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Aquatic Toxicology: Past, Present, and Prospects

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Aquatic organisms have played important roles as early warning and monitoring systems for pollutant burdens in our environment. However, they have significant potential to do even more, just as they have in basic biology where preparations like the squid axon have been essential tools in establishing physiological and biochemical mechanisms. This review provides a brief summary of the history of aquatic toxicology, focusing on the nature of aquatic contaminants, the levels of contamination in our waters, and the origins of these agents. It considers the features of the aquatic environment that determine the availability of xenobiotics to aquatic life and the fate of foreign chemicals within the organism. Finally, toxic effects are considered with primary emphasis on the potential of aquatic models to facilitate identification of the underlying mechanisms of toxicity.

Introduction

The waters of our planet constitute the ultimate sink for many of the chemicals produced and used by man. Aquatic toxicology determines the fate and effects of chemicals in organisms inhabiting these waters. Over the years, aquatic toxicology has played a number of important roles in our attempts to understand the consequences of xenobiotic release into the environment. In the 1940s, the evolution of aquatic toxicology as a formal discipline was tied closely to the development and use of the organochlorine pesticide DDT [1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethane] for it was soon observed that DDT application could result in fish and wildlife mortality (*1–3*). Over the next 2 decades, it became clear that acute toxicity was not the only concern and that low-level exposure could lead to marked accumulation of persistent pesticides with associated toxic symptoms, particularly in sessile organisms, e.g., oysters and mussels, that were unable to move away from a contaminated site. Indeed, "biological magnification" of 70,000-fold over the ambient water concentration was observed in controlled laboratory exposure of oysters to DDT (*2*). Such observations led to the initiation of a concerted effort to monitor bioavailable pollutants in the field through measurement of residues in oysters and mussels. This effort continues today in the Mussel Watch program of the National Oceanic and Atmospheric Administration (NOAA), although the types of chemicals measured and the analytical capabilities available have expanded significantly (*4*). Thus, for 25 years aquatic toxicology has played a central role in assessing environmental chemical exposure and, like the canary in the coal mine, has provided an early warning of toxic threat to the organisms, their ecosystem, and man.

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Acute and chronic toxicity studies in aquatic species have not only documented the susceptibility of individual species to a wide variey of pollutants (*5*), but also served to highlight a number of fundamental principals, e.g., bioaccumulation within the individual organism, biomagnification along the food chain, and the importance of the physical and chemical properties of each agent in determining the extent of both processes (*6*). More recently, research emphasis has changed somewhat as the search for "biomarkers" indicative of environmental hazards turned to biochemical and physiological indicators of pollutant stress and/or toxicity such as the formation of DNA adducts and induction of P-450 isozymes or metallothioneins (*5,7,8*). At the same time, there has been an increased awareness of the potential of some aquatic test systems to provide more rapid feedback on the effects of pollutants over the complete life cycle of an organism (*9,10*) and to assess population effects within an ecosystem, mesocosm, or microcosm (*11*). Finally, aquatic toxicology has begun to contribute to the important area of risk assessment. One aspect of this effort has been aimed at assessment of the risks posed by human consumption of contaminated aquatic foodstuffs (*12*). There has also been considerable interest in the use of aquatic organisms as alternatives for traditional carcinogenicity testing because the aquatic models have the potential to save both time and money (*13,14*).

Each of these areas has received considerable attention over the years. However, there is an additional area in which aquatic organisms have a great deal of untapped potential, i.e., as models with which to define the mechanisms through which xenobiotics exert their toxic effects. As stated by Lederberg (*15,16*) more than a decade ago, unless the mechanisms of toxic action are understood, prediction of environmental and human toxicity must remain largely an empirical, hit-or-miss proposition. He argued that utilization of a greater variety of species and models would facilitate identification of the underlying general principles that determine how various agents, or families of agents, exert

their toxicity. Thus, comparative toxicology in general, and aquatic toxicology in particular, have a great deal to offer in the search for toxic mechanisms. With the unique specializations that permit them to cope with ion regulation, respiration, chemical signaling, and reproduction in the aquatic environment, aquatic organisms provide us with an array of models with which to address fundamental mechanistic questions about toxic agents and ease the task of extrapolating effects in a few species to effects in the environment at large and man in particular.

Such a notion is by no means new to biology where use of nonmammalian models has long proven vital to research in such diverse fields as neurophysiology, renal function, and developmental biology (*17–20*). However, in toxicology less attention has been given to the potential of such nonstandard models. To be sure, a substantial body of data has been generated on the acute toxicity of many agents in fish and aquatic invertebrates (*5*), but the focus of the bulk of this work has been largely on the extent and nature of toxicity, rather than on the underlying mechanisms of toxicity. Fortunately, this is changing. For example, McKim (*21*) has begun a systematic assessment of structure–activity relationships in the toxicity of several classes of agents toward teleost fish.

The sections that follow examine *a*) the nature, origins, and extent of contamination found in our streams, lakes, and oceans; *b*) the accumulation of foreign chemicals by aquatic organisms and their fate within the organism; and *c*) the nature of toxic effects observed in aquatic life, focusing on a few specific examples that illustrate the usefulness, or potential, of aquatic organisms in the search for the underlying mechanisms that ultimately determine toxicity. Because of the breadth of these topics, each area has been summarized briefly, providing representative data and citing reviews where possible to facilitate exploration of specific topics in greater depth.

Exposure and Accumulation

The aquatic environment has been the recipient of a vast array of chemicals. These include the halogenated hydrocarbons (e.g., polychlorinated biphenyls [PCBs], dioxins, hexachlorobenzene), an array of pesticides (DDT, dieldrin, mirex), a wide spectrum of polycyclic compounds (particularly polycyclic aromatic hydrocarbons [PAHs]), and a number of metals (lead, mercury, copper, arsenic) (*4,5,22*). For example, Figure 1 depicts the spectrum of PAHs found in coastal sediments from around the United States and in the mollusks associated with them. In addition, these sediments contained many of the other pollutants listed above (*4*). As new chemicals are used and as analytical techniques are refined, still more agents are routinely detected. Although there is little doubt about their presence in the water column, the sediments, and even the air–water interface, it is far less certain how they get there and how available they are to the plants and animals of the aquatic environment. Certainly, point source discharges are important for many chemicals, but runoff from urban and agricultural areas also make major contributions. Atmospheric deposition is also important for a number of organic and inorganic pollutants (*23*). Indeed, it was estimated that in recent years, as point sources have been reduced, as much as 50% of the PCBs that reach the Great Lakes may now come from the atmosphere (*24*). Similarly, much of the lead reaching the open oceanic waters was carried by the air, apparently be-

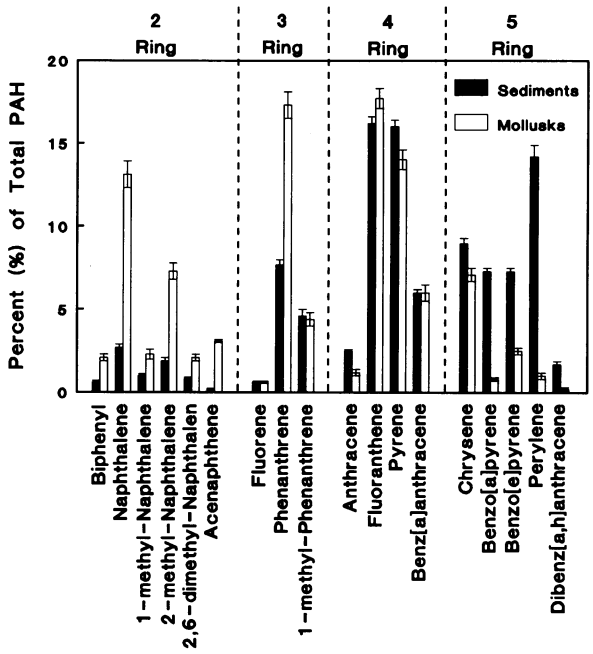

FIGURE 1. The average (± SEM) contribution of a spectrum of individual polyaromatic hydrocarbons to the total burden of marine sediments (n = 1032) and mollusks (n = 990) living in those sediments. Data are from the National Status and Trends database. Adapted from O'Connor (*4*).

cause lead binds readily to particulates and may be carried into the atmosphere bound to components in dust and smoke, whereas other metals, (e.g., chromium and nickel), which did not bind to particulates so effectively, could not use this route as effectively. Other studies indicated that approximately one-third of the cadmium delivered to the ocean was carried by the atmosphere, with the remainder being carried by the rivers (*25*). The dynamics of pollutant input and the impact of physical and chemical properties on this process are the subject of considerable current interest and uncertainty.

Once chemicals reach the aquatic environment, what determines their availability to aquatic organisms? This is far from a simple question because the form of a given agent may be greatly modified by physical, chemical, and/or biological events once it reaches the aquatic environment. For example, salinity, pH, and temperature, as well as types and quantities of dissolved organics (humates, hydroxamates) and particulates (clays, detritus) all affect the speciation of metals, giving rise to a dynamic equilibrium between metal ions, dissolved metal, and organic and inorganic complexes (Fig. 2) (*26*). In turn, because metal toxicity apparently derives largely, if not exclusively, from the free metal ions, speciation greatly alters both uptake and toxicity of metals (*27–29*). Similarly, organic chemicals partition between dissolved and particulate forms (*30*), and concentrations of many organic pollutants in the sediments exceed those in the water column by several orders of magnitude (*4,5*). Thus, factors that influence this relationship (e.g., salinity, pH, competing chemicals) may profoundly alter toxicity. Likewise, alteration of the chemical form of contaminants by biological or physical means, such as methylation of metals or photooxidation of organic xenobiotics, may greatly alter their availability through changes

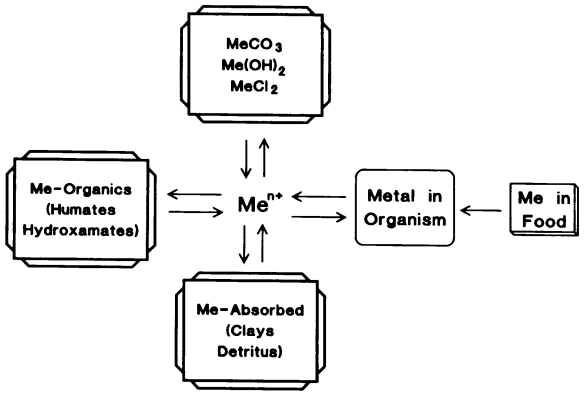

FIGURE 2. Factors influencing the availablility of metals (Me) to aquatic life. Adapted from Engel et al. (*26*).

in their solubility or reactivity (*30*). These considerations make the physical chemistry of the particulate–water interface a prime determinant of the availability and resulting toxicity of waterborne chemicals, albeit one which, because of the complexity of the microenvironment within the sediments and the shear number of chemicals involved, is as yet incompletely understood. Nevertheless, considerable progress has been made in this area as investigators have begun to develop quantitative relationships between variables including water solubility, lipid solubility, octanol/water partition coefficient (K_{ow}), and organic collid concentrations in the water column or interstitial water to predict concentrations of free and bound xenobiotics [see Farrington (*30*) for a review of this complex area]. Recently, it has been shown that the air–water interface is another site at which pollutants may be concentrated (as much as 1000 times the concentration in the rest of the water column), with resulting increased risk of toxicity in those organisms or larval stages inhabiting this microlayer (*5,31,32*). Finally, because so much of the pollutant burden of an aquatic ecosystem is associated with its sediments, seasonal or climatic events such as floods and storms may greatly alter their availability, as can the activity of biota that may physically disturb the sediments or may, as prey, themselves serve as pollutant vectors through the food chain (*30*).

Many of the same features that influence the distribution of toxicants between the water column and the sediments also play significant roles in determining their bioconcentration within aquatic organisms (*30*). For organic molecules, our current understanding of accumulation derives from the equilibrium exchange hypothesis of Hamelink et al. (*6,33*), which proposed that partitioning of chemicals between water and organism was determined by the ease with which they cross biological membranes, particularly gill and integument. Because membranes are composed largely of lipids, this hypothesis predicted that, in general, the higher the lipid solubility, the greater the uptake. Thus, bioconcentration factors, i.e., the concentration in the organism divided by the water concentration, should bear some predictable relationship to the lipid solubility of the chemical. As summarized in recent reviews (*6,30*), this approach has proven to predict the accumulation of a number of organic chemicals, giving rise to specific equations relating bioconcentration to lipid solubility, usually expressed as the K_{ow}. Of course, as discussed in these reviews, other factors such as availability in the sediments, chemical form, excretion rate, and metabolism all modify the basic relationship between K_{ow} and bioconcentration. Nevertheless, this notion provides a very useful first approximation of likely body burden in aquatic organisms and thus of the potential toxicity. Indeed, both theoretical predictions and actual measured values demonstrate that accumulation of lipophilic xenobiotics such as the PCBs may be as great as 10^5 times the water column concentration (*5*). Even when compared to sediment xenobiotic concentrations, tissue levels are often considerably greater for some classes of chemicals. For example, in mollusks, PCBs averaged 9 times higher than the sediments upon which they were reared. Total DDT-related chemicals were concentrated even more, 22 times, whereas tissue concentrations of total PAHs were essentially identical to those found in the sediments, and for high molecular weight PAHs, tissue levels were only 64% of the sediment values (*4*). For metals, it appears that both organic and inorganic metal complexes are poorly absorbed, apparently because they are either too large or too polar to cross membranes readily (*29*). Thus, both uptake and toxicity reflect the free ion concentration in the water. However, events such as methylation by microorganisms may greatly increase their lipid solubility, thus augmenting their absorption and altering their distribution and toxicity within the organism (*34,35*). Overall, even though the details of the uptake of specific chemicals from the aquatic environment may vary, and, indeed, in some cases are as yet unknown, it is clear that biological uptake is primarily determined by the physical and chemical properties of the chemicals themselves and the barrier function of biological membranes. Anything that increases the free concentration of the agents in water or reduces the effectiveness of the membrane barriers will promote uptake and increase the potential for toxicity.

Direct exposure from the water is not the only means of xenobiotic uptake. Consumption of contaminated food organisms by predators carries with it the potential for increased pollutant exposure and accumulation. This phenomenon, termed biomagnification, was observed by Woodwell (*36*) in an aquatic ecosystem 25 years ago (Fig. 3), and there are now numerous examples of increased pollutant burdens higher in the food chain (*5*). A classic example of bioconcentration in aquatic ecosystems and its potential for serious toxicological consequences at higher trophic levels was the accumulation of DDT and its metabolites by fish-eating birds, including the bald eagle (*37,38*). Not only were residue levels in these top predators much greater than in their prey, but a number of eagles, hawks, and marine birds began to suffer severe population declines. The basis for this decline was subsequently shown to be the production of thin-shelled eggs, which broke easily, markedly reducing reproductive success in the affected birds. Much of the data supporting this sequence of events were recently reviewed in a comprehensive environmental risk assessment analysis by Colburn (*39*). This example is of particular historic significance, for it provided the first documented example of the effects of chronic, relatively low-level contamination of the environment at large, as opposed to acute toxicity following isolated local release of chemicals. As such, eggshell thinning gave an early warning that played a major role in increasing both scientific and public awareness of, and concern for, environmental issues.

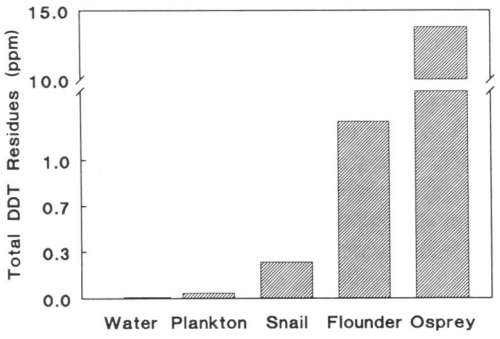

FIGURE 3. Relative tissue concentrations of total DDT in four organisms representative of increasing trophic levels within a salt march ecosystem. Note that tissue concentrations rise at higher levels in the food chain, an example of biomagnification. Data from Woodwell et al. (*36*).

Upon uptake into the organism, whether from the water or the food, foreign chemicals first enter plasma water. Their subsequent distribution within aquatic animals is determined by the same pharmacokinetic principles that govern distribution in mammals (*40–42*). Initially, the rate of distribution to specific tissues is determined by the regional blood flow through each tissue and the ease with which the agent enters the cells. Thus, organs with high blood flow such as liver and kidney tend to accumulate xenobiotics most readily. Likewise, small water- soluble molecules or those with significant lipid solubility penetrate most easily. In addition, factors such as plasma protein binding, affinity for specialized cellular uptake mechanisms, metabolism, and excretion all affect the ultimate pattern of distribution, retention, and toxicity. Although some of these parameters are known for specific aquatic animals and xenobiotics, many gaps in our knowledge remain. The cogent summary of Malins and Ostrander in their recent review is particularly apt (*5*):

> ... both bioconcentration and biomagnification of xenobiotics in aquatic species are multifaceted due to the myriad of compounds, their potential synergisms and antagonisms, and routes of exposure. Based on the weight of similar mammalian studies, the resultant high body burdens of these compounds can be expected to predispose organisms to a variety of potentially deleterious biological effects. Regrettably, among aquatic species, very little is known about these processes. Consequently, future studies should attempt to relate body burden(s) of xenobiotics and their biotransformation products to specific toxicological effects.

Metabolism and Excretion

In general, biotransformation of foreign chemicals serves two purposes: to render them less toxic and/or to make them easier to excrete. Particularly for lipid soluble xenobiotics, such as PCBs and PAHs, that accumulate to high levels in aquatic animals, metabolism is critical. Unless they are metabolized to more polar, more water-soluble forms, their elimination from the animal will be extremely limited, increasing expression of their toxicity (*43,44*). Nevertheless, it was widely held, as late as 1960, that aquatic organisms had little or no capacity to metabolize foreign chemicals. However, the pioneering work of Williams (*45,46*) set this notion to rest. Since that time, virtually every pathway used by terrestrial mammals in the metabolic transformation of foreign chemicals has been demonstrated in both aquatic vertebrates and invertebrates (*40,47,48*). Furthermore, the extent and nature of biotransformation reactions often profoundly influence the distribution, retention, and toxicity of xenobiotics in aquatic species, just as in terrestrial mammals (*5,40,49*).

In all animals, biotransformation of foreign chemicals may be divided into two components. The phase I reactions catalyze the addition (via oxidation or reduction) or unmasking (via hydrolysis) of functional groups. The cytochrome P-450 mixed-function oxidase (MFO) system and the related flavine monooxygenase (FMO) system are by far the most important of the phase I enzymes (*5,47*). In general, phase I reactions render the xenobiotic more polar, increasing its water solubility and potential for excretion. These oxidation products may also be less toxic than the parent compounds (i.e., detoxication). However, in a significant number of instances, the products may be even more reactive and more toxic than the parent [i.e., metabolic activation or toxication (*50*)]. Thus, the primary role of phase II, or conjugation, reactions is to reduce toxicity through addition of a variety of chemical moieties to reactive functional groups, masking or altering their activity (*48*). Phase II reactions include glycosylation, sulfation, mercapturic acid formation, amino acid conjugation, and acetylation. Not only are most phase II metabolites less toxic in their own right, but in many instances they are substrates for carrier-mediated excretion by the liver or kidney (*51,52*). Nevertheless, as is the case for phase I reactions, some conjugated xenobiotics are toxic to aquatic organisms [reviewed in James (*48*)].

In these general features, there are few differences between aquatic species and mammals. Thus, the primary research need in the biotransformation of xenobiotics by aquatic animals is a broader understanding of the tissue distribution, rates, specificities, and products of the responsible enzymes and their relationship to specific toxic effects. In particular, the recent advances in the purification of specific isozymes and molecular biological characterization of their properties, distribution, and development should greatly facilitate such studies (*7,47,53*). In addition, however, there are several specific features of aquatic organisms that may be exploited to increase our understanding of xenobiotic biotransformation and its impact on toxicity to the animals themselves or those predators that may consume them. One obvious difference is that invertebrates and fish are cold blooded. Thus, temperature change will alter rates of xenobiotic metabolism, facilitating assessment of the impact of metabolism on toxicity in the field and the laboratory. Additional more specific differences have begun to be exploited in the study of cytochrome P-450-mediated xenobiotic metabolism. It has been known for some time that teleost fish metabolize benzo[*a*]pyrene to an array of metabolites that differs from mammals in the proportionately greater production of epoxides at the 7,8 and 9,10 positions (*47,50,54–56*), metabolites proximate to the putative ultimate carcinogenic form of benzo[*a*]pyrene (*57*). These studies implied a significantly greater probability of cancer in those fish and those who consume the fish, including man. Consistent with this possiblity was the greater frequency of DNA adduct formation in fish than in rats following equivalent doses of benzo[*a*]pyrene (*58*). It now appears that these differences in metabolite pattern relate to the relative proportions of specific P-450 isozymes. The major P-450 isozyme that is induced in fish by the 3-methylcholanthrene (3-MC) class of inducers (3-MC,

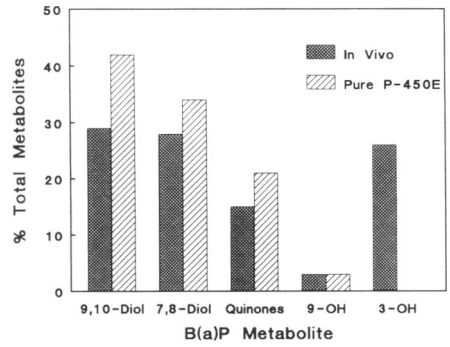

FIGURE 4. A comparison of the metabolite profile obtained in fish liver following *in vivo* exposure to benzo[*a*]pyrene with that produced by metabolism of benzo[*a*]pyrene by purified P-450E *in vitro*. Epoxide hydrolase was added to the *in vitro* reaction mixture to hydrolyse epoxides, since this occurs *in vivo*. Note the preponderance of metabolites at the 7,8 and 9,10 positions on the benzo[*a*]pyrene ring, both *in vivo* and *in vitro*. Data from Stegeman and Lech (*50*).

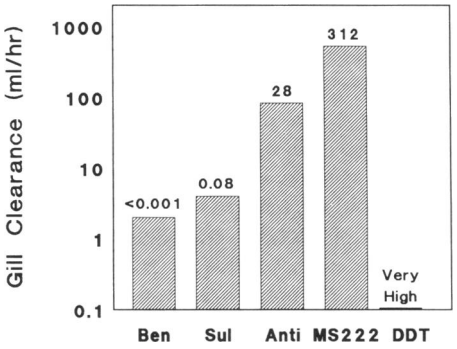

FIGURE 5. Influence of increasing lipid solubility on the gill excretion of several drugs by the dogfish shark. Ben, benzolamide; Sul, sulfanilamide; Anti, antipyrine; MS222, tricaine methane sulfonate. As discussed in the text, increasing lipid solubility increases the ease with which the drugs cross gill epithelial membranes. However, DDT has such a low water solubility that its clearance is essentially zero, since it cannot leave the gill and enter the surrounding water. Values at the top of each bar indicate relative lipid solubility. Data were obtained from Maren and associates (*63,64*).

PCB, β-naphthoflavone), is P-450E [as characterized in scup, which is apparently equivalent to P-450 LM_{4b} of trout and P-450c of cod; reviewed Stegeman and Kloepper-Sams (*47*)]. This isozyme is present at low levels in uninduced fish, but increases sharply upon exposure to 3-MC type inducers. As judged by the metabolites formed by the purified enzyme *in vitro* (Fig. 4), it appears to be responsible for production of the toxic epoxides of benzo[*a*]pyrene in fish (*47,53*). In mammals, the equivalent isozyme (e.g., P-450c of rat) is also found. However, other isozymes are present. Furthermore, induction with phenobarbital-type inducers (which do not induce effectively in fish) produces a different array of isozymes and of metabolites, many of which are not as reactive as those produced by P-450E or its mammalian counterpart (*47,50,59*). Thus, studies in fish promise to be useful in attempts to define the roles of specific isozymes and the ways in which their functions are regulated.

Like many of the processes discussed above, excretion of xenobiotics or xenobiotic metabolites is once again critically dependent on the physical and chemical properties of the molecule (*43,44,60,61*). This is as true for passive excretion across the gill as it is for active transport in the liver or the kidney. Because the integument of aquatic species is in intimate contact with the water, it has the potential to play a significant role in xenobiotic excretion. In practice, however, most surface membranes in aquatic species are specialized to minimize water and solute flux (*62*). Thus, the bulk of xenobiotic uptake or depuration across the surface of aquatic organisms takes place across the respiratory surfaces of the gill where such barriers are reduced to permit efficient gas exchange. Respiratory excretion of CO_2 and NH_3 is very efficient because it takes advantage of unique features of these gases. Both are sufficiently small and lipid soluble so that they diffuse rapidly across the epithelium of the gitl, yet they are water soluble as well, so they are able to enter the aqueous environment around the gill (*60*). Few xenobiotics have the necessary solubility in both phases to effectively use this route. Large, water-soluble agents do not cross the gill membrane readily. More lipophilic agents, like DDT, can cross the membrane, but are not water soluble enough to partition readily into the surrounding water. There are a few agents, e.g., the fish anesthetic tricaine methane sulfonate, which are adequately soluble in both media. They readily cross the gill in either direction (*63*). As shown in Figure 5, the clearance of this anesthetic across the gills of the dogfish shark was several hundred times greater than that of more polar drugs (e.g., benzolamide) and more than five times that of drugs of intermediate water solubility (antipyrine). On the other hand, in the same experimental system, clearance of DDT (very hydrophobic) by the gill was so limited that it could not be detected (*64*).

For most xenobiotics, hepatic and renal excretion are much more effective than the gill clearance (*40*). Although the basic mechanisms of biliary excretion in fish are not yet as well characterized as in mammals (*65*), it is clear that xenobiotics, particularly those over 500 in molecular weight are excreted into the bile. In fact, pollutants and their metabolites are often highly concentrated in fish bile and several authors have proposed the use of bile as means to monitor the extent of environmental contamination (*40*). In contrast, the invertebrate hepatopancreas does not appear to be an effective route of xenobiotic excretion (*66*). Lipophilic xenobiotics do accumulate in hepatopancreas, but it apparently retains them. This may relate to the anatomy of the gland, which is a diverticulum off the stomach. It is composed of a branching series of blind ductules, whose primary function seems to be absorption of nutrients (*67*). Although there is flux of gastric contents into this system of tubules, apparently much of this fluid is absorbed there. The quantitative contribution of fluid reflux back to the stomach is uncertain. Thus, it is not clear whether it could carry xenobiotics excreted by the hepatopancreas back into the main axis of the gastrointestinal tract for excretion from the animal. In addition, preliminary experiments using isolated luminal membranes from lobster hepatopancreas indicated that carrier-mediated secretion did not move anionic xenobiotics into the lumen of the ductular system (*66*). Instead, weak acids appeared to be sequestered within the cells of the hepatopancreas by virtue of the steep pH gradient [$pH4_{out} \leq 4$ vs $pH_{in} > 7$; (*68*)] that traps the ionized form of the xenobiotic in the cell and promotes passive reabsorption of those anionic xenobiotics that were able to reach the lumen of the hepatopancreatic tubular system.

Renal excretion of xenobiotics by fish and mammals has been reviewed several times in recent years (*43,61,69*). Compounds to be excreted enter the lumen of the renal tubule via ultrafiltration of plasma at the glomerulus. Additional modification of the primary filtrate by active secretion of organic anions into the lumen or passive reabsorption of lipophilic molecules occurs during its passage through the tubule. Because the kidney acts only on the fraction of the xenobiotic present in the plasma, several factors limit excretion of lipid-soluble chemicals. First, lipid-soluble chemicals tend to be sequestered in the tissues; thus, their plasma concentration is low. Furthermore, even the small quantities of lipophilic agents that are present in the plasma are often tightly bound to plasma proteins, further reducing their availability for filtration and/or active tubular secretion. Finally, because they cross plasma membranes so readily, any lipid-soluble chemical that does reach tubular fluid may be passively reabsorbed as its concentration in tubular fluid rises secondary to the reabsorption of water. On the other hand, if the xenobiotic or its metabolites are substrates for active tubular secretion, it may be cleared at much greater rates. In fact, clearance of a good substrate for either renal organic anion or organic cation secretion may approach the entire renal blood flow (*17*). Because metabolism increases both the water solubility of lipophilic agents and in many instances converts them to anions or cations that may be actively secreted, metabolism must play a central role in determining the efficacy of excretion for many xenobiotics.

Recent studies in flounder comparing the renal handling of several phase I metabolites of benzo[*a*]pyrene (BaP) illustrate the impact of these interactions (*52*). The flounder was chosen for these experiments because it, like other teleost fish, has a renal portal system that increases relative perfusion of its renal tubules and amplifies the contribution of tubular secretion, greatly facilitating assessment of the renal handling of specific metabolites (*52*). These studies demonstrated that individual BaP metabolites were excreted at very different rates (BaP-phenols were cleared 10 times faster than BaP itself, and BaP-7,8-dihydrodiol was cleared 10 times faster still [~30 times the glomerular filtration rate) (Fig. 6). The basis for the rapid excretion of certain metabolites was tubular secretion (as their sulfate and glucuronide conjugates) via the renal organic anion transport system. Because excretion rate was determined by organic anion transport, inhibitors of this system, including other anionic xenobiotics, were able to markedly reduce excretion of BaP and its metabolites. Thus, these results raise the possibility that one pollutant, even if relatively nontoxic itself, may reduce excretion of another, enhancing its retention and possibly its toxicity. Clearly, accurate prediction of xenobiotic retention and toxicity are possible only when both metabolism and excretion of a given xenobiotic and its metabolites are known.

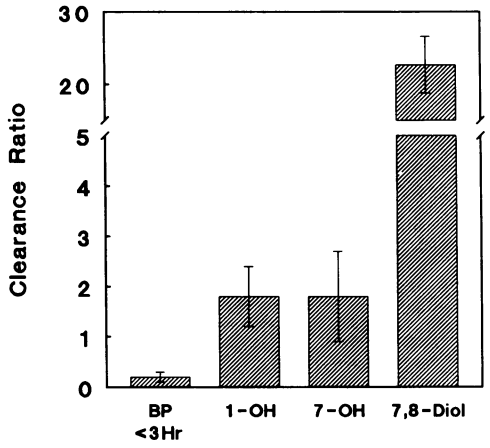

FIGURE 6. Relative renal clearance of benzo[*a*]pyrene (BaP) and its metabolites by southern flounder. Results are expressed as the clearance ratio (clarance of the metabolite/glomerular filtration rate). A clearance ratio of less than 1 indicates net reabsorption of the compound. Clearances greater than 1 indicate net tubular secretion. Note the widely different excretion of the different metabolites. BaP clearance was determined over the first 3 hr to minimize the contribution of its conversion to other, more rapidly excreted metabolites. See Pritchard and Bend (*52*) for details. 1-OH, BaP-1-phenol; 7-OH, BaP-7-phenol; 7,8-diol, BaP-7,8-*trans*-dihydrodiol.

Effects and Experimental Models

At its outset, aquatic toxicology was primarily concerned with the acute toxicity of chemicals. Its focus later broadened to include chronic toxicity and sublethal effects as well, particularly in the search for reliable markers for the extent and nature of pollution in the field. As recently reviewed by Malins and Ostrander (*5*), each of these aspects continues today, but with an increased emphasis on end points other than death and on determining the mechanisms of the effects observed. What does not seem to be widely appreciated is the potential of aquatic organisms as models to speed the search for the general principles and mechanisms of toxicity (*15,16*). As summarized by Kleinzeller (*70*) in his recent review, many of the most significant advances in our understanding of basic physiological principles have been intimately tied to the selection of an appropriate experimental model. This experience argues that there are particular animals in which any given function (or disruption of that function) may be studied more easily and decisively. Our challenge is to find those model systems. Aquatic organisms provide numerous opportunities for fruition of such a search.

In general, the aquatic model systems that have been used in studies of basic biology or toxicology have taken advantage of unique features of the model that facilitate experimental manipulation. Some have used anatomical specializations of the tissue, such as the giant axons of the squid and crayfish for neurophysiology (*71*) and the huge muscle fibers of the barnacle for studies of ion and pH regulation (*72*). Similarly, the aglomerular kidney of certain marine teleosts (*17*) and the rectal gland of the shark (*70*) have provided effective models for evaluation of the mechanisms and control of epithelial transport. In other systems the novel advantage may be the accessibility of the site of interest, e.g., external location of purinergic receptors in the lobster (*73*) and the external fertilization and development of urchins, ascidians, and teleosts (*74–76*). In the remainder of this paper, I discuss examples in which aquatic organisms have contributed to the search for toxic mechanisms and/or where they have significant potential. Only a few examples can be treated in detail here, but recent publications highlight additional examples (*77–80*).

Chemical Carcinogenesis

The presence of tumors in some wild-caught fish have been reported with increasing frequency since the 1940s (*81–93*).

In the late 1950s, hatchery trout were found with liver tumors. Subsequent study demonstrated that their food was contaminated with a potent natural carcinogen, aflatoxin, and that tumors could be produced in rainbow trout upon laboratory exposure to aflatoxin [reviewed in Sinnhuber et al. (*84*)]. The finding of tumors in the field and the laboratory led to the suggestion by Dawe (*85*) in 1964 that at least some of the fish cancers in the wild might have been caused by environmental pollutant(s). Since that time there has been a great deal of interest in this area. Known carcinogens and promoters were concentrated in the tissues of wild-caught fish from contaminated areas, and these fish had higher incidences of tumors than fish from less polluted areas (*22,55,56,86*). Laboratory studies have demonstrated the ability of many of these same agents to cause or promote the development of cancers in fish (*87*). Similarly, exposure to extracts prepared from polluted sediments caused cancer development in cultured fish (*55,56*). Furthermore, fish exposed to specific carcinogens or extracts from polluted sediments in the laboratory or to a spectrum of chemicals in the wild also demonstrated both induction of the drug metabolizing enzymes (*50*) and increased formation of DNA and protein adducts (*56,88*).

Clearly, these results are in keeping with the tradition of aquatic organisms as early warning systems of danger to our environment. They raise concerns about the health of aquatic ecosystems and the potential for consumption of contaminated aquatic foodstuffs to serve as a vector for xenobiotics and their activated metabolites to man (*12*). Such concerns have led a number of groups to suggest that the presence of induced levels of P-450 isozymes [reviewed in Stegeman and Lech (*50*)] or of DNA adducts (*88*) might serve as monitoring tools indicating elevated levels of dangerous chemicals in the environment. These suggestions, like those for the use of fish bile as a monitoring tool, deserve serious consideration in any strategy to identify biomarkers that warn of dangerous levels of pollutants. However, one must be careful in attempting to use such markers. As noted by Dunn (*88*), although the presence of activated metabolites and DNA adducts may indicate a serious threat to that organism, consumption of those animals as food may not pose a similar threat. Indeed, a variety of evidence indicates that once reactive metabolites bind to DNA or proteins, they are no longer readily available to subsequent consumers. Thus, paradoxically, it may well be that the mollusks that metabolize foreign chemicals more slowly than fish (and show fewer reactive products and fewer adducts), may have a greater potential to pass along bioavailable forms of pollutants (*88*).

Aquatic organisms also provide special advantages for both carcinogenicity testing and more basic investigations into cancer mechanisms. As discussed above, differences in isozyme pattern and induction have significant potential in efforts to understand the relationship between metabolism and carcinogenesis. A number of special features also facilitate their use in testing (*13,14,84,87*). Fish are sensitive to chemical carcinogens, developing tumors in a variety of tissues. They are easy to breed and maintain, both reducing cost and providing access to all stages in their life cycles. Fish also offer unique opportunities to evaluate genetic contributions to carcinogen sensitivity (*14*). Finally, fish have a number of practical advantages for application of transgenic technology. Fish and aquatic invertebrates produce large numbers of semitransparent eggs. Both fertilization and development are external, greatly easing technical problems faced by those working with mammalian systems (*89–91*). The possibilities for addition of specific functional genes (e.g., oncogenes) or genetic constructs designed for specific purposes such as monitoring of mutation frequency are myriad and exciting.

Membrane Toxicity

Because of their exposed location and functional importance, biological membranes are likely targets for toxic agents (*92*). Aquatic organisms provide several examples of this important toxic mechanism. As discussed above, eggshell thinning of top predators in aquatic ecosystems (bald eagles, pelicans) was an early indicator of the potential detrimental effects of persistent organochlorine compounds. Membrane toxicity apparently underlies this effect. In sensitive species, as shown by Kinter and colleagues (*93,94*), DDT metabolites effectively inhibited calcium-ATPase, an integral membrane transport protein that is required for shell deposition by the avian oviduct epithelium. In contrast, Ca-ATPase from the chicken, a species that does not show eggshell thinning, was far less sensitive. Similarly, a variety of studies indicate that the Na,K-ATPase, or sodium pump, is also sensitive to xenobiotics (*5*). Because the sodium pump is responsible for osmoregulatory salt transport in fish and invertebrates, osmoregulatoy failure has often been observed in xenobiotic-exposed aquatic animals [reviewed by Health (*95*)]

Recent studies provide interesting variations on the theme of membrane toxicity. The data of Exley et al. (*96*) suggest that aluminum toxicity has two components, both membrane related. Initial binding to the apical (external) face of gill epithelial cells leads to an increase in apical membrane permeability (loss of barrier function), followed by increased aluminum penetration into the cell interior where it inhibits Na,K-ATPase in the basolateral membrane. The combined effects of barrier loss and sodium pump inhibition lead to loss of osmoregulation (and ion regulation), with cell sloughing from the gill epithelium and, ultimately, death of the animal.

Cell signaling processes depend on membrane events at the cell surface (e.g., receptor binding and ion transport), as well as at internal organelles (e.g., release of calcium in response to inositol trisphosphate). As reviewed by Rossi et al. (*97*), these events may be disrupted by pollutants. Alteration of cell signaling has just begun to be considered as a toxic mechanism in any system, but it clearly has the potential for profound impact. Studies using sea urchin eggs figured prominently in development of our current understanding the cell signalling process (*74,97*). Thus, once again, aquatic models should prove to be effective tools for assessing the impact of xenobiotics on signalling, just as they have been in documenting the effects of ATPase inhibition.

Conclusions

Ever since its inception, aquatic toxicology has provided critical insights into the state of our environment and early warning of the hazards posed by environmental pollutants. These roles will certainly continue. However, thanks to the sheer diversity of aquatic life and its unique requirements, greater attention to aquatic animals as models should pay substantial dividends

in the form of increased understanding of the fundamental principles which underlie toxicity in all species.

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