thereof) may be used as an indicator of environmental pollution, both substrate and tissue specificities of P-450-dependent MO activities in a given indicator species must be carefully characterized and appropriate tissues and pro-

Table 10. Hepatic monooxygenase activity in fish from different areas.

Species	Type enzyme activity[a]	Source of fish	Enxyme activity units/ unit protein[b,c]	Reference
Carp	EROD	Hatchery	0.21 ± 0.10 (15)	(*112*)
		"Polluted" area	4.72 ± 2.99 (13)	
Brown trout	AHH	"Clean" lake	26.5 ± 19.4 (8)	(*46*)
		"Polluted" lake	362 ± 51 (3)	
Fundulus	AHH	"Clean" area	89 ± 28 (64)	(*115*)
		"Clean" area	74 ± 33 (69)	
		"Polluted" area	109 ± 19 (53)	
		"Polluted" area	109 ± 32 (148)	
Fundulus	AE	"Clean" area	0.34 ± 0.07 (3)	(*70*)
		"Polluted" area	0.63 ± 0.08 (5)	
Cunner	AHH	"Clean" area	16.0 ± 7.6 (10)	(*116*)
		"Clean" area	19.9 ± 6.6 (9)	
		Refinery area	53.2 ± 25.2 (10)	
		"Polluted" area	46.6 ± 6.7 (8)	
Northern pike	AHH	"Clean" lake	18 ± 12 (6)	(*114*)
		"Polluted" lake	3 ± 2 (7)	

[a] AHH, aryl hydrocarbon hydroxylase; AE, aldrin epoxidation; EROD, ethoxyresorufin-*O*-deethylase.
[b] ± Standard deviation.
[c] Numbers in parentheses indicate number of fish.

Table 11. Hepatic microsomal monooxygenase induction by low doses of polychlorinated biphenyls and polycyclic aromatic hydrocarbons.

Inducing agent	Species	Monooxygenase activity[a]	Minimum effective dose, mg/kg	Reference
Clophen A50	Rainbow trout	PNAOD	10	(*72*)
3,4,3,4′-Tetrachlorobiphenyl	Rainbow trout	ECOD, EROD	0.01	(*121*)
Benzo(a)-pyrene	Rainbow trout	AHH	0.3	(*120*)
Aroclor 1254	Rainbow trout	ECOD, EROD	0.2	(*121*)
Aroclor 1254	Carp	EROD	0.2	(*121*)

[a] PNAOD, *p*-nitroanisole *O*-demethylase; ECOD, ethoxycoumarin-*O*-deethylase; EROD, ethoxyresorufin-*O*-deethylase; AHH, aryl hydrocarbon hydroxylase.

totype substrates selected for analysis. Finally, as indicated earlier in this review, temperature, sex, and age may profoundly affect the induction process. Thus, one must also be concerned that attenuation of the inductive response because of varying environmental and physiological factors could make it difficult to assess exposure in free ranging fish living in slightly to moderately contaminated waters.

As an alternative approach, early investigations suggested that determination of xenobiotic biotransformation products in fish bile might provide a means for monitoring waterborne chemicals (*124*). Several studies have indicated that xenobiotics present at low levels in the environment (or their metabolites) might be concentrated in fish bile to levels facilitating their analytical detection (*109,125*). Furthermore, monitoring of biliary biotransformation products permits detection of polar metabolites, which might otherwise go undetected in conventional residue monitoring studies.

In conclusion, it is clear that relationship(s) between biotransformation and enzyme induction, on the one hand, and toxicity, bioaccumulation and monitoring of xenobiotic chemicals in aquatic species, on the other hand, requires further investigation. The significance of induction to the disposition and toxicology of pollutants in the environment, the possible use of induction as an environmental monitoring tool, and the relationship of biotransformation to specific P-450 isozymes are several of the newer areas in aquatic toxicology that may further our understanding of chemicals in the aquatic environment.

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