Relationship Between Biotransformation and the Toxicity and Fate of Xenobiotic Chemicals in Fish

by John J. Lech* and John R. Bend†

Many of the biotransformation reactions which have been described for xenobiotic substances in mammals have been demonstrated in fish in both in vitro and in vivo experiments. Several of these biotransformation reactions have been shown to occur in fish at rates which are sufficient to have significant effects on the toxicity and residue dynamics of selected chemicals. Inhibition of these reactions can lead to increased toxicity and bioaccumulation factors for certain chemicals. Several classes of compounds, including some polychlorinated biphenyls, are metabolized slowly, and their disposition in fish may not be influenced to any great extent by biotransformation. Metabolites of compounds which are biotransformed rapidly may appear in certain fish tissues, and in many instances these are not accounted for by conventional residue analysis methods. Microsomal mixed-function oxidases in several species of fish have been demonstrated to be induced by specific polycyclic aromatic hydrocarbons and by exposure of fish to crude oil. Induction of these enzymes in fish can result in both qualitative and quantitative differences in the metabolic disposition of xenobiotics to which fish are exposed.

The notion that fish did not oxidize or conjugate foreign chemicals was popular among students of drug metabolism in the early 1960's. This idea persisted for quite some time, despite the fact that several investigators (1-5) had demonstrated the presence of mixed-function oxidase-like activity in livers of fish. Over the past ten years, research in this area has accelerated, and the presence and properties of mixed-function oxidases and conjugating enzymes in a variety of tissues of several species of fishes have been reported, and this has been the subject of several recent reviews (6-8). While these in vitro studies are important in themselves, investigations over the past few years have been directed toward elucidation of the functional significance of mixed function oxidases and conjugating enzymes in aquatic species.

The knowledge of the rates and pathways of biotransformation of xenobiotic chemicals in fish and other aquatic species is as germane to the discipline of aquatic toxicology as it is to mammalian toxicology. Information concerning the biotransformation, distribution, and elimination of xenobiotic chemicals in fish is pertinent to the mission of many industrial and federal research programs, whether their ultimate aim is the protection of aquatic species themselves or the human consumer of products from the aquatic environment.

Since much progress has been made over the past few years in the study of the behavior of xenobiotic chemicals in aquatic species, the purpose of this discussion is to review some of the more recent developments and show by specific examples, the relationship between biotransformation and the toxicity, persistence and bioaccumulation of xenobiotic chemicals in fish.

In Vivo Metabolism of Xenobiotics in Fish

Although it has been known for some time that various species of fish possess the enzymes involved in the biotransformation of xenobiotic chemicals,
this area of research did not enjoy the intensity of effort in the past as did its mammalian counterpart. Recent interest in the aquatic environment and in aquatic toxicology has led to investigations with \textit{in vitro} tissue preparations from several species of fish, and it is now well documented that fish possess mixed-function oxidase enzymes and conjugating enzymes, and that the former are inducible by many of the agents which induce mixed-function oxidases in mammals (9-15). While many of these enzyme systems were found to be qualitatively similar to those in mammals, the question concerning the functional relevance of these biotransformation pathways in the intact animal has only been addressed in recent years. Table 1 lists several biotransformation reactions which have been demonstrated to occur in species of fish \textit{in vivo}. This information serves to illustrate that most of the biotransformation reactions that are known to occur in mammals and which have been previously demonstrated by using \textit{in vitro} preparations from fish have now been shown to occur in the intact animal. It is difficult to assess the quantitative aspects of these biotransformation reactions from the current literature since few experiments have been designed solely to compare the rate of biotransformation of xenobiotics in fish with the rates seen in mammals. From the limited amount of information available, it appears as if oxidation, in general, proceeds more slowly than either hydrolysis or conjugation, but this interpretation must be tempered by species differences or variations in experimental conditions.

**Biotransformation and Toxicity of Chemicals to Fish**

While it is a well known concept that the rate of biotransformation of a given chemical or drug, along with its intrinsic activity as a pharmacological or toxicological agent, is one of the prime determinants of its toxic or effective dose, the importance of this concept when dealing with fish has not been fully explored. One of the approaches to this subject in mammalian toxicology has been to use inhibitors of specific biotransformation reactions and to observe the effect of this inhibition on the dose-response relationship for the pharmacological or toxicological agent under consideration.

Equation (1) is a simplified expression of the rate of change of a chemical in an exposed fish ([F]) and assumes a first-order rate constant, $k_1$, for uptake and another, $k_2$, for removal by, for example, biotransformation.

\[
\frac{d[F]}{dT} = k_1[W] - k_2[F] \tag{1}
\]

\[
[F] = \left( \frac{k_1}{k_2} \right) [W] \left( 1 - e^{-k_2t} \right) \tag{2}
\]

\[F]_{ss} = \left( \frac{k_1}{k_2} \right) [W] \tag{2a}
\]

**Table 1. Biotransformation reactions demonstrated in fish \textit{in vivo}**

| Reaction                          | Species            | Compound                        | Reference                        |
|----------------------------------|--------------------|---------------------------------|----------------------------------|
| Glycine, glucuronide conjugation | Flounder, goosefish| Aminobenzoic acid               | Huang and Collins (16)           |
| Glucuronide conjugation          | Rainbow trout      | 3-Trifluoromethyl-4 nitrophenol | Lech (17)                        |
|                                 | Rainbow trout      | Pentachlorophenoil              | Glickman et al. (18)             |
| Taurine conjugation              | Flounder           | 2,4D                            | James and Bend (19)              |
| Sulfate conjugation              | Goldfish           | Pentachlorophenoil              | Akitake and Kobayashi (20)       |
| Glutathione conjugation          | Carp               | Molinate                        | Lay and Menn (21)                |
| Hydrolysis                       | Catfish, bluegills | 2,4D-esters                     | Rodgers and Stalling (22)        |
|                                 | Rainbow trout      | Diethylhexyl phthalate          | Melancon and Lech (23)           |
|                                 | Pinfish            | Malathion                       | Cook and Moore (24)              |
| Acetylation                      | Dogfish shark      | Ethyl \textit{m}-aminobenzoate | Maren et al. (25)                |
|                                 | Rainbow trout      | Ethyl \textit{m}-aminobenzoate | Hunn et al. (26)                 |
| Oxidation                        | Mud sucker, sculpin| Naphthalene, benzo(â)pyrene     | Lee et al. (27)                  |
|                                 | Coho salmon        | Naphthalene                     | Collier et al. (28)              |
|                                 | Rainbow trout      | Methylnaphthalene               | Melancon and Lech (29)           |
|                                 | Carp               | Rotenone                        | Fukami et al. (30)               |
|                                 | Bluegills          | 4-(2,4-DB)                      | Gutenmann and Lisk (31)          |
|                                 | Mosquitofish       | Aldrin, dieldrin                | Yarbrough and Chambers (32)      |
| O-Dealkylation                   | Fathead minnow     | \textit{p}-Nitrophenylethers    | Hansen et al. (33)               |
|                                 | Rainbow trout      | Pentachloroanisole              | Glickman et al. (18)             |
|                                 | Rainbow trout      | Fenitrothion                     | Miyamoto et al. (34)             |
| N-Dealkylation                   | Carp               | Dinitramine                     | Olson et al. (35)                |

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The steady-state expression is given in Eq. (2); it is apparent that [F] is directly proportional to $k_1$ and the water concentration of the chemical [W], and inversely related to the biotransformation rate constant, $k_2$. This analysis indicates that the concentration of a chemical in an exposed fish at steady state will be determined by the concentration of the chemical in water and its rate of biotransformation by the fish. One could predict then, that perturbation of the rate of biotransformation of a given chemical should affect its LC$_{50}$, and the extent to which the LC$_{50}$ is changed is a reflection of the toxicological significance of the metabolic pathway of the chemical under study. For the purpose of illustration only, one process ($k_2[F]$) was used for elimination; however, there certainly are at least two processes which will be discussed later.

Several studies utilizing inhibitors of biotransformation have been done with fish, and the results indicate that glucuronide conjugation and sulfoxide formation in rainbow trout and mosquitofish respectively, may be rapid enough to significantly affect the toxicity of certain chemicals (36, 37). Figure 1 illustrates the effect of salicylamide, an inhibitor of glucuronide formation on the acute toxicity of 3-trifluoromethyl-4-nitrophenol (TFM) to fingerling rainbow trout. In this study salicylamide, 25 mg/L, produced no effects on the test fish alone but decreased the LC$_{50}$ of the phenol to approximately one-third of the control value (36). This same concentration of salicylamide lowered the amount of TFM glucuronide in blood of the test animals and at the same time elevated the levels of unconjugated TFM in blood (Fig. 2). Figure 3 illustrates that this concentration of salicylamide also reduced the amount of conjugated TFM which was excreted in the bile. The data in Table 2 also show that at all exposure times indicated, salicylamide elevated the levels of unconjugated 3-trifluoromethyl-4-nitrophenol in blood, muscle, heart, and brain of rainbow trout. From this information one can con-

![Figure 1](image1.png)

**Figure 1.** Effect of salicylamide on the acute toxicity of 3-trifluoromethyl 4-nitrophenol (TFM) to rainbow trout (36).

![Figure 2](image2.png)

**Figure 2.** Effect of salicylamide on TFM glucuronide and unconjugated TFM in blood of rainbow trout exposed to 3-trifluoromethyl-4-nitrophenol (TFM) for the indicated times (36).

![Figure 3](image3.png)

**Figure 3.** Effect of salicylamide on biliary excretion of TFM glucuronide in rainbow trout exposed to 3-trifluoromethyl 4-nitrophenol (TFM) for the indicated times (36).
clude that the rate of glucuronide formation proceeds rapidly enough to be a significant factor in the acute toxicity of this phenol to rainbow trout. A similar phenomenon has been described in mosquitofish with the use of the mixed-function oxidase inhibitor, sesamex, and the organophosphorus insecticide, parathion (37). Current evidence indicates that parathion must be activated by mixed-function oxidases to paraoxone, which is the active cholinesterase inhibitor. It follows that the magnitude of toxicity of parathion is directly related to its rate of activation as well as inactivation. Figure 4 illustrates that when mosquitofish were pretreated with 2 mg/l. of sesamex for 24 hr, the 48-hr LC50 for parathion was increased from 0.11 mg/l. to 1.2 mg/l. Although the LC50 curves shown in Figure 4 are not parallel, which may indicate a further interaction of sesamex with parathion, the data clearly indicate that sesamex did reduce the acute toxicity of parathion. Extracts of livers from fish pretreated with sesamex activated little or no parathion, while similar extracts from untreated fish produced enough paraoxone to inhibit 60% of brain cholinesterase activity. Although studies of this type are rare in fish, these examples serve to illustrate that biotransformation may be an important determinant of the toxicity of certain chemicals in fish and may play a role in species sensitivity to chemicals.

Bioaccumulation and Persistence of Chemicals in Fish

Observations of bioaccumulation of persistent chemicals in fish and other members of the food chain have been an important factor in the inspiration and development of research concerning the behavior of chemicals in aquatic species. Although the introduction of this concept was of great significance to ecological interests, its importance to human health was not fully appreciated until the appearance of methylmercury and polychlorinated biphenyls in humans was linked to the bioaccumulation of these compounds in fish. It is important to recognize, however, that while bioaccumulation is important when considering the magnification of certain chemicals within aquatic and human food chains, it is not the sole determinant of the hazard of chemicals to aquatic species themselves, and therefore, bioaccumulation and persistence are not necessarily synonymous with toxicity.

While bioaccumulation and persistence have often

| Tissue | Exposure time, hr. | Control | Salicylamide | p |
|--------|--------------------|---------|--------------|---|
| Blood  | 1.5                | 4.46 ± 0.4 | 7.86 ± 0.71  | < 0.01 |
|        | 3.0                | 3.79 ± 0.95 | 13.56 ± 1.86 | < 0.01 |
|        | 4.5                | 4.60 ± 0.32 | 20.97 ± 1.73 | < 0.01 |
| Muscle | 1.5                | 1.31 ± 0.21 | 2.58 ± 0.33  | < 0.01 |
|        | 3.0                | 1.33 ± 0.29 | 4.80 ± 0.54  | < 0.01 |
|        | 4.5                | 1.71 ± 0.52 | 6.64 ± 0.89  | < 0.01 |
| Brain  | 1.5                | 4.06 ± 0.33 | 7.57 ± 1.01  | < 0.01 |
|        | 3.0                | 4.66 ± 0.64 | 15.39 ± 3.74 | < 0.01 |
|        | 4.5                | 6.09 ± 2.06 | 17.87 ± 2.13 | < 0.01 |
| Heart  | 1.5                | 4.44 ± 0.31 | 16.87 ± 8.75 | NSb |
|        | 3.0                | 5.90 ± 1.16 | 20.59 ± 4.32 | < 0.01 |
|        | 4.5                | 8.70 ± 2.68 | 26.63 ± 1.80 | < 0.01 |

*Data from Lech (36).

bNot significant.
been treated as a physical-chemical problem, it is obvious from the mammalian literature that the role of biotransformation as a determinant of persistence and bioaccumulation cannot be ignored. A modification of Eq. (2) yields a form [Eq. (3)] which has been used to advantage in experimental attempts to predict bioconcentration factors of potentially persistent chemicals in fish by

\[
[F]/[W] = \frac{k_1}{k_2} \quad (3)
\]

as accelerated method (38). Since the bioaccumulation factor \([F]/[W]\) is achieved at steady-state conditions, the use of \(k_1\) (uptake) and \(k_2\) (elimination) may allow for a more rapid estimate of bioaccumulation factors without having to extend an experiment until the steady state is reached. \(k_2\) is often composed of at least two constants: \(k_2\) (excretion of undamaged molecule) and \(k_3\) (elimination by biotransformation) which when substituted in Eq. (2) yields Eqs. (4) and (5):

\[
[F]_\infty = \frac{[k_1/(k_2 + k_3)]}{[W]} \quad (4)
\]

\[
[F]/[W] = \frac{k_1}{k_2 + k_3} = \text{Bioaccumulation factor at steady state} \quad (5)
\]

\[
t_1 = \frac{0.69}{k} = \frac{k_2 + k_3}{k} \quad (6)
\]

While these analyses are admittedly unsophisticated toxicokinetically, it is readily apparent that the \(k\) for biotransformation is a potentially important constant in the determination of the final bioaccumulation factor [Eq. (5)]. In addition, since the half-life \(t_1\) is equal to 0.69/\(k\) for a first-order elimination reaction, the relationship between the half-life of a compound and its bioaccumulation factor should become apparent [Eq. (6)]. As alluded to earlier, if this concept is valid, perturbations of the biotransformation rate should be accompanied by changes in the bioaccumulation factor, and the magnitude of the changes should reflect the relative importance of the biotransformation process.

Although it should be obvious that \(k_1\) and \(k_2\) are as important as \(k_3\) in determining the bioaccumulation factor in even this simple analysis, there is limited evidence to suggest that biotransformation rates may affect the bioaccumulation of certain compounds.

In a series of short- and long-term exposures of various organisms to benzo(a)pyrene in a mixed aquatic ecosystem, it was found that benzo(a)pyrene was rapidly metabolized by mosquitofish and slowly metabolized by snails (39). The data in Table 3 show the bioaccumulation factors for benzo(a)pyrene by these species in an aquatic system for 3 days and an established aquatic ecosystem after 33 days. It can be seen that the bioaccumulation factor in mosquitofish was much lower than that for snails in both systems, and the higher values obtained after 33 days in the ecosystem were attributed to food web biomagnification. Inclusion of the mixed-function oxidase inhibitor, piperonyl butoxide, in both systems increased the bioaccumulation factor considerably in the mosquitofish, where metabolism of benzo(a)pyrene was most rapid, but had little effect in the snail. It is interesting that while piperonyl butoxide increased the bioaccumulation factor in the mosquitofish, presumably by inhibiting metabolism, the bioaccumulation factor in the snail was much higher than that in the mosquitofish despite the “equalization” of the rates of metabolism of benzo(a)pyrene by the inhibitor. Although several explanations for this are plausible and can be illustrated by manipulations of Eq. (5), the point that the bioaccumulation factor is determined by processes in the organism, biotransformation being but one, cannot be overemphasized.

Within a given species, biotransformation of chemicals to forms which can be excreted by active processes (glucuronides, sulfates) can greatly influence compartmentalization within the animal and hence bioaccumulation factors in various tissues. This concept has been illustrated using pentachlorophenol and its O-methyl derivative, pentachloroanisole, in rainbow trout (18). When trout were exposed in water to 0.02 \(\mu g/ml\) of \(^{14}\)C pentachlorophenol, the concentration in adipose tissue rose to approximately 3.5 \(\mu g/g\) in 8 hr. However, the concentration of \(^{14}\)C in the gall bladder bile in these

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**Table 3. Bioaccumulation of benzo(a)pyrene in aquatic systems.**

| Exposure                  | Aquatic system (3 days) | Aquatic terrestrial ecosystem (33 days) |
|---------------------------|-------------------------|----------------------------------------|
|                           | Mosquitofish | Snail       | Mosquitofish | Snail |
| Benzo(a)pyrene            | <1*         | 2177        | 30          | 4860 |
| Benzo(a)pyrene + piperonyl Butoxide | 22          | 3056        | 140         | 7520 |

*Data from Lu et al. (39).

Numbers indicate organism/water benzo(a)pyrene ratios at the end of the stated time periods.
exposures was equivalent to over 250 \( \mu g/ml \) pentachlorophenol. These concentrations represent bioaccumulation factors of approximately 200 for fat and over 12,000 for bile. Adipose tissue contained only free pentachlorophenol, while bile contained pentachlorophenol glucuronide. On the other hand, when a similar experiment was done with the \( O \)-methyl derivative, pentachloroanisole, the concentration in fat rose to approximately 60 \( \mu g/g \), while the concentration in bile amounted to approximately 10 \( \mu g/g \). These values yield bioconcentration factors of approximately 3000 for fat and under 500 for bile. These data are shown for two exposure periods with both compounds in Figure 5.

In the pentachloroanisole exposure, as in the pentachlorophenol exposure, the \( ^{14}C \) labeled material in bile was almost all pentachlorophenol glucuronide, indicating some demethylation of pentachloroanisole with subsequent conjugation with glucuronic acid (Fig. 6). The half-lives of both of these compounds are shown in Table 4, and it is apparent that pentachloroanisole has a much greater half-life than does pentachlorophenol in all tissues shown. The data in Table 5 show that pentachloroanisole is dealkylated in rainbow trout and as it is excreted in bile as a glucuronide conjugate, a treatment of the fish with piperonyl butoxide decreased the amount of pentachlorophenol glucuronide excreted in bile while concomitantly increasing the amount of unchanged pentachloroanisole and this effect may be due to its inhibition of both dealkylation and conjugation. In view of the relatively rapid rate of conjugation and elimination of pentachlorophenol in these studies, a possible reason for the longer half-life and higher bioaccumulation factor of pentachloroanisole may be its slow rate of dealkylation to the free phenol. A potentially important parameter, the excretion of unchanged pentachlorophenol and pentachloroanisole via the gills, was not measured in these cited experiments. This route of elimination may be an important factor in determining the bioaccumulation factor and half-life of chemicals, depending upon the particular compound under study and its ability to diffuse across the gills unchanged into the surrounding aquatic environment. This situation is not usually incurred in comparable mammalian studies unless the compound in question is volatile and pulmonary excretion is significant.

![Figure 6](image)

**Figure 6.** Thin-layer chromatographic profiles of radioactivity in bile from rainbow trout exposed to \( ^{14}C \) labeled pentachloroanisole. The striped circle represents the mobility of authentic pentachloroanisole, and the open circle represents the mobility of authentic pentachlorophenol (18).

![Figure 5](image)

**Figure 5.** Levels of pentachlorophenol and pentachloroanisole equivalents in various tissues of rainbow trout exposed to \( ^{14}C \)-labeled pentachlorophenol and pentachloroanisole for the indicated times (18).
Thus disposition or chlorinated compounds may be administered that the animals have received much attention in recent years. Available data indicate that most of these compounds are slowly metabolized, if at all, by species of fish which have been studied (40, 41). It is clear, however, from studies in mammals, that persistence or lack of persistence may be determined by the rate of metabolism of a particular congener (42).

Thus it appears that congeners which are rapidly metabolized have shorter half-lives than those which are slowly metabolized, and there may be a range of species specificity in this respect. Studies on the disposition and persistence of mixtures of polychlorinated biphenyls in mammals have indicated that the storage patterns of specific congeners within the animals may vary from the pattern of the mixture administered to the animal (43). This "enrichment" or retention pattern has been attributed to selective metabolism and elimination of specific congeners.

In studies with fish, the pattern of stored PCB is similar to that seen in the mixture administered (40). It has been shown that 2,5,2',5'-tetrachlorobiphenyl (TCB) which is rapidly metabolized in mammals, is metabolized little if at all in rainbow trout and estimates of its metabolism range to as low as 0.1% of the accumulated amount in rainbow trout (41). In an extended study of the behavior of TCB in rainbow trout, it was found that after an initial redistribution of this compound at early time periods after exposure, the whole body half-life was estimated to be approximately 1.75 years in female rainbow trout (44). Although rates of biotransformation and excretion via the gills were not measured, it was obvious that the long half-life pointed towards little or no elimination via these mechanisms. The data in Figure 7, however, illustrate an important means of elimination of TCB and possibly other similar compounds which may have previously been overlooked in short term studies. During the period of egg development in the female trout, a considerable amount of 14C-labeled TCB appeared in the de-

![Figure 7. Radioactivity in visceral fat, periorbital fat, and developing eggs of female trout exposed to 14C-2,5,2',5'-tetrachlorobiphenyl (TCB) (44).]

**Table 4. Half-life t½ of pentachlorophenol and pentachloroanisole in rainbow trout tissues.**

| Chemical                  | t½  |
|---------------------------|-----|
| Pentachlorophenol         | 6.2 hr | 9.8 hr | 23.7 hr | 6.9 hr | 10.3 hr | 6.9 hr |
| Pentachloroanisole        | 6.3 days | 6.9 days | 23.4 days | 6.3 days | ---     | ---    |

*Data from Glickman et al. (18).

As a class of very persistent chemicals, the polychlorinated biphenyls have received much attention in recent years. Available data indicate that most of these compounds are slowly metabolized, if at all, by species of fish which have been studied (40, 41). It is clear, however, from studies in mammals, that persistence or lack of persistence may be determined by the rate of metabolism of a particular congener (42). Thus it appears that congeners which are rapidly metabolized have shorter half-lives than those which are slowly metabolized, and there may be a range of species specificity in this respect. Studies on the disposition and persistence of mixtures of polychlorinated biphenyls in mammals have indicated that the storage patterns of specific congeners within the animals may vary from the pattern of the mixture administered to the animal (43). This "enrichment" or retention pattern has been attributed to selective metabolism and elimination of specific congeners.

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**Table 5. Effect of piperonyl butoxide on distribution of 14C in bile of rainbow trout exposed to 14C pentachloroanisole.**

| Conditions            | N   | Bile volume, ml | 14C concentration as PCA, μg/ml | Total excreted as PCA, ng | Metabolite distribution |
|-----------------------|-----|-----------------|---------------------------------|---------------------------|-------------------------|
| Control               | 8   | 0.082 ± 0.02    | 3.78 ± 0.57                    | 310                       | 257(83) | 6(2) | 47(15) |
| Piperonyl butoxide    | 7   | 0.098 ± 0.01    | 2.06 ± 0.21                    | 202                       | 85(42)  | 10(5) | 107(53) |

*Data of Glickman et al. (18).*
developing eggs. Over this period, approximately 5% of the initial total body burden of TCB was eliminated in the egg mass, and this was correlated with a change in slope of the adipose tissue elimination curves. Although not shown here, a similar phenomenon was observed in male trout, and a portion of the body burden of TCB was eliminated in sperm. Although the mechanisms for these translocations are not known at present, an analogy may be drawn between these phenomena and those observed in the transfer of persistent polychlorinated biphenyls into breast milk in mammals.

It is important to note from these observations that when evaluating the persistence of chemicals in fish, multiple mechanisms of elimination may exist, and while metabolism is important for certain compounds, alternate mechanisms may predominate for others. It may be expected that such alternate mechanisms will be particularly important for compounds that are metabolized slowly in vivo.

### Biotransformation, Residues, and Monitoring

Since it has been clearly established that biotransformation of xenobiotic chemicals does occur in fish in vivo, it is appropriate to consider these findings and their implications with regard to monitoring and residue analysis programs. While monitoring and residue analysis for xenobiotic chemicals in fish have a common base analytically, the purposes for which these analyses are done may be quite diverse. The data obtained from such studies may serve several needs, ranging from a survey of the aquatic environment for ecological purposes to the determination of the suitability of tissues of fish for animal or human consumption. However, most analytical methods for monitoring purposes are designed to look for unchanged xenobiotic chemicals and not their biotransformation products. While this is sufficient for many purposes and will indicate how much of chemical X is in a given fish, it does not consider if chemical X was in a given fish previously or the fact that biotransformation products can be more toxic than the starting material.

The data in Table 6 illustrates the former point quite vividly. In this study (24), pinfish were exposed to 75 μg/l. of malathion in sea water for 24 hr. Various organs were then excised and analyzed for malathion content as well as three possible biotransformation products of malathion. It can clearly be seen from these data that if the residue analysis method were designed to determine only malathion, the conclusion could only be that the fish contained no malathion. However, the very high concentrations of malathion metabolites found (MCA and DCA), indicate that the fish were probably exposed to malathion. If MCA and DCA were more toxic materials than malathion or if the analysis had been done to locate a point source of malathion, the entire analytical exercise would have been misleading unless these were assayed.

Since we have only reached a basal level of awareness concerning the biotransformation of chemicals in fish in recent years, it is difficult to predict the extent of this phenomenon in the environment or how far one should really go in designing residue analysis methodology. However, it is clear from a limited number of studies that certain chemicals are biotransformed rapidly, and that residues of metabolites may appear in tissues of the exposed animals.

It has been shown by several investigators that high concentrations of metabolic products of xenobiotics are excreted in the gall bladder of certain species of fish (27, 45). The nature of the biotransformation products varies considerably depending upon the structure of the starting xenobiotic, but, in general, the materials found in the gall bladder bile and possibly urine, appear to be conjugates. The data shown in Figure 8 illustrate the high-pressure liquid chromatographic profile of the bile from coho salmon fed 3H-naphthalene (28). It can be seen that approximately eight product peaks are present, several of which have been designated as 1-naphthol, 1,2-dihydro-1,2-dihydroxynaphthalene, and their conjugates. Oxidation products of several aromatic hydrocarbons including naphthalene have been found in tissues of fish and other aquatic organisms exposed to these materials (46).

While the enterohepatic circulation of xenobiotic substances after gall bladder emptying has been known for some time in mammals, the extent to which this occurs in fish is unknown at present. It is quite likely, however, that chemicals and their metabolites which are excreted into bile reenter the systemic circulation via an enterohepatic circulation.
products which were more polar than the starting materials. The data in Table 8 show the bile to water ratios in rainbow trout exposed to a variety of xenobiotic substances which differ widely in their chemical properties. From these and other data, the trend appears to be that compounds which are conjugated directly or readily metabolized to materials which can be conjugated with either glucuronic acid, sulphate or glutathione accumulate to a greater extent in the bile than compounds which are more slowly metabolized.

It is not meant to imply that the concentration of these materials in bile can be directly related to their concentration in water, but this information is presented to suggest that the bile may be a convenient sample source for the qualitative determination of the biotransformation products which may be formed in fish.

Since bile may be a source of high concentrations of metabolites of xenobiotic chemicals, this fluid should not be ignored in instances where it is critical to determine evidence of a previous exposure. On a practical basis, it is obvious from this information that residue analysis done on extracts of whole undissected fish should be interpreted with caution.

A question which is often and justifiably posed concerns the relevance of data gathered in the laboratory to actual environmental conditions. Table 9 shows the concentrations of pentachlorophenol (PCP) and pentachlorophenol-related products in water and several tissues taken from three species of fish which were collected after an accidental spill of technical pentachlorophenol (48). Several important pieces of information are illustrated. First, when pentachlorophenol was determined in tissue samples from these fish, the concentrations were found to be highest in bile followed by liver and muscle, and the bile to water ratio was as high as 100,000. The concentrations in both liver and bile were much higher

![Figure 8. High-pressure liquid chromatographic profile of radioactivity in bile from coho salmon fed 14C-labeled naphthalene](28).

Table 7. Biliary 14C following exposure of fish to 14C-naphthalene or 14C-2-methyleneaphthalene.*

| Species       | Average fish weight, g | Initial water level, mg/l | Exposure duration | Bile level of parent compound and metabolites, mg/ml | Ratio Bile level H2O level |
|---------------|------------------------|---------------------------|-------------------|---------------------------------------------------|--------------------------|
| Naphthalene   |                        |                           |                   |                                                   |                          |
| Trout         | 11 g                   | 0.005                     | 24 hr             | 0.002                                            | 370                      |
| Trout         | 6 g                    | 0.005                     | 8 hr*             | 0.002                                            | 384                      |
| Trout         | 5 g                    | 0.017                     | 16 days           | 0.327                                            | 19200                    |
| Trout         | 11 g                   | 0.005                     | 24 hr             | 0.013                                            | 2600                     |
| 2-methyl-naphthalene |                      |                           |                   |                                                   |                          |
| Trout         | 4 g                    | 0.023                     | 14 days           | 0.434                                            | 18900                    |
| Trout         | 74 g                   | 0.494                     | 24 hr             | 0.104                                            | 217                      |
| Carp          | 4 g                    | 0.013                     | 8 days            | 1.835                                            | 141200                   |
| Carp          | 25 g                   | 0.337                     | 24 hr             | 0.654                                            | 1940                     |
| Sheephead     | 450-550 g              | 0.072                     | 48 hr             | 0.026                                            | 361                      |

*Data from Melancon and Lech (47).

*Exposure 8 hr, followed by 24 hr in fresh (naphthalene-free) water.
than those found in muscle and would have been ideal tissues to sample had the residue in muscle been below the analytical capabilities of the procedure used. The tetrachlorophenol isomers which were present in the technical mixture spilled were also found in relatively high concentrations in the tissues sampled and the analytical methods used indicate that these phenols were present in bile primarily as conjugates (48). It is interesting to note that pentachloroanisole was also found during the analysis of these tissues since pentachloroanisole is more persistent than pentachlorophenol, and it is not a component in the technical mixture of pentachlorophenol, which was involved in the spill. Pentachloroanisole has been identified in fish tissues in several surveys, but the precise source in aquatic systems has not been determined, although there is some evidence to suggest that pentachlorophenol can be methylated in soil systems (49).

**Induction of Biotransformation Enzymes in Fish**

Studies concerning the *in vivo* and *in vitro* biotransformation of xenobiotic chemicals in fish have been extended to include an examination of the inducibility of mixed-function oxidases. Early work, largely with inducers of the phenobarbital class, were not definitive with regard to the inducibility of mixed-function oxidase activity in fish (50, 51). There has been a rapid increase in interest in this area recently, and it is apparent from several lines of

### Table 8. Biliary concentration of various xenobiotics by rainbow trout (*Salmo gairdnerii*).\(^{a,b}\)

| Compound | Concentration in H<sub>2</sub>O, mg/l | Radioactivity, dpm/ml | Ratio (H<sub>2</sub>O<sup>14</sup> C to bile<sup>14</sup>C) |
|----------|-----------------------------------|----------------------|-----------------|
|          |                                    | H<sub>2</sub>O (0 hr) | H<sub>2</sub>O (24 hr) | Bile (24 hr) |
| 2', 5-Dichloro-4'-nitrosalicylanilide | 0.05 | 3,010 | 30,500,000 | 10,100 | 1 |
| Pentachlorophenol (PCP) | 0.1 | 4,070 | 21,800,000 | 5,360 | 2? |
| Methylnaphthalene | 0.005 | 310 | 796,000 | 2,570 | ? |
| 3-Trifluoromethyl-4-nitrophenol (TFM) | 0.5 | 2,020 | 2,150,000 | 1,064 | 1 |
| 1-Naphthyl-N-methylcarbamate | 0.25 | 1,030 | 975,000 | 947 | 3 |
| Naphthalene | 0.005 | 305 | 127,000 | 414 | 2 |
| Di-2-ethylhexyl phthalate (DEHP) | 0.5 | 1,070 | 265,000 | 247 | 5? |
| 1,1,1-Trichloro-2,2-bis-(p-chlorophenyl)ethane | 0.1 | 180 | 22,500 | 124 | 1 |
| 2,5,2',5'-Tetrachlorobiphenyl | 0.5 | 3,640 | 39,000 | 11 | 2? |

\(^{a}\)Exposures were made at 12° C for 24 hr. Water hardness was 134 ppm, measured by the CaCO<sub>3</sub> method, and pH was 7.2. Radioactivities are expressed as disintegrations per minute (dpm) per milliliter; each value of the 24-hr bile radioactivity is the mean of a minimum of five animals from at least two separate exposures.

\(^{b}\)Data from Statham et al. (45).

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### Table 9. PCP and PCP degradation products in lake water and fish tissue.*

| Date       | Fish  | Chemical | Water, ng/ml | Tissue level, ng/g* |
|------------|-------|----------|--------------|---------------------|
|            |       |          | Muscle       | Liver               | Bile                 |
| January 1977 | Sunfish | PCP      | 24           | 8,000               | 1 x 10<sup>4</sup> | N.A. c |
|            |       | PCP-OCH<sub>3</sub> | 0.08 | 60       | 560 | N.A. |
|            |       | TCP<sup>d</sup> | 1            | 70        | 1,300 | N.A. |
|            | Bass  | PCP      | 24           | 1 x 10<sup>4</sup> | 2 x 10<sup>4</sup> | 2 x 10<sup>4</sup> |
|            |       | PCP-OCH<sub>3</sub> | 0.08 | 170      | 600 | 200 |
|            |       | TCP      | 1            | 230       | 6,000 | 1.2 x 10<sup>4</sup> |
| April 1977 | Sunfish | PCP      | 5            | 1,000    | 1.5 x 10<sup>4</sup> | N.A. |
|            |       | PCP-OCH<sub>3</sub> | 0.03 | 30       | 150 | N.A. |
|            |       | TCP      | 1            | 20        | 200 | N.A. |
|            | Catfish | PCP      | 5            | 5,000    | 3.5 x 10<sup>4</sup> | 1.5 x 10<sup>6</sup> |
|            |       | PCP-OCH<sub>3</sub> | 0.03 | 140      | 350 | 200 |
|            |       | TCP      | 1            | 60        | 1,000 | 2,000 |

*Data from Pierce (48).

*Weight per wet weight tissue.

*Not analyzed.

<sup>d</sup>TCP represents total of 2,3,4,5-, 2,3,4,6- and 2,3,5,6-TCP isomers.

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investigation that compounds such as benzo(a)-pyrene and 3-methylcholanthrene, inducers of Pi-450 cytochrome(s) in mammals, are capable of elevating mixed-function oxidase activity from 10 to 50-fold in several species of fish (12-15, 52-56). Figure 9 illustrates the effect of phenobarbital, 3-methylcholanthrene, benzantracene, and β-naphthoflavone on the specific activity of glucose-6-phosphatase, a microsomal marker enzyme, glucuronyl transferase, and benzo(a)pyrene hydroxylase in microsomes prepared from liver of rainbow trout treated with these compounds. It can be seen that while phenobarbital, under these experimental conditions, did not affect the level of the benzo(a)pyrene hydroxylase, 3-methylcholanthrene, benzantracene, and β-naphthoflavone all significantly increased benzo(a)pyrene hydroxylase activity without significantly increasing other microsomal marker enzymes.

Recent evidence from several laboratories has indicated that compounds of environmental interest, including petroleum components and some crude oils themselves, are capable of elevating mixed-function oxidase activity in fish (57, 58). Figure 10 illustrates the effects of crude (Tia Juana Medium) oil exposure (0.2-2 mg/l) on the levels of arylhydrocarbon hydroxylase (AHH) activity in cunners. It can be seen that hydroxylase activity is increased after several days of exposure, with two different substrates for the AHH assay, and that the degree of induction reliably follows the concentration of oil in the water (58). These data serve to illustrate that hepatic AHH activity can be increased in fish with direct water exposure as well as by injection of potential inducers.

The data in Table 10 summarize the effect of several inducers on five different monooxygenase reactions in trout liver which have been used to characterize the nature of induction in similar mammalian studies. These data along with information from several sources in the literature all seem to point to a particular pattern of induction in fish. In general, it appears that inducers of the cytochrome(s) Pi-450 (i.e., β-naphthoflavone) are capable of elevating those enzyme activities in trout which are induced by this type of inducer in mammals, i.e., benzo(a)pyrene hydroxylase, and ethoxyresorufin deethylase. On the other hand, inducers of cytochrome(s) P-450 (phenobarbital) have not proved to be effective inducers in fish when used in similar doses and under similar experimental conditions as the Pi-450 type inducers (52, 59). The reasons for these observations remain unresolved at present.

The effects of pure congeners of the polychlorinated biphenyls and polybrominated biphenyls on
mixed-function oxidase activity and cytochrome(s) P-450 have not been extensively studied in fish, although there is some evidence to suggest that the coplanar congeners are β-naphthoflavone-like inducers while the noncoplanar congeners appear to be incapable of inducing at equivalent dosage levels (59).

**Implications of Induction**

Much of the work that has been done to characterize the induction phenomenon in fish has been done with intraperitoneal injections of the potential inducing agent. While this approach is justified in order to describe and assess the responsiveness of the hepatic system to the various inducers, questions concerning the response of fish to inducers in the environment, and the consequences of induction need to be answered.

Studies with mammalian species have clearly demonstrated the presence of multiple forms of hepatic (60, 61) and extrahepatic (62) cytochrome P-450 and have shown that various cytochrome P-450 isozymes have divergent substrate specificities and are under different regulatory control. For example, the administration of polycyclic hydrocarbon-type inducing agents, such as 3-methylcholanthrene (3MC), to rats results in the formation of a major form of the hemoprotein, cytochrome P1-450, which is primarily responsible for differences in the metabolism of certain substrates in 3-MC treated vs. control animals (60, 61).

Induction of the microsomal mixed-function oxidase system can influence the metabolism and toxicity of a pollutant in several ways. If a single major form of cytochrome P-450 is induced, the rate of metabolism of a chemical that is a poor substrate or a nonsubstrate for this particular cytochrome P-450 isozyme may be lower in induced than in untreated animals. Should metabolism of the chemical be required for toxicity, induction resulting in a reduced rate of biotransformation might actually protect against the toxic response. If, however, the chemical is a good substrate (high turnover number) for the form(s) of cytochrome P-450 induced, significant increases in the rate of oxidative metabolism of the compound are to be anticipated. These changes in metabolic rate can be quite dramatic, especially if the chemical is a poor substrate for the predominant form(s) of cytochrome P-450 in liver of uninduced animals or if only very small amounts of the induced form(s) of the cytochrome are present in untreated animals. Induction of the microsomal mixed-function oxidase system can alter the metabolism of xenobiotics in another manner which may also be an important determinant of chemical-mediated toxicity. Different forms of cytochrome P-450 catalyze preferential metabolism at certain positions of various molecules. For example, hepatic microsomes from rats treated with 3-MC convert biphenyl to significant amounts of 2-hydroxybiphenyl, whereas hepatic microsomes from untreated or phenobarbital (PB)-treated rats metabolize biphenyl predominantly to 4-hydroxybiphenyl (63); highly purified hepatic cytochrome P-450 (from 3-MC-treated rats) preferentially metabolizes testosterone at the 7α-position, whereas cytochrome P450 purified from livers of PB-treated rats produces primarily 16α-hydroxytestosterone (64); hepatic microsomes from 3-MC-treated rats produce relatively greater amounts of benzo(a)pyrene 7,8-dihydriodiol and benzo(a)pyrene 9,10-dihydriodiol, but not benzo(a)pyrene 4,5-dihydriodiol, from benzo(a)pyrene, whereas microsomes from PB-treated rats do not (65, 66). Where one particular oxidation product is especially toxic or is further metabolized to a very toxic product, subtle changes in the metabolic profile can have profound toxicological effects.

Although considerably less information is avail-

### Table 10. Induction of hepatic microsomal monooxygenation in rainbow trout.*

| Treatment of fish       | Dose, mg/kg | EMD | BeND | AHH | ECOD | EROD |
|-------------------------|-------------|-----|------|-----|------|------|
| Corn oil                |             | 100 | 100  | 100 | 100  | 100  |
| Phenobarbital           | 150         | 105 | 98   | 133 | 104  | 64   |
| Aroclor 1242            | 150         | 89  | ND   | 700 | 547  | 1564 |
| Aroclor 1254            | 150         | 89  | ND   | 4081| 1178 | 4455 |
| Firemaster BP6          | 100         | 88  | ND   | 104 | 64   | 1564 |
| β-Naphthoflavone        |             | 100 | 100  | 100 | 100  | 100  |

*Data from Elcombe et al. (59).

*Ethylmorphine-N-demethylation.

*Benzphetamine-N-demethylation.

*Arylhydrocarbon [benzo(a)pyrene] hydroxylation.

*Ethoxyresorufin-O-deethylation.

*Ethoxycoumarin-O-deethylation.
able in aquatic species concerning induction, different forms of cytochrome P-450, and position-specific metabolism of xenobiotics, such studies are relevant to the chemical nature and amount of pollutant residues in fish and the toxicity of the pollutant and its metabolites to fish as well as to those species, including man, which utilize fish for food. Moreover, detailed investigations of this type will be necessary for determining whether or not induction of the hepatic mixed-function oxidase system of fish can be used as an efficient indicator for pollution by certain classes of chemicals in aquatic environments.

Along these lines, a form of cytochrome P-450 having its absorption maximum at 448 nm in the reduced and CO-ligated state was identified in liver microsomes from 1,2,3,4-dibenzoanthracene (DBA)-treated little skates (Raja erinacea) after solubilization and partial purification (67). The hepatic cytochrome P1-450 in this marine elasmobranch was associated with DBA treatment, but it was not the only major form of hemoprotein present in the induced animals, accounting for 30-60% of the total cytochrome P-450 in both male and female fish. The other major form of cytochrome P-450 (absorption maximum at 451 nm) in livers from DBA-induced skates was very similar, if not identical, to the major form of hepatic cytochrome P-450 in untreated skates. The presence of cytochrome P1-450 was associated with dramatic increases in benzo(a)pyrene hydroxylase activity of DBA-treated skate hepatic microsomes when assayed by fluorescence or HPLC (total metabolite quantitation) techniques. Similarly, large increases in benzo(a)pyrene hydroxylase activity of hepatic microsomes from rainbow trout (Salmo gairdneri) treated with β-naphthoflavone, a polycyclic hydrocarbon-type inducer, were associated with a large increase in a microsomal hemoprotein which had a monomeric molecular weight of 57,000 as demonstrated by polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate (52).

As discussed previously, several environmental pollutants, including benzo(a)pyrene, are converted by the microsomal mixed-function oxidase system to a number of metabolites, some of which are known to be preferentially produced by various forms of cytochrome P-450. Consequently, the metabolic profiles of 14C-benzo(a)pyrene were compared in hepatic microsomes from DBA-treated and control male little skates (Table 11). The metabolites produced were qualitatively very similar, if not identical, in control and induced fish, although the rate of formation was much greater (about 17-fold) in the DBA-treated skates. The radioactivity co-eluting with the 9-hydroxybenzo(a)pyrene and the 3-hydroxybenzo(a)pyrene standards (i.e., the phenolic metabolites) accounted for about 50% of the total biotransformation products in both control (68%) and induced (50%) skates. The major quantitative differences in the metabolic profiles were the relatively greater amounts of radioactivity co-chromatographing with the standards in the quinones [benzo(a)pyrene-1,6-, -3,6-, and -6,12-quinones] plus benzo(a)pyrene 4,5-oxide region of the chromatograms in induced microsomal incubation mixtures (97-fold higher than in controls) and the relatively lower amounts of benzo(a)pyrene 4,5-dihydrodiol formed (only 3.3-fold greater than control) by hepatic microsomes from DBA-treated skates.

From a toxicological viewpoint it was interesting that significant amounts of benzo(a)pyrene 7,8-dihydrodiol were formed by hepatic microsomes of both untreated (15% of total metabolites) and DBA-induced (13%) skates, since this dihydrodiol is the metabolic precursor for the isomeric benzo(a)pyrene 7,8-dihydrodiol-9,10-epoxides, which are ultimate carcinogenic and mutagenic forms of benzo(a)-pyrene (68, 69). Of course, formation of the 7,8-dihydrodiol was much more rapid (14-fold control values) in microsomes of the DBA-induced skates.

Studies utilizing rainbow trout and the inducer β-naphthoflavone have indicated that the state of

| Table 11. HPLC identification and quantitation of metabolites obtained upon incubation of 14C-benzo(a)pyrene with hepatic microsomes from control or 1,2,3,4-dibenzoanthracene-treated little skates (Raja erinacea).* |
|---------------------------------------------------------------|
| **Metabolite** | **Fraction** | **DBA-treated skates** | **Control skates** | **DBA/Control** |
| Benzo(a)pyrene 9,10-dihydrodiol | 1.2 | 0.06 | 20.0 |
| Benzo(a)pyrene 4,5-dihydrodiol | 0.1 | 0.03 | 3.3 |
| Benzo(a)pyrene 7,8-dihydrodiol | 1.4 | 0.10 | 14.0 |
| Benzo(a)pyrene 4,5-oxide + quinones | 2.9 | 0.03 | 96.7 |
| 9-Hydroxybenzo(a)pyrene | 1.9 | 0.12 | 15.8 |
| 3-Hydroxybenzo(a)pyrene | 3.6 | 0.33 | 10.9 |
| Total metabolites | 11.1 | 0.66 | 16.8 |

*Data from Bend et al. (67).
induction may influence the disposition and metabolism of certain chemicals in vivo (47). The data in Table 12 illustrate the effect of administration of $\beta$-naphthoflavone on the disposition of $^{14}$C-labeled naphthalene, 2-methylnaphthalene, and 1,2,4-trichlorobenzene in rainbow trout. In the induced trout, biliary excretion of metabolites of all three compounds was increased several fold over control values, and muscle and blood contained lower total $^{14}$C residues than did the control trout.

Although detailed studies are still required before enzyme induction in fish is used as a monitoring system for aquatic pollution by certain classes of chemicals, or before the relationships between enzyme induction and chemical toxicity are fully understood for even a single fish species, there is considerable interest and promise in these fields of endeavor. There is now no doubt that several species of freshwater and marine fish (both teleosts and elasmo-branchs) exhibit induction of the hepatic microsomal mixed-function oxidase system when exposed to certain polycyclic hydrocarbons, polychlorinated biphenyls, polybrominated biphenyls or dioxins. Compounds in each of these chemical classes are known to be very toxic and demonstrating their presence at trace concentrations in the aquatic environment is certainly important for both animal and human health. This is especially relevant since fish bioaccumulate lipophilic compounds from the water and can be expected to show enzyme responses after prolonged exposure to even very low concentrations of pollutant. The most sensitive parameters to use for demonstrating enzyme induction in wild populations may vary from fish species to species. Consequently, a good approach is to compare several properties of the hepatic or extrahepatic mixed-function oxidase system, including enzyme activities with several substrates that are known to show preferential metabolism with different forms of mammalian cytochrome P-450 (see Table 10), in control versus maximally induced fish (i.e., fish pre-treated with a known inducer). The use of differential inhibitors or activators of enzyme activity is currently of value in this comparison (67). Field observations with the highest validity should come from species that have first been very well characterized under laboratory conditions. Eventually, as different types of cytochrome P-450 have been highly purified from various fish species and monospecific antibodies are available, inhibition of microsomal enzyme activity by these specific antibodies should also be an excellent method for assessing enzyme induction in field studies.

A recent investigation, which demonstrated that approximately 50% of the winter flounder (Pseudo-pleuronectes americanus) captured in Maine during June, July, and August 1978, had induced microsomal mixed-function oxidase systems (67), suggests that polycyclic hydrocarbon-like inducing agents may be widely distributed in the marine environment. However, it must be pointed out that environmental pollution has not yet been conclusively linked to this enzyme response in these Maine flounder. On the other hand, Stegeman (70) has shown

| Table 12. Effect of pre-administration of $\beta$-naphthoflavone (BNF) on the metabolism and disposition of $^{14}$C-labeled chemicals in rainbow trout.* |
|-------------------------------------------------|-------------------------------------------------|
| **Chemical** | **Tissue** | **Control** | **BNF-treated** |
| | | **Tissue level** | **Parent chemical metabolites, µg/g or ml** | **% Metabolites** | **Tissue level** | **Parent chemical metabolites, µg/g or ml** | **% Metabolites** |
| | | | | | | |
| 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 | 6.5 ± 0.2 | 0.97 ± 0.08 | |
| 2-Methylnaphthalene | 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 | |
| 1,2,4-Trichlorobenzene | Bile | 14.7 ± 0.8 | 65 | 87.5 ± 5.5 | 98 |
| | Muscle | 575 µg | 0.8 | 299 µg | 2.1 |
| | Liver | 22 µg | 3.7 | 42 µg | 6.2 |
| | Blood | 2.01 ± 0.12 | | 1.03 ± 0.04 | |

*Data from Melancon and Lech (47).
higher levels of benzo(a)pyrene hydroxylase activity and cytochrome P-450 in killifish (*Fundulus heteroclitus*) from Wild Harbor Marsh, the site of a 1969 oil spill, than in fish from "uncontaminated" reference marshes. In this instance, the elevated enzyme activities are presumed to be related to the oil spill which occurred 8 years before the enzyme assays. In any event, such observations as these support the hypothesis that induction of the microsomal mixed-function oxidase system may eventually be used to detect certain classes of xenobiotics in the aquatic environment.

Due to the similarities between xenobiotic metabolism (including induction by polycyclic hydrocarbons) in fish and mammalian species, it is not surprising that similar toxicological responses are observed with some chemicals in fish and mammals. Thus, aflatoxin B1, a mycotoxin produced by various strains of *Aspergillus flavus*, is a potent hepatotoxin and hepatocarcinogen in rainbow trout (71) and the rat (72). Moreover, aflatoxin B1-2,3-oxide appears to be an ultimate carcinogen in both trout and rats (73), since aflatoxin B1 (2,3-dihydroaflatoxin B1), which cannot be metabolized directly to the epoxide derivative, is much less hepatotoxic in trout (71). Other potent precarcinogens for mammals, such as 2-acetylaminofluorene, 4-dimethylaminoazobenzene, and dimethylnitrosamine, also induce tumor formation in fish (74). Consequently, it would appear that certain types of neoplasia in wild fish populations may be related to exposure to environmental contaminants, which are also toxic to mammals. It should be stressed that carcinogenesis is only one possible end point for such studies. For example, fin erosion occurs in wild and captive populations of both freshwater and marine fishes, and at least in the case of the Dover sole (*Microstomus pacificus*) high incidences of the lesion are associated with polychlorinated pollutant discharge (75). In order for pathological conditions of wild fish species to be used as sentinel or early warning indicator systems for dangerous chemical pollutants in the aquatic environment, it will be necessary to characterize thoroughly the etiology of the disease process and to determine which chemicals cause the toxic response under carefully controlled experimental conditions. The dramatic alterations that enzyme induction can exert upon the rate of formation of both toxic and nontoxic metabolites, including changes in the metabolic profile, can be especially important in this context. Thus, induction of the microsomal mixed-function oxidase system can potentiate the toxicity by increasing the steady-state level of an electrophilic metabolite or exert an antagonistic (protective) effect by decreasing the steady-state level of the reactive intermediary metabolite(s).

The precise relationships between metabolism and toxicity, and metabolism, toxicity, and enzyme induction for specific xenobiotics in aquatic species will only be understood through detailed investigation. However, using similar studies in mammalian species as a precedent, it is quite clear that many interesting observations will be made due to the similarities between biotransformation systems in fish and mammals and because of the great number of aquatic species available for study.

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